ASPECTS OF THE BIOLOGY OF

BOOPHILUS DECOLORATUS (KOCH, 1844)

(ACARINA : IXODIDAE)

by

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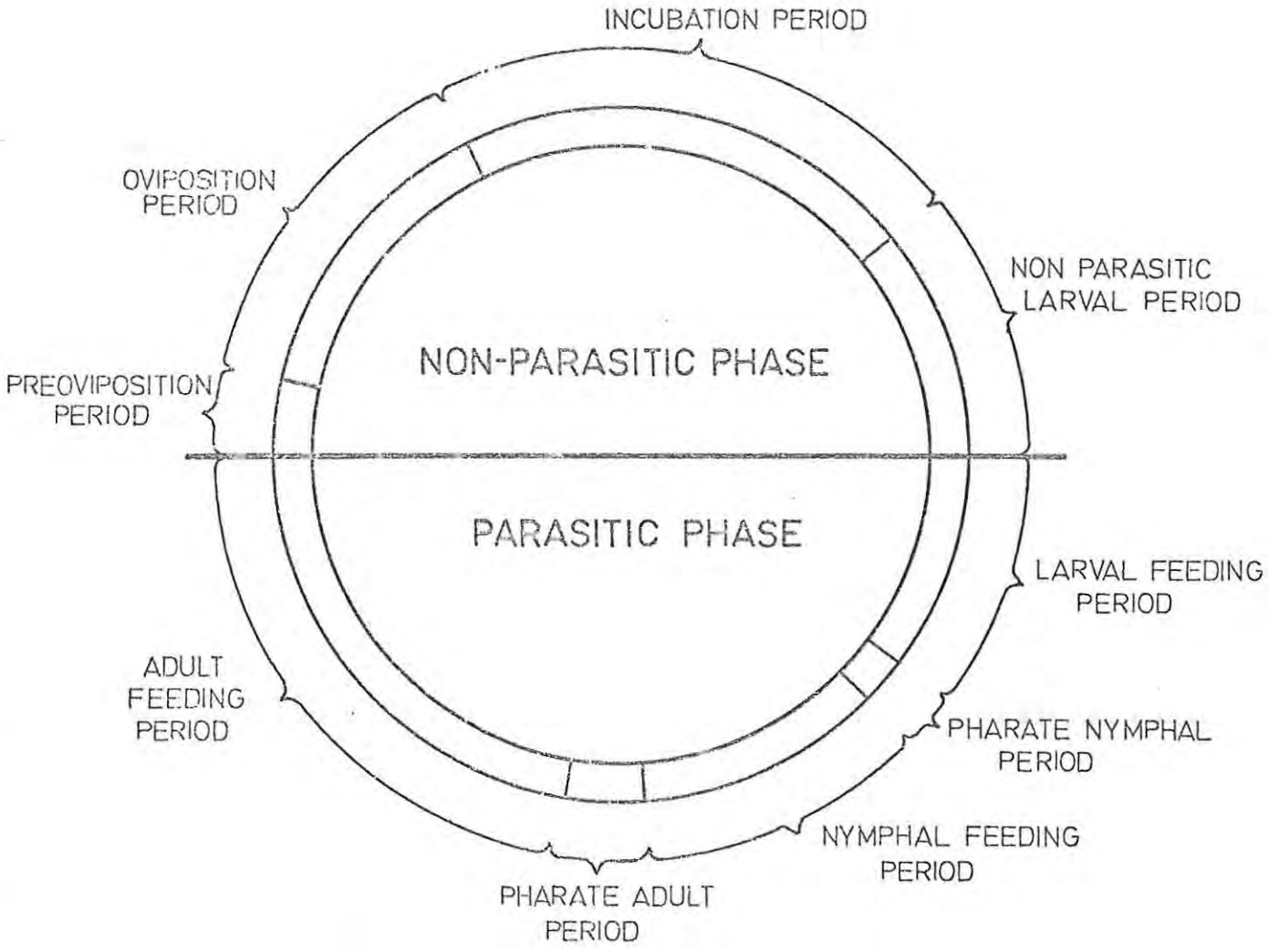
FRONTISPIECE

A diagramatic representation of the various phases which make up the life cycle of Boophilus decoloratus (Koch)



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Londt, J.G.H. and Whitehead, G.B. (1972) Ecological studies of larval ticks in South Africa (Acarina:Ixodidae). Parasitology 65: 469-490.

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1. INTRODUCTION

Southern Africa has a large number of economically important tick species. The eastern Cape Province is particularly rich in ticks, 47 being listed by Arthur (unpublished data) following a survey of the taxonomic literature on South African Ixodoidea. Of these 47 species only a relatively few appear to be of any real concern to livestock farmers. Among these are Boophilus decoloratus (Koch, 1844), Amblyomma hebraeum Koch 1844, Rhipicephalus evertsi Neumann 1897, Rhipicephalus appendiculatus Neumann 1901, Hyalomma rufipes Koch 1844, Ixodes rubicundus Neumann 1904 and Margaropus winthemi Karsch 1879. These species may transmit pathogenic organisms, cause paralysis or damage leather. The high costs of acaricides, coupled with the evolution of resistant tick populations, has made it imperative that biological and ecological work be undertaken on all known economically important tick species in an attempt to devise alternative methods of control. Despite the recognized significance of ticks as an economic problem in South Africa there is a lack of biological and ecological data of a quantitative nature. Apart from the work of Stampa (1959, 1969), Goldsmid (1967), Kraft (1961, 1971), Londt (1970), Londt and Whitehead (1972)[1], Bezuidenhout and Schneider (1972) and Arthur and Londt (1973) there has been little progress since the work of C.P. Lounsbury just before the turn of the century (eg. 1899). Theiler and her associates at Onderstepoort have produced extensive work on the taxonomy of a number of species and her Zoological Survey of ticks in the Republic of South Africa has formed an excellent basis for further ecological investigations.

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1. A copy of this paper, which resulted from the work of the author submitted for the M.Sc. degree at Rhodes University, has been included with this thesis (see inside back cover).

It is however qualitative and based largely on records from veterinary officers and there is an obvious need for adopting a quantitative approach to the many problems raised by this excellent study.

B.decoloratus, the Blue Tick, was selected for study for a number of reasons. B.decoloratus is one of the chief transmitters of piroplasms, Gall sickness (Anaplasmosis), Spirochaetosis and Tick Bite Fever in South Africa. Damage to leather caused by the scars following their bites is also of considerable importance to the leather industry. This tick completes its parasitic life cycle on a single host and is consequently an easy species to breed for experimental purposes. Being a 'one host species' implies, in theory, that it may be readily controlled by regular application of acaricides but this 'virtue' has been cancelled by the rapid evolution of acaricidal resistance. A feature paralleled by a similar phenomenon in the closely allied species Boophilus microplus (Canestrini, 1888) particularly in Australia. 'Strains' of B.decoloratus are now known which are resistant to arsenicals, chlorinated hydrocarbons and a number of the more recently used organophosphorus acaricides. Although the species can still be adequately kept under control by existing chemical controlling agents the threat of resistance is always present.

Studies on the egg stage were initially undertaken as an extension of previous work (Londt 1970, Londt and Whitehead 1972). Previously it was found that larvae of a number of different species including B.decoloratus were able to survive under laboratory conditions which were far more 'unfavourable' than the microclimatic conditions experienced in the field. This suggested that the distribution of larvae in the field was not dependent entirely on their own tolerance levels to environmental vicissitudes but that

microclimatic conditions might exercise significant limitations on the survival of egg laying females, their capabilities to produce eggs or on the eggs themselves. The present study was therefore largely concerned with the tolerances of these stages to the physical or abiotic factors of temperature and humidity. Apart from the non-parasitic larval stage, the egg stage and the period during which the engorged female tick produces eggs, all the stages in the life cycle of B.decoloratus occur in direct association with the host. Accordingly it was thought desirable to consider briefly certain aspects of the parasitic cycle to place the non-parasitic phases in perspective.

PART ONE

THE PARASITIC LIFE CYCLE OF *BOOPHILUS DECOLORATUS* (KOCH)

2. THE PARASITIC LIFE CYCLE[1]

Synopsis

Detailed studies of the morphology of the immature stages of B.decoloratus are sparse. The present work, using scanning electron microscopy, aims at clearing up misconceptions and considers homologies between larvae, nymphs and adults. The biological activities of this tick during its parasitic cycle on the host are examined with special reference to the course of feeding of all stages, and to the influence of the condition of the pharate nymph and pharate adult in accelerating the completion of the life cycle. The pattern of dropping of replete females is considered in relation to their weight and their time of drop-off.

2.1 INTRODUCTION

A number of workers (Minning 1934, Bedford 1934, Theiler 1943, Hoogstraal 1956, Arthur 1960, Gothe 1967a) have described and illustrated the adults and immature stages of B.decoloratus using conventional light microscopy. From experience gained by using scanning microscope techniques on the immature stages of the Bont Tick, A.hebraeum, (Arthur, 1973) and on representatives from other ixodid genera (Arthur, unpublished) it was considered expedient to apply them to studying all stages of B.decoloratus. These techniques are valuable in tick taxonomy in that the necessity for clearing and mounting specimens is eliminated and they reduce distortion, which occurs when either the whole or parts of the body are flattened. The need for this approach was emphasized when attempting to use the descriptions and illustrations prepared by Gothe (1967a) to separate

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1. Written in collaboration with Professor D.R. Arthur, who kindly agreed to assist in describing all the stages of B.decoloratus for inclusion in this thesis. The biological work was that of the author. This chapter has been published (Arthur and Londt, 1973), in an almost identical form, and a copy of this publication has been bound into this volume at the end of this chapter.

For the scanning electron micrographs used in the description of all stages of B. decoloratus (ie. Figs 1, 2, 6, 7, 8, 9, 10, 11 and 12) see reprint.

the immature stages of B.decoloratus from those of B.microplus, particularly as the distributions of these two species overlaps in parts of South Africa.

Information on the biology of B.decoloratus during its parasitic phase is sparse. Previous authors have shown that, except for digestion of the 'blood meal' by the female, the maturation of her gonads and egg laying, the life cycle is completed entirely on the host. The times given for this period on the host ranges from three weeks (Theiler, 1911), to a month (Lewis, 1939). Lounsbury (1905 - cited by Theiler, 1943) reported that the larva moults after 7 days on the host and the succeeding nymph moults after a further 7 days. The fully fed female leaves the host at about 23 days from the time of its attachment as a larva. Theiler (1943) repeats this information. There are neither data on the feeding period nor qualitative or quantitative assessments correlating size and age during the periods for which the various stages are feeding, as there is for B.microplus (Legg 1930, as B.australis; Hitchcock, 1955a; Kitaoka and Yajima 1958, as B.caudatus; Roberts, 1968). The use of tick length as a measure of size change in relation to age has been advocated by Roberts (1968), although he recognized that engorging and engorged nymphs were substantially affected by fixation and processing in hot alkali. More importantly, however, tick length does not take into account the periods of time during which the nymphs and the adults are in a pharate condition. To determine this requires more exact information on moulting periods.

2.2 MATERIALS AND METHODS

All the stages of B.decoloratus, used for description, were obtained from a culture in the Tick Research Unit of Rhodes University.

The specimens were preserved in 70% ethanol, dried in air and fixed to the specimen stubs either by colloidal silver paint or by 'Sellotape' dissolved in chloroform. No additional adhesives were necessary. A layer of gold (100-150 Å) was plated onto specimens in a Hitachi HUS 3B vacuum evaporator. The specimens were examined in a JEOL JSM U3 (Japan Electron Optics Ltd.) scanning electron microscope. Accelerating voltages of 15kV and 25kV were used, and the cathode ray tube images were recorded in a Graflex 2¼" x 2¼" camera using Ilford FP4 negative film and developed in Microphen developer. The nomenclature of the body setae adopted follows that of Clifford and Anastos (1960) and the palpal setae that of Arthur (1973).

For biological studies, larvae were reared from fully-fed females maintained in the laboratory at 26°C and about 90% relative humidity (R.H.). The larvae were kept under these conditions for about a month before being used to infest a Guernsey calf. Each infestation of the calf involved the use of about twenty thousand larvae, and these were distributed as uniformly as possible along the length of the calf's spine from behind the head to just above the base of the tail.

Daily observations were made on the length of time taken for each parasitic stage to moult on six occasions from March to October 1972. Before the fourth infestation a random sample of fifty larvae were weighed to ascertain the weight of unfed larvae. After the initial infestation a variable number of ticks were removed daily from the neck region of the calf, weighed, inserted in appropriate vials and placed in an incubator at 26°C and about 90% R.H. Daily collections of ticks were made up to the time when the first engorged females dropped from the calf. Thereafter only the dropped females were collected. Ticks, removed from the host, were placed in an

incubator and examined 10 days later. The numbers of moulted larvae and moulted nymphs were counted. For weighing larvae, nymphs and adult males a Cahn G-2 electro-balance was used and for adult females a Sartorius single pan balance. Temperatures and relative humidities in the stable, housing the calf, were recorded on a thermohygrograph throughout the investigation.

For determining the pharate condition, daily samples of the nymphs and adults were taken from the calf and cleared in lactic acid. Pharate nymphs and adults were recognized when their legs and coxae were readily discernible and when separation of the nymphal or adult cuticle from the preceeding stage was clearly defined under a light microscope.

2.3 OBSERVATIONS AND RESULTS

2.31 Description of larva

Unfed specimens rounded, about one and a third times as long as maximum breadth at about level of third pair of coxae (Fig. 1(1)).

Capitulum - dorsal. (Fig. 1(1)). Basis length (as measured from base of external cheliceral sheath to posterior margin) to maximum width ratio about 1:2; posterior margin of basis, behind level of basis sensilla, as a flattened bow-shape, thence straight for a very short distance becoming indented, at origin of 'palpiger'. Surface generally flattened except for an antero-lateral triangular elevation immediately behind palpal insertion; two widely separated sensilla at about one third of length of basis.

Palpi. (Fig. 2(1)). Short, from 0,084-0,091 mm long, tendency to be bulbous. No suture line between segments 1 and 2. Ratio of length of segment 2, measured along dorsal surface from its postero-mesial protuberance to 'suture' with segment 3, to maximum width about 0,9:1,0; that of segment 3 1,2:1,0. Lateral profile of combined segments 1 and 2 sinuous, mesial profile of 2 mildly convex; both lateral and mesial profiles of segment 3 almost straight and converging to broadly rounded apex. Surface of segment 2 strongly tumescent, protuberant meso-posteriorly, this continued obliquely antero-laterally and mesial of the basal lateral seta of segment 1 and terminating baso-laterally of anterior lateral seta of same segment; surface of segment 3 smooth, evenly and gently curved to the midline and laterally, but more steeply to its junction with segment 4. Setae arranged in three longitudinal series: mesial series (M) consisting of two setae, M1 on segment 2 barbed on outer face, sub-parallel and shorter than similarly barbed M2 seta on segment 3;

dorsal sub-median series (D) of four setae, D1 (vertical in position and accordingly much foreshortened in Fig. 2(1)) on segment 2, and D2 and D3 on segment 3 are multi-barbed, D4 of 'fine' tapered type; lateral series (L) of three setae, all single barbed, of which two (L1 and L2) located on segment 2 and one (L3) on segment 3.

Capitulum - ventral. (Fig. 2(2)). Basis transversely ovate; surface curved, sloping gently to hypostomal base and dipping more strongly to shallow, broad oblique depressions antero-laterally, beyond which the surface becomes slightly elevated to straight lateral margins.

Palpi. (Fig. 2(2)). No clear sutures between palpal segments 1 to 3; lateral profile of palps broadly arcuate, mesial profile only slightly so. Surface of palp with shallow irregular furrows basally; distal margin of segment 3 thickened and broader laterally than ventrally where it is flange-like; relative to the long axis this margin oblique and flange lacks any distinctive spur; extensive area of intersegmental membrane on mesial and distal face of segment 3 on which palpal segment 4 is mounted. Two setae on segment 2 of which ventral-mesial (VM) is 'feather-like' under light microscope, under scanning electron microscope consists of a stout base from which successive layers of barbs arise, deepest layer (i.e. the most dorsal) tapering to setal apex, and strongly serrated marginally (Fig. 2(3)); mid-ventral seta (MV) on this segment single barbed with bifid or trifid apex; third ventral seta lies on mid-ventral line adjacent to the distal thickening of segment 3, and is of the 'fine' type. Column of segment 4 bears three tapering setae, apex carries 1 dorsal, 3 sub-dorsal, 3 supra-ventral and 1 ventral setae, all rounded apically.

According to Gothe (1967a) the segments, other than the fourth, have "eight setae dorsally and four setae ventrally" and in this he is, doubtless, following the classification of Clifford and Anastos (1960). The reasons for the present palpal nomenclature are discussed elsewhere (Arthur, 1973).

Hypostome. (Fig. 2(2)). Length 0,082-0,090 mm, spatulate, apex broadly rounded extending beyond palpal apices; dentition behind small sub-apical corona 2/2 files with 6 denticles per file, distal three rows sharply pointed, proximal three rows rounded; two basal medial depressions, in line with two widely separated post-hypostomal setae.

Extrascutal region. (Fig. 1(1)). One pair of sensilla sagittiformia (S) peripherally on postero-lateral margin; seven or eight pairs of marginal-dorsal setae (Md1-7 or 8) of which Md1 to Md3 in a sub-marginal linear series, Md4-Md7 (or Md8 when present) in a progressively marginal series, Md1-Md5 in front of sensillum; two pairs of centro-dorsal setae (Cd1, 2) of which posterior pair significantly longer than anterior pair, and whose intersegmental distance is greater.

Scutum. (Fig. 1(1)). 1,26-1,28 times as broad as long, greatest width across level of eyes, latter bulging, protruding beyond edge and surrounded by well defined orbit; posterior margin narrowly rounded, postero-lateral margins very weakly concave or sometimes straight, antero-lateral margins convex, scapulae broad; emargination well defined. Surface reticulately patterned; cervical grooves short, shallow, parallel sided with two circular depressions mesial of each, surface between grooves mildly elevated

and beyond them sloping gradually to periphery. Scutal setae in three pairs (Sc1-3), Sc1 located anteriorly and laterally of cervical grooves, Sc3 in median field at about or slightly behind level of eyes, Sc2 at about two thirds the length of antero-lateral margin and a short distance inwards from it, in length Sc1 > Sc3 > Sc2. Two pairs of depressions, one pair in angle formed by scapular margins, the other more medianly placed in a line connecting eye and Sc2.

Ventral surface. (Fig. 1(2)). Surface, with fine epicuticular folds, longitudinal depression dorsal to coxal insertions and extending back to one third the distance from posterior edge. First pair of sternal setae (St1) on a level with first intercoxal space, second pair (St2) level with second intercoxal space, third pair (St3) posterior to third pair of coxae; relative lengths being St3 > St2 > St1, distance between setae of each pair increasing from St1 to St3. Two pairs of preanal setae (Pa1, 2), distance between anterior pair (Pa1) greater than that between posterior pair (Pa2). Pre-marginal setae (Pm) arranged in two groups, more anterior pairs (Pm1 and Pm2) about level with pre-anal setae, more posterior pairs (Pm3 and Pm4) located postero-laterally of anus and almost in line with fourth and fifth ventral marginal setae. Five pairs of ventral marginal setae (Vm1-Vm5), Vm1 in line with lateral groove and slightly behind level of Pm2, thence successive setae (Vm2-Vm5) more marginally located. One pair of anal setae (An).

Legs. (Fig. 1(1) and (2)). Tarsus I from 0,160-0,168 mm long, somewhat swollen in front of pseudo-articulation thence tapering gradually to apex; claws slightly longer than pulvilli. Dorsal setal alignment on Tarsus I as a single seta on the declivity, one pre-halleral, and single pairs of halleral, post-halleral, antero-dorsal and postero-dorsal setae. Trough of Haller's organ (Fig. 2(5)) contains one long, curved seta, two moderately long setae with narrowly rounded apices, two setae tapering to sharp points; capsule apertures triadiate with transverse section more widely open than short longitudinal section.

Coxae. (Fig. 1(2)). Coxae I sub-triangular, mesial margin salient, extending to postero-internal face (not as figured by Gothe (1967a) Fig. 14), not protruding over its posterior margin but following the contour of coxal margin for a short distance. Following the nomenclature of Clifford and Anastos (1960) three coxal setae, C1.al with single face barbed, C1.mm and C1.pl fine and tapered. Coxa II elongate, sub-rectangular, mesial margin broadly rounded, setae C2.al and C2.pl present, as well as coxal sense organ on its anterior distal angle, posterior margin bearing a long narrow salience. Coxa III sub-triangular with mesial margin narrowly rounded and continued into posterior divergent margin; no salience or spur. Three pairs of sensilla sagittiformia present, the first dorsal to insertion of coxa I, the second and third in line with the postero-external angles of coxae II and III respectively.

2.32 Larval feeding and nymphal emergence

Larval feeding. The average weight of unfed larvae of *B.decoloratus* was 0,024 mg and, on the basis of their cumulative

weight increases over time, the feeding period extended over about five days (Fig. 3). Initially, the increase in body weight was relatively slow; on the first day after attachment this showed only a slight increase over that of the unfed larva, on the second day it almost doubled and on the third day was nearly trebled. The greatest increase occurred between the third and fourth days when imbibition of 'blood' and tissue fluids[1] was between two and three-fold, and average maximum intake of 0,225 mg was attained twenty-four hours later. In other words, the fully engorged larva was about ten times the weight of the unfed stage. Cleared specimens of larvae almost up to the fifth day of larval attachment (Fig. 4a) showed no evidence of visible developmental changes.

Pharate nymph. Larvae retained their external form for a further two or three days after completion of feeding i.e. from about the sixth to the eighth day after larval attachment. The mean weight of these 'larvae' during this period decreased (Fig. 3). Such larvae, when cleared, showed substantial nymphal development within their cuticle (Fig. 2(4)) over three days (Fig. 4a). Thus, it would appear that larvae are capable of rapid digestion of the 'blood meal' and of almost immediate utilization of metabolic resources. It can also be inferred that histolysis (e.g. of larval body muscles) and histogenesis (e.g. the development of the nymphal spiracle and of the fourth pair of legs) occur concomitantly during the latter phases of feeding.

Nymphal emergence. The periods of nymphal metamorphosis and of moulting extended over three days and were normally completed by

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1. The actual constituents of the 'blood meal' are not known and the words 'blood' or 'blood meal' have been used extensively to denote the nutritive matter imbibed by ticks during their feeding phases.

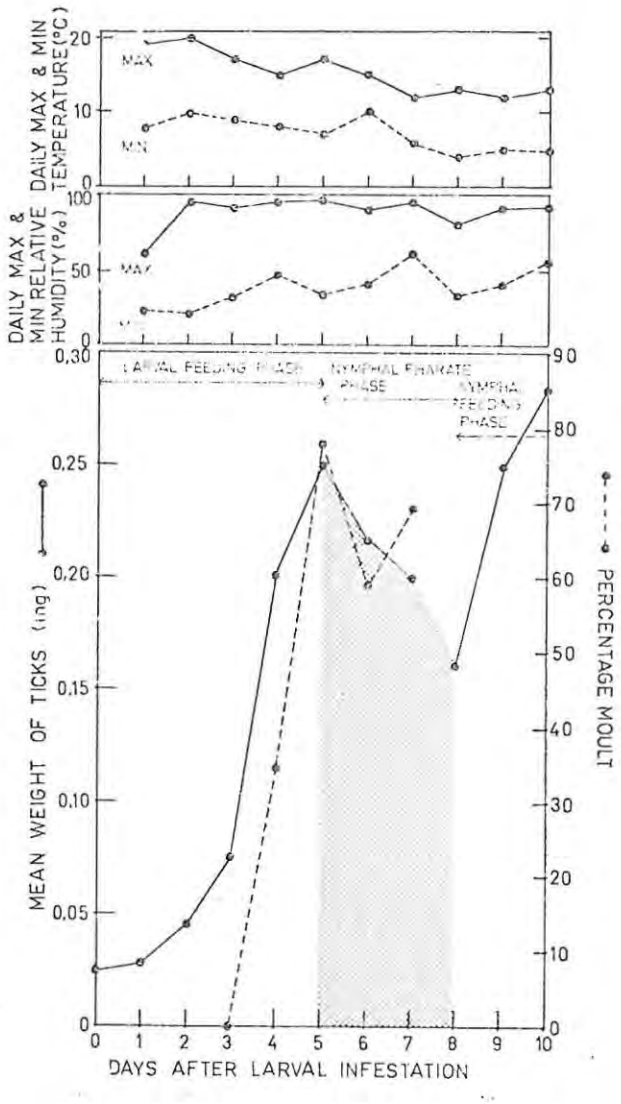

Fig. 3. The changes in weight of feeding and moulting Boophilus decoloratus larvae in relation to time, and the percentage number of larvae moulting after removal from the host on each successive day of the larval parasitic cycle. Daily maximum and minimum temperatures and relative humidities as recorded in the stable are shown separately above.

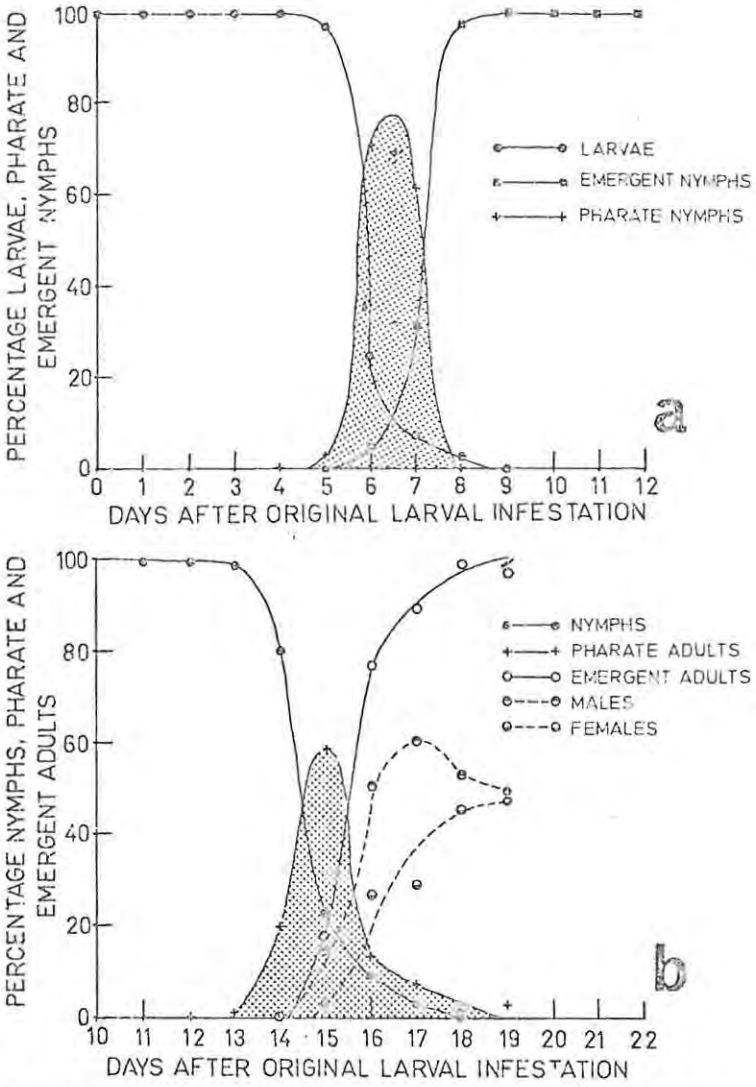

Fig. 4. (a). The percentage of larvae, pharate nymphs and emergent nymphs of *Boophilus decoloratus* present on each successive day of the parasitic cycle between the first and the twelfth days after the original larval infestation of the host. (b) The percentage of nymphs, pharate adults and emergent adults of *B. decoloratus* present on each successive day of the parasitic cycle between the tenth and the nineteenth day after the original larval infestation of the host.

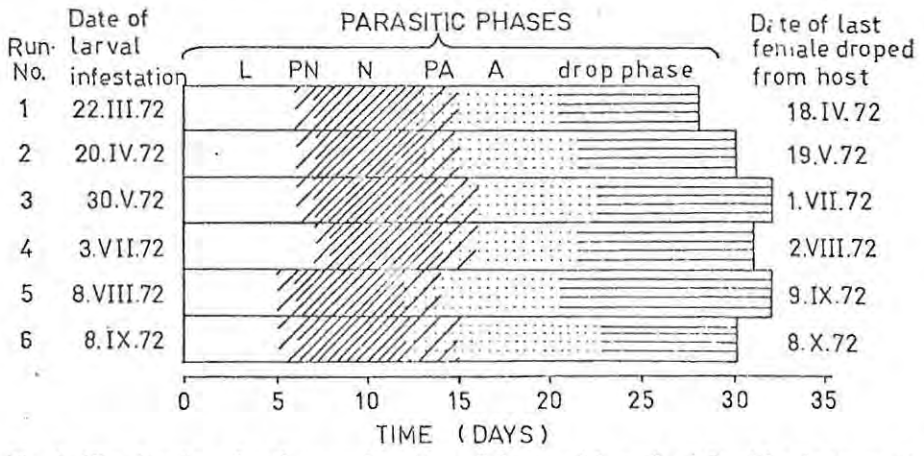

Fig. 5. The duration of each successive phase of the parasitic cycle of *Boophilus decoloratus* in six successive runs on the same host. L—Larval phase, PN—Pharate nymph phase, N—Nymphal phase, PA—Pharate adult phase, A—Adult phase.

the eighth day after infestation with larvae. Reference to Fig. 5 shows that over six runs, extending from March to October, larvae and nymphs occurred together on the hosts on the sixth day in two runs, on the seventh day in three and on the eighth day in one. On days previous to, and succeeding these days only larvae and nymphs respectively were present.

The series of infestations ranged through from summer to winter, and though there was variation in the diurnal and mean environmental factors of the ambient environment in the stable, there was little evidence of these fluctuations interfering with the pattern of the parasitic cycle. This was probably attributable to the fact that maintenance of water balance in the ticks was derived from the 'blood' intake from the host, and that the temperature of the host's body probably fluctuated less than the macroclimatic conditions in the stable.

Larvae removed from the host between the fifth and seventh days after the original infestation and transferred to an incubator ($26^{o}C$, about 90% R.H.) for ten days, gave a percentage moult of nearly 80% (Fig. 3). Of those removed from the host on the fourth day less than 25% moulted. No moulting of larvae occurred when they were removed from the hosts within the first three days of attachment. This indicated that a threshold quantity of 'blood' intake was required, not only for cuticle synthesis to be completed, but also to allow for the 'stimulation' of developmental changes.

Larvae fed for three days do not moult. Since a smaller proportion of those feeding for four days and a substantially larger proportion of those feeding for five days do, then the minimum 'blood' intake to effect moulting is about 0,176 mg. As

only about one-third of the four-day attached ticks moulted, the chances are that those larvae which did so after four days were those which had fed faster.

2.33 Description of nymph

Unfed specimens elongate oval (Fig. 6 (1)), about twice as long as maximum width; fully fed specimens broader anteriorly at about level of scutum, where margins strongly undulate, thence narrowing to sharply rounded posterior margin.

Capitulum - dorsal. (Fig. 7(1)). Posterior margin straight or slightly indented in the middle, merging by way of strongly divergent, postero-lateral 'angles' with mildly sinuous posterior-lateral margins to maximum width, thence through about a right angle to straight, convergent antero-lateral margins to furrowed region, adjacent to insertion of palps. Surface smooth and curving gently to the periphery, except antero-laterally where it is elevated and longitudinally furrowed; a pair of widely separated basis sensilla slightly behind level of greatest width.

Palpi. (Fig. 7(1)). Palpal segment 1 not separated from segment 2, combined length of both measured along mid-longitudinal axis from 0,077-0,081 mm (mean 0,079 mm), with greatest breadth from 0,061-0,065 mm (mean 0,063 mm) at about half way along segment 2; palpal segment 3 measures from 0,040-0,045 mm (mean 0,042 mm) long and greatest width from 0,056-0,059 mm (mean 0,057mm) just beyond its base. Lateral profile concave in vicinity of the presumptive segment 1 thence, beyond a slight dilatation, straight to suture between segments 2 and 3, mesial profile of segment 2 arcuate with greatest width about mid-length; mesial profile of segment 3 convex becoming sharply rounded sub-apically, thence to a somewhat flattened apex which passes into lateral profile by a broadly curved margin. Segment 2 tumescent, gently curved except baso-laterally, where it curves steeply downwards; at two-thirds the distance from base a transverse irregular crevice-like groove, segment 3 curves gently from mid-dorsal line. Setae absent on segment 1, setal pattern and numbers on segments 2 and 3 as in larva, but morphological form of setae different, all approximate to straight 'fine' type. One palpal sensilla between M1 and D1 and a second posterior of M2.

Capitulum - ventral. (Fig. 7(2)). Posterior and postero-lateral margins of basis markedly convex converging to greatest width, thence sharply angled to short, straight, convergent antero-lateral margins; interrupted only by a shallow depression associated with short, transverse furrow; region subtending palpal insertion bears short, shallow depressions. At greatest width surface sharply ridged for a short distance transversely, at this level a pair of widely separated basis setae; a pair of closely set post-hypostomal setae of similar length, with two deep pits between them at hypostomal base. Pair of setae on declivitous posterior face of basis.

Palpi. (Fig. 7(2)). No separation between palpal segments 1 and 2; no setae arise from the presumed site of former; lateral profile of combined segments 1 and 2 biconcave, being separated by an intervening transverse, elevated ridge continuous across most of ventral surface of segment, mesial profile of combined segments

1 and 2 concave; lateral profile of 3 short and arcuate, mesial profile shorter and straight, distal margin as a flanged and cushion-like thickening, but not drawn out into a pronounced spur; placed obliquely to long axis of segment. Segment 4 cone-like and borne on an extensive area of intersegmental membrane. Presumed segment 1 lacking setae; segment 2 with three setae, of which two are probably homologous with larval 'feathered' ventral-mesial (VM) and with mid-ventral setae (MV); the third more laterally located on ridge; two setae on segment 3 one being the counterpart of the larval mid-ventral and the other is short and ventral-mesial; segment 4 with one dorsal, three sub-dorsal, three supra-ventral and one ventral setae with rounded tips apically, and three finer tapered setae on column.

Hypostome. (Fig. 7(2)). Length from post-hypostomal setae to broadly and shallowly indented apex from 0,14-0,15 mm; spatulate, tapering only gradually for about two-thirds its length from apex, broadening basally; toothed for more than two-thirds of length, dentition behind apex of 2 rows of 5/5 very small teeth with some having bifid tips, 1 row of 4/4 teeth (this may be variable and irregular), succeeded by three files on either side of midline of which lateral one bears 8 teeth, intermediate one 7 teeth and mesial one 6 teeth, distal rows of teeth sharply pointed becoming more rounded proximally and merging imperceptibly into crenulations.

Post-scutal area. (Fig. 6(1)). Surface with few, small, deep punctations. Setae arranged in two pairs in position of larval Cd1 and 2; other setae present as shown in Fig. 6(1), but we are unable to correlate these with the larval condition.

Scutum. (Fig. 6(1)). In unfed nymphs length, measured from tips of scapulae to posterior margin 0,45-0,47 mm; greatest width 0,44-0,45 mm across eyes, i.e. slightly longer than broad; eyes oval, protuberant over scutal margin. Posterior margin narrowly rounded, postero-lateral margin rectilinear, or weakly concave, thence mildly undulate and convergent to prominent scapulae with rounded apices; emargination deep. Cervical grooves very shallow, convergent at first then divergent and reaching back to postero-lateral margins. Setae present in comparable situations to Sc1, Sc2 and Sc3 of larva, supplemented by further pairs on scapulae, adjacent to Sc2 and between Sc2 and Sc1. Surface smooth with few, small shallow punctations.

Ventral - surface. (Fig. 6(2)). In nearly moulted nymphs longitudinal divergent grooves extend from level of first intercoxal space almost to posterior margin. Surface with widely dispersed shallow pits; supra-coxal groove, seen in other ixodid nymphs, absent. Some variation involving reduction or duplication of setae but following broad pattern of setal distribution emerges: Sternal setae (St) - one pair at level of first intercoxal space, one pair at level with second intercoxal space, one pair at level of third coxae and two pairs arranged transversely at level of fourth coxae; pre-anal setae (Pa) - an anterior pair of widely separated setae succeeded by two groups of three setae in position of second pair of larval pre-anal setae (Pa2); lateral anal setae - 2 pairs; pre-marginal setae (Pm) - a group of setae (3-5 pairs usually) positionally equivalent to first two pairs of larval pre-marginals (Pm1, 2), and retention of two pairs of larval pre-marginals postero-lateral of the anus (Pm3, 4); ventral marginals (Vm) - five pairs as in larva, circum-spiracular setae - variable in number.

Spiracular plate. (Fig. 6(4)). Circular in surface view, elevated above body surface, margins steep sided and not sunk into cuticle; surface produced into about twenty protuberances, each having an apical apperture.

Legs. (Fig. 6(2)). Tarsus I short and stumpy from 0,128-0,147 mm long, tapering rapidly to a blunt point, claws longer than pulvillus; Haller's organ with six sensillae in trough and aperture of capsule as figured (Fig. 6(3)). Tarsus IV short and stumpy from 0,132-0,141 mm tapering rapidly towards distal end. Coxa I sub-triangular with setae C1 al, C1 mm and C1 pl of about equal length; internal spur broad, originating from the antero-mesial margin extending back to overlap the posterior edge for about a third of its length, postero-internal angle sharply rounded; external spur narrower tapered apically, not overlapping posterior edge of coxae; II elongate with antero-mesial angle more broadly convex than postero-mesial angle, distal half of edge of posterior margin very much broader than proximal half due to pronounced salient ridge, Setae C2 mm and C2 pl of about equal length; III shorter than II, but as broad or even slightly broader, postero-external salience less clearly defined than in II, setae C3 mm and C2 pl of about equal length; IV sub-triangular with only C4 pl present and lacking spurs. Sensilla sagittiformia lacking.

2. 34 Nymphal feeding and adult emergence

Feeding. During the period of moulting the average weight of the fully engorged larva was reduced from 0,249 mg to 0,161 mg for the emergent nymph. This in part, was due to the loss of the larval cuticle, the release of guanine and possibly of water. The feeding pattern of the nymphs (Fig. 8) paralleled that of the larva and, as judged by continued weight increases to a maximum, takes five days. Over the first two days the average uptake of 'blood' was 0,12 mg, which was less than the average body weight of an unfed nymph. On the third day the average amount of 'blood' taken in was 0,404 mg. This was followed by rapid uptake on the fourth day when the ticks weighed ten times their original weight and the average quantity of 'blood' ingested was 1,44 mg. On the fifth day, feeding slowed down and the average weight of engorged nymphs was 1,90 mg. The apparent mean weight increase in nymphs fourteen days after the original larval infestation was not real as moulting had already occurred in part of the tick population. If the mean

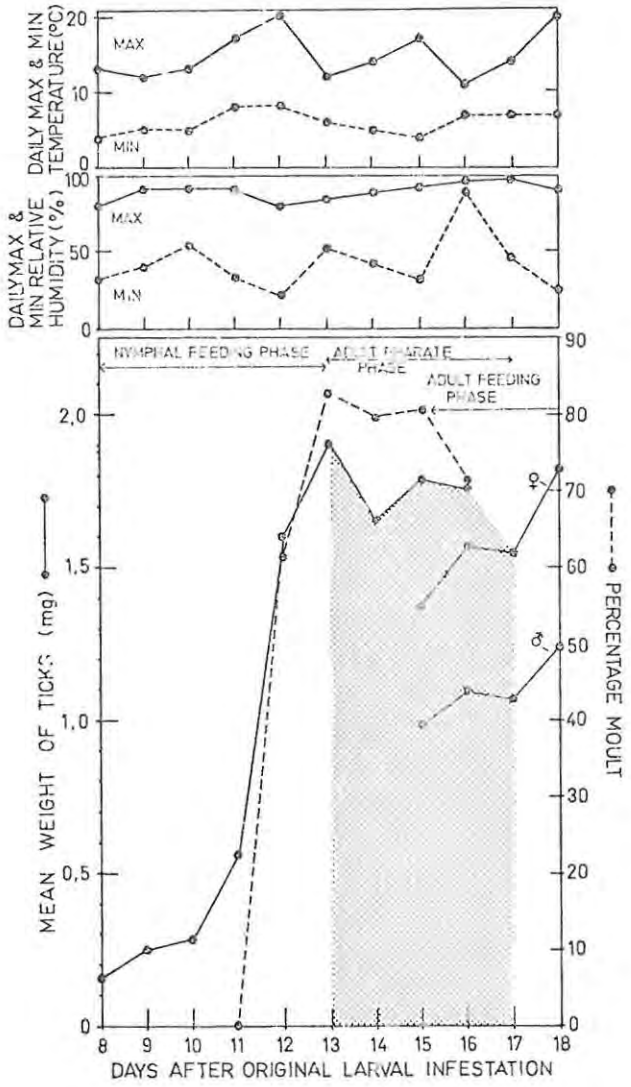

Fig. 8. The changes in weight of feeding and moulting Boophilus decoloratus nymphs in relation to time, and the percentage number of nymphs moulting after removal from the host on each successive day of the nymphal parasitic cycle. Daily maximum and minimum temperatures and relative humidities as recorded in the stable are shown separately above.

weights of all the ticks, nymphs and adults, was to be calculated for the fifteenth and sixteenth days, the values would be lower than that recorded for nymphs on the fourteenth day.

Emergence of adults. Analysis of six successive runs of infestation of the calf by B.decoloratus beginning on 22 March 1972 and ceasing on 8 October 1972 (Fig. 5) showed that nymphs alone, as judged by their external form, were present on hosts for 6, 6, 7, 6, 6 and 6 days giving an average of 6,2 days. After the ultimate day of occurrence of only nymphs on the hosts, nymphs and adults were recorded in five runs for two days, and in one for four days following which only adults were collected.

The results presented in Fig. 4b show the components of random samples of the population on the host, on a daily basis after clearing the nymphs in lactic acid. From the seventh to the twelfth day after the original infestation by larvae the treated nymphs showed no evidence of differentiation into adults. On the thirteenth day 0,7% of the nymphal population sample yielded pharate adults, this percentage increased to 20% on the fourteenth day, to a maximum of 59-60% on the fifteenth day and thereafter declined to 13% and 7% on the sixteenth and seventeenth days. Only occasionally were pharate specimens collected on the eighteenth and nineteenth days.

Nymphs collected from cattle after feeding for one, two or three days, and placed in an incubator failed to moult (Fig. 8). Those removed on the fourth and fifth days of feeding and similarly treated yielded substantial numbers of adults. Correlating successful moulting of nymphs to adults with mean 'blood' uptake on successive days, indicates that about 1,5 mg of 'blood' is a

necessary prerequisite for complete development.

The first emergence of adults was on the fifteenth day, i.e. on the same day as the maximum occurrence of pharate adults. Initially, the males outnumbered the females by about 5:1, but on the two succeeding days the ratio altered to 2:1, and within a further two days males and females were present in equal numbers in samples of the population (Fig. 4b).

Since (i) the first appearance of pharate nymphs in the population occurred two days before the first emergence of adults and (ii) the period of greatest pharate incidence was up to four days it appears likely that the 'blood meal' is rapidly metabolised, and that this is accompanied by rapid, concurrent development to the succeeding stage.

2.35 Description of female

Elongate oval (Fig. 9(1)) about 4,8 mm long, about twice as long as broad with greatest width of about 2,4 mm, at about the level of spiracles, when engorged bluish in colour and measuring approximately 12,0 mm long and 8,0 mm wide.

Capitulum - dorsal. (Fig. 10(1)). Posterior margin of basis straight with median indentation, in most specimens cornua absent, if present only faintly indicated, postero-lateral angles broadly rounded, straight or mildly concave postero-lateral margins, strongly divergent to greatest width, thence sharply angled and convergent to palpiger. Length (measured from bifurcation of external cheliceral sheaths to posterior margin) to breadth ratio 1:2,5. Porose areas elevated, oval, oblique to long axis, separated by an interval either equal to, or greater than maximum length of one of them. From elevated region around porose areas, surface slopes almost declivitously to irregular postero-lateral surface, and more gradually to an anterior median depressed region; two or three pairs of hairs antero-laterally.

Palpi. (Fig. 9(3)). Length of combined segments 1 to 3, when measured mid-dorsally between 0,23 and 0,25 mm; segment 1 broader than long, with straight mesial margin, lateral profile concave, setae absent; segment 2 with mildly arcuate mesial profile, longer than straight converging lateral profile; mesial profile of segment 3 mildly convex terminates in sharply rounded apex, thence continuous with mildly curved lateral profile to near origin of segment, where it is seen dorsally as a lateral spur; seen end-on this spur is a flange continuous with ventral ridge

of segment 3 (Fig. 9(4)). Surface curves gently from mesial margin laterally, except for declivitous proximal edge of the basal lateral spur on segment 3, and along base of segment 2. Setae arranged in four series, mesial series of 2 on segment 2 and 2 on segment 3; dorsal sub-median with 2 on segment 2, 4 on segment 3; dorsal sub-lateral series of 2 on segment 2, 2 on segment 3; the lateral series of 2 on segment 2, 0 on segment 3; all of fine setal type.

Capitulum - ventral. (Fig. 10(2)). Posterior edge declivitous with groups of short, stumpy setae laterally; osterior and postero-lateral margins broadly curved to greatest width, thence by weakly concave antero-lateral margins to palpal insertions. Surface generally flattened except for two semi-lunar elevations, with steep posterior faces adjacent to regions of greatest width, a sharp slope to hypostomal base between palps, longitudinally oblique furrows antero-laterally. Three or four setae at about level of greatest width and in proximity with antero-lateral margins, 5 or 6 shorter setae adjacent to semi-lunar elevations.

Palpi. (Fig. 9(4)). Segment 1 with concave lateral profile, mesial profile with basal half weakly demi-concave, then angled to straight distal edge, separated from segment 2 mesially by transverse V-shaped cleft; one pair of fine setae antero-laterally, a single short, mid-ventral, apically serrated seta, one or two pairs of stout bifid, multi-pointed setae (Fig. 9(5)) on distal straight margin. Segment 2 with strong irregular transverse elevation lateral of median flattened spur, posterior face declivitous; lateral profile sharply angled and projecting beyond margin of segment 1, mesial profile arcuate, convergent distally; surface very irregular; one basal lateral seta, a transverse row of setae on anterior face of ridge, two or three stout, multipointed setae on lmesial margin as shown in Fig. 9(4). Segment 3 with declivitous posterior face bearing two or three fine setae, which are separated sub-apically; towards the midline posterior margin extended into a triangular flange, before straightening to strongly projecting basal-lateral extremity, beyond which lateral profile gently arcuate; mesial profile short, broadly rounded with two or three short, broad, tapered setae interposed between it and triangular flange (Fig. 9(4)), distal margin obliquely curved, with extensive area of intersegmental membrane between it and base of segment 4. Segment 4 cone-like, with fine tapered setae on column and eight round tipped setae on the apex, arranged as in nymph.

Hypostome. (Fig. 10(2)). Length from single pair of post-hypostomal setae to apex 0,27-0,29 mm; spatulate with broad, shallow indentation mid-apically; pronounced sub-apical corona of minute denticles followed by either one row of 5/5 or 4/4 teeth, then by up to nine or ten rows of 3/3 teeth; may become irregular basally where teeth merge imperceptibly with crenulations.

Post-scutal area. (Fig. 9(1)). With dense covering of setae of moderate length, except in the depressions of the posterior median and paramedian grooves; punctations small and shallow.

Scutum. (Fig. 9(1)). Length, measured from tips of scapulae to posterior margin 0,94-1,10 mm, greatest breadth in front of mid-length across the eyes 0,82-1,03 mm; length/breadth ratio about 1,15:1,0. Posterior margin narrowly rounded, postero-lateral

margins almost rectilinear and divergent to level of eyes, latter mildly protuberant, antero-lateral margins arcuate to sharply rounded apices, emargination well defined. Cervical grooves broad, shallow, convergent at first, then divergent to end at about midway along postero-lateral margins. Setae of moderate length, arranged along the antero-lateral margins, between cervical grooves anteriorly and as a postero-median group.

Ventral surface. (Fig. 9(2)). Setae of moderate length well distributed over ventral surface, absent only from coxal and inter-coxal regions of I, around genital orifice, along genital grooves and posterior to anus. Genital opening on level with second inter-coxal space. Anal groove obsolete.

Legs. (Fig. 9(2)). Pale yellow, longer but not as stout as in male. Coxa I sub-triangular, with two well defined apically rounded spurs, outer spur broader and shorter, discernibly separated by narrow but deep inverted V-shaped cleft, do not usually protrude beyond coxal margin, coxa II sub-rectangular, with broadly rounded external spur, coxa III sub-rectangular lacking external spur but slight salience may be present, coxa IV broad, sub-triangular, usually lacking salience postero-externally, all supplied with fine setae most of which are longer than those on the ventral integument. Tarsus I short, from 0,40-0,43 mm long, tapers rapidly to rounded apex, with ventral sub-apical spur; tarsus IV from 0,37-0,39 mm, tapering fairly gradually towards rounded apex with two ventral retrograde spurs. Insertion of pulvillus apical. Haller's organ as figured (Fig. 10(4)).

Spiracular plate. (Fig. 10(3)). Almost circular in surface view, peripheral walls vertical, not sunk into surrounding cuticle; macula elevated, antero-basally placed in relation to long axis of body, surrounded by about 50-60 aperture bearing protuberances on surface.

2.36 Description of male

An examination of samples of males of B.decoloratus during biological investigations makes it clear that there is a particularly wide range of sizes and that within a single population there may be two discrete peaks in size. Concomitant with this, certain morphological characters may be reduced or enhanced. As a consequence assigning a dimension, or a range of dimensions, to particular features in the males of this species is fraught with risk.

Capitulum - dorsal. (Fig. 12(1)). Posterior margin concave with variably developed cornua, lateral margins arcuate or undulate to level of palpiger, where they are indented. Surface flattened mid-dorsally, slightly elevated, curving downwards peripherally; slightly behind mid-length a transverse arc of eight or nine conspicuous setae leading to a group of about four peripheral setae.

Palpi. (Fig. 11(3)). Short, broad, suture lacking between segments 1 and 2; lateral profile of presumed segment 1 straight except distally where it contributes to proximal ridge of segment 2, mesial profile shorter and curved; mesial profile of segment 2 undulate, almost straight, lateral profile either rectilinear, or may be undulate and irregular, shorter than mesial margin; distal

edge straight and horizontal in resting palp, proximal margin broadly curved at postero-internal angle whence slightly obliquely and antero-laterally to palpal margin; posterior face short, declivitous, bears two setae; segment 3 with straight posterior margin and steep face at junction with segment 2, especially at its projection beyond distal lateral edge of segment 2 to spur-like extension, mesial profile gently convex to broadly rounded apex, thence to straight lateral profile to basal spur-like extension. Setae in four linear series, mesial series of two setae on each of segments 2 and 3; dorsal sub-median series of two setae on segment 2, 4 on segment 3, dorsal sub-lateral series of two setae on segment 2, and two on segment 3, and a lateral series of two on segment 2.

Capitulum - ventral. (Fig. 12(2)). Posterior margin broadly curved, postero-lateral angles rounded, divergent lateral margins curved to greatest width slightly behind palpal insertion, thence convergent gradually to latter. Surface strongly down-curved laterally, otherwise gently convex. Mesial of antero-lateral group of setae, longitudinal rows of setae.

Palpi. (Fig. 11(4)). Lateral margin of segment 1 short, straight, mesial margin angled and longer than lateral margins (Fig. 11(4)); dorsal-median surface thickened to form salient curved flange posteriorly (Fig. 12(3)); one fine seta on median edge of thickened area, either one or two stout, multi-pointed setae arise from distal mesial face. Segment 2 broader than long, posterior edge salient; at junction with segment 1 face of segment 2 steep; proximal margin arcuate and approximately parallel with, but longer than distal margin, lateral profile straight, mesial margin irregular; transverse row of three setae at about mid-length, one fine seta on steep posterior face, two or three stout, milti-pointed setae on mesial face; mesial face of segment 3 forming a triangular spur overlapping distal margin of 2 (Fig. 12(3)), beyond this curved laterally, flattened and extended into dorsal ridge of 3, projects beyond lateral margin of 2 (Fig. 11(4)); lateral margin sinuous or arcuate to apex; two setae on steep posterior face, one of which at outer base of spur and three stout mesial setae subtending inner base of spur. Segment 4 columnar with apical setal distribution as in female.

Hypostome. (Fig. 12(2)). Single pair of closely-set, short post-hypostomal setae, with two shallow depressions between them. Length from post-hypostomal setae to rounded apex 0,17-0,20 mm, spatulate shaped; corona of small denticles occupying up to one quarter length of toothed region, followed by one row of 4/4, then 6 rows of 3/3, with occasionally some rows of 3,5/3,5.

Dorsal surface. (Fig. 11(1)). Body widest about level of fourth coxae, yellow to brown, weakly sclerotized, gut caecae as dark outlines through translucent cuticle. Small triangular caudal appendage on posterior margin.

Scutum. (Fig. 11(1)). Broadly rounded behind, with irregular outline at caudal base, indented at spiracular level thence broadens to level with fourth coxae, before narrowing gradually by undulating margins to sub-triangular scapulae, emargination deep; eyes large, yellow, circular, at level of coxae II, not overlapping scutal margin. Cervical grooves shallow, diverging posteriorly and reaching back beyond eye-level. Pair of shallow depressions at third intercoxal space and coxae IV; pair of posterior-lateral

grooves in line with depressions, shallow, broad and reaching posterior margin; postero-median groove extending from in front of spiracular level to caudal base. Setae absent from grooves and depressions, giving setal pattern as shown in Fig. 11(1).

Ventral surface. (Fig. 11(2)). Genital aperture level with second pair of coxae. Surface uniformly and abundantly setose except between first pair of coxae, between the ad-anal shields behind anus, on caudal appendage and posterior to genital grooves. Considerable variation in size and development of ad-anal and accessory shields (Fig. 10 (5) and (6)); ad-anal shields elongate and, when well developed, internal spur strong, sub-triangular separated either by shallow concavity or a cleft from smaller external spur, latter may be obsolescent; in large specimens internal spur may project beyond posterior margin, in small specimens frequently fails to reach margin; accessory shields drawn out to single points, whether they protrude beyond posterior margin or not depends on overall size of the male; both shields sparsely punctate.

Spiracular plate. (Fig. 12(6)). Similar to female, but with fewer goblets.

Legs. (Fig. 11(1) and (2)). Stout. Coxae large, with dense covering of setae; I sub-triangular, drawn out postero-internally into narrowly rounded spur, external spur triangular, protruding beyond coxal margin; II sub-rectangular with antero-internal angle broadly curved, postero-internal angle salient, external spur short and broadly rounded; III similar in shape to II, but broader, external spur shorter; IV broader than either II or III, sub-triangular with postero-external corner drawn out but lacking external spur. Tarsus I from 0,33-0,36 mm long, tapering fairly rapidly to rounded apex, small sub-apical spur, tarsus IV from 0,35-0,36 mm long, two retrograde sub-apical spurs; insertion of pulvillus apical. Haller's organ as illustrated (Fig. 12(5)).

2.37 Adult feeding and female drop

Emergent males and females were lighter in weight than the gorged nymphs from which they developed, probably for the same reasons that emergent nymphs were lighter than gorged larvae. Emerging males were lighter than emerging females; males weighed an average of about 0,98 mg and females 1,37 mg. Both males and females attached immediately after moulting. This is in contrast to the information given by Legg (1930), for the closely allied B.australis Fuller (= B.microplus Canestrini), where male ticks were reported not to attach but searched for unfertilized females.

Over the first three days (days 16, 17, 18 in Fig. 13) of

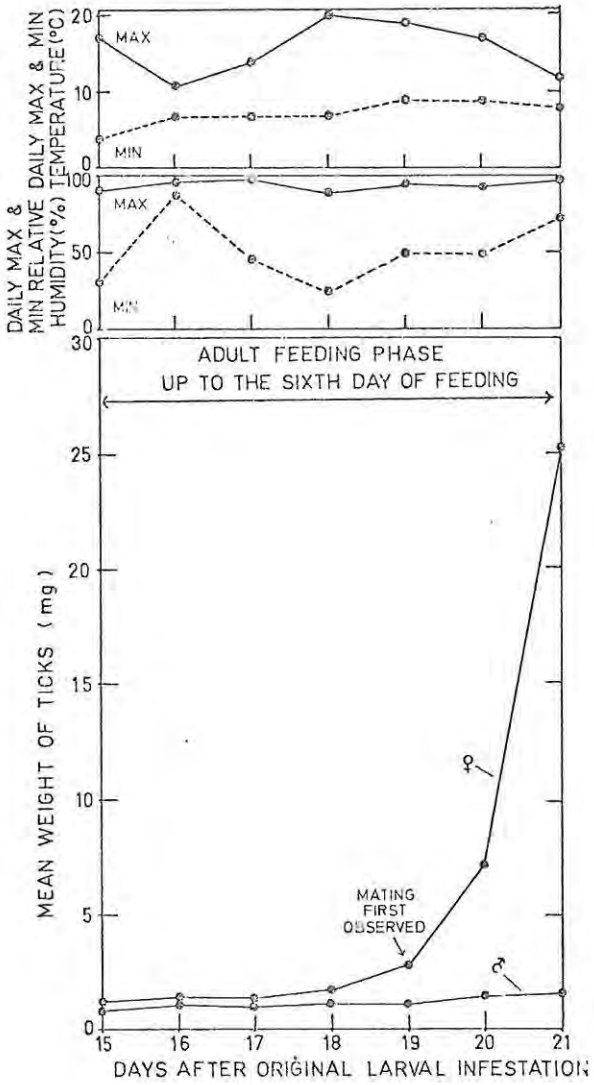

Fig. 13. The changes in weight of feeding Boophilus decoloratus adults in relation to time, up until the day before the first females dropped from the host. Daily maximum and minimum temperatures and relative humidities as recorded in the stable are shown separately above.

attachment the weight increases of males and females were almost in parallel and up to the sixth day (day 21 in Fig. 13) of feeding there was a relatively insignificant increase in the weight of males. The weight of females began to increase on the fourth day of feeding (day 19 in Fig. 13) at which time, too, mating was first observed. By the sixth day (day 21) the quantity of 'blood' imbibed was a mean of 23,9 mg per female. The first satiated females dropped from the host on the seventh day after adult emergence, having attained weights of four to eight times greater than those of the average weights of the sixth day. Fully fed females continued to drop off for up to twelve days after this, with the majority detaching within two to five days (Fig. 14d). Comparison of Fig. 14c and d for weights of engorged females and their dropping-off times shows no definite relationship, except that in infestations 1, 2 and 4 there is a tendency for heavier females to fall at the peak period of drop-off.

2.38 Adult male population structure[1]

Adult males were collected from the host in large numbers on a single day prior to the beginning of the female drop period on three different occasions. This was done by locating engorging female ticks on the host and male ticks found beneath them collected. By this means any bias relating to the selection of males was eliminated. Each male so removed was weighed and the weights of the population recorded as a histogram. From Fig. 15, which is indicative of replicated results, two size groups are seen to exist within the male population. The two, slightly overlapping, weight classes have means of approximately 2,0 mg and 0,9 mg respectively.

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1. Not included in publication by Arthur and Londt (1973).

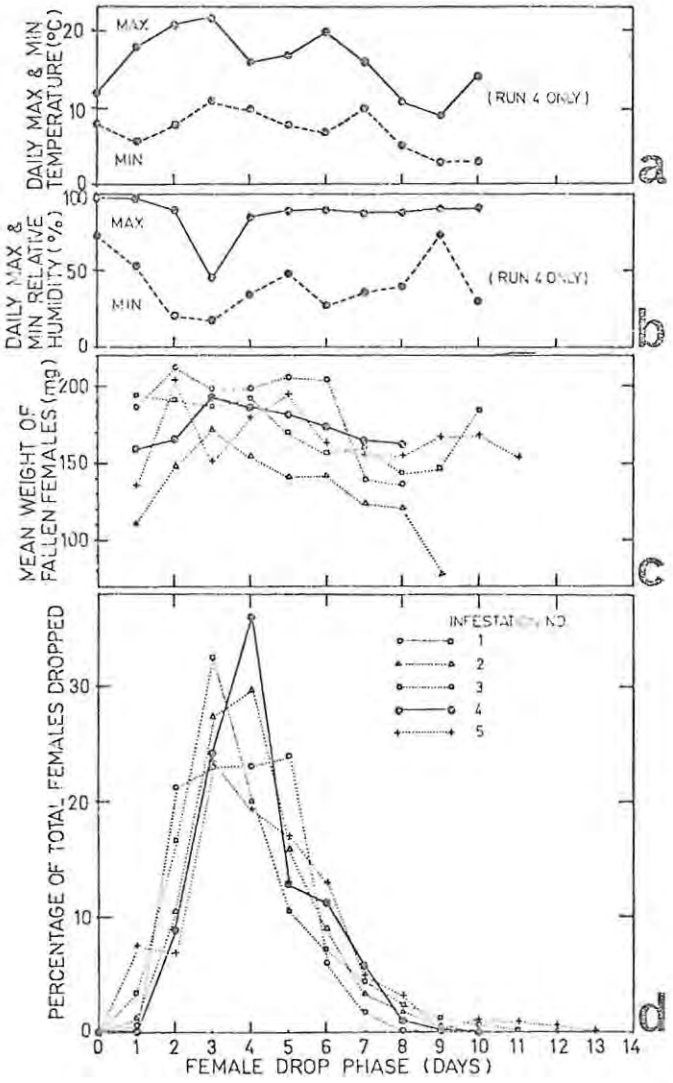

Fig. 14. (a-b). Daily maximum and minimum temperatures (a) and relative humidities (b) recorded in the stable during the fourth run which represents a continuation of the cycle shown in Figs 3, 8 and 13. (c). The mean weight of engorged adult females of Boophilus decoloratus on each successive day of the female drop phase. The results of five runs are shown, the fourth of which represents a continuation of the cycle shown in Figs 3, 8 and 13. (d). The percentage number of females dropped from the host on each successive day of the female drop phase. The results of five runs are shown, the fourth of which represents a continuation of the cycle shown in Figs 3, 8 and 13.

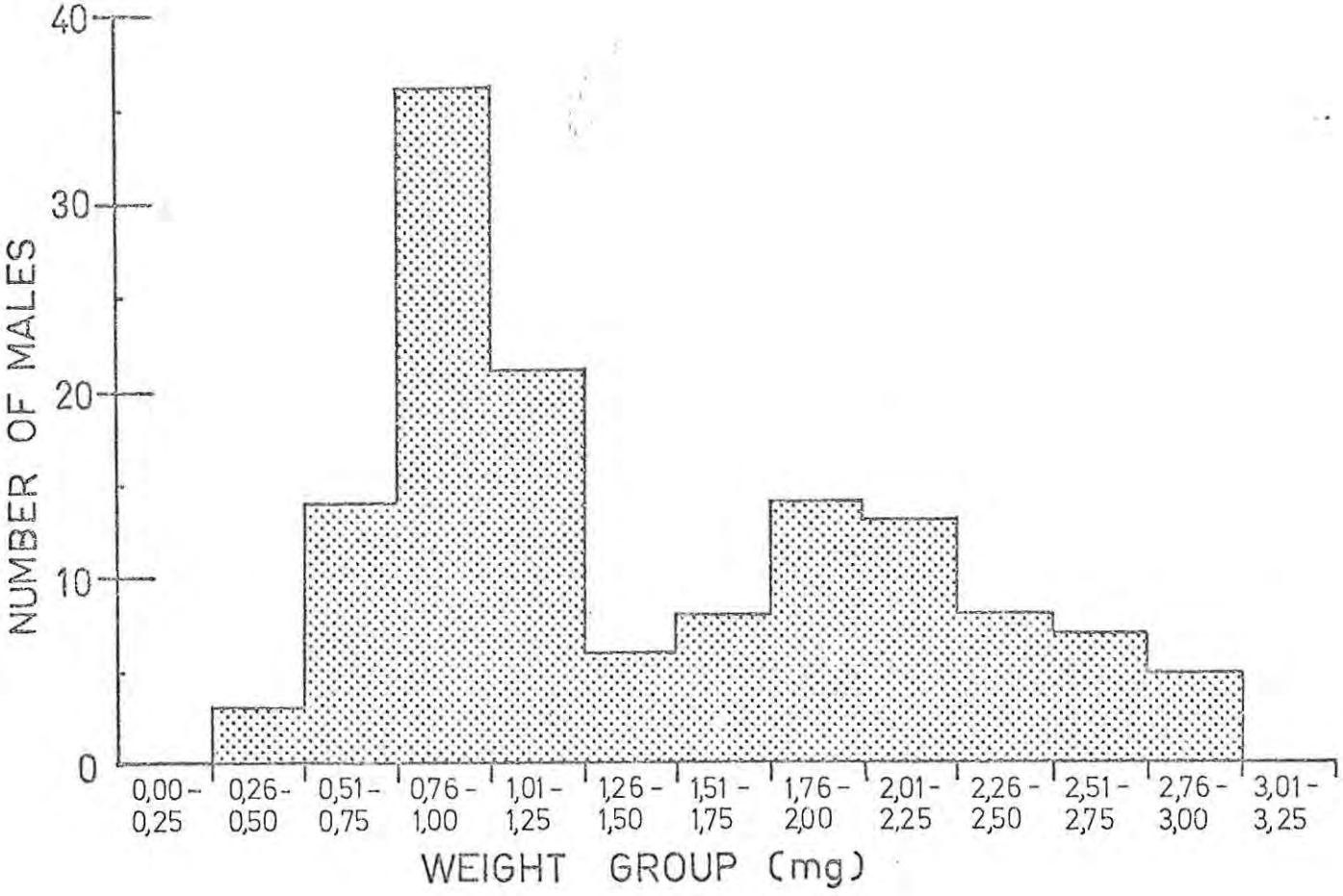

Fig. 15: Histogram constructed after the weighing of 135 randomly collected Boophilus decoloratus males taken from the host on the 20th day of the parasitic cycle.

Probit analysis of the percentage males falling within each weight group (Fig. 16) shows that two distinct types of males are present. The biological significance of this is not understood at present but is worthy of further elucidation. It is however clear that taxonomically it emphasizes the necessity for examining the whole range of male organization derived from the products of a single female and from an extensive range of females from restricted geographical locations and from widely dispersed areas. The genetical approach to this should also be employed. What is clear from this preliminary investigation is that certain morphological features may be enhanced in large ticks and reduced in small ticks. For example the anal plates in large males project beyond the hind margin of the idiosoma (Fig. 10(6)) and hardly reach it in small males (Fig. 10(5)). A more detailed examination of the variation in morphological features in the males of B.decoloratus is currently being undertaken by Londt and Arthur.

2.4 DISCUSSION

One of the features of the life cycle of one-host ticks, such as B.decoloratus, when compared with three-host ticks, in particular, is the rapidity with which the parasitic life cycle, from larval attachment to the fully engorged female, is completed. In B.decoloratus, up to the time of the first drop-off of females ready for egg-laying, this is between 21 and 23 days, in B.australis (= B.microplus) it is generally 20-21 days (more exceptionally 23 or 25 days) according to Legg (1930), and for the same species is approximately 18 days, according to Roberts (1968).

No attempt has been made to account for the differences in the length of time taken for one-host and three-host ticks to complete

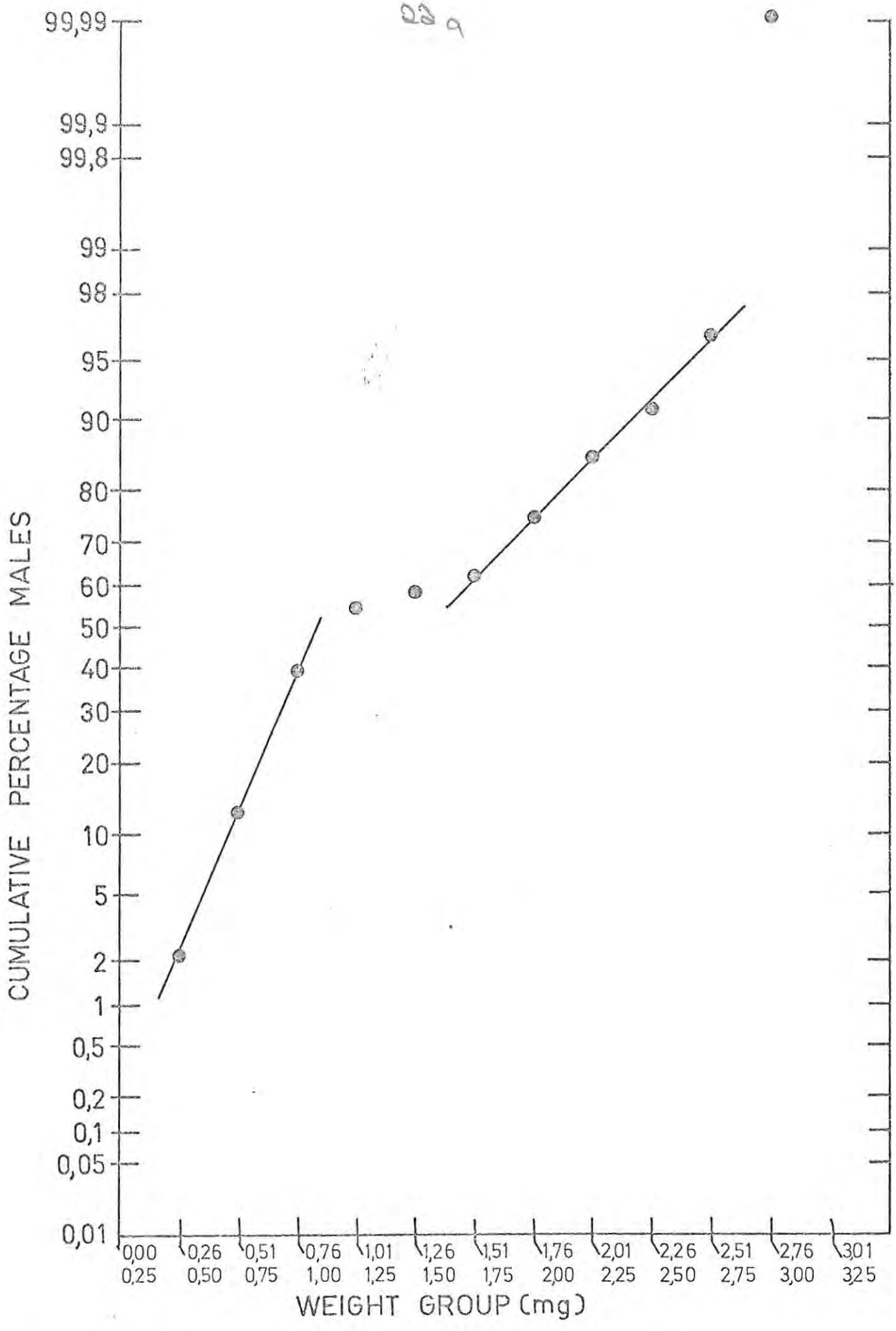

Fig. 16: Probits of the percentage male Boophilus decoloratus specimens in each weight group shown in Fig. 15.

their parasitic cycles. One reason for this has been the lack of information on the lengths of the feeding times of larvae and of nymphs of one-host ticks in relation to the metamorphic changes which occur, when these stages remain attached *in situ*, after cessation of feeding.

Roberts (1968) selected length as a means of analysing age structure in the different stages of tick populations. This is inadequate because it fails to distinguish between the feeding larval and nymphal stages in *sensu stricto* from those in which pharate nymphs and pharate adults are developing rapidly. These later stages are more truly referable to the succeeding instars rather than to those in which their development is taking place. This becomes clear by reference to Figs 4 and 5. From this point of view tick weights, coupled with clearing techniques, are not only a better indication of 'blood' intake but also of visible developmental changes taking place in the attached immature stages.

To analyse changes between the lengths of the life cycles of one-host and three-host ticks, concurrent investigations were carried out by Norval (unpublished data) on the life history of the three host tick, *A.hebraeum*, under the same macroenvironmental conditions as those used for *B.decoloratus*. The larva of *A.hebraeum* feeds on rabbits for 4-10 days, with most of the larvae becoming fully engorged between the sixth and the eighth day. In an incubator at 26°C and ca. 90% R.H. the period between detachment of the larvae and moulting ranged from 14 to 21 days with the majority moulting on the fifteenth day. Nymphs of *A.hebraeum* fed for 5-13 days on rabbits and 6-9 days on sheep to become replete. It is thus possible that the larval feeding period may be shorter on some hosts than on others. Again in the incubator the pre-moulting

period of adults extended from 21-29 days with most nymphs moulting on or about the twenty-third day.

Moulting time is influenced by temperature within the survival limits imposed on the ticks by relative humidity. In one-host ticks, such as B.decoloratus, and two-host ticks, like R.evertsi, the temperature regime during the moulting periods approximates to that prevailing during feeding by the immature stages. The various instars of three-host ticks on satiation and detachment from the hosts are, however, subject to considerably more variable temperatures in the vegetation complex into which they drop. The body temperatures of cattle range from 101°F to 103°F with skin temperatures varying from 95°F to 105°F (Rippon: pers.commun.). Londt and Whitehead (1972) have shown that the larvae of A.hebraeum are found predominantly in medium protected vegetation, and it may be that all stages in the life history are found here. The temperatures recorded in these situations in the eastern Cape Province are on average lower and more variable than those used in our incubator experiments. The experiments on the emergence of nymphs from larvae and of adults from nymphs of A.hebraeum and B.decoloratus were carried out at 26°C (= 78,8°F). Under these circumstances it is likely that B.decoloratus would take longer to emerge than they do on cattle, and that the immatures of the bont tick (A.hebraeum) proceed to their subsequent stages in a shorter time than they would have done under field conditions. Under natural conditions it is likely then that the life history of a one-host tick will be shorter than that of a three-host tick and to a lesser extent that of a two-host tick.

There is, however, a more fundamental consideration. In the life history of one-host ticks the fully fed larval stage is succeeded

immediately by the immobile pharate nymphal stage, which gives rise within three days to the active nymph. This nymph is ready to feed almost immediately. Similarly, the immobile pharate adult appears within two or three days of cessation of feeding of the nymph and is capable of attaching to the host on emergence. These considerations also apply to the transformation which occurs between the larva and the nymph in such two-host ticks as R.evertsi. In three-host ticks, however, when the fully fed larva or the fully fed nymph detach from the host they are extremely mobile for two or three days (Arthur, 1962) before entering a quiescent phase which may last for two or three weeks, prior to emergence. This is also so in the nymph-adult metamorphosis of the two-host ticks.

During the transformation to the pharate state the appendages of the succeeding stage of any ixodid tick, whether it be nymph or adult, do not develop internally to those of the preceeding stages, but appear de novo. In such three-host ticks as A.hebraeum the rudiments of the appendages of the nymph and the adult are visible through the larval and nymphal integuments respectively even before detachment from the host and there are indications of the pharate condition. But the musculature and nervous system of the larva and the nymph remain operative for two or three days after the ticks drop off. Thus the change from the larval to the nymphal stages and from the nymphal to the adult stages in three-host ticks is extended when compared to what occurs in one-host ticks. This may be attributed in part to a delay in histolysis of tissues in three-host ticks or an acceleration in these processes in one-host ticks. A question which is probably answerable only when we learn which is the primitive condition.

On the evidence available from life-history studies it appears

that the meal is more rapidly digested and metabolized in one-host ticks than it is in three-host ticks. This may account for the apparent delay in the development of the embryological precursors for the succeeding stages in the latter. The fact that such a three-host tick as A.hebraeum may feed concurrently on the same host as the one-host B.decoloratus and for the same length of time and under similar conditions without alteration of the one- or three-host pattern indicates that each of these species may employ different biochemical or hormonal pathways to attain the same ends.

The evidence from the literature that the two- and three-host tick patterns are interchangeable in relation to different hosts has been applied to H.rufipes. The minimum time for this tick to complete its life history is between four and five months, but this may be doubled under local conditions, according to Hoogstraal (1956). Howard (1908) considered H.rufipes to be a two-host tick in South Africa; Jack (1928) reported that it had a two-host and a three-host cycle in Rhodesia; Brassey-Edwards (1932) that it behaves as a two-host tick on hares and a three-host tick on sheep, cattle and domestic fowls. Theiler's (1943) data suggest three-host behaviour although stating without qualification that they are "as a rule two-host ticks; the larvae may drop-off". Preliminary results (Arthur, unpublished) show that larval ticks of this species bred on rabbits have a pharate nymphal condition at the end of feeding; this being similar to that observed in B.decoloratus. Biological studies on the life-histories of H.rufipes (Arthur, Norval and Knight, unpublished) show that a proportion of the ticks which drop-off do so as partially fed larvae, fully fed larvae, and partially fed nymphs, but that the vast bulk of the population remains on the host and there undergo the change from larvae to nymphs. The removal from the host of immature ticks in various stages of

engorgement suggest an active de-ticking process by the host. To accept interchangeability of tick-host pattern implies possible changes in the biochemical pathways leading to metamorphosis and, in the case of H.rufipes, the replacement of an immobile pharate nymphal condition with an active larval state on completion of feeding. Although not impossible it is unlikely, and has not hitherto been observed in other ticks having a catholic choice of hosts. Like B.decoloratus and H.rufipes, the cuticle of fully fed larvae of R.evertsi also enshrouds a pharate nymph and on detachment in this condition remains immobile.

According to Legg (1930) the development of the tick B.australis (= B.microplus) during its parasitic life shows considerable uniformity in timing in December, January, May (2 runs), July (1 run) and August. This is supported by the present results extending from 9 February 1972 to 8 October 1972, and these data are summarized in Fig. 5. Reference to the plotted temperatures and relative humidities of the macro-environment of the host for one run (Figs 3, 8, 13, 14a, b) shows that these factors vary quite widely. Macro-climate conditions thus do not appear to affect the length of the various phases of the life cycle of B.decoloratus on the host.

These data may be of value in approaching the problem of acaricidal treatment of blue tick populations on stock because of the constancy of timing, and the effects of acaricides applied to stock when the ticks are in the pharate condition might bear further examination.

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The parasitic cycle of *Boophilus decoloratus* (Koch 1844) (Acarina: Ixodidae)

by

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Detailed studies on the morphology of the immature stages of *Boophilus decoloratus* are sparse. The present work, using scanning electron microscopy, aims at clearing up existing misconceptions and considers homologies between larvae and nymphs. The biological activities of this tick during its parasitic cycle on the host are examined with special reference to the course of feeding of all stages, and to the influence of the condition of the pharate nymph and the pharate adult in accelerating the completion of the life cycle. The pattern of dropping of replete females is considered in relation to their weight and to their time of drop-off.

INTRODUCTION

A number of workers (Minning, 1934; Bedford, 1934; Theiler, 1943; Hoogstraal, 1956; Arthur, 1960; Gothe, 1967) have described and illustrated the adults and immature stages of *Boophilus decoloratus* (Koch) using conventional light microscopy. From experience gained by using scanning electron microscope techniques on the immature stages of the Bont tick, *Amblyomma hebraeum* Koch (Arthur, 1973) and on representatives from other ixodid genera (Arthur, unpublished) it was considered expedient to apply them to studying all stages of *B. decoloratus*. These techniques are valuable in tick taxonomy in that the necessity for clearing and mounting specimens is eliminated and they reduce distortion, which occurs when either the whole or parts of the body are flattened. The need for this approach was emphasized when attempting to use the descriptions and illustrations prepared by Gothe (1967) to separate the immature stages of *B. decoloratus* from those of *B. microplus* (Canestrini, 1887), particularly as the distribution of these two species overlaps in parts of South Africa.

Information on the biology of *B. decoloratus* during its parasitic phase on the host is sparse. Previous authors have shown that, except for digestion of the blood meal by the female, the maturation of her gonads and egg laying, the life cycle is completed entirely on the host. The times given for this period on the host ranges from three weeks (Theiler, 1911), to a month (Lewis, 1939). Lounsbury (1905—cited by Theiler, 1943) reported that the larva moults after 7 days on the host and the succeeding nymph moults after a further 7 days. The fully fed female leaves the host at about 23 days from the time of its attachment as a larva. Theiler (1943) repeats this information. There are neither data on the feeding period nor qualitative or quantitative assessments correlating size and age during the periods for which the various stages are feeding, as there are for *B. microplus* (Legg, 1930, as *B. australis;* Hitchcock, 1955; Kitaoka and

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Yajima, 1958, as *B. caudatus;* Roberts, 1968). The use of tick length as a measure of size change in relation to age has been advocated by Roberts (1968), although he recognized that engorging and engorged nymphs are substantially affected by fixation and processing in hot alkali. More importantly, however, tick length does not take into account the periods of time during which the nymphs and the adults are in a pharate condition. To determine this requires more exact information on moulting periods.

MATERIALS AND METHODS

All the stages of *B. decoloratus,* used for description, were obtained from a breeding culture in the Tick Research Unit of Rhodes University. The specimens were preserved in 70% ethanol, dried in air and fixed to the specimen stubs either by colloidal silver paint or by "Sellotape" dissolved in chloroform. No additional adhesives were necessary. A layer of gold (100–150 Å) was evaporated onto specimens in a Hitachi HUS 3B vacuum evaporator. The specimens were examined in a JEOL JSM U3 (Japan Electron Optics Laboratory Ltd.) scanning electron microscope. Accelerating voltages of 15 kV and 25 kV were used, and the cathode ray tube images were recorded in a Graflex 2¼″ × 2¼″ camera using Ilford FP4 negative film and developed in Microphen developer. The nomenclature of the body setae adopted follows that of Clifford & Anastos (1960) and the palpal setae that of Arthur (1973).

For biological studies, larvae were reared from fully-fed females maintained in the laboratory at 26°C and about 90% relative humidity. The larvae were kept under these conditions for about a month before being used to infest a Guernsey calf. Each infestation of the calf involved the use of about twenty thousand larvae, and these were distributed as uniformly as possible along the length of the calf's spine from behind the head to just above the base of the tail.

Daily observations were made on the length of time taken for each parasite stage to moult on six occasions from March to October 1972. Before the fourth infestation a random sample of fifty larvae were weighed to ascertain the weight of unfed larvae. After the initial infestation a variable number of ticks were removed from the neck region of the calf daily, weighed, inserted in appropriate vials and placed in an incubator at 26°C and 90% R.H. Daily collections of ticks were made up to the time when the first engorged females dropped from the calf. Thereafter only the dropped females were collected. Ticks, removed from the host, were placed in the incubator and examined ten days later. The numbers of moulted larvae or moulted nymphs were counted. For weighing larvae, nymphs and males a Cahn G-2 electro-balance was used and for females a Sartorius single pan balance. Temperatures and relative humidities in the stable, housing the calf, were recorded throughout the investigation by a thermohygrograph.

For determining the pharate condition of the nymphs and of the adults daily samples, taken from the calf, were cleared in lactic acid. Pharate nymphs and adults were recognized as such when their legs and coxae were readily discernible and when separation of the nymphal or adult cuticle from the preceding stage was clearly defined.

OBSERVATIONS AND RESULTS

Description of larva

Unfed specimens rounded, about one and a third times as long as maximum breadth at about level of third pair of coxae (fig. 1 (1)).

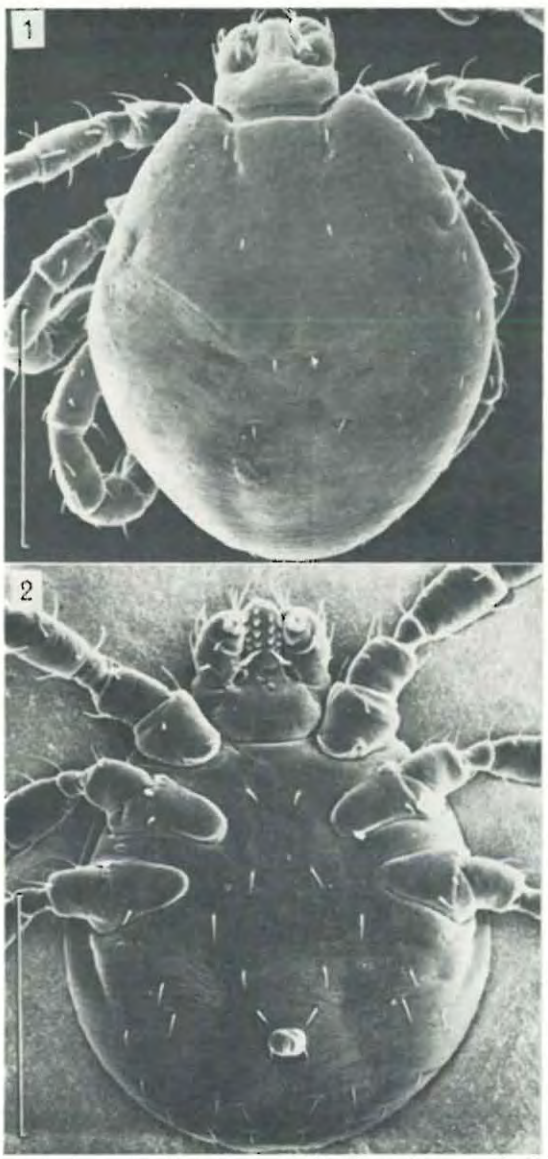

Fig. 1. *Boophilus decoloratus* larva. (1). Dorsal aspect. (2). Ventral aspect. (Scale lines = 0,25 mm).

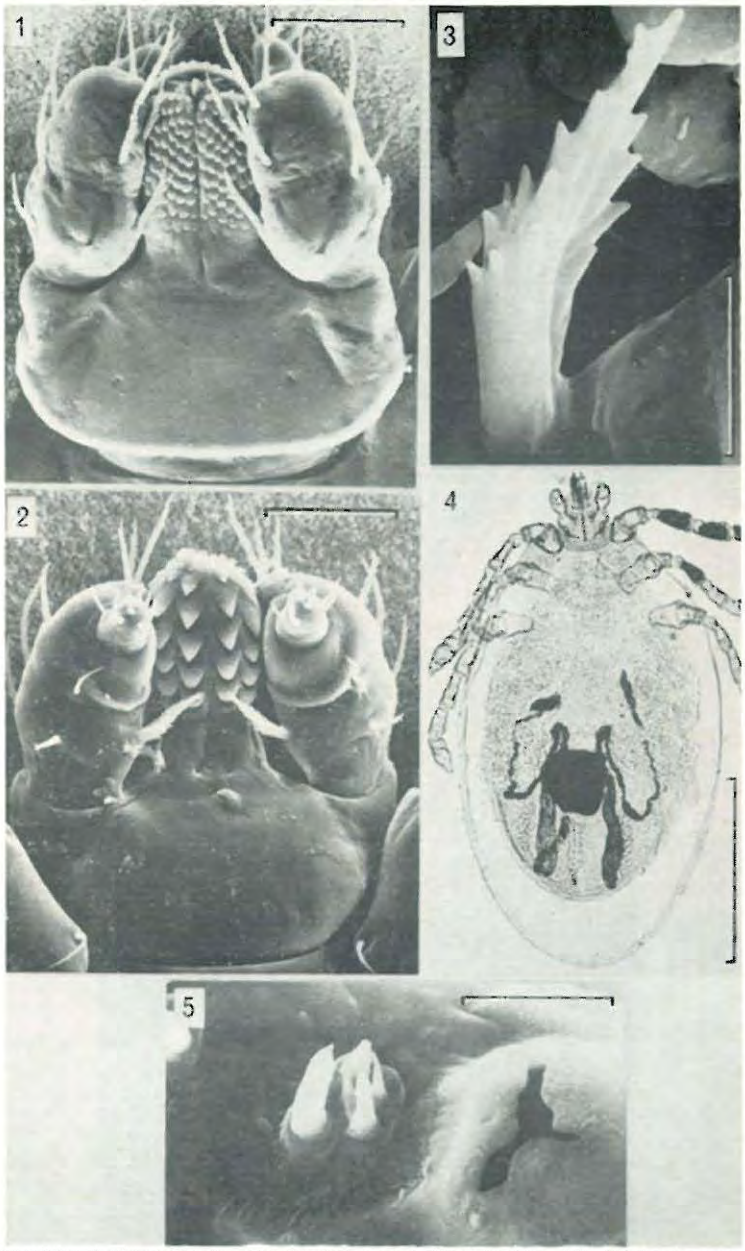

Fig. 2. *Boophilus decoloratus* larva. (1). Dorsal aspect of capitulum. (2). Ventral aspect of capitulum. (3). Ventral medial seta. (4). Ventral aspect of seventh day larva showing pharate nymph. (5). Haller's organ. (Scale lines: 1–2 = 0,05 mm, 3 and 5 = 0,01 mm, 4 = 0,5 mm).

CAPITULUM—DORSAL. (fig. 1 (1)). Basis length (as measured from base of external cheliceral sheath to posterior margin) to maximum width ratio about 1:2; posterior margin of basis, behind level of basis sensilla, as a flattened bow-shape, thence straight for a very short distance becoming indented, at origin of 'palpiger'. Surface generally flattened except for an antero-lateral triangular elevation immediately behind palpal insertion; two widely separated sensilla at about one third of length of basis.

PALPI. (fig. 2 (1)). Short, from 0,084 to 0,091 mm long, tendency to be bulbous. No suture line between segments 1 and 2. Ratio of length of segment 2, measured along dorsal surface from its postero-mesial protuberance to 'suture' with segment 3, to maximum width about 0,9 : 1,0; that of segment 3 1,2 : 1,0. Lateral profile of combined segments 1 and 2 sinuous, mesial profile of 2 mildly convex; both lateral and mesial profiles of segment 3 almost straight and converging to broadly rounded apex. Surface of segment 2 strongly tumescent, protuberant meso-posteriorly, this continued obliquely antero-laterally and mesial of the basal lateral seta of segment 1 and terminating baso-laterally of anterior lateral seta of same segment; surface of segment 3 smooth, evenly and gently curved to the midline and laterally, but more steeply to its junction with segment 4. Setae arranged in three longitudinal series: mesial series (M) consisting of two setae, M1 on segment 2 barbed on outer face, sub-parallel and shorter than similarly barbed M2 seta on segment 3; dorsal sub-median series (D) of four setae, D1 (vertical in position and accordingly much foreshortened in fig. 2 (1)) on segment 2, and D2 and D3 on segment 3 are multi-barbed, D4 of "fine" tapered type; lateral series (L) of three setae, all single barbed, of which two (L1 and L2) located on segment 2 and one (L3) on segment 3.

CAPITULUM—VENTRAL. (fig. 2 (2)). Basis transversely ovate; surface curved, sloping gently to hypostomal base and dipping more strongly to shallow, broad oblique depressions antero-laterally, beyond which the surface becomes slightly elevated to straight lateral margins.

PALPI. (fig. 2 (2)). No clear sutures between palpal segments 1 to 3; lateral profile of palps broadly arcuate, mesial profile only slightly so. Surface of palp with shallow irregular furrows basally; distal margin of segment 3 thickened and broader laterally than ventrally where it is flange-like; relative to the long axis this margin oblique and flange lacks any distinctive spur; extensive area of inter-segmental membrane on mesial and distal face of segment 3 on which palpal segment 4 is mounted. Two setae on segment 2 of which ventral-mesial (VM) is 'feather' like under light microscope, under scanning electron microscope consists of a stout base from which successive layers of barbs arise, deepest layer (i.e. the most dorsal) tapering to setal apex, and strongly serrated marginally (fig. 2 (3)); mid-ventral seta (MV) on this segment singly barbed with bifid or trifid apex; third ventral seta lies on mid-ventral line adjacent to the distal thickening of segment 3, and is of the 'fine' type. Column of segment 4 bears three tapering setae, apex carries 1 dorsal, 3 sub-dorsal, 3 supra-ventral and 1 ventral setae, all rounded apically.

According to Gothe (1967) the segments, other than the fourth, have "eight setae dorsally and four setae ventrally" and in this he is, doubtless, following the classification of Clifford & Anastos (1960). The reasons for the present palpal nomenclature are discussed elsewhere (Arthur, 1973).

HYPOSTOME. (fig. 2 (2)). Length 0,082—0,090 mm, spatulate, apex broadly rounded extending beyond palpal apices; dentition behind small sub-apical corona

2/2 files with six denticles per file, distal three rows sharply pointed, proximal three rows rounded; two basal medial depressions, in line with two widely separated post-hypostomal setae.

EXTRASCUTAL REGION. (fig. 1 (1)). One pair of sensilla sagittiformia (S) peripherally on postero-lateral margin; seven or eight pairs of marginal dorsal setae (Md1–7 or 8) of which Md1 to Md3 in a sub-marginal linear series, Md4 to Md7 (or Md8, when present) in a progressively marginal series, Md1–Md5 in front of sensillum; two pairs of central-dorsal setae (Cd1, 2) of which posterior pair significantly longer than anterior pair, and whose intersetal distance is greater.

SCUTUM. (fig. 1 (1)). 1,26–1,28 times as broad as long, greatest width across level of eyes, latter bulging, protruding beyond edge and surrounded by well defined orbit; posterior margin narrowly rounded, postero-lateral margins very weakly concave or sometimes straight, antero-lateral margins convex, scapulae broad; emargination well defined. Surface reticulately patterned; cervical grooves short, shallow, parallel sided with two circular depressions mesial of each, surface between grooves mildly elevated and beyond them sloping gradually to periphery. Scutal setae in three pairs (Sc1–3), Sc1 located anteriorly and laterally of cervical grooves, Sc3 in median field at about or slightly behind level of eyes, Sc2 at about two-thirds the length of antero-lateral margin and a short distance inwards from it, in length Sc1 > Sc3 > Sc2. Two pairs of depressions, one pair in angle formed by scapular margins, the other more medianly placed of a line connecting eye and Sc2.

VENTRAL SURFACE. (fig. 1 (2)). Surface, with fine epicuticular folds, longitudinal depression dorsal to coxal insertions and extending back to one third the distance from posterior edge. First pair of sternal setae (St1) on a level with first intercoxal space, second pair (St2) level with second intercoxal space, third pair (St3) posterior to third pair of coxae; relative lengths being St3 > St2 > St1, distance between setae of each pair increasing from St1 to St3. Two pairs of pre-anal setae (Pa1, 2), distance between anterior pair (Pa1) greater than that between posterior pair (Pa2). Pre-marginal setae (Pm) arranged in two groups, more anterior pairs (Pm1 and Pm2) about level with pre-anal setae, more posterior pairs (Pm3 and Pm4) located postero-laterally of anus and almost in line with fourth and fifth ventral marginal setae. Five pairs of ventral-marginal setae (Vm1–Vm5), Vm1 in line with lateral groove and slightly behind level of Pm2, thence successive setae (Vm2–Vm5) more marginally located. One pair of anal setae (An).

LEGS. (fig. 1 (1) & (2)). Tarsus I from 0,16–0,168 mm long, somewhat swollen in front of pseudo-articulation thence tapering gradually to apex; claws slightly longer than pulvilli. Dorsal setal alignment on tarsus I as a single seta on the declivity, one pre-halleral, and single pairs of halleral, post-halleral, antero-dorsal and postero-dorsal setae. Trough of Haller's organ (fig. 2 (5)) contains one long, curved seta, two moderately long setae with narrowly rounded apices, two setae tapering to sharp points; capsule aperture triadiate with transverse section more widely open than short longitudinal section.

COXAE, (fig. 1 (2)). Coxa I sub-triangular, mesial margin salient, extending to postero-internal face (not as figured by Gothe (1967) fig. 14), not protruding over its posterior margin but following the contour of coxal margin for a short distance. Following the nomenclature of Clifford & Anastos (1960) three coxal setae, Cl.al with single face barbed, Cl.mm and Cl.pl fine and tapered. Coxa II elongate, sub-rectangular, mesial margin broadly rounded, setae C2.al and C2.pl present, as well

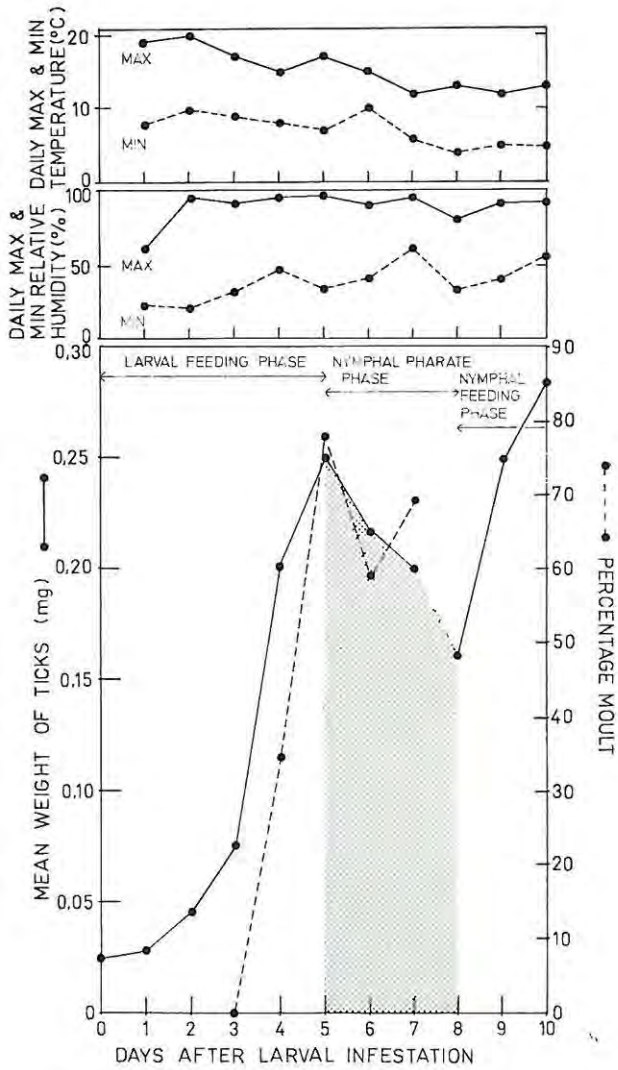

Fig. 3. The changes in weight of feeding and moulting *Boophilus decoloratus* larvae in relation to time, and the percentage number of larvae moulting after removal from the host on each successive day of the larval parasitic cycle. Daily maximum and minimum temperatures and relative humidities as recorded in the stable are shown separately above.

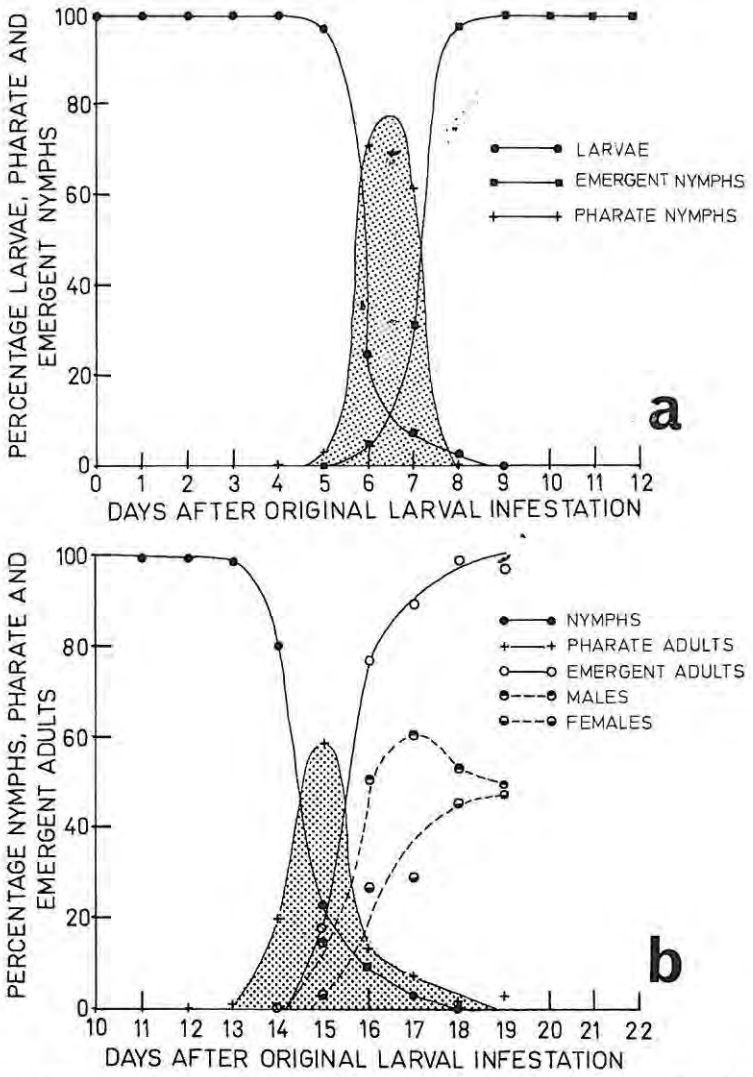

Fig. 4. (a). The percentage of larvae, pharate nymphs and emergent nymphs of *Boophilus decoloratus* present on each successive day of the parasitic cycle between the first and the twelfth days after the original larval infestation of the host. (b) The percentage of nymphs, pharate adults and emergent adults of *B. decoloratus* present on each successive day of the parasitic cycle between the tenth and the nineteenth day after the original larval infestation of the host.

as coxal sense organ on its anterior distal angle, posterior margin bearing a long narrow salience. Coxa III sub-triangular with mesial margin narrowly rounded and continued into posterior divergent margin; no salience or spur. Three pairs of sensilla sagittiformia present, the first dorsal to insertion of coxa I, the second and third in line with the postero-external angles of coxae II and III respectively.

Larval feeding and nymphal emergence

LARVAL FEEDING. The average weight of unfed larvae of *B. decoloratus* was 0,024 mg and, on the basis of their cumulative weight increases over time, the feeding period extended over about five days (fig. 3). Initially, the increase in body weight was relatively slow; on the first day after attachment this showed only a slight increase over that of the unfed larva, on the second day it almost doubled and on the third day was nearly trebled. The greatest increase occurred between the third and fourth days when imbibition of blood and tissue fluids was between two and three-fold, and average maximum intake of 0,225 mg was attained twenty-four hours later. In other words, the fully engorged larva was about ten times the weight of the unfed stage. Cleared specimens of larvae almost up to the fifth day of larval attachment (fig. 4a) showed no evidence of visible developmental changes.

PHARATE NYMPH. Larvae retained their external form for a further two or three days after completion of feeding i.e. from about the sixth to the eighth day after larval attachment. The mean weight of these 'larvae' during this period decreased (fig. 3). Such larvae, when cleared, showed substantial nymphal development within their cuticle (fig. 2 (4)) over three days (fig. 4a). Thus, it would appear that larvae are capable of rapid digestion of the blood meal and of almost immediate utilization of metabolic resources. It can also be inferred that histolysis (e.g. of larval body muscles) and histogenesis (e.g. the development of the nymphal spiracle and of the fourth pair of legs) occur concomitantly during the latter phases of feeding.

NYMPHAL EMERGENCE. The periods of metamorphosis and of moulting extended over three days and were normally completed by the eighth day after infestation with larvae. Reference to fig. 5 shows that over six runs, extending from March to October, larvae and nymphs occurred together on the hosts on the sixth day in two 'runs', on the seventh day in three and on the eighth day in one. On days previous to, and succeeding these days only larvae and nymphs respectively were present.

The series of infestations ranged through from summer to winter, and though there was variation in the diurnal and mean environmental factors of the ambient environment in the stable, there was little evidence of these fluctuations interfering with the pattern of the parasitic cycle. This was possibly attributable to the fact that maintenance of water balance in the ticks was derived from the blood intake from the host, and that the temperature of the host's body probably fluctuated less than the macroclimatic conditions in the stable.

Larvae removed from the host between the fifth and seventh days after the original infestation and transferred to an incubator (26°C, about 90% R.H.) for ten days gave a percentage moult of nearly 80% (fig. 3). Of those removed from the host on the fourth day less than 35% moulted. No moulting of larvae occurred when they were removed from the hosts within the first three days of attachment. This indicated that a threshold value of blood intake was required, not only for cuticle synthesis to be completed, but also to allow for the 'stimulation' of developmental changes.

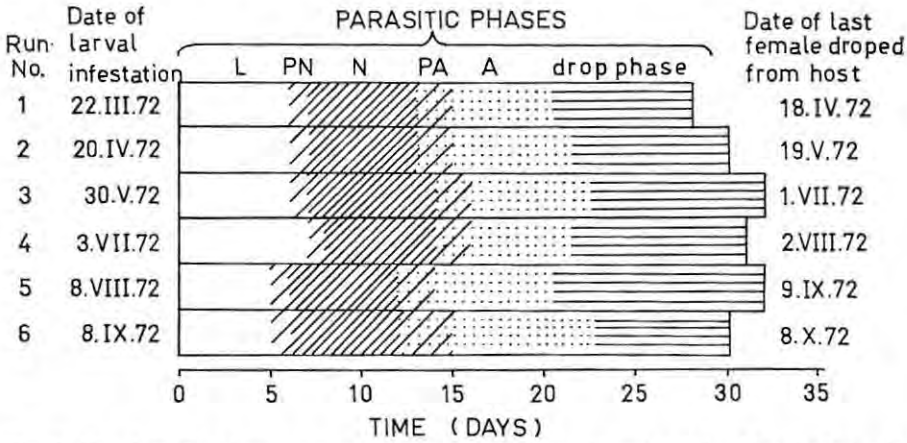

Fig. 5. The duration of each successive phase of the parasitic cycle of *Boophilus decoloratus* in six successive runs on the same host. L—Larval phase, PN—Pharate nymph phase, N—Nymphal phase, PA—Pharate adult phase, A—Adult phase.

Larvae fed for three days do not moult. Since a smaller proportion of those feeding for four days and a substantially larger proportion of those feeding for five days do, then the minimum blood intake to effect moulting is about 0,176 mg. As only about one-third of the four-day attached ticks moulted, the chances are that those larvae which did so after four days were those which had fed faster.

Description of nymph

Unfed specimens elongate oval (fig. 6 (1)), about twice as long as maximum width; fully fed specimens broader anteriorly at about level of scutum, where margins strongly undulate, thence narrowing to sharply rounded posterior margin.

Capitulum—dorsal. (fig. 7 (1)). Posterior margin straight or slightly indented in the middle, merging by way of strongly divergent, postero-lateral "angles" with mildly sinuous posterior-lateral margins to maximum width, thence through almost a right angle to straight, convergent antero-lateral margins to furrowed region, adjacent to insertion of palps. Surface smooth and curving gently to the periphery, except antero-laterally where it is elevated and longitudinally furrowed; a pair of widely separated basis sensilla slightly behind level of greatest width.

Palpi. (fig. 7 (1)). Palpal segment 1 not separated from segment 2, combined lengths of both measured along mid-longitudinal axis from 0,077–0,081 mm (mean 0,079 mm), with greatest breadth from 0,061–0,065 mm (mean 0,063 mm) at about halfway along segment 2; palpal segment 3 measures from 0,040–0,045 mm (mean 0,042 mm) long and greatest width from 0,056–0,059 mm (mean 0,057 mm) just beyond its base. Lateral profile concave in vicinity of the presumptive segment 1 thence, beyond a slight dilatation, straight to suture between segments 2 and 3, mesial profile of segment 2 arcuate with greatest width about mid-length; mesial profile of segment 3 convex becoming sharply rounded sub-apically, thence to a somewhat flattened apex which passes into lateral profile by a broadly curved margin. Segment 2 tumescent, gently

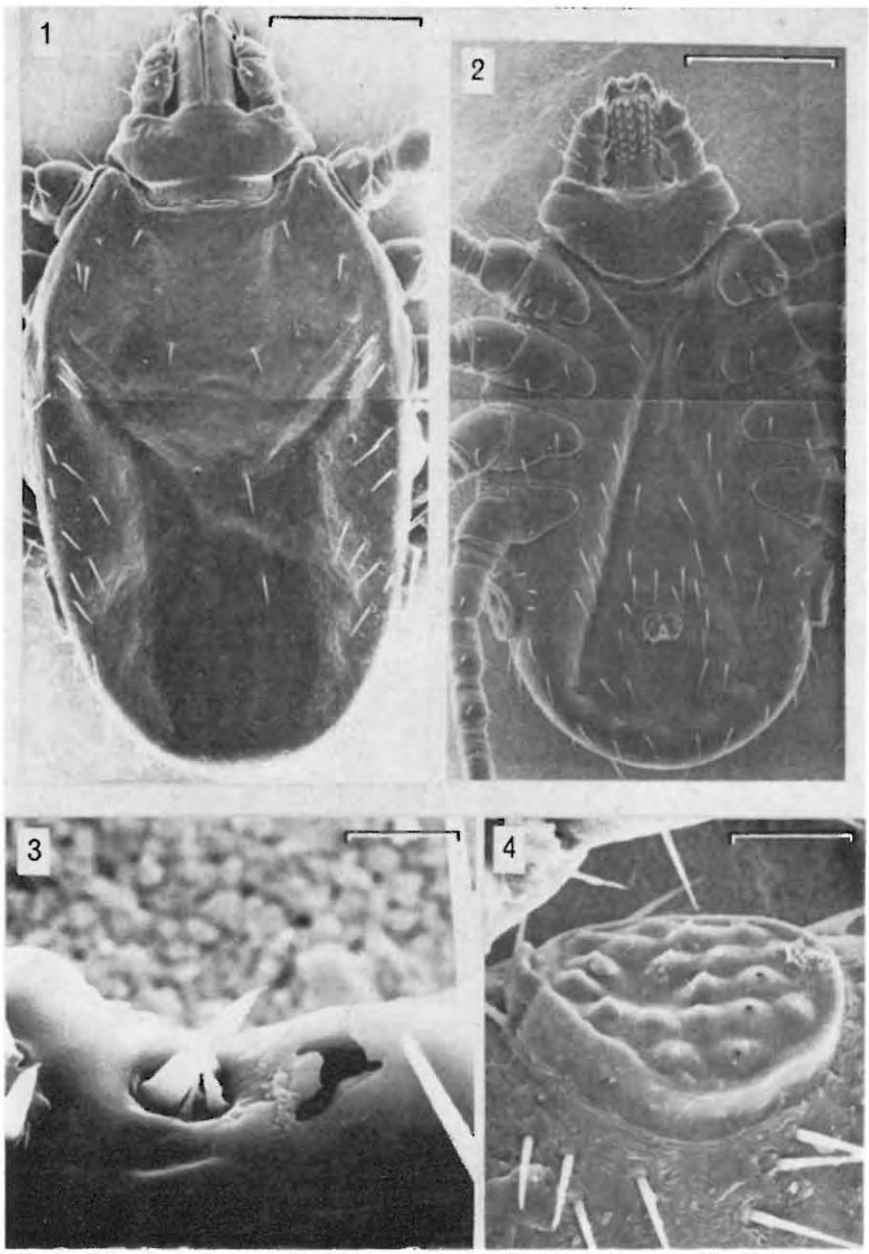

Fig. 6. *Boophilus decoloratus* nymph. (1). Dorsal aspect. (2). Ventral aspect. (3). Haller's organ. (4). Spiracular plate. (Scale lines: 1–2 = 0,25 mm, 3 = 0,01 mm, 4 = 0,05 mm).

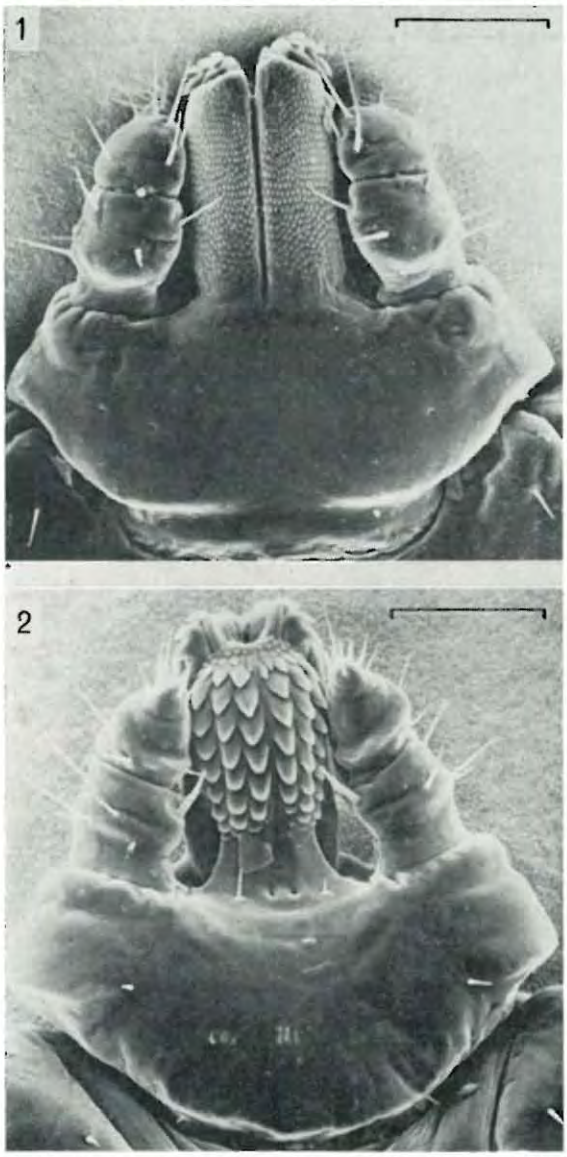

Fig. 7. *Boophilus decoloratus* nymph. (1). Dorsal aspect of capitulum. (2). Ventral aspect of capitulum. (Scale lines = 0,1 mm).

curved except baso-laterally, where it curves steeply downwards, at two-thirds the distance from base a transverse irregular crevice-like groove; segment 3 curves gently from mid-dorsal line. Setae absent on segment 1, setal pattern and numbers on segments 2 and 3 as in larva, but morphological form of setae different, all approximate to straight "fine" type. One palpal sensilla between M1 and D1 and a second posterior of M2.

CAPITULUM—VENTRAL. (fig. 7 (2)). Posterior and postero-lateral margins of basis markedly convex diverging to greatest width, thence sharply angled to short, straight, convergent antero-lateral margins, interrupted only by a shallow depression associated with short, transverse furrow; region subtending palpal insertion bears short, shallow depressions. At greatest width surface sharply ridged for short distance transversely, at this level a pair of widely separated basis setae; a pair of closely set post-hypostomal setae of similar length, with two deep pits between them at hypostomal base. Pair of setae on declivitous posterior face of basis.

PALPI. (fig. 7 (2)). No separation between palpal segments 1 and 2; no setae arise from the presumed site of former; lateral profile of combined segments 1 and 2 biconcave, being separated by an intervening transverse, elevated ridge continuous across most of ventral surface of segment, mesial profile of combined segments 1 and 2 concave; lateral profile of 3 short and arcuate, mesial profile shorter and straight, distal margin as a ventral flanged and cushion-like thickening, but not drawn out into a pronounced spur; placed obliquely to long axis of segment. Segment 4 cone-like and borne on an extensive area of intersegmental membrane. Presumed segment 1 lacking setae; segment 2 with three setae, of which two are probably homologous with larval "feathered" ventral-mesial (VM) and with mid-ventral setae (MV); the third more laterally located on ridge; two setae on segment 3 one being the counterpart of the larval mid-ventral and the other is short and ventral-mesial; segment 4 with one dorsal, three sub-dorsal, three supra-ventral and one ventral setae with rounded tips apically, and three finer tapered setae on column.

HYPOSTOME. (fig. 7 (2)). Length from post-hypostomal setae to broadly and shallowly indented apex from 0,14–0,15 mm; spatulate, tapering only gradually for about two-thirds its length from apex, broadening basally; toothed for more than two-thirds of length, dentition behind apex of 2 rows of 5/5 very small teeth with some having bifid tips, 1 row of 4/4 teeth (this may be variable and irregular), succeeded by three files on either side of midline of which lateral one bears 8 teeth, intermediate one 7 teeth and mesial one 6 teeth, distal rows of teeth sharply pointed becoming more rounded proximally and merging imperceptibly into crenulations.

POST-SCUTAL AREA. (fig. 6 (1)). Surface with few, small, deep punctuations. Setae arranged as two pairs in position of larval Cd 1 and 2; other setae present as shown in fig. 6 (1), but we are unable to correlate these with the larval condition.

SCUTUM. (fig. 6 (1)). In unfed nymphs length, measured from tips of scapulae to posterior margin, 0,45–0,47 mm; greatest width 0,44–0,45 mm across eyes, i.e. slightly longer than broad; eyes oval, protuberant over scutal margin. Posterior margin narrowly rounded, postero-lateral margins rectilinear, or weakly concave, thence mildly undulate and convergent to prominent scapulae with rounded apices; emargination deep. Cervical grooves very shallow, convergent at first then divergent and reaching back to postero-lateral margins. Setae present in comparable situations to Sc1, Sc2 and Sc3 of larva, supplemented by further pairs on scapulae, adjacent to Sc2 and between Sc2 and Sc1. Surface smooth with few, small shallow punctuations.

VENTRAL SURFACE. (fig. 6 (2)). In newly moulted nymphs longitudinal divergent grooves extend from level of first intercoxal space almost to posterior margin. Surface with widely dispersed shallow pits; supra-coxal groove, seen in other ixodid nymphs, absent. Some variation involving reduction or duplication of setae but following broad pattern of setal distribution emerges: Sternal setae (St)—one pair at level of first intercoxal space, one pair at level of second intercoxal space, one pair at level of third coxae and two pairs arranged transversely at level of fourth coxae; pre-anal setae (Pa)—an anterior pair of widely separated setae succeeded by two groups of three setae in position of second pair of larval pre-anal setae (Pa2); lateral anal setae—two pairs; pre-marginal setae (Pm)—a group of setae (3–5 pairs usually) positionally equivalent to first two pairs of larval pre-marginals (Pm1, 2), and retention of two pairs of larval pre-marginals postero-lateral of the anus (Pm3, 4); ventral marginals (Vm)—five pairs as in larva, circum-spiracular setae—variable in number.

SPIRACULAR PLATE. (fig. 6 (4)). Circular in surface view, elevated above body surface, margins steep sided and not sunk into cuticle; surface produced into about twenty protuberances, each having an apical aperture.

LEGS. (fig. 6 (2)). Tarsus I short and stumpy from 0,138–0,147 mm long, tapering rapidly to a blunt point, claws longer than pulvillus; Haller's organ with six sensillae in trough and aperture of the capsule as figured (fig. 6 (3)). Tarsus IV short and stumpy from 0,132–0,141 mm tapering rapidly towards distal end. Coxa I sub-triangular with setae C1 al, C1 mm and C1 pl of about equal length; internal spur broad, originating from the antero-mesial margin extending back to overlap the posterior edge for about a third of its length, postero-internal angle sharply rounded; external spur narrower tapered apically, not overlapping posterior edge of coxa; II elongate with antero-mesial angle more broadly convex than postero-mesial angle, distal half of edge of posterior margin very much broader than proximal half due to pronounced salient ridge, setae C2 mm and C2 pl of about equal length; III shorter than II, but as broad or even slightly broader, postero-external salience less clearly defined than in II, setae C3 mm and C2 pl of about equal length; IV sub-triangular with only C4 pl present and lacking spurs. Sensilla sagittiformia lacking.

Nymphal feeding and adult emergence

FEEDING. During the process of moulting the average weight of the fully engorged larva was reduced from 0,249 mg to 0,161 mg for the emergent nymph. This in part, was due to the loss of the larval cuticle, the release of guanine and possibly of water. The feeding pattern of the nymphs (fig. 8) paralleled that of the larva and, as judged by continued weight increases to a maximum, takes five days. Over the first two days the average uptake of blood was 0,12 mg, which was less than the average body weight of an unfed nymph. On the third day the average amount of blood taken in was 0,404 mg. This was followed by rapid uptake on the fourth day when the ticks weighed ten times the original weight and the average quantity of blood ingested was 1,44 mg. On the fifth day, feeding slowed down and the average weight of gorged nymphs was 1,90 mg. The apparent mean weight increase in nymphs fourteen days after the original larval infestation was not real as moulting had already occurred in part of the tick population. If the mean weights for all the ticks, nymphs and adults, was to be calculated for the fifteenth and sixteenth days, the values would be lower than that recorded for nymphs on the fourteenth day.

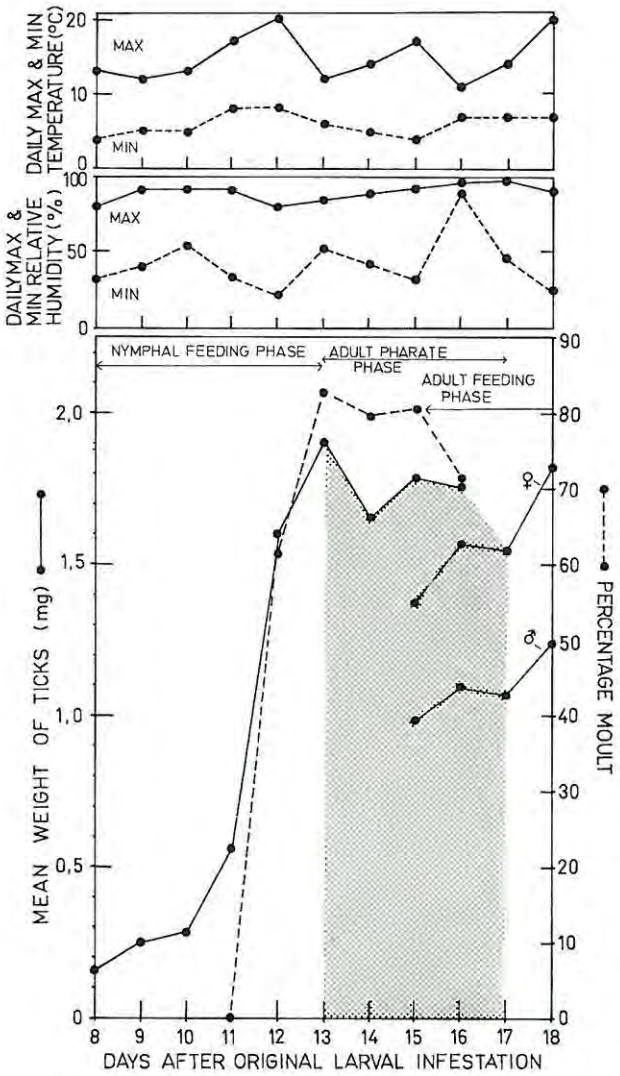

Fig. 8. The changes in weight of feeding and moulting *Boophilus decoloratus* nymphs in relation to time, and the percentage number of nymphs moulting after removal from the host on each successive day of the nymphal parasitic cycle. Daily maximum and minimum temperatures and relative humidities as recorded in the stable are shown separately above.

EMERGENCE OF ADULTS. Analysis of six successive runs of infestation of cattle by *B. decoloratus* beginning on 22 March 1972 and ceasing on 8 October 1972 (fig. 5) showed that nymphs alone, as judged by their external form, were present on hosts for 6, 6, 7, 6, 6 and 6 days giving an average of 6,2 days. After the ultimate day of occurrence of only nymphs on the hosts, nymphs and adults were recorded in five "runs" for two days, and in one for four days following which only adults were collected.

The results presented in fig. 4b show the components of random samples of the population on the host, on a daily basis after clearing the nymphs in lactic acid. From the seventh to the twelfth day after the original infestation by larvae the treated nymphs showed no evidence of differentiation into adults. On the thirteenth day 0,7% of the nymphal population sample yielded pharate adults, this percentage increased to 20% on the fourteenth day, to a maximum of 59–60% on the fifteenth day and thereafter declined to 13% and 7% on the sixteenth and seventeenth days. Only occasional pharate specimens were collected on the eighteenth and nineteenth days.

Nymphs collected from cattle after feeding for one, two or three days, and placed in an incubator failed to moult (fig. 8). Those removed on the fourth and fifth days of feeding and similarly treated yielded substantial numbers of adults. Correlating successful moulting of nymphs to adults with mean blood uptake on successive days indicates that about 1,5 mg of blood is a necessary pre-requisite for complete development.

The first emergence of adults was on the fifteenth day, i.e. on the same day as the maximum occurrence of pharate adults. Initially, the males outnumbered females by about 5 : 1, but on the two succeeding days the ratio altered to 2 : 1, and within a further two days males and females were present in equal numbers in samples of the population (fig. 4b).

Since (i) the first appearance of pharate nymphs in the population occurred two days before the first emergence of adults and (ii) the period of greatest pharate incidence was up to four days it appears likely that the blood meal is rapidly metabolised, and that this is accompanied by rapid, concurrent development to the succeeding stage.

Description of female

Elongate oval (fig. 9 (1)) about 4,8 mm long, about twice as long as broad with greatest width of about 2,4 mm, at about the level of spiracles, when engorged bluish in colour and measuring approximately 12,0 mm long and 8,0 mm wide.

CAPITULUM—DORSAL. (fig. 10 (1)). Posterior margin of basis straight with median indentation, in most specimens cornua absent, if present only faintly indicated, postero-lateral angles broadly rounded, straight or mildly concave postero-lateral margins, strongly divergent to greatest width, thence sharply angled and convergent to palpiger. Length (measured from bifurcation of external cheliceral sheaths to posterior margin) to breadth ratio 1 : 2,5. Porose areas elevated, oval, oblique to long axis, separated by an interval either equal to, or greater than maximum length of one of them. From elevated region around the porose areas, surface slopes almost declivitously to irregular postero-lateral surface, and more gradually to an anterior median depressed region; two or three pairs of hairs antero-laterally.

PALPI. (fig. 9 (3)). Length of combined segments 1 to 3, when measured mid-dorsally, between 0,23 and 0,25 mm; segment 1 broader than long, with straight mesial margin, lateral profile concave, setae absent; segment 2 with mildly arcuate mesial profile, longer than straight converging lateral profile; mesial profile of segment 3

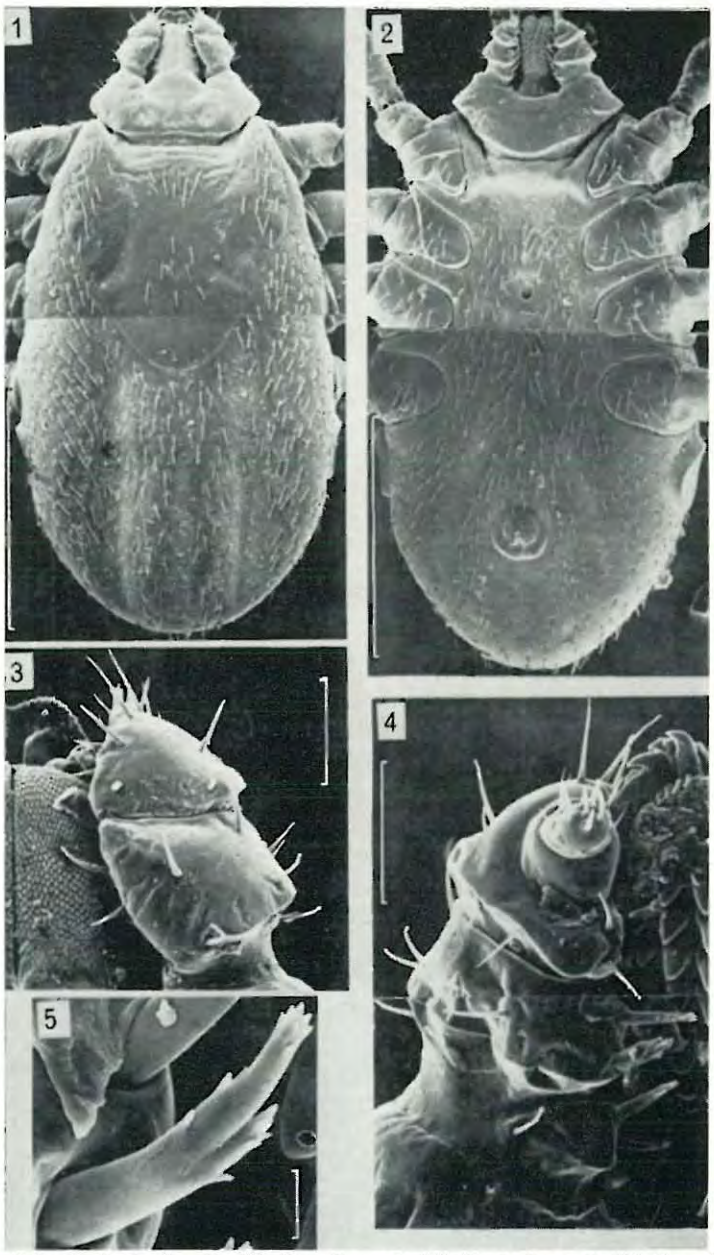

Fig. 9. *Boophilus decoloratus* female. (1). Dorsal aspect. (2). Ventral aspect. (3). Dorsal aspect of palp. (4). Ventral aspect of palp. (5). Multi-pointed ventral medial seta. (Scale lines: 1–2 = 1,0 mm, 3–4 = 0,1 mm, 5 = 0,01 mm).

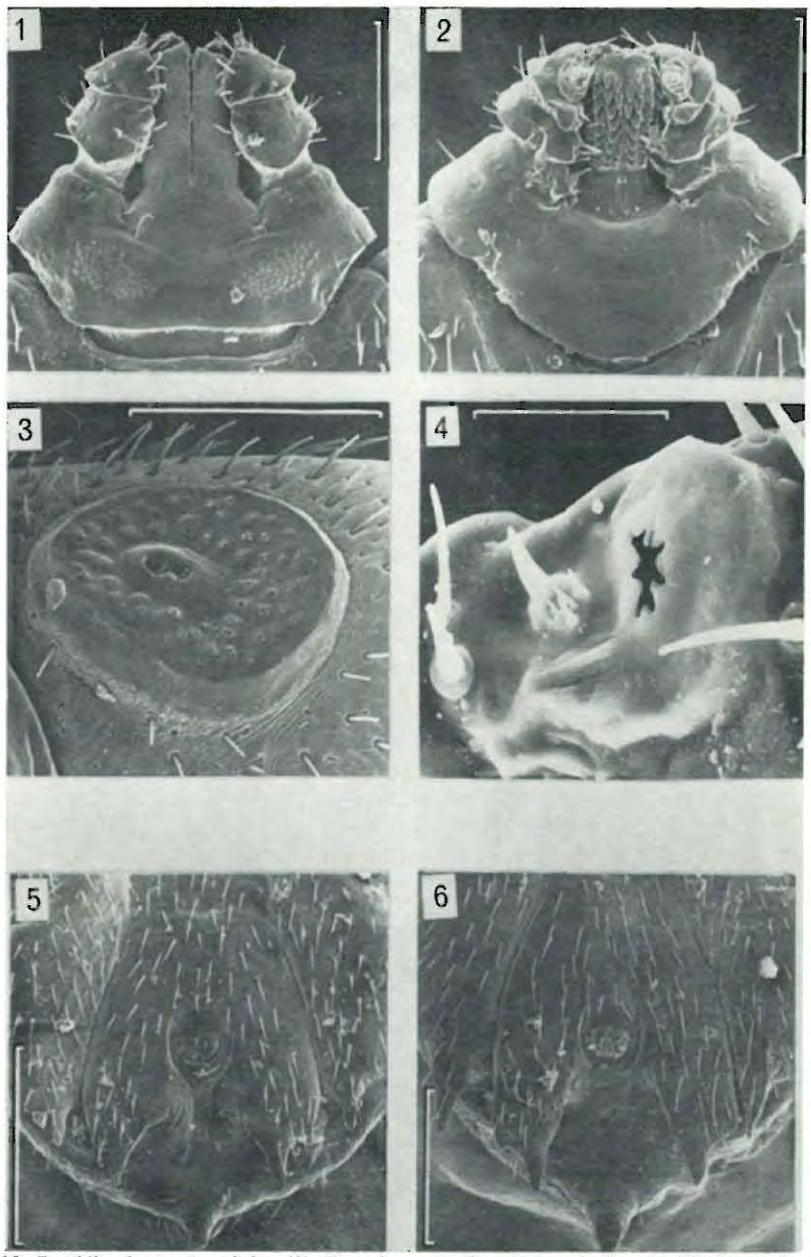

Fig. 10. *Boophilus decoloratus* adults. (1). Dorsal aspect of capitulum in female. (2). Ventral aspect of capitulum in female. (3). Spiracular plate of female. (4) Haller's organ of female. (5) and (6). Ventral aspects of anal areas of a small and a large male. (Scale lines: 1–3 = 0,25 mm, 4 = 0,1 mm, 5–6 = 0,5 mm).

mildly convex terminates in sharply rounded apex, thence continuous with mildly curved lateral profile to near origin of segment, where it is seen dorsally as a lateral spur; seen end-on this spur is a flange continuous with ventral ridge of segment 3 (fig. 9 (4)). Surface curves gently from mesial margin laterally, except for declivitous proximal edge of the basal lateral spur on segment 3, and along base of segment 2. Setae arranged in four series, mesial series of 2 on segment 2 and 2 on segment 3; dorsal sub-median with 2 on segment 2, 4 on segment 3; dorsal sub-lateral series of 2 on segment 2, 2 on segment 3; the lateral series of 2 on segment 2, 0 on segment 3; all of 'fine' setal type.

CAPITULUM—VENTRAL. (fig. 10 (2)). Posterior edge declivitous with groups of short, stumpy setae laterally; posterior and postero-lateral margins broadly curved to greatest width, thence by weakly concave antero-lateral margins to palpal insertions. Surface generally flattened except for two semi-lunar elevations, with steep posterior faces adjacent to regions of greatest width, a sharp slope to hypostomal base between palps, longitudinally oblique furrows antero-laterally. Three or four pairs of setae at about level of greatest width and in proximity with antero-lateral margins, 5 or 6 shorter setae adjacent to semi-lunar elevations.

PALPI. (fig. 9 (4)). Segment 1 with concave lateral profile, mesial profile with basal half weakly demi-concave, then angled to straight distal edge, separated from segment 2 mesially by transverse V-shaped cleft; one pair of fine setae antero-laterally, a single short, mid-ventral, apically serrated seta, one or two pairs of stout bifid, multi-pointed setae (fig. 9 (5)) on distal straight margin. Segment 2 with strong irregular transverse elevation lateral of large median flattened spur, posterior face declivitous; lateral profile sharply angled and projecting beyond margin of segment 1, mesial profile arcuate, convergent distally; surface very irregular; one basal lateral seta, a transverse row of setae on anterior face of ridge, two or three stout, multi-pointed setae on mesial margin as shown in fig. 9 (4). Segment 3 with declivitous posterior face bearing two or three fine setae, which are serrated sub-apically; towards the mid-line posterior margin extended into a triangular flange, before straightening to strongly projecting basal-lateral extremity, beyond which lateral profile gently arcuate; mesial profile short, broadly rounded with two or three short, broad, tapered setae interposed between it and triangular flange (fig. 9 (4)), distal margin obliquely curved, with extensive area of intersegmental membrane between it and base of segment 4. Segment 4 cone-like, with fine tapered setae on column and eight round tipped setae on the apex, arranged as in nymph.

HYPOSTOME. (fig. 10 (2)). Length from single pair of post-hypostomal setae to apex 0,27–0,29 mm; spatulate with broad, shallow indentation mid-apically; pronounced sub-apical corona of minute denticles followed either by one row of 5/5 or 4/4 teeth, then by up to nine or ten rows of 3/3 teeth; may become irregular basally where teeth merge imperceptibly with crenulations.

POST-SCUTAL AREA. (fig. 9 (1)). With dense covering of setae of moderate length, except in the depressions of the posterior median and paramedian grooves; punctations small and shallow.

SCUTUM. (fig. 9 (1)). Length, measured from tips of scapulae to posterior margin 0,94–1,1 mm, greatest breadth in front of mid-length across the eyes 0,82–1,03 mm; length/breadth ratio about 1,15 : 1,0. Posterior margin narrowly rounded, postero-lateral margins almost rectilinear and divergent to level of eyes, latter mildly protuberant, antero-lateral margins arcuate to sharply rounded apices, emargination well defined. Cervical grooves broad, shallow, convergent at first, then divergent to end at

about midway along postero-lateral margins. Setae of moderate length, arranged along the antero-lateral margins, between cervical grooves anteriorly and as a postero-median group.

VENTRAL SURFACE. (fig. 9 (2)). Setae of moderate length well distributed over ventral surface, absent only from coxal and intercoxal regions of I, around genital orifice, along genital grooves and posterior to anus. Genital opening on level with second intercoxal space. Anal groove obsolete.

LEGS. (fig. 9 (2)). Pale yellow, longer but not as stout as in male. Coxa I sub-triangular, with two well defined apically rounded spurs, outer spur broader and shorter, discernibly separated by narrow but deep inverted V-shaped cleft, do not usually protrude beyond coxal margin, coxa II sub-rectangular, with broadly rounded external spur, coxa III sub-rectangular lacking external spur but slight salience may be present, coxa IV broad, sub-triangular, usually lacking salience postero-externally, all supplied with fine setae most of which are longer than those on the ventral integument. Tarsus I short, from 0,40 to 0,43 mm long, tapers rapidly to rounded apex, with ventral sub-apical spur; tarsus IV from 0,37 to 0,39 mm, tapering fairly gradually towards rounded apex with two ventral retrograde spurs. Insertion of pulvillus apical. Haller's organ as figured (fig. 10 (4)).

SPIRACULAR PLATE. (fig. 10 (3)). Almost circular in surface view, peripheral walls vertical, not sunk into surrounding cuticle; macula elevated, antero-basally placed in relation to long axis of body, surrounded by about 50 to 60 aperture bearing protuberances on surface.

Description of male

An examination of samples of males of *B. decoloratus* during biological investigations makes it clear that there is a particularly wide range of sizes and that within a single population there may be two discrete peaks in size. Concomitant with this, certain morphological characters may be reduced or enhanced. As a consequence assigning a dimension, or a range of dimensions, to particular features in the males of this species is fraught with risk.

CAPITULUM—DORSAL. (fig. 12 (1)). Posterior margin concave with variably developed cornua, lateral margins arcuate or undulate to level of palpiger, where they are indented. Surface flattened mid-dorsally, slightly elevated, curving downwards peripherally; slightly behind mid-length a transverse arc of eight or nine conspicuous setae leading to a group of about four peripheral setae.

PALPI. (fig. 11 (3)). Short, broad, suture lacking between segments 1 and 2; lateral profile of presumed segment 1 straight except distally where it contributes to proximal ridge of segment 2, mesial profile shorter and curved; mesial profile of segment 2 undulate, almost straight, lateral profile either rectilinear, or may be undulate and irregular, shorter than mesial margin; distal edge straight and horizontal in resting palp, proximal margin broadly curved at postero-internal angle whence slightly obliquely and antero-laterally to palpal margin; posterior face short, declivitous, bears two setae; segment 3 with straight posterior margin and steep face at junction with segment 2, especially at its projection beyond distal lateral edge of segment 2 to spur-like extension, mesial profile gently convex to broadly rounded apex, thence to straight lateral profile to basal spur-like extension. Setae in four linear series, mesial series of two setae on each of segments 2 and 3; dorsal sub-median series of two setae on segment 2, 4 on segment 3,

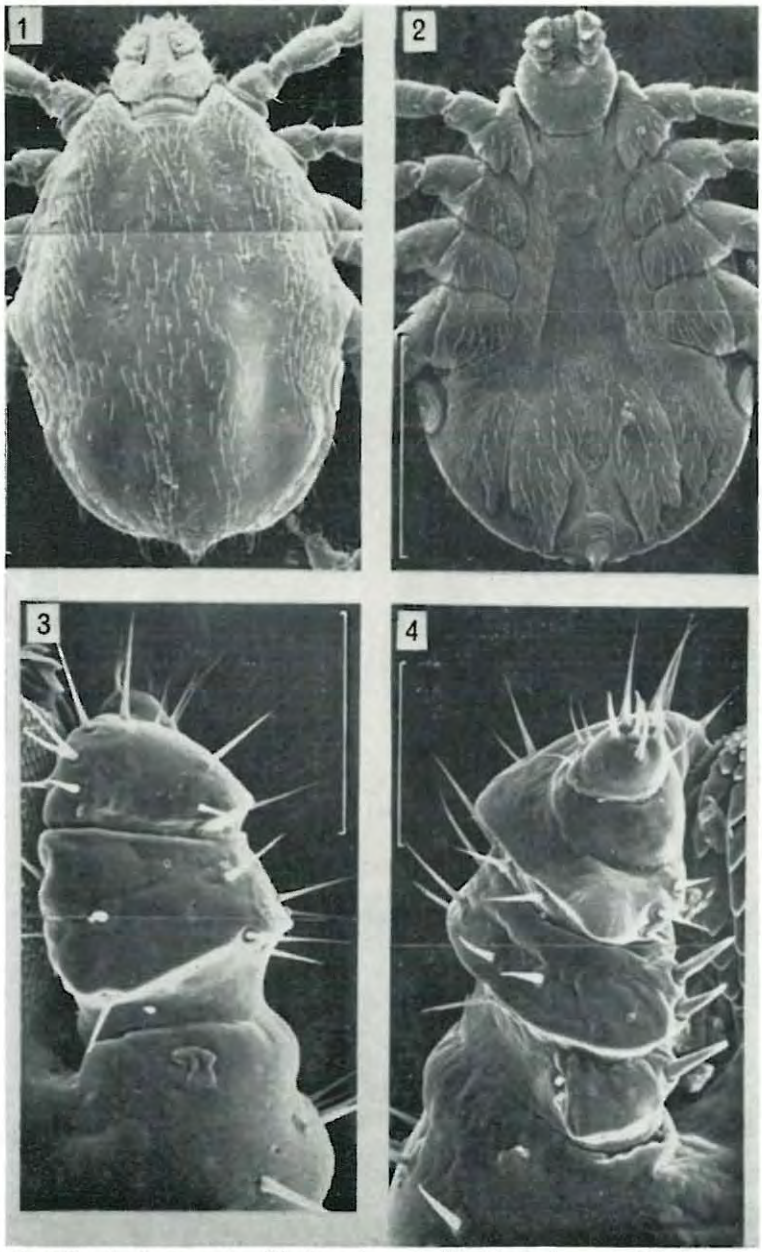

Fig. 11. *Boophilus decoloratus* male. (1). Dorsal aspect. (2). Ventral aspect. (3). Dorsal aspect of palp. (4). Ventral aspect of palp. (Scale lines: 1–2 = 1,0 mm, 3–4 = 0,1 mm).

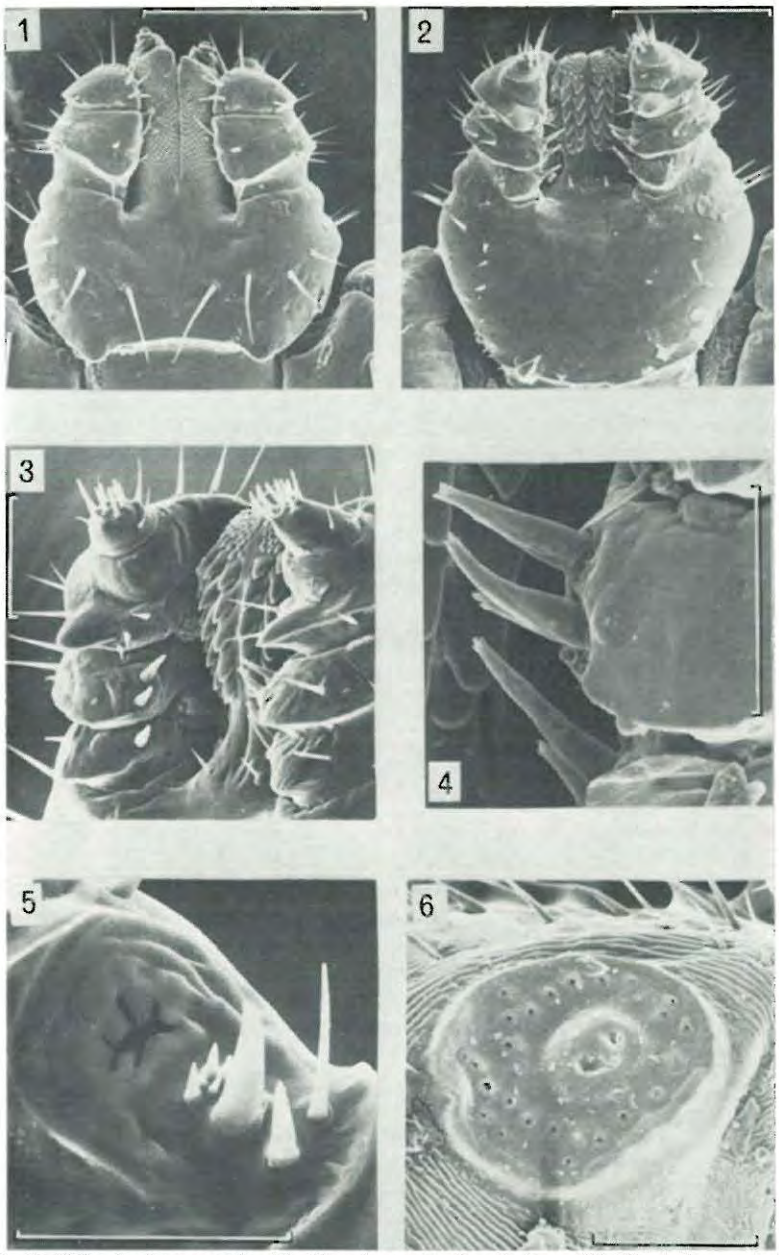

Fig. 12. *Boophilus decoloratus* male. (1). Dorsal aspect of capitulum. (2). Ventral aspect of capitulum. (3). Ventro-lateral aspect of palps and hypostome. (4). Multi-pointed ventral medial setae. (5). Haller's organ. (6). Spiracular plate. (Scale lines: 1–2 = 0,25 mm, 3 and 6 = 0,1 mm, 4–5 = 0,05 mm.)

dorsal sub-lateral series of two setae on segment 2, and two on segment 3, and a lateral series of two on segment 2.

CAPITULUM—VENTRAL. (fig. 12 (2)). Posterior margin broadly curved, postero-lateral angles rounded, divergent lateral margins curved to greatest width slightly behind palpal insertion, thence convergent gradually to latter. Surface strongly down-curved laterally, otherwise gently convex. Mesial of antero-lateral group of setae, longitudinal rows of shorter setae.

PALPI. (fig. 11 (4)). Lateral margin of segment 1 short, straight, mesial margin angled and longer than lateral margins (fig. 11 (4)); dorsal-median surface thickened to form salient curved flange posteriorly (fig. 12 (3)); one fine seta on median edge of thickened area, either one or two stout, multi-pointed setae arise from distal mesial face. Segment 2 broader than long, posterior edge salient; at junction with segment 1 face of segment 2 steep; proximal margin arcuate and approximately parallel with, but longer than distal margin, lateral profile straight, mesial margin irregular; transverse row of three setae at about mid-length, one fine seta on steep posterior face, two or three stout, multi-pointed setae on mesial face; median face of segment 3 forming a triangular spur overlapping distal margin of 2 (fig. 12 (3)), beyond this curved laterally, flattened and extended into dorsal ridge of 3, projects beyond lateral margin of 2 (fig. 11 (4)); lateral margin sinuous or arcuate to apex; two setae on steep posterior face, one of which at outer base of spur and three stout mesial setae subtending inner base of spur. Segment 4 columnar with apical setal distribution as in female.

HYPOSTOME. (fig. 12 (2)). Single pair of closely-set, short post-hypostomal setae, with two shallow depressions between them. Length from post-hypostomal setae to rounded apex 0,17 to 0,20 mm, spatulate shaped; corona of small denticles occupying up to one quarter length of toothed region, followed by one row of 4/4, then 6 rows of 3/3, with occasionally some rows of 3,5/3,5.

DORSAL SURFACE. (fig. 11 (1)). Body widest about level of fourth coxae, yellow to brown, weakly sclerotized, gut caecae as dark outlines through translucent cuticle. Small triangular caudal appendage on posterior margin.

SCUTUM. (fig. 11 (1)). Broadly rounded behind, with irregular outline at caudal base, indented at spiracular level thence broadens to level of fourth coxae, before narrowing gradually by undulating margins to sub-triangular scapulae, emargination deep; eyes large, yellow, circular, at level of coxae II, not overlapping scutal margin. Cervical grooves shallow, diverging posteriorly and reaching back beyond eye-level. Pair of shallow depressions at level of third intercoxal space and coxae IV; pair of posterior-lateral grooves in line with depressions, shallow, broad and reaching posterior margin; postero-median groove extending from in front of spiracular level to caudal base. Setae absent from grooves and depressions, giving setal pattern as shown in fig. 11 (1).

VENTRAL SURFACE. (fig. 11 (2)). Genital aperture level with second pair of coxae. Surface uniformly and abundantly setose except between first pair of coxae, between the ad-anal shields behind anus, on caudal appendage and posterior of genital grooves. Considerable variation in size and development of ad-anal and accessory shields (fig. 10 (5) & (6)); ad-anal shields elongate and, when well developed, internal spur strong, sub-triangular separated either by shallow concavity or a cleft from smaller external spur, latter may be obsolescent; in large specimens internal spur may project beyond posterior margin, in small specimens frequently fails to reach margin;

accessory shields drawn out to single points, whether they protrude beyond posterior margin or not depends on overall size of the male; both shields sparsely punctate.

SPIRACULAR PLATE. (fig. 12 (6)). Similar to female, but with fewer goblets.

LEGS. (fig. 11 (1) & (2)). Stout. Coxae large, with dense covering of setae; I sub-triangular, drawn out postero-internally into narrowly rounded spur, external spur triangular, protruding beyond coxal margin; II sub-rectangular with antero-internal angle broadly curved, postero-internal angle salient, external spur short and broadly rounded; III similar in shape to II, but broader, external spur shorter; IV broader than either II or III, sub-triangular with postero-external corner drawn out but lacking external spur. Tarsus I from 0,33 to 0,36 mm long, tapering fairly rapidly to rounded apex, small sub-apical spur, tarsus IV from 0,35 to 0,36 mm long, two retrograde sub-apical spurs; insertion of pulvillus apical. Haller's organ as illustrated (fig. 12 (5)).

Adult feeding and female drop

Emergent males and females were lighter in weight than the gorged nymphs from which they developed, probably for the same reasons that emergent nymphs were lighter than gorged larvae. Emerging males were lighter than emerging females; males weighed an average about 0,98 mg and females 1,37 mg. Both males and females attached immediately after moulting. This is in contrast to the information given by Legg (1930), for the closely allied *B. australis* Fuller (=*B. microplus* Canestrini), where male ticks were reported not to attach but searched for unfertilized females.

Over the first three days (days 16, 17, 18 in fig. 13) of attachment the weight increases of males and females were almost in parallel and up to the sixth day (day 21 in fig. 13) of feeding there was a relatively insignificant increase in the weight of males. The weight of females began to increase on the fourth day of feeding (day 19 in fig. 13) at which time, too, mating was first observed. By the sixth day (day 21) the quantity of blood imbibed averaged 23,9 mg per female. The first satiated females dropped from the host on the seventh day after adult emergence, having attained weights of four to eight times greater than those of the average weights of the sixth day. Fully-fed females continued to drop off for up to twelve days after this, with the majority detaching within two to five days (fig. 14d). Comparison of fig. 14c and d for weights of engorged females and their dropping-off times shows no definite relationship, except that in infestations 1, 2 and 4 there is a tendency for heavier females to fall at the peak period of drop-off.

DISCUSSION

One of the features of the life cycle of one-host ticks, such as *B. decoloratus*, when compared with three-host ticks, in particular, is the rapidity with which the parasitic life cycle, from larval attachment to the fully engorged female, is completed. In *B. decoloratus*, up to the time of the first drop-off of females ready for egg-laying, this is between 21 and 23 days, in *B. australis* (= *B. microplus*) it is generally 20–21 days (more exceptionally 23 or 25 days) according to Legg (1930), and for the same species is approximately 18 days, according to Roberts (1968).

No attempt has been made to account for the differences in the length of time taken for one-host and three-host ticks to complete their parasitic cycles. One reason for this has been the lack of information on the length of the feeding times of larvae and

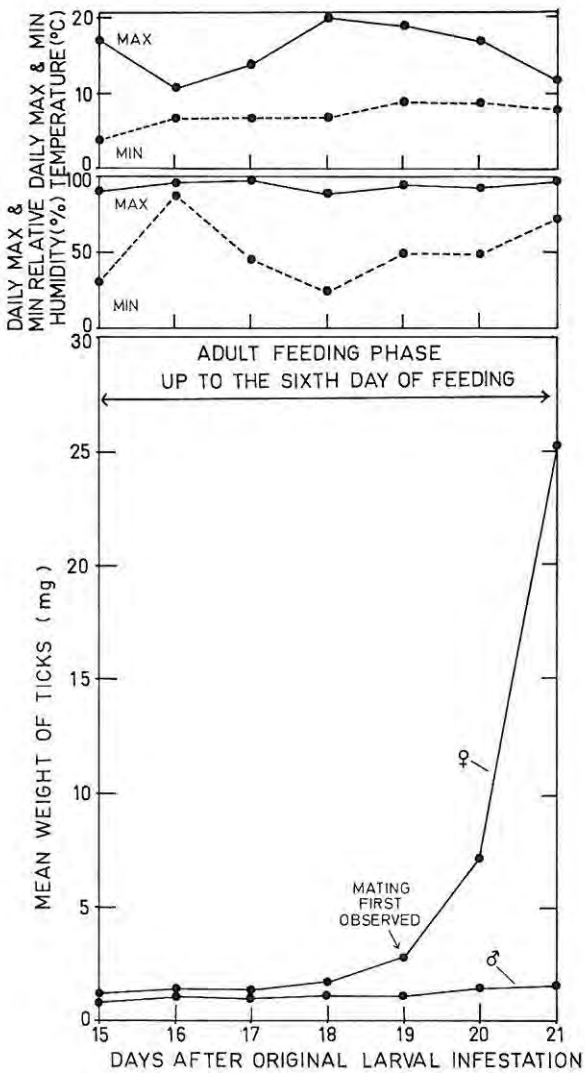

Fig. 13. The changes in weight of feeding *Boophilus decoloratus* adults in relation to time, up until the day before the first females dropped from the host. Daily maximum and minimum temperatures and relative humidities as recorded in the stable are shown separately above.

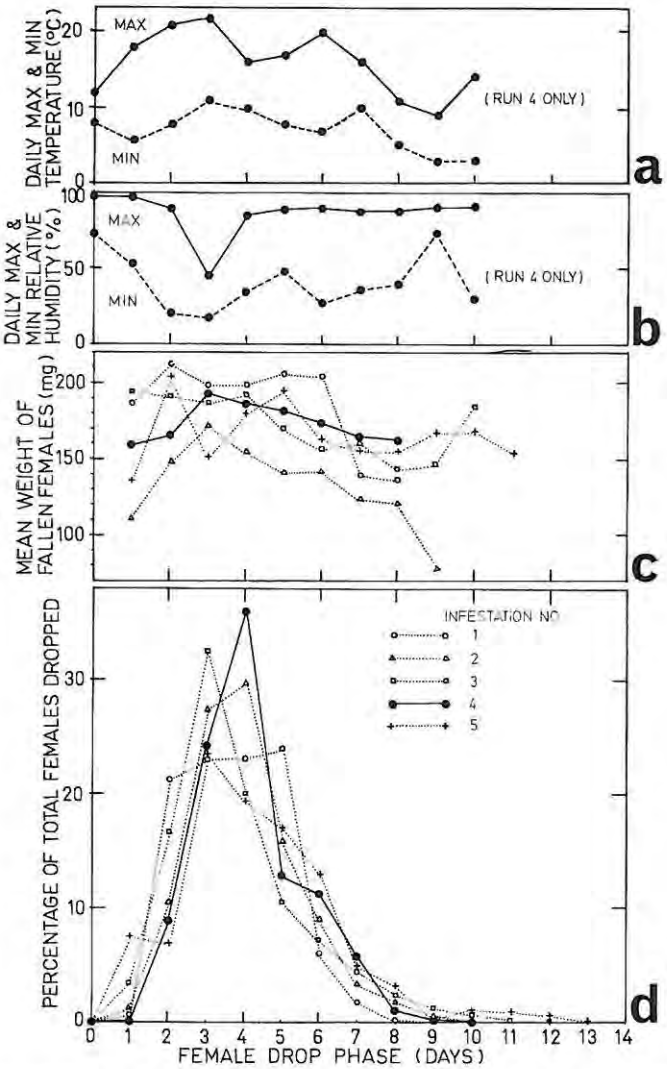

Fig. 14. (a–b). Daily maximum and minimum temperatures (a) and relative humidities (b) recorded in the stable during the fourth run which represents a continuation of the cycle shown in figs 3, 8 and 13 (c). The mean weight of engorged adult females of *Boophilus decoloratus* on each successive day of the female drop phase. The results of five runs are shown, the fourth of which represents a continuation of the cycle shown in figs 3, 8 and 13 (d). The percentage number of females dropped from the host on each successive day of the female drop phase. The results of five runs are shown, the fourth of which represents a continuation of the cycle shown in figs 3, 8 and 13.

of nymphs of one-host ticks in relation to the metamorphic changes which occur, when these stages remain attached *in situ*, after cessation of feeding.

Roberts (1968) selected length as a means of analysing age structure in the different stages of tick populations. This is inadequate because it fails to distinguish between the feeding larval and nymphal stages in *sensu strictu* from those in which pharate nymphs and pharate adults are developing rapidly. These later stages are more truly referable to the succeeding instars rather than to those in which their development is taking place. This becomes clear by reference to figs 4 and 5. From this point of view tick weights, coupled with clearing techniques, are not only a better indication of blood intake but also of visible developmental changes taking place in the attached immature stages.

To analyse changes between the lengths of the life cycles of one-host and three-host ticks, concurrent investigations were carried out by Norval (unpublished data) on the life history of the three-host tick *Amblyomma hebraeum* Koch 1844, under the same macro-environmental conditions as those used for *B. decoloratus*. The larva of *A. hebraeum* feeds on rabbits for 4–10 days, with most of the larvae becoming fully engorged between the sixth and the eighth day. In an incubator at 26°C and 90% R.H. the period between detachment of the larvae and moulting ranged from 14 to 21 days with the majority moulting on the fifteenth day. Nymphs of *A. hebraeum* fed for 5–13 days on rabbits and for 6–9 days on sheep to become replete. It is thus possible that the larval feeding period may be shorter on some hosts than on others. Again in the incubator the pre-moulting period of adults extended from 21 to 29 days with most nymphs moulting on or about the twenty-third day.

Moulting time is influenced by temperature within the survival limits imposed on the ticks by relative humidity. In one-host ticks, such as *B. decoloratus*, and two-host ticks, like *Rhipicephalus evertsi* Neumann 1897, the temperature regime during the moulting periods approximates to that prevailing during feeding by the immature stages. The various instars of three-host ticks on satiation and detachment from the hosts are, however, subject to considerably more variable temperatures in the vegetation complex into which they drop. The body temperatures of cattle range from 101°F to 103°F with skin temperatures varying from 95°F to 105°F (Rippon: pers. commun.). Londt & Whitehead (1972) have shown that the larvae of *A. hebraeum* are found predominantly in medium protected vegetation, and it may be that all stages in the life history are found here. The temperatures recorded in these situations in the Eastern Cape Province are on average lower and more variable than those used in our incubator experiments. The experiments on the emergence of nymphs from larvae and of adults from nymphs of *A. hebraeum* and *B. decoloratus* were carried out at 26°C (= 78,8°F). Under these circumstances it is likely that *B. decoloratus* would take longer to emerge than they do on cattle, and that the immatures of the bont tick (*A. hebraeum*) proceed to their subsequent stages in a shorter time than they would have done under field conditions. Under natural conditions it is likely then that the life history of a one-host tick will be shorter than that of a three-host tick and to a lesser extent than that of a two-host tick.

There is, however, a more fundamental consideration. In the life history of one-host ticks the fully fed larval stage is succeeded immediately by the immobile pharate nymphal stage, which gives rise within three days to the active nymph. This nymph is ready to feed almost immediately. Similarly, the immobile pharate adult appears within two or three days of cessation of feeding of the nymph and is capable

of attaching to the host on emergence. These considerations also apply to the transformation which occurs between the larva and the nymph in such two-host ticks as *R. evertsi*. In three-host ticks, however, when the fully fed larva or the fully fed nymph detach from the host they are extremely mobile for two or three days (Arthur, 1962) before entering a quiescent phase which may last for two or three weeks, prior to emergence. This is also so in the nymph-adult metamorphosis of the two-host ticks.

During the transition to the pharate state the appendages of the succeeding stage of any ixodid tick, whether it be nymph or adult, do not develop internally to those of the preceding stages, but appear *de novo*. In such three-host ticks as *A. hebraeum* the rudiments of the appendages of the nymph and the adult are visible through the larval and nymphal integuments respectively even before detachment from the host and there are indications of the pharate condition. But the musculature and nervous system of the larva and the nymph remain operative for two or three days after the ticks drop off. Thus the change from the larval to the nymphal stages and from the nymphal to the adult stages in three-host ticks is extended when compared with what occurs in one-host ticks. This may be attributed in part to a delay in histolysis of tissues in three-host ticks or an acceleration in these processes in one-host ticks. A question which is probably answerable only when we learn which is the primitive condition.

On the evidence available from life-history studies it appears that the meal is more rapidly digested and metabolized in one-host ticks than it is in three-host ticks. This may account for the apparent delay in the development of the embryological precursors for the succeeding stages in the latter. The fact that such a three-host tick as *A. hebraeum* may feed concurrently on the same host as the one-host *B. decoloratus* and for the same length of time and under similar conditions without alteration of the one- or three-host pattern indicates that each of these species may employ different biochemical or hormonal pathways to attain the same ends.

The evidence from the literature that the two- and three-host tick patterns are interchangeable in relation to different hosts has been applied to *Hyalomma rufipes* (Koch 1844). The minimum time for this tick to complete its life history is between four and five months, but this may be doubled under local conditions, according to Hoogstraal (1956). Howard (1908) considered *H. rufipes* to be a two-host tick in South Africa; Jack (1928) reported that it had a two-host and a three-host cycle in Rhodesia; Brassey-Edwards (1955) that it behaves as a two-host tick on hares and a three-host tick on sheep, cattle and domestic fowls. Theiler's (1943) data suggest three-host tick behaviour although stating without qualification that they are "as a rule two-host ticks; the larvae may drop-off". Preliminary results (Arthur, unpublished) show that larval ticks of this species bred on rabbits have an immobile pharate nymphal condition at the end of feeding; this being similar to that observed in *B. decoloratus*. Biological studies on the life histories of *H. rufipes* (Arthur, Norval & Knight, unpublished) show that a proportion of the ticks which drop-off do so as partially fed larvae, fully fed larvae, and partially fed nymphs, but that the vast bulk of the population remain on the host and there undergo the change from larvae to nymphs. The removal from the host of immature ticks in various stages of engorgement suggests an active de-ticking process by the host. To accept interchangeability of the tick-host pattern implies possible changes in the biochemical pathways leading to metamorphosis and, in the case of *H. rufipes*, the replacement of an immobile pharate nymphal condition with an active larval state on completion of feeding. Though not impossible it is unlikely, and has not hitherto been observed in other ticks having a catholic choice of hosts. Like *B. decoloratus* and *H.*

rufipes, the cuticle of fully fed larvae of *R. evertsi* also enshrouds a pharate nymph and on detachment in this condition remains immobile.

According to Legg (1930) the development of the tick *B. australis* (= *B. microplus*) during its parasitic life shows considerable uniformity in timing in December, January, May (2 runs), July (1 run) and August. This is supported by the present results extending from 9 February 1972 to 8 October 1972, and these data are summarized in fig. 5. Reference to the plotted temperatures and relative humidities of the macro-environment of the host for one run (fig. 3, 8, 13, 14a, b) shows that these factors vary quite widely. Macro-climate conditions thus do not appear to affect the length of the various phases of the life cycle of *B. decoloratus* on the host.

These data may be of value in approaching the problem of acaricidal treatment of blue tick populations on stock because of the constancy of timing, and the effects of acaricides applied to stock when the ticks are in the pharate condition might bear further examination.

ACKNOWLEDGEMENTS

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PART TWO

THE PREHATCH PERIOD OF

BOOPHILUS DECOLORATUS (KOCH)

3. THE PREOVIPOSITION PERIOD

Synopsis

The preoviposition period of B.decoloratus was studied under laboratory and field conditions and found to be temperature dependent. Effects of humidity on the duration of the preoviposition period was found to be negligible. Female ticks placed in a Stephenson's screen in the field demonstrated very long preoviposition periods and the biological implications are discussed.

3.1 INTRODUCTION

The preoviposition period of any ixodid tick is biologically significant in that it is this period during which the engorged female tick must find a suitable habitat in which to lay eggs. Milne (1950) has shown that Ixodes ricinus L. is capable of 'burrowing' a few centimetres below the surface in areas where leaf litter and other debris is present. Hunter and Hooker (1907) have shown similar behaviour patterns in Margaropus annulatus Say (= B.annulatus). This behavioural pattern places the female tick in an environment suitable for oviposition and subsequent incubation of the eggs.

Most of the published work on the preoviposition phase of ixodid ticks has been concerned primarily with some of the factors influencing the duration of the period under laboratory or field conditions. These data are summarised in tabular form in Table 1. One of the earliest references to the preoviposition period was that of Lounsbury (1899) who recorded that the preoviposition period of A.hebraeum was longer in 'cold' weather than in 'warm' weather, thus implying temperature dependence. Since this early observation other workers have found that the preoviposition period is directly

TABLE 1.

Factors influencing the duration of the preoviposition period in ixodid ticks.

Species	Factors investigated							Reference
	H	T	P	E	W	N	I	
Amblyomma americanum (L)	+			-	-	- +		Lancaster and McMillan (1955) Sactor et al (1948) Gladney and Drummond (1970)
Amblyomma hebraeum Koch		+						Lounsbury (1899)
Amblyomma maculatum Koch					-			Drummond and Whetstone (1970)
Boophilus annulatus (Say)		+ +						Hunter and Hooker (1907) Graybill (1911)
Boophilus decoloratus (Koch)	-	+			+			This study
Boophilus microplus (Canestrini)	-	+ +					+ +	Hitchcock (1955b) Legg (1930) Hall and Wilkinson (1960) Sutherst (1971)
Dermacentor marginatus Sulz.			+					Belozerov and Kvikto (1965)
Dermacentor variabilis (Say)	-[1]		+					Smith et al (1946)
Hyalomma aegyptium (L.)	-[2]		+					Sweatman (1968)
Ixodes hexagonus Leach	+	+						Arthur (1951)
Ixodes ricinus L.	+	+						Macleod (1935)
Rhipicephalus sanguineus Latreille	+	+				-		Sweatman (1967)

Abbreviations: + = positive correlation; - = negative correlation; H = humidity; T = temperature; P = photoperiod; E = engorgement time of adult female; W = initial weight of engorged female; N = number of eggs produced; I = immersion in water.

1 = Inferred from general statements made by author.
2 = Tendency seen but not statistically significant.

dependent on the prevailing temperature conditions (Table 1). There appears to be some 'disagreement' as to whether atmospheric humidity has any direct influence on the duration of the preoviposition period. Macleod (1935), Arthur (1951) and Sweatman (1967), working on the species indicated in Table 1, considered humidity to be of importance in that dry conditions would cause an extension of the preoviposition period. Smith et al. (1946) and Hitchcock (1955b), however, were unable to show positive correlations with humidity (Table 1). Sweatman (1968) demonstrated a slight correlation using Hyalomma aegyptium (L.) but this was found to be statistically not significant.

Apart from studies involving temperature and humidity only a few other environmental factors have been investigated in relation to their influence on the duration of the preoviposition period. Belozerov and Kvikto (1965), for example, demonstrated that the duration of the preoviposition period in Dermacentor marginatus Sulz. shows a positive correlation with photoperiod while both Hall and Wilkinson (1960) and Sutherst (1971) report a positive effect of immersion in water (such as might be expected during rain or heavy dew) on the length of the preoviposition period of B.microplus.

The present investigation was concerned chiefly with the effects of female size (semi and fully engorged), temperature and humidity on the duration of the preoviposition period of B.decoloratus.

3.2 MATERIALS AND METHODS

To assess the effect of female size, as presumed by their weights, on the duration of the preoviposition period of B.decoloratus,

twenty one female ticks, selected to have a wide range of weights, were removed from the host (a Guernsey calf) and placed in an incubator at 26°C. The tubes in which the ticks were kept were placed in an atmosphere of approximately 95% relative humidity (i.e. conditions of approximately 1,30 mm Hg saturation deficit) which was maintained in a glass container by using the appropriate concentration of potassium hydroxide solution (Peterson 1953). This, and other relative humidity levels used in other experiments reported in this work, was checked at weekly intervals using cobalt thiocyanate paper indicators in the manner discussed by Solomon (1957).

In determining the effects of different constant temperatures and relative humidities, and therefore constant saturation deficits,[1] six different temperatures and three different relative humidities were used. In Table 2 the combinations of temperature and relative humidity used and the corresponding saturation deficit values are shown. Table 2 also shows the number of females studied in each temperature and relative humidity combination as well as the means employed in the maintenance of the temperatures used. The three different relative humidity levels (90%, 70%, and 50% R.H.) were selected on the basis of work done on both egg (discussed later in this thesis) and larval tolerance levels (Londt and Whitehead 1972). 90% Relative humidity was considered as a 'favourable' environmental atmosphere for B.decoloratus because both larvae and eggs of this species were able to survive and develop normally in this environment. 50% Relative humidity was, by the same token, considered 'unfavourable' as larvae, when exposed to this atmosphere for more than five days died from desiccation and eggs failed to hatch. 70% Relative humidity was selected as it represents the approximate 'critical humidity'[2] or

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1. See section 6.2 headed: Assessment of the effects of climatic conditions on ticks.

2. See section 5.23 in which this term is defined.

'equilibrium humidity' (Lees, 1946) of eggs and larvae respectively.

TABLE 2.

Combinations of temperature, relative humidity, and saturation deficit used in the study of these parameters on the durations of the preoviposition, oviposition and incubation periods, the mass of eggs produced by females and the percentage hatch of eggs of Boophilus decoloratus.

Temperature (°C)	Relative Humidity (%)	Saturation Deficit (mmHg)	Means by which Temp. achieved	No. ticks used
10	90	0,91	waterbath	4
10	70	2,74	kept in cold	4
10	50	4,57	room (4°C)	4
15	90	1,26	waterbath	4
15	70	3,80	kept in cold	4
15	50	6,33	room (4°C)	4
20	90	1,73	waterbath	4
20	70	5,21	kept in cold	4
20	50	8,68	room (4°C)	4
26	90	2,55	incubator	3
26	70	7,53	kept in	3
26	50	12,51	laboratory	3
32	90	3,64	incubator	3
32	70	10,68	kept in	3
32	50	17,72	laboratory	3
38	90	8,07	incubator	3
38	70	14,89	kept in	3
38	50	24,71	laboratory	3

The effects of macroclimatic fluctuations in temperature, relative humidity and saturation deficit were studied by placing tubes of female ticks in a Stephenson's screen, together with a thermohygrograph. The Stephenson's screen was placed at ground level so that the closest approximation of microclimatic conditions would be achieved. Detailed observations of the microclimate were not possible as the necessary recording equipment for such work was not available.

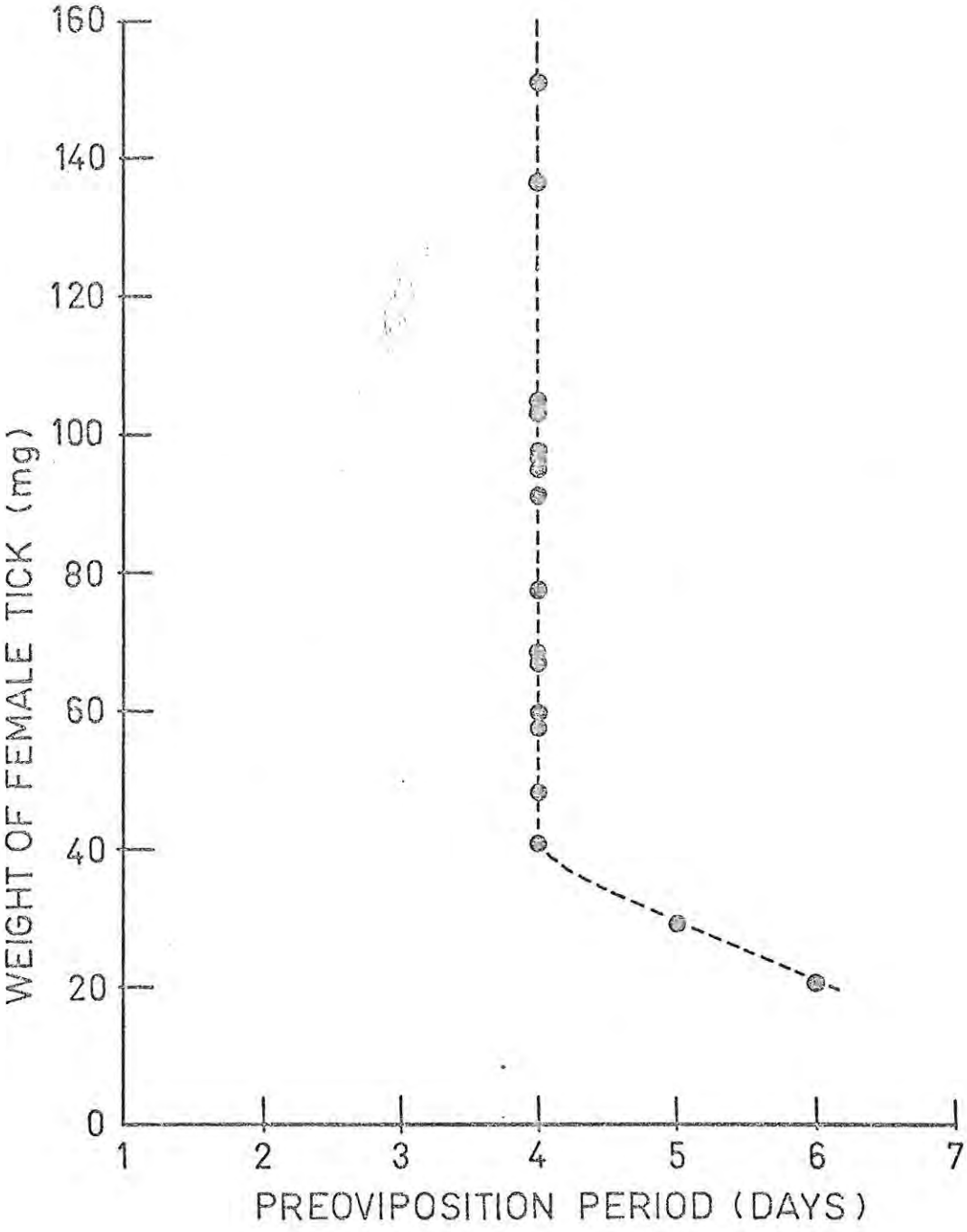

Fig. 17: The influence of female weight on the duration of the preoviposition period of Boophilus decoloratus.

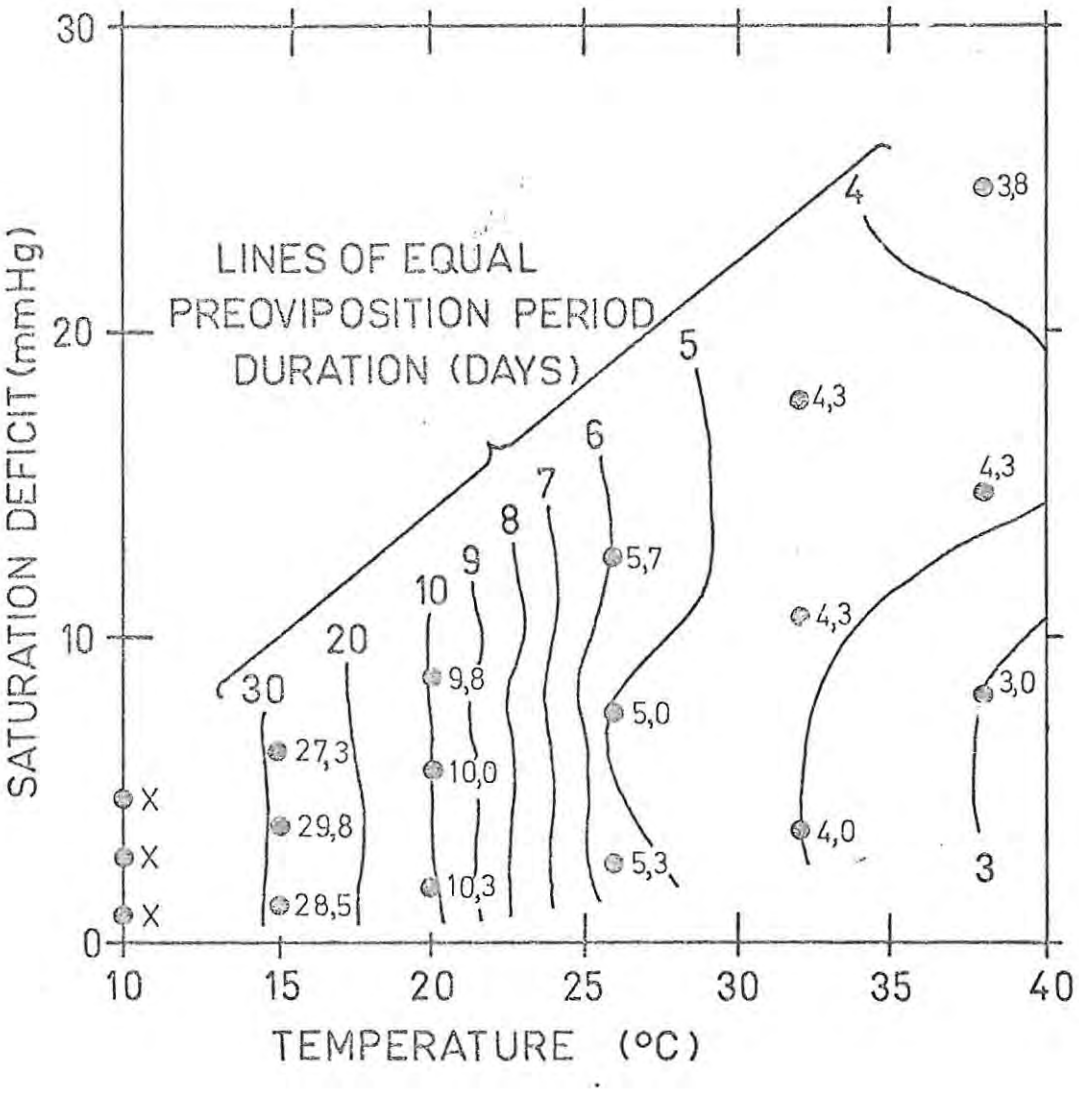

Fig. 18: A contour diagram showing the relationships between temperature, saturation deficit and the duration of the preoviposition period of Boophilus decoloratus.
X = no oviposition took place.

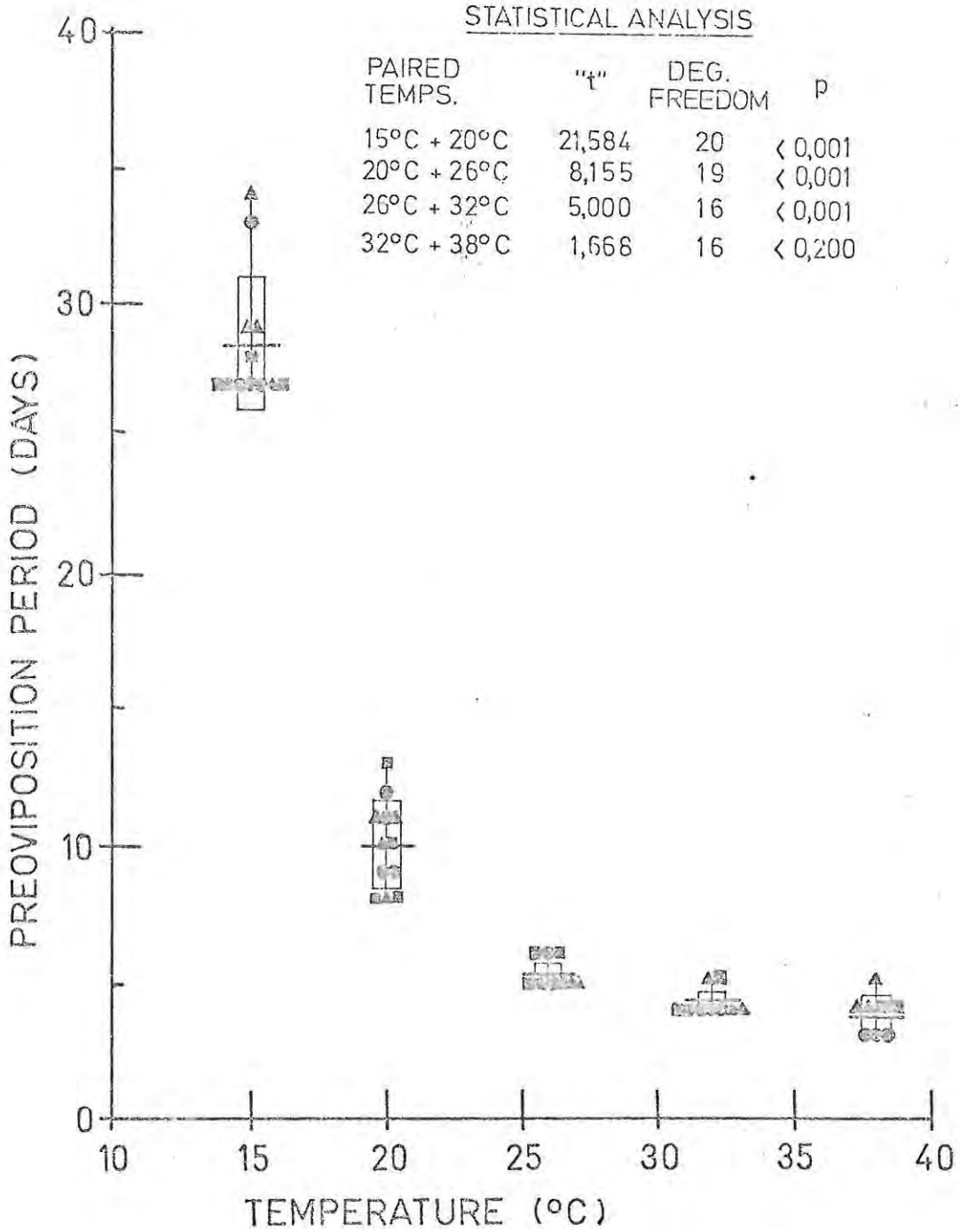

Fig. 19: The relationship between temperature and the duration of the preoviposition period of Boophilus decoloratus ignoring any humidity effects. ● = data obtained at 90% R.H., ▲ = data obtained at 70% R.H., ■ = data obtained at 50% R.H. The horizontal line indicates the mean preoviposition period at each temperature, the open boxes, one standard deviation about the mean and the vertical line, the range.

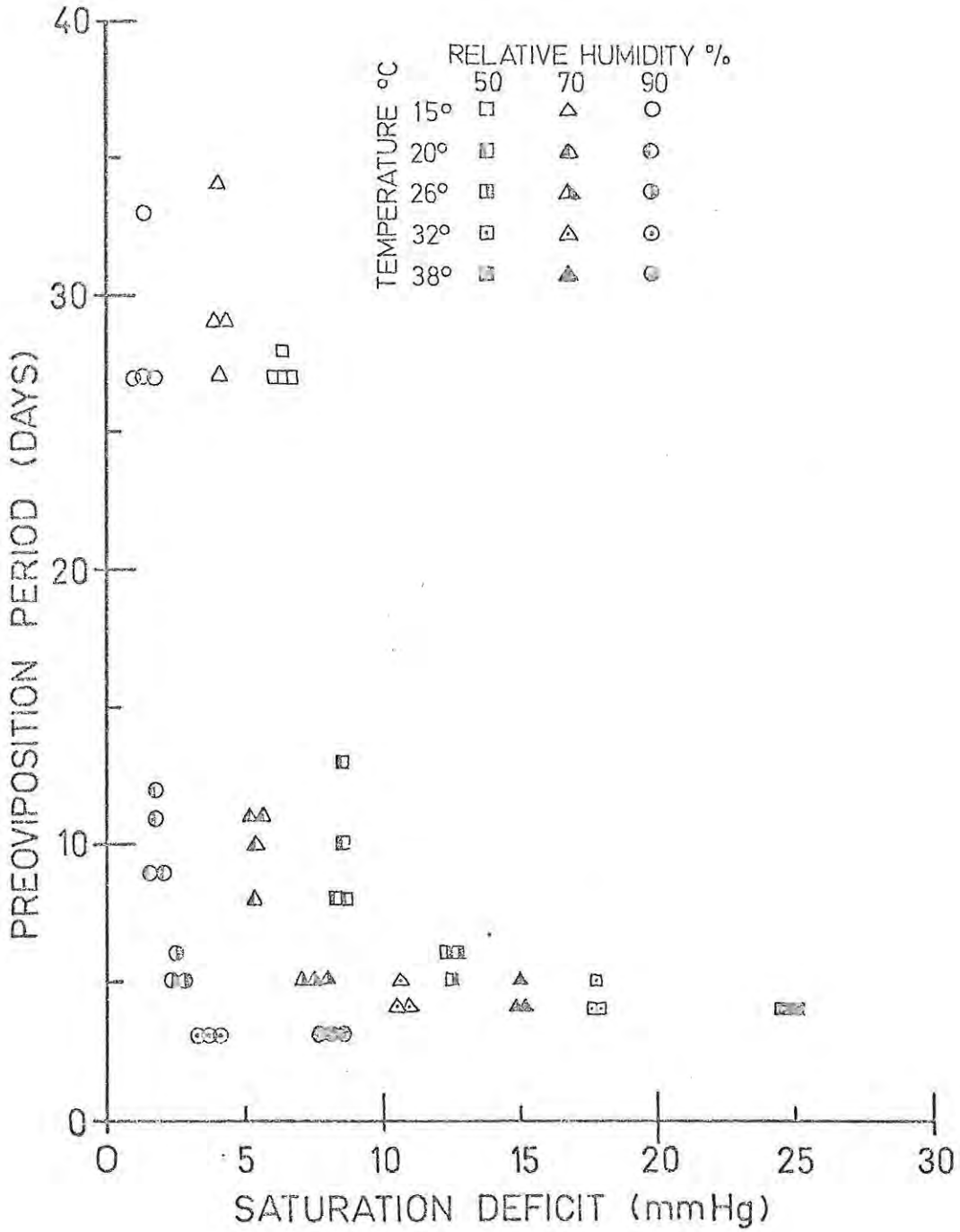

Fig. 20: The relationship between saturation deficit and the duration of the preoviposition period of Boophilus decoloratus ignoring any effects of temperature.

3.3 RESULTS

The duration of the preoviposition periods of twenty one engorged female ticks of different weights ranging from 10,0-151,0 mg are shown in Fig. 17. All females weighing more than 40 mg displayed a preoviposition period of four days under the experimental conditions (26°C; ca. 95% R.H.; 1,3 mm.Hg.). One female (29,0 mg) had a preoviposition period of five days while another (20,5 mg) took six days before commencing oviposition. Four females, all less than 20 mg in weight, failed to lay. As the mean weight of completely unengorged female ticks was approximately 1,4 mg it is suggested that the minimum weight of 'blood' uptake necessary to maintain basic metabolism is therefore in the order of approximately 19 mg.

Fig. 18, a contour diagram[1], shows the relationships which exist between temperature, saturation deficit and the duration of the preoviposition period. As the lines of equal preoviposition period lie almost parallel to the saturation deficit axis, it is suggested that humidity does not have any effect on the duration of the preoviposition period. Temperature, however, is clearly of importance in that the duration of the preoviposition period is shortened with temperature increase. This marked temperature effect is shown more clearly in Fig. 19 in which the effects of saturation deficit have been ignored. The relationship between preoviposition period duration and saturation deficit is shown in Fig. 20. It appears that there is a definite correlation between these parameters in that the length of the preoviposition period is increased with decrease in saturation deficit. This apparent

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1. The use of contour diagrams in assessing the effects of physical factors of the environment on ticks is discussed later (Section 6.2).

relationship is however due to the overriding effects of temperature, which is used in the calculation of saturation deficit. If the data at each different temperature level are studied separately it becomes apparent that there is little or no effect of saturation deficit on the duration of the preoviposition period.

The mean duration of the preoviposition period in two batches of female ticks placed in a Stephenson's screen under naturally fluctuating macroclimatic conditions was 36,6 days and 41,6 days respectively. In order to get an estimate of the mean daily temperature experienced by these females, daily hour degrees were calculated (above a baseline of 10°C as no laboratory held female produced eggs at this temperature) for each day of the preoviposition periods of each batch. The mean daily number of hour degrees was calculated for both batches of females and found to be 69,4 h°C and 104,5 h°C for batch one (26 July - 1 September 1972) and batch two (5 August - 21 September 1972) respectively. These values, if plotted on a standard curve of the number of hour degrees per day relative to constant temperature (Fig. 21), show that the mean daily temperature values experienced by the two batches of female ticks were approximately 13,0°C and 14,5°C respectively. These values fall between the temperatures 10°C and 15°C used in the laboratory work and by referring to Fig. 19 the preoviposition period which would have been expected at these two temperatures under laboratory conditions would have been approximately 42 and 39 days respectively. These values approximate fairly closely to those actually recorded in the field (i.e. 36,6 and 41,6 days).

3.4 DISCUSSION

The cool winter conditions under which the field work was done

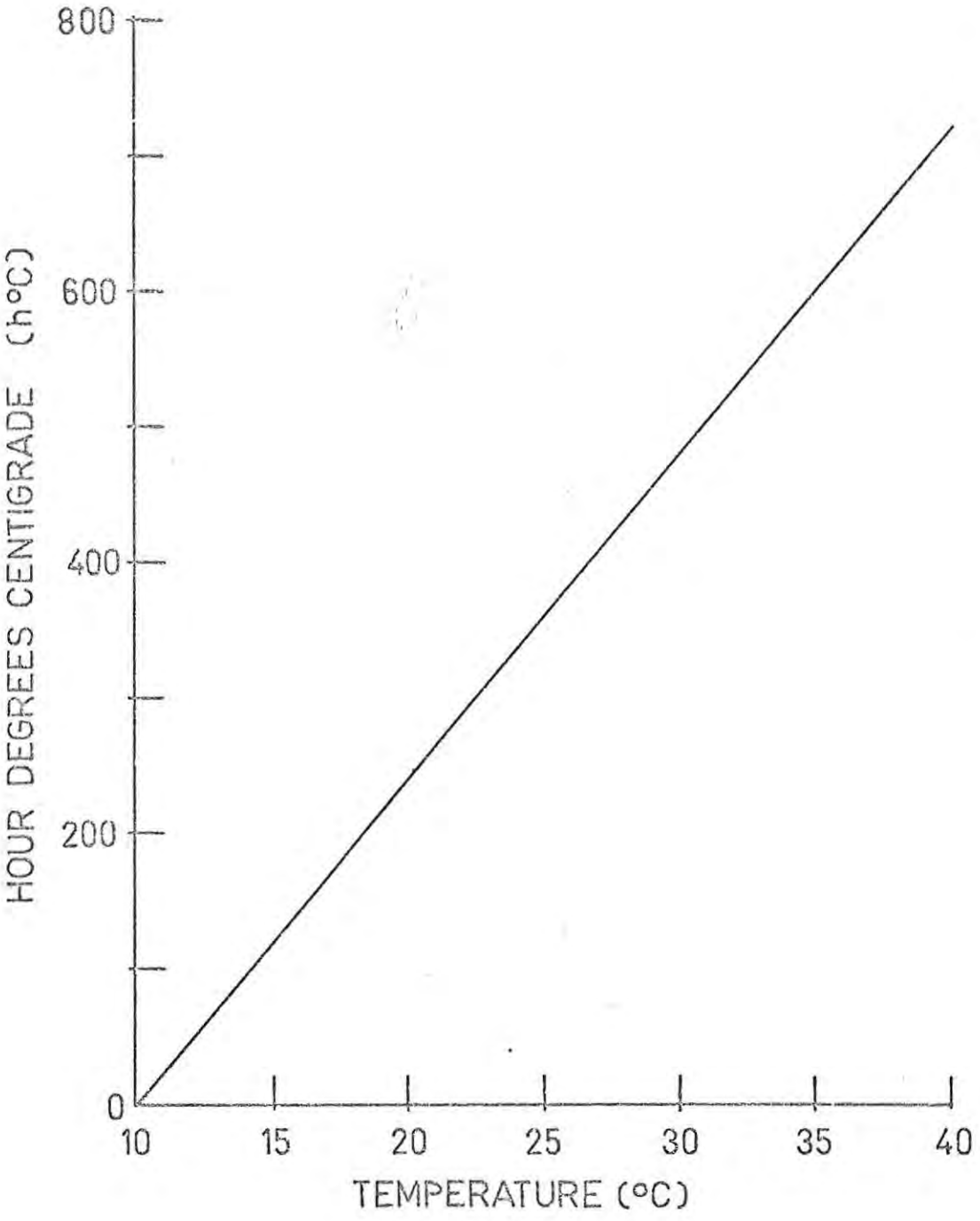

Fig. 21: A standard curve of the relationship between the number of hour degrees per twenty-four hours and constant temperature. Hour degrees being calculated above 10°C.

resulted in long preoviposition periods as was expected from the results of the laboratory work and the findings of previous workers in this field (Table 1). At 10°C female ticks in the laboratory died without laying eggs. This suggests that protracted periods of cold weather may inhibit oviposition long enough to result in the death of the ticks before oviposition takes place. Unfortunately it was not possible to study the duration of the preoviposition of B.decoloratus under microclimatic conditions because of the unavailability of sufficiently accurate instrumentation for continuous recording of temperatures and relative humidities at a microclimatic level. This work will obviously have to be undertaken before relationships between microclimatic conditions and the duration of the preoviposition period are elucidated. The results of laboratory work indicate that female ticks detaching from the host at regular intervals over a long period of cold weather might be expected to commence oviposition at approximately the same time. This suggestion is supported by the work of Snowball (1957), on B.microplus, and Kraft (1961) on various ixodid ticks including B.decoloratus. Kraft (1961) demonstrated that females placed in the field during the winter not only tended to commence egg laying at about the same time but also tended to be less successful in producing viable eggs than females placed in the field during the summer months. The work of Kraft (1961) and the results reported in this investigation help to explain the greater incidence of B.decoloratus during the summer months (Theiler 1969).

The situation concerning the effects of atmospheric humidity on the duration of the preoviposition period needs clarification. An examination of past findings (Table 1) reveals that there is some 'disagreement' regarding the influence of this physical factor.

The reasons for this may be quite varied and therefore need further consideration. Workers who have reported a positive correlation between preoviposition period duration and humidity include Lancaster and McMillan (1955), Arthur (1951), Macleod (1935) and more recently Sweatman (1967). Sweatman (1968) demonstrated a tendency towards a saturation deficit dependent preoviposition period in H.aegyptium, but this was shown to be statistically not significant. Sweatman (1967) critically examined all previous work in this field and came to the conclusion that the findings of Lancaster and McMillan (1955) need reaffirmation. Sweatman demonstrated that Arthur's (1951) data, pertaining to Ixodes hexagonus Leach, when subjected to statistical analysis show a significant correlation. This correlation may however have been affected by the fact that Arthur's data were obtained from ticks in various states of semi-engorgement. Macleod (1935) presented scanty information on the preoviposition period of I.ricinus and states that "all that can be safely inferred....is that at the extreme limits of temperature and humidity....the period is longer than at median temperatures and high humidities. At many of the extremes, particularly of humidity, oviposition was abortive, only some few dozen eggs being laid". This means that further work on I.ricinus is necessary before a positive correlation between humidity and preoviposition period duration can be finally accepted. It appears, therefore, that the work preceeding Sweatman (1967, 1968) is not particularly conclusive with the possible exception of that of Arthur (1951) who demonstrated that the preoviposition period duration increases with increase in saturation deficit. Sweatman's (1967) analysis of Arthur's work is reproduced as Fig. 22a. This finding, however, is at variance with those of Sweatman (1967, 1968) who demonstrated that in both H.aegyptium (results not statistically significant) and Rhipicephalus

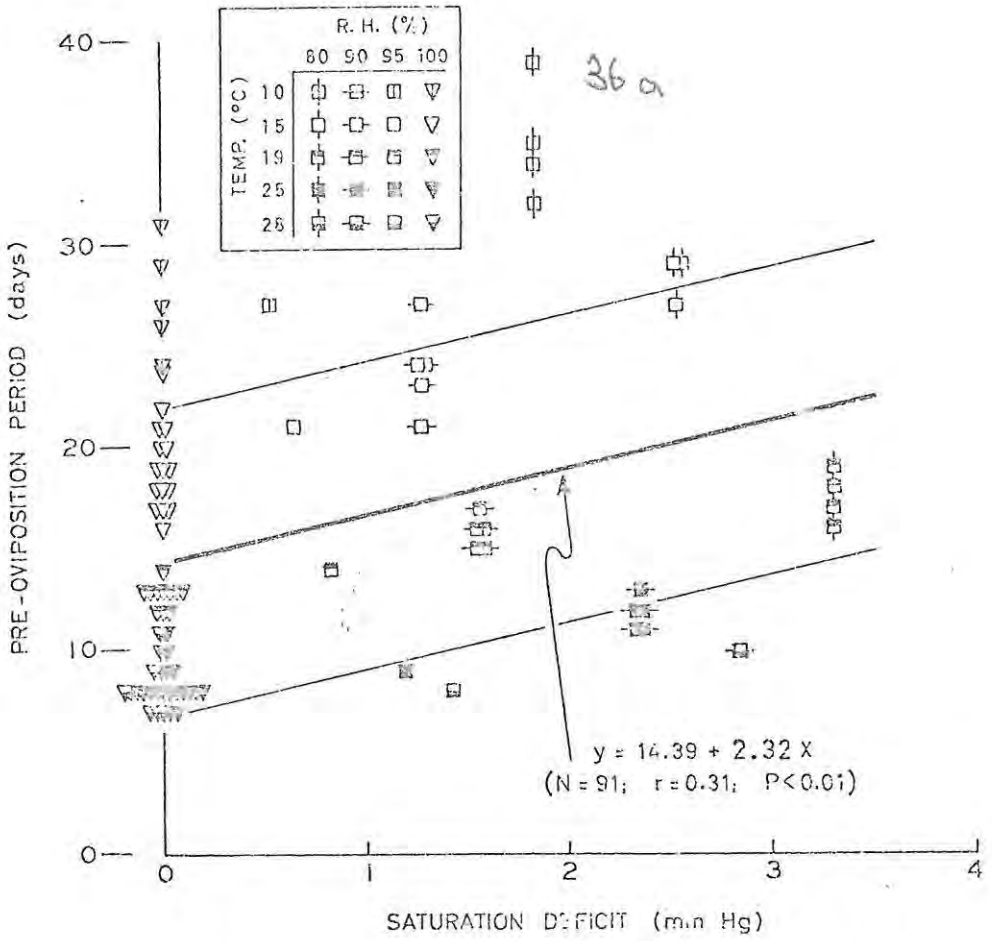

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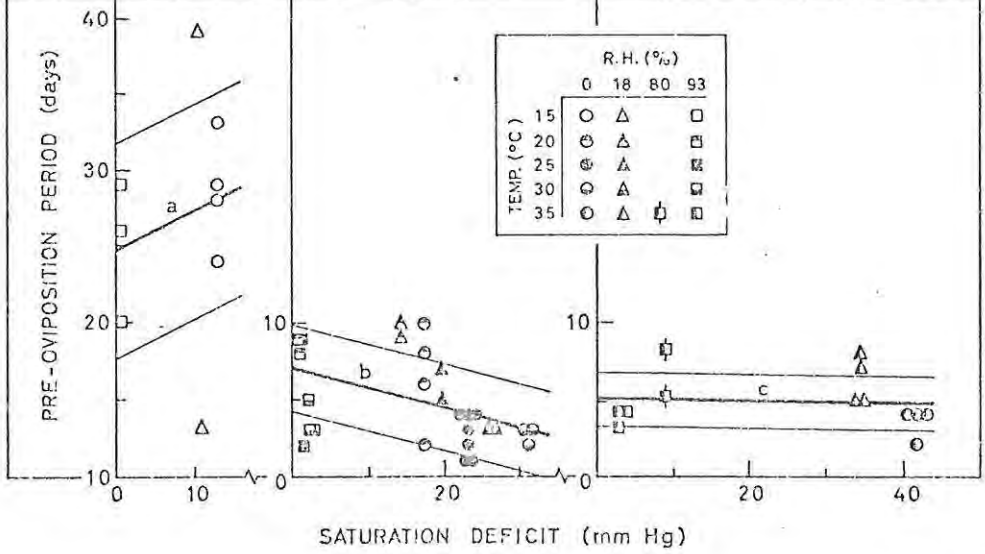
FIGURE 14. Relationships between pre-oviposition period and saturation deficit of *Ixodes hexagonus* held at constant temperatures between 10 and 28 C and at constant relative humidities between 80% and 100%. Data from Table 1 in Arthur, 1951. The observations may be somewhat distorted because some ticks on experiment were removed from their hosts before complete engorgement. Details as in Figure 4.

TEMP. (°C)	15	20, 25, 30	35
LINE	a	*b*	c
EQUATION	Y = 24.69+0.25X	Y = 7.07 - 0.13 X	Y = 4.95 - 0.01 X
N	9	27	13
r	0.19	*-0.47*	-0.04
P	>0.1	*<0.02*	>0.1



b

FIGURE 9. Pre-oviposition periods of female *R. sanguineus* at different saturation deficits for different temperatures. Abbreviations as in Figure 4.

Fig. 22: (a). A copy of Sweatman's (1967) figure 14 with accompanying legend. (b). A copy of Sweatman's (1967) figure 9 with accompanying legend.

sanguineus Latreille the preoviposition period duration tends to decrease with increase in saturation deficit. Sweatman subjected all his results to rigorous statistical analyses and, at least in the case of R.sanguineus, has demonstrated that increase in saturation deficit decreases the duration of the preoviposition period at temperatures of 20°C, 25°C and 30°C.

The results presented in this chapter (Figs 18, 19 and 20) suggest that the type of positive correlation which Sweatman (1967, 1968) proposes between saturation deficit and preoviposition period duration is not real. Temperature has a very powerful influence over preoviposition period duration and as temperature conditions are taken into account when determining saturation deficit it is very probable that the positive correlation between saturation deficit and the preoviposition period is a direct result of a temperature effect. It has been shown in Fig. 20 that even though the preoviposition period is longer at low saturation deficit values, these values were all derived from conditions of low temperature. If Sweatman's (1967) data (His Fig. 9 reproduced here as Fig. 22b) are examined it will be noticed that his data demonstrate the same overriding effects of temperature but not as clearly as is seen in B.decoloratus (Fig. 20). This observation implies that saturation deficit does not have a clearly positive correlation with preoviposition period duration and as the actual effects of saturation deficit on ticks is concerned with water loss it is suggested that fully engorged female ticks, clearly possessing large water reserves and possibly means of taking up water vapour from moist air, are unlikely to be influenced by a very wide range of saturation deficit levels. It is therefore suggested that atmospheric humidity does not have any effect on the preoviposition period of B.decoloratus (Table 1).

Hitchcock (1955b), working on B.microplus, supports this contention in a single statement: "Within the range tested (45-90 per.cent.), relative humidity had no influence on the duration of the pre-oviposition period". Smith et al. (1946), working on Dermacentor variabilis (Say), although not actually presenting any evidence state: "Moisture is essential to the survival of all stages.... neither relative humidity nor precipitation shows any correlation with activity or length of developmental periods".

From this discussion it becomes evident that previously published information on the effects of humidity on the duration of the preoviposition period of ixodid ticks should be viewed with caution and due regard taken of the marked effect of temperature made on this period.

4. THE OVIPOSITION PERIOD

Synopsis

The duration of the oviposition period of B.decoloratus and the number (or weight) of eggs produced during this period were studied under laboratory and field conditions. Both these parameters were found to be temperature dependent and uninfluenced by humidity. The minimum amount of 'blood meal' required by laboratory maintained females for egg production was approximately 16 mg. The oviposition behaviour of female ticks has been briefly described. The significance of temperature dependent oviposition, and the oviposition period of ixodid ticks in general, is discussed.

4.1 INTRODUCTION

Apart from the published observations of Lounsbury (1899) on the behaviour of A.hebraeum during oviposition, little more has been added to the information for southern African Ixodid tick species. Information concerning species occurring elsewhere in the world is more abundant. The two major aspects which have received attention are the factors influencing the duration of the oviposition period and the factors influencing the number (or weight[1]) of eggs produced during the oviposition period. A survey of the literature and the main findings reported are summarized in Tables 3 and 4. The general conclusions resulting from this survey are that there is a positive correlation between temperature, photoperiod, female disturbance and the duration of the oviposition period. Delay in mating, physical environmental conditions during the preoviposition period and the initial weight of the female tick, on the other hand, do not appear to influence the duration of the oviposition period. Similarly there is evidence that such factors as the initial weight of the female tick, disturbance of ovipositing females and submersion

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1. The relationship between number and weight of B.decoloratus eggs is linear as is the case in all previously studied ixodids.

TABLE 3.

Factors influencing the duration of the oviposition period in ixodid ticks.

Species	Factors investigated								References
	H	T	P	D	M	N	Pr	W	
Amblyomma americanum (L)	+ -								Lancaster and McMillan (1955) Sonenshine and Tigner (1969)
Amblyomma hebraeum Koch		+		+					Lounsbury (1899)
Amblyomma maculatum Koch						+		-	Drummond and Whetstone (1970)
Boophilus annulatus (Say)		+ +				 +		 +	Hunter and Hooker (1907) Graybill (1911)
Boophilus decoloratus (Koch)	-	+				+		+	This study
Boophilus microplus (Canestrini)	 -	+ +							Legg (1930) Hitchcock (1955b)
Dermacentor marginatus Sulz.			+						Belozerov and Kvikto (1965)
Dermacentor variabilis (Say)	-				-				Sonenshine (1967) Sonenshine and Tigner (1969)
Hyalomma aegyptium (L)	-	-[1]				-		-	Sweatman (1968)
Hyalomma anatolicum (Koch)	+	+	+				-		Snow and Arthur (1966)
Ixodes hexagonus Leach	-	+							Arthur (1951)
Ixodes ricinus L.	-	+							Macleod (1935)
Rhipicephalus sanguineus Latr.	+	+				-		-	Sweatman (1967)

Abbreviations: + = positive correlation; - = negative correlation; H = humidity; T = temperature; P = photoperiod; D = disturbance of ovipositing female; M = delay in mating; N = number of eggs produced; Pr = conditions during preoviposition period; W = initial engorged weight of female.

1 = A tendency observed but found to be statistically not significant.

TABLE 4.

Factors influencing the number (or mass) of eggs produced by ixodid ticks.

Species	Factors investigated						References
	H	T	D	C	I	W	
Amblyomma americanum Koch						+	Gladney and Drummond (1970)
Amblyomma hebraeum Koch			+				Lounsbury (1899)
Amblyomma maculatum Koch			-			+	Drummond and Whetstone (1970)
Anocentor nitens (Neumann)			-	-		+	Drummond et al (1969a) Thompson (1970)
Boophilus annulatus (Say)					+		Hunter and Hooker (1907)
Boophilus decoloratus (Koch)	-	+	+			+	This study
Boophilus microplus (Canestrini)	-	+			+	+	Sutherst (1971) Hitchcock (1955b)
Dermacentor albipictus (Packard)			+		+		Drummond et al (1969b)
Dermacentor variabilis (Say)	-		+				Sonenshine and Tigner (1969)
Hyalomma aegyptium (L)	-	-				+	Sweatman (1968)
Hyalomma anatolicum (Koch)						+	Snow and Arthur (1966)
Ixodes hexagonus Leach	+	-					Arthur (1951)
Ixodes ricinus L.	+	-					Macleod (1935)
Rhipicephalus sanguineus Latreille	-	+				+	Sweatman (1967)

Abbreviations: H = humidity; T = temperature; D = disturbance of female; C = crowding of females; I = immersion in water; W = initial engorgement weight of female; + = positive correlation; - = negative correlation.

of females in water directly influence the numbers of eggs produced. Relative humidity and crowding of females during oviposition, however, do not appear to be of any importance. There is disagreement over whether a correlation between temperature and the number of eggs produced exists. These 'disagreements' do not necessarily reflect the quality or validity of the conclusions arrived at by the different workers involved but may be the result of species specific responses. The investigations reported in this study were primarily designed to determine the relationships between some of the factors considered in earlier reports by other workers in relation to the oviposition period of B.decoloratus. The work has, for convenience, been reported under the following main headings:

i. Preliminary experiments. Including a description of oviposition; the general pattern of oviposition under constant laboratory conditions; the weight changes which take place during oviposition; the effects of handling females during oviposition.

ii. The influence of constant temperature and humidity on the oviposition period.

iii. The influence of naturally fluctuating temperatures and humidities on oviposition.

4.2 MATERIALS AND METHODS

The ticks used were cultured on young Guernsey calves. Two different 'strains'[1] were used; one originally from Lesotho, the

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1. Dr G.B. Whitehead (pers.commun.) has shown differences in the degree to which these 'strains' are resistant to various insecticides. Morphologically, physiologically and behaviourally the 'strains' have been considered to be identical.

other from East London (Cape Province of South Africa). Larvae were placed on the calves and allowed to develop through to the adult stage. Engorged female ticks were then collected either by hand picking them from the host or allowing them to fall from the host naturally. These semi-engorged or fully engorged females were placed in glass tubes enclosed in air-tight containers in which particular humidity levels, if required, could be maintained. Different relative humidity values were obtained by using potassium hydroxide solutions of different concentrations (Peterson, 1953). The air-tight glass containers were placed either in incubators in the laboratory or in water-baths kept in a cold room (4°C) depending on the temperature requirements of the experiments undertaken (see Table 2). Further technical details of special requirements are discussed under the relevant sections.

4.3 OBSERVATIONS AND RESULTS

4.31 Preliminary experiments

4.311 Oviposition behaviour

The oviposition behaviour of B.decoloratus females, with clean mouth-parts, was observed and photographed. The important stages in this behaviour are as follows:-

(i) Towards the end of the preoviposition period the female tick appears to have a slightly concave appearance in the region between the forelegs. The capitulum, which lies near the centre of this region, appears slightly withdrawn.

(ii) The first sign of egg laying involves a downward movement of the capitulum against the anterior end of the body (Fig. 23a), where it comes to lie in a small depression. The dorsal surface of

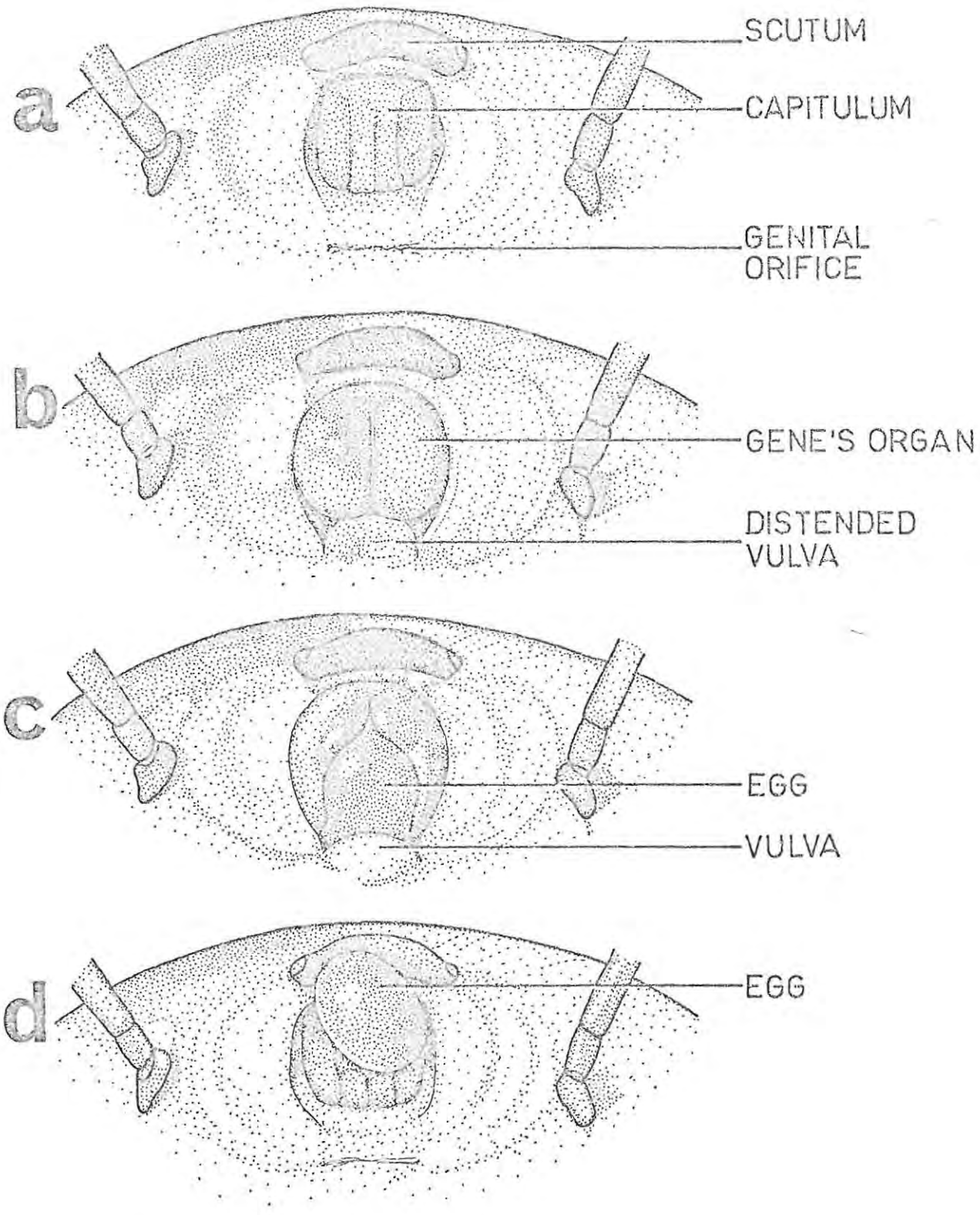


Fig. 23: Antero-lateral view of various stages of the oviposition behaviour of Boophilus decoloratus.

the capitulum then is either flush with or slightly below the integument.

(iii) Gene's organ, only slightly visible when the capitulum is so positioned, then becomes 'inflated' from the membranous region between the scutum and capitulum until it overlies the depressed capitulum. Concomitantly the vulva distends, presumably because of the bulk of the egg in the genital tract (Fig. 23b). The egg, on emerging from the genital orifice, is embraced by the lateral horns of Gene's organ, and at this stage, though visible through the translucent organ, is not exposed to the atmosphere.

(iv) The vulva then assumes its normal appearance, meanwhile the two horns of Gene's organ recede slowly over the surface of the egg (Fig. 23c) until completely retracted. On completion of these movements the egg is left adhering to the dorsal surface of the capitulum (Fig. 23d), presumably due to the 'sticky' nature of the soft 'wax' covering.

(v) The capitulum is then slowly elevated and the egg is carried on to the front edge of the scutum, to which it usually adheres. This process is repeated in respect of each egg and after some time an egg cluster accumulates antero-dorsally and in front of the capitulum.

This pattern parallels the observations of Hunter and Hooker (1907) for Margaropus annulatus (= Boophilus annulatus) and Lees and Beament (1948) for Ornithodorus moubata Murray, an argasid tick.

The rate of laying individual eggs was not determined but since a female tick may produce about 400 eggs within a single twenty-four hour period, and assuming a constant rate of production, each egg probably takes about four minutes to be produced. Hunter and Hooker

(1907) record that, in M.annulatus (= B.annulatus), "the actual time consumed by the tick in laying a single egg is about 30 seconds, while the removal of the egg and the resting period consume from one to several minutes, a much longer resting period being taken at intervals between lots of from 10 to 50 eggs". It is probable that B.decoloratus possesses very similar behaviour.

Periodically some female ticks were collected either with damaged mouth parts or with tissue or cement adhering to the base of the hypostome. Observations were made on the oviposition behaviour of such specimens. Females with tissue adhering to the hypostome were unable to fully depress the capitulum which resulted in the eggs not receiving a 'wax' coating from Gene's organ. Such eggs invariably became desiccated within a short time of being laid. Females with artificially damaged mouth-parts, produced by crushing them between the tips of a pair of fine forceps, produced eggs in the normal manner. The efficiency of forcing the eggs back onto the scutum may have been affected but this was not substantiated from the observations.

4.312 The pattern of oviposition under constant temperature and humidity conditions

The general pattern of oviposition in fully and partially fed B.decoloratus females was studied by removing and by either counting or weighing daily collections of eggs. For this purpose ten fully engorged females, which had dropped from the host naturally, and ten semi-engorged females, which were hand picked off the host, were used. All these fameles possessed clean, undamaged mouth-parts. The results, at constant temperature of 26°C and constant relative humidity of 95% R.H. (i.e. Saturation deficit conditions

of 1,3 mm Hg), are illustrated in Fig. 24a. The graphs show that there is an initial and rapid increase in the numbers of eggs produced by both sets of females producing a 'peak' on approximately the second or third day of the oviposition period. This peak, which was higher for naturally fallen females, was followed by a gradual decrease in egg production until, by the last few days of the oviposition period, very few eggs were laid. The mean duration of the oviposition period was slightly shorter for females which had fallen from the host naturally (i.e. mean: 17 days) than for 'hand picked' females (mean: 21 days). This difference in the duration of the oviposition periods is probably of little significance and is undoubtedly related to the weight differences of the two groups of females, as is clear from the next paragraph.

Females pulled off the host were lighter (mean weight: 172,5 mg) than those which had been allowed to fall naturally (mean weight: 277,5 mg). The smaller females also produced fewer eggs (mean number: 2033) than did the larger ones (mean number: 3739). As the results obtained for females which had fallen naturally from the host (Fig. 24a) demonstrated two peaks in egg production (i.e. on the 3rd and 7th days), a further six naturally fallen females were examined in order to clarify this observation. The results (Fig. 24b) indicate that only a single peak exists (on day 4), as was the case with the hand picked females (peak on day 2).

The relationship between the numbers of eggs produced and the initial weights of the female ticks studied above is shown in Fig. 25a. This is a linear relationship similar to that now known to occur in other ixodid species (Table 4). The numbers of eggs produced is directly related to the weight of the engorged adult female and therefore to the amount of 'blood meal' ingested.

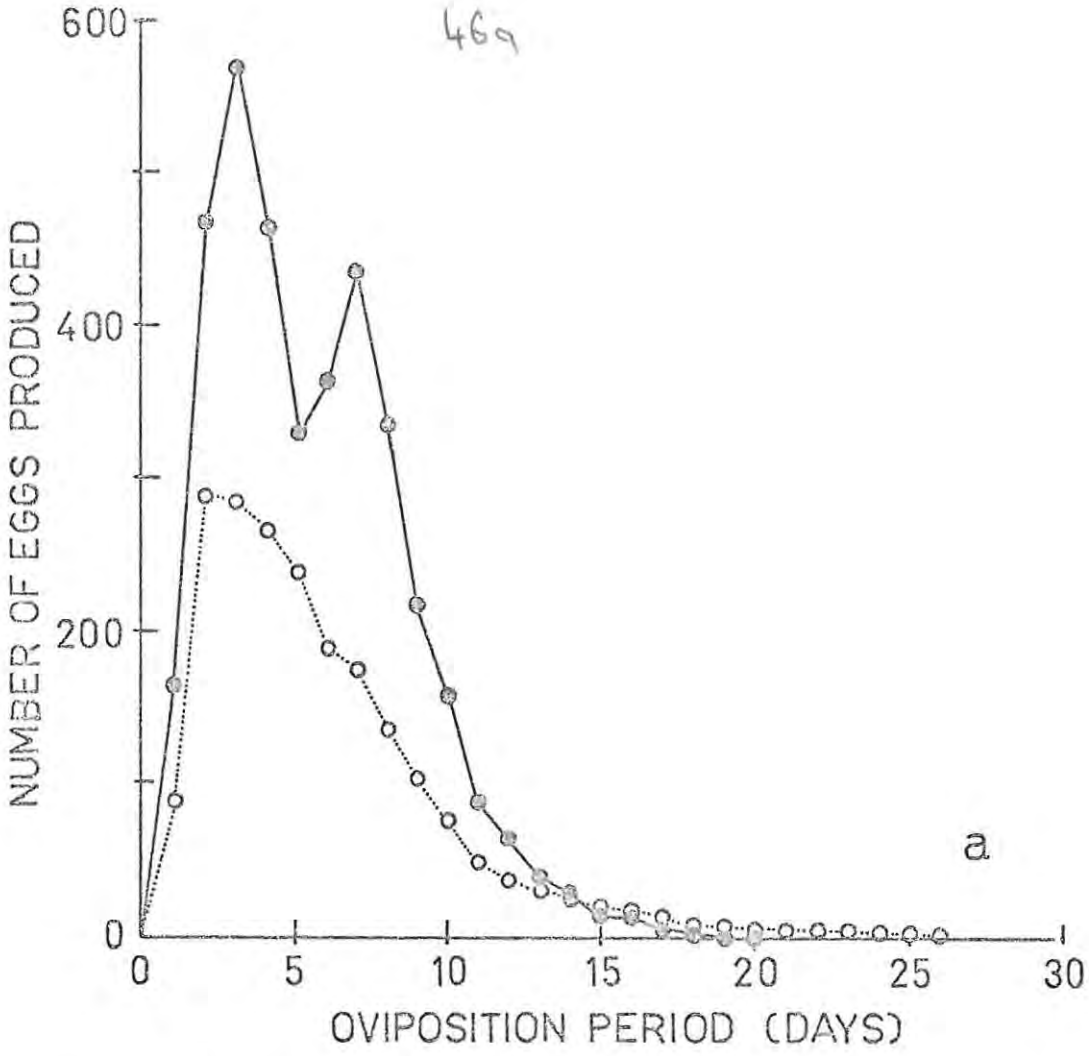
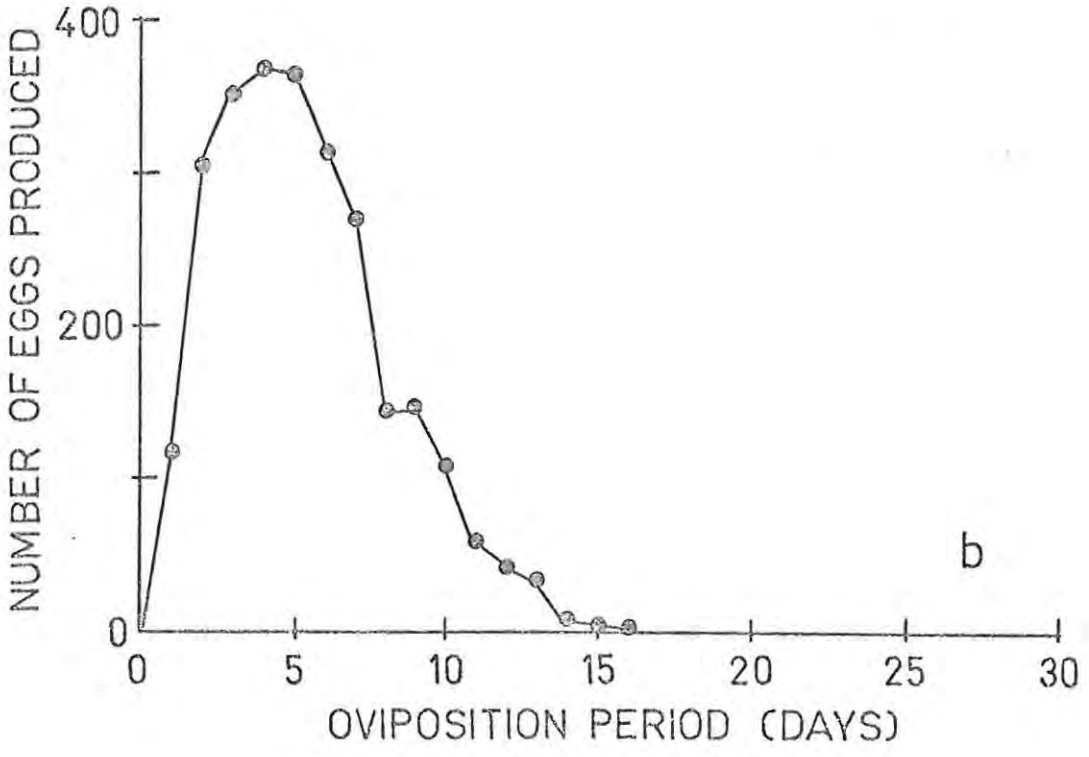


Fig. 24: (a-b). The pattern of oviposition of (a) ten naturally fallen Boophilus decoloratus females (●——●), ten females hand picked off the host (o·····o) and (b) six naturally fallen females.



In order to ascertain the minimum weight of 'blood meal' which must be imbibed by a female tick before egg production is possible, twenty-one semi-engorged females, selected to show a wide range in size, were pulled off the host, weighed, and allowed to oviposit in an incubator held at 26°C and 95% R.H. (saturation deficit 1,3 mmHg). The relationship between initial female weight and mass of eggs produced is shown in Fig. 25b. The minimum weight at which a female will produce eggs was approximately 17 mg which implies that, as unengorged females possess a mean weight of approximately 1,4 mg, a 'blood meal' of approximately 16 mg is required for egg production. The relationship between the initial weight of the female tick and the duration of the oviposition period was also ascertained (Fig. 26) and although there was some spread of the data points, there appears to be a relationship, which has been indicated by a dotted line. Ticks weighing less than 20 mg did not produce eggs. Between weights of 20 mg and 152,0 mg (i.e. the heaviest female) the relationship is exponential. When the individual oviposition patterns of all the females were examined it was found that a series of curves were obtained. Six of these curves are illustrated in Fig. 27. Females between the weights of 40 mg and 140 mg demonstrated only slight differences in length of oviposition period but marked differences in the magnitude of the peak weight of eggs produced. This implies that the rate of egg production is dependent on the amount of 'blood' imbibed by the tick and that the actual period required to produced the eggs is not dependent on the amount of food taken in.

4.313 Weight changes taking place during the preoviposition and oviposition periods.

By daily weighing each of the twenty-one female ticks mentioned

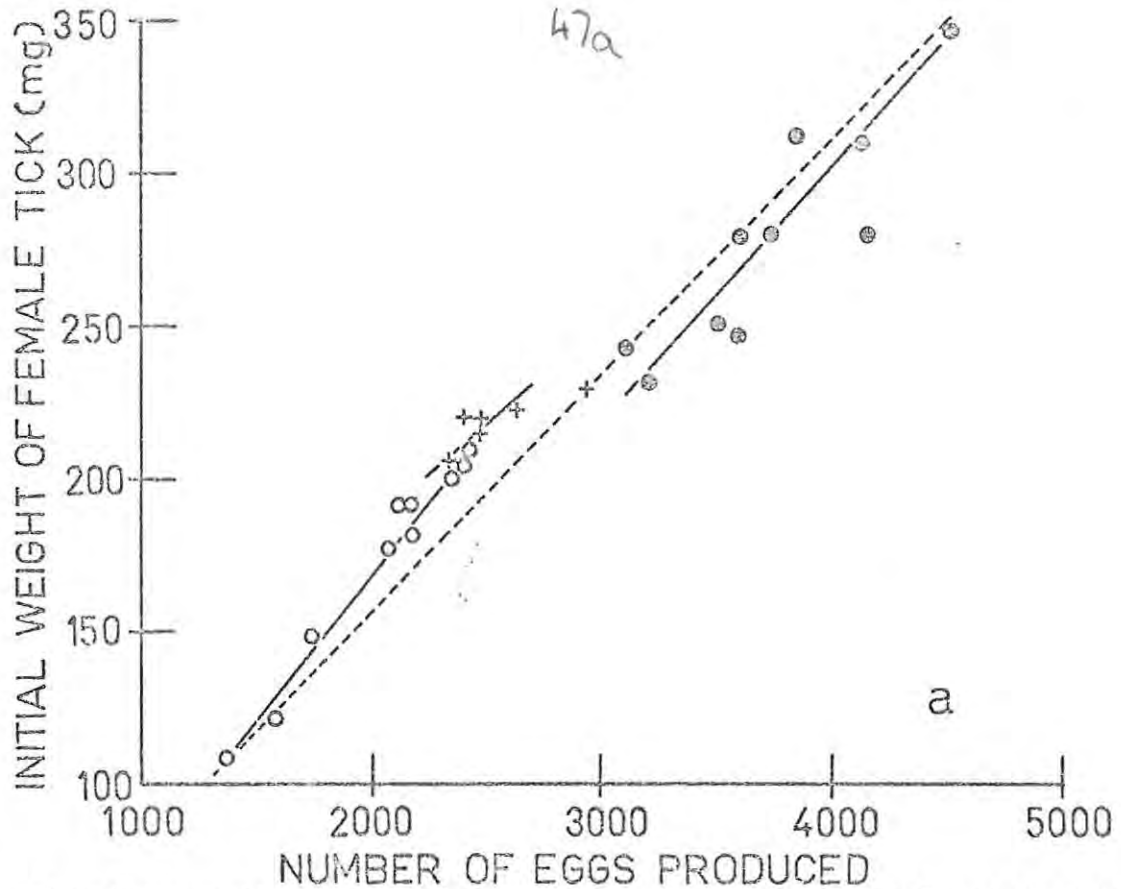


Fig. 25a:
The relationship between the number of eggs produced and the initial weight of ten naturally fallen females of Boophilus decoloratus (●), ten females removed from the host prior to their natural fall(⊙) and a further sample of six naturally fallen females (+). The broken line represents an approximate relationship based on all the females.

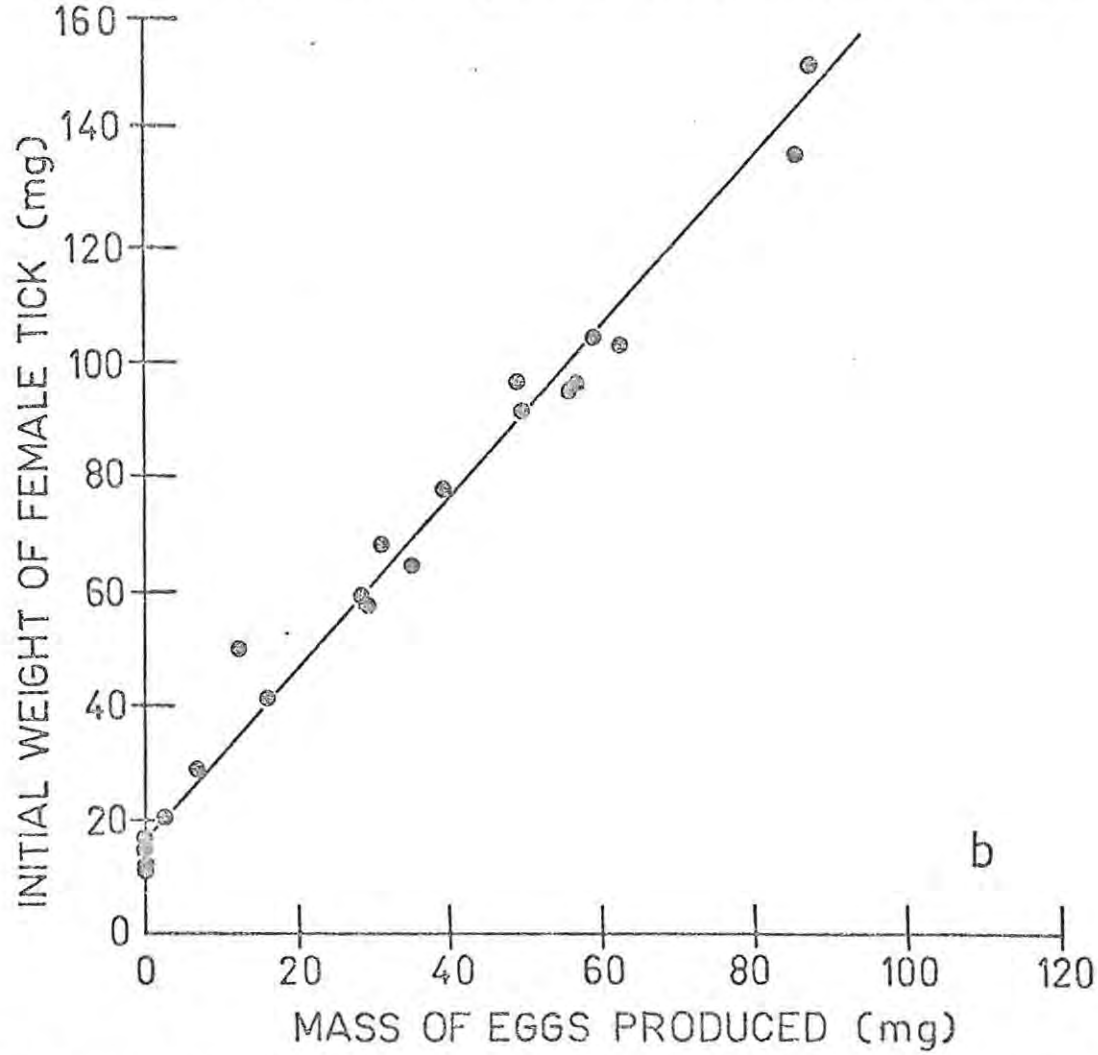


Fig. 25b: The relationship between the initial weight of twenty-one B.decoloratus females and the mass of eggs they produced.

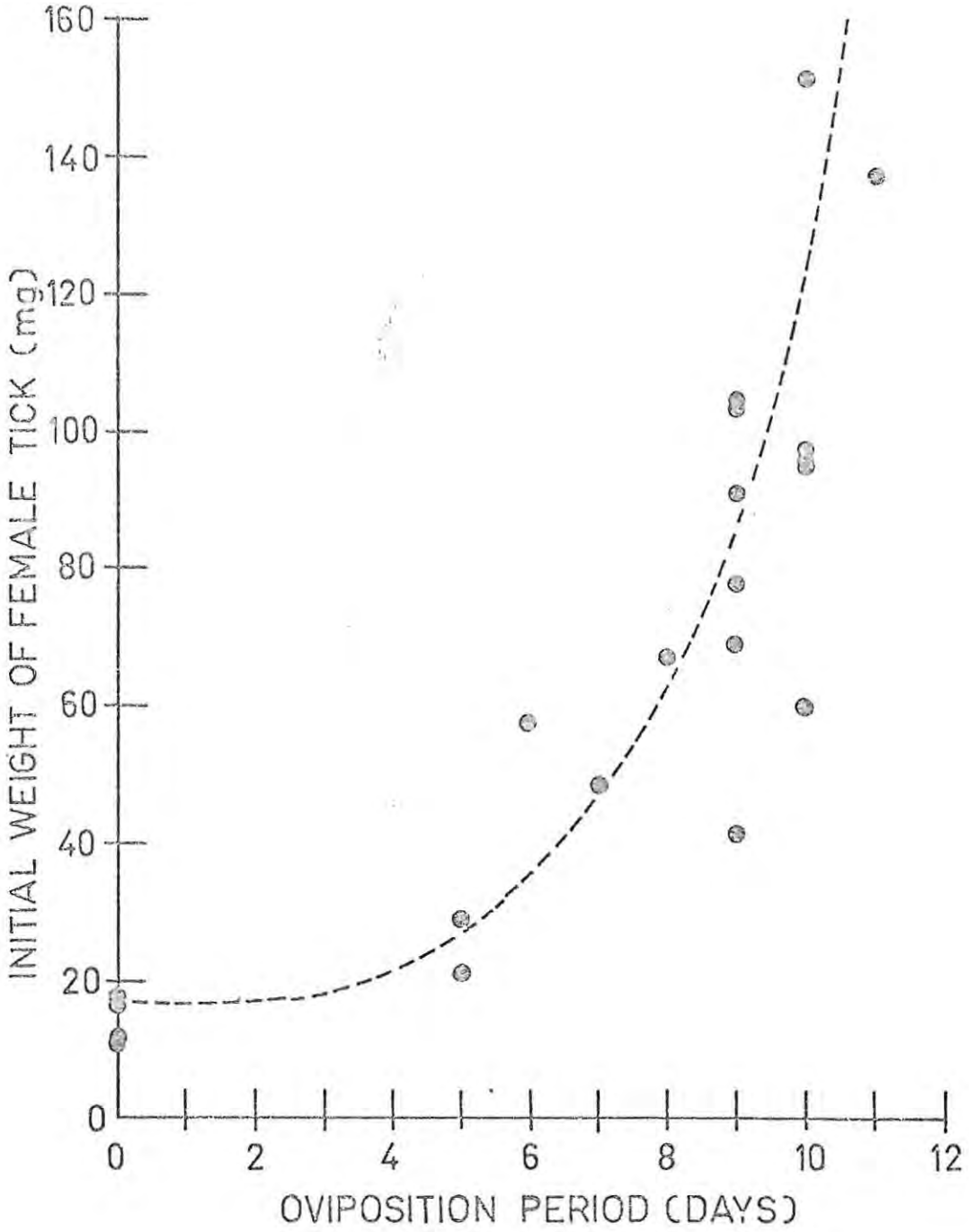


Fig. 26: The possible relationship between the initial weight of female Boophilus decoloratus ticks and the duration of the oviposition period.

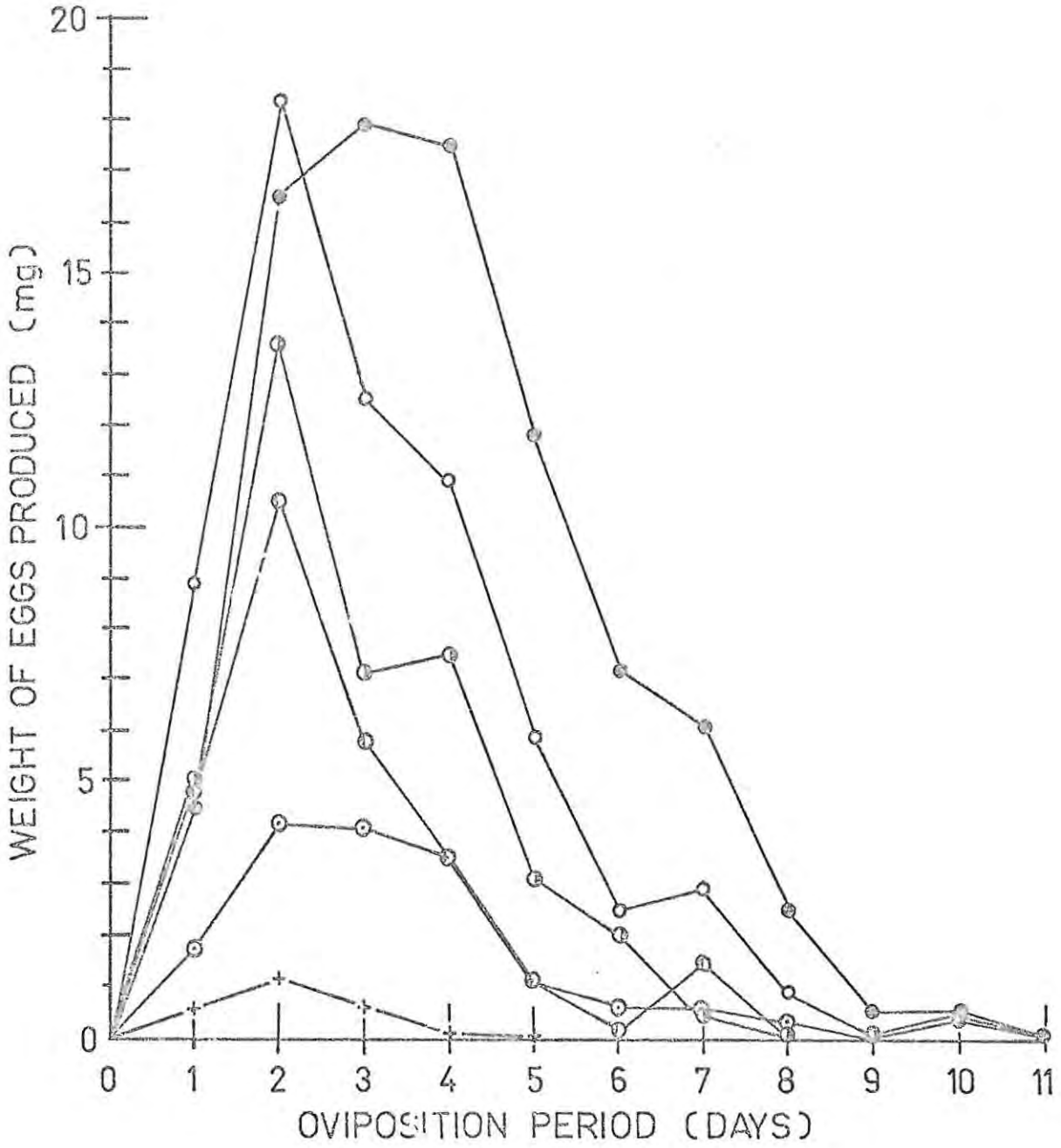
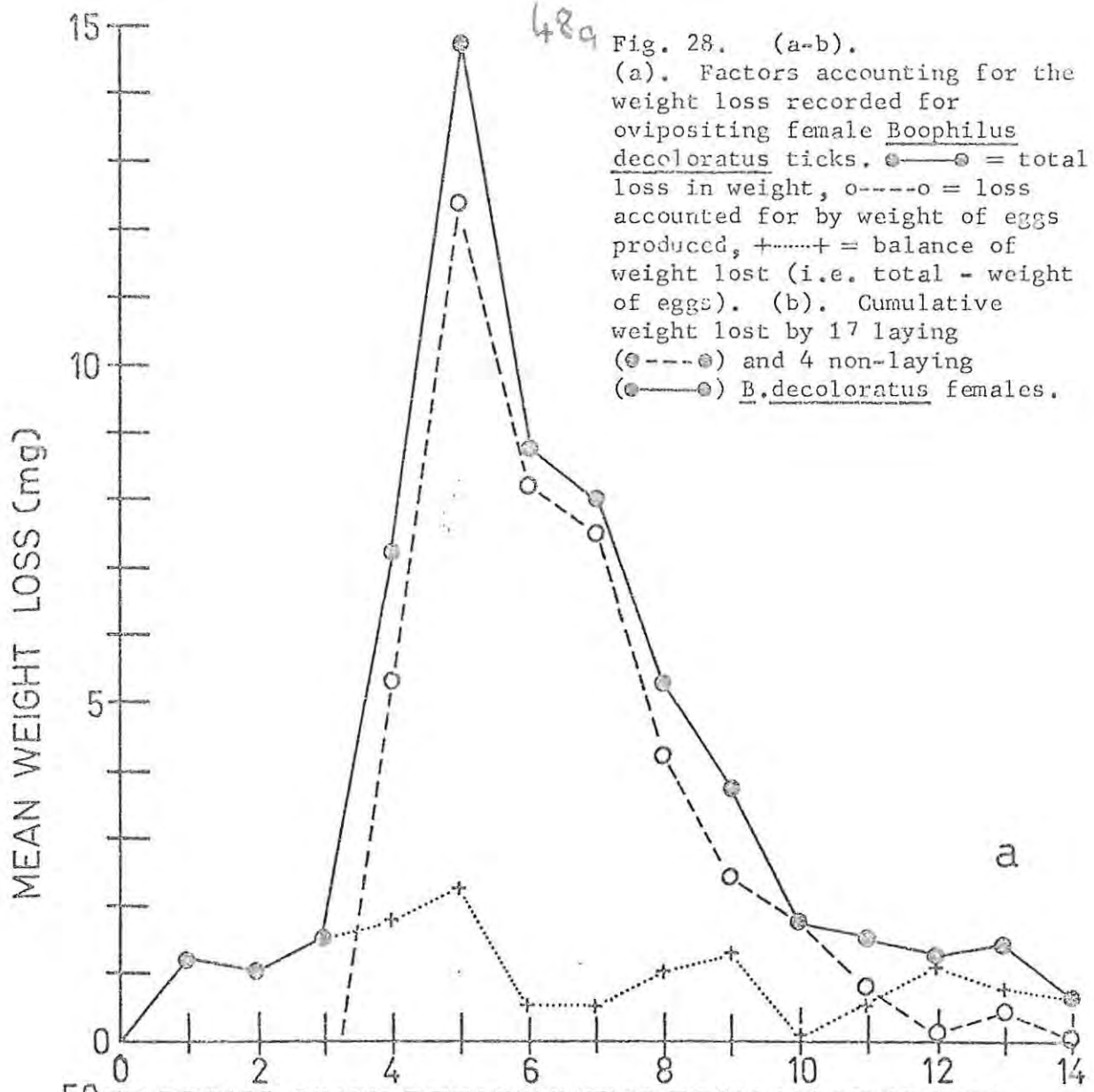


Fig. 27: The oviposition patterns of six Boophilus decoloratus females of different initial weight. + = 20,5 mg, ⊙ = 41,0 mg, ◐ = 59,8 mg, ◉ = 77,9 mg, ○ = 103,7 mg, ● = 137,0 mg.

above (4.312), together with any eggs which they may have produced over each twenty-four hour period, information was gained concerning the weight changes of both ovipositing and non-ovipositing ticks. Fig. 28a shows the changes in mean weight recorded for egg producing females during their preoviposition and oviposition periods. The actual liberation of eggs accounted for most of the weight lost by females once they had entered the oviposition period (i.e. the 4th day after removal from host), as would be expected, the balance, however, must be due to some other factor. The following suggestions could be made to account for this weight loss:-

(i) Weight loss from the female ticks.

(ii) Weight loss from the egg masses produced every 24 hours. While weight loss from the eggs could only be due to water loss through evaporation, there are many avenues through which weight could be lost by the engorged female tick. Some of these are, water loss (through cuticle or from the main body openings such as anus, genital opening, spiracles and mouth) and the release of excretory products such as guanine or undigested 'blood'. The weight changes of non-ovipositing females and ovipositing females, over and above the losses sustained through egg liberation, followed the same general pattern (Fig. 28b). This implies that the actual process of oviposition does not result in any increase in weight loss. Such weight loss would be expected in laying females as there is probably an increase in the metabolic rate, coupled with an increase in respiration, which suggests a possible increase in water loss from the spiracles, and an increase in the rate of water loss from the genital opening and the membranous Gene's organ. This implies that female ticks must possess various means of compensating for these various different avenues resulting in weight loss during the oviposition period. Certainly the behaviour of covering the



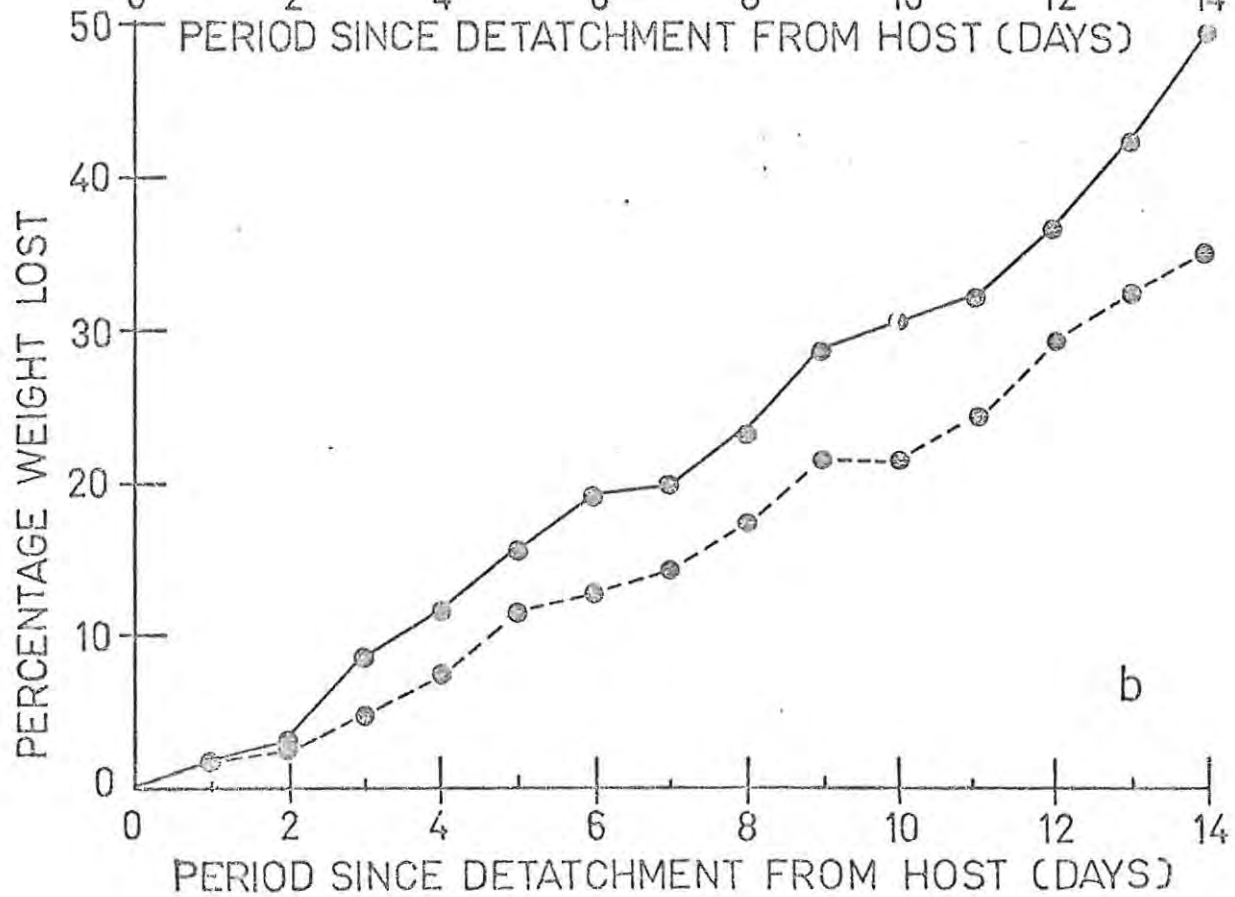


Fig. 28. (a-b).
(a). Factors accounting for the weight loss recorded for ovipositing female Boophilus decoloratus ticks. ●——● = total loss in weight, o-----o = loss accounted for by weight of eggs produced, +·····+ = balance of weight lost (i.e. total - weight of eggs). (b). Cumulative weight lost by 17 laying (●----●) and 4 non-laying (●——●) B.decoloratus females.

whole egg and genital opening with Gene's organ during egg liberation could be such a compensating device. The fact that non-laying females demonstrated a greater rate of weight loss than laying females (not taking into account the loss due to egg liberation) (Fig. 28b), can be explained by the greater surface to volume ratio of the non-laying females, all of which weighed less than 20 mg.

4.314 The effects of handling on total egg output

Previous workers (Lounsbury, 1899; Drummond et al 1969a; Sonenshine and Tigner, 1969), have shown a positive correlation between egg output and mechanical disturbance of the female tick during oviposition. Other workers (Drummond and Whetstone, 1970; Drummond et al. 1969b) have shown the effects of handling female ticks to be insignificant on egg production. As experiments involving egg production of B.decoloratus frequently entailed handling females in order to remove their eggs at various intervals during the oviposition period it was thought advisable to attempt to assess the effects of 'handling' on total egg output. It is fully appreciated that this type of study is likely to be subjective and therefore an attempt was made to handle each female in a similar manner.

To assess the effects of daily removal of eggs from B.decoloratus females, six females were weighed and allowed to lay their eggs without any disturbance. A comparison was then made of the total egg output of these females with six females which were handled daily when their eggs were being removed (Table 5). Although there appears to be only a slight difference between the egg outputs of disturbed and undisturbed females this difference is statistically significant

which means that the daily handling of females does influence egg output in that fewer eggs are produced by disturbed females.

TABLE 5.

A comparison of the egg output of disturbed and undisturbed Boophilus decoloratus females.

	Disturbed females			Undisturbed females		
	Initial wt. of female. (mg)	Total No. eggs produced.	No. eggs per mg of tick	Initial wt. of female. (mg)	Total No. eggs produced.	No. eggs per mg of tick.
	222,9	2627	11,79	233,7	3045	13,03
	205,8	2309	11,22	243,2	2784	11,45
	214,0	2466	11,52	205,1	2511	12,24
	227,2	2957	13,02	264,6	3348	12,67
	218,3	2465	11,29	230,3	3007	13,06
	219,3	2381	10,86	216,7	2764	12,76
Means	217,9	2534	11,62	232,2	2910	12,53

't' test on No. eggs/mg tick: 't' = 2,31
p $\Rightarrow$0,025 -$\Leftarrow$0,050*

4.32 The influence of constant temperature and humidity on the oviposition period

The influence of different constant temperatures and humidities on the oviposition period of B.decoloratus was studied. Six different temperature and three different relative humidity conditions were used (see Table 2), these were 10°C, 15°C, 20°C, 26°C, 32°C and 38°C; 90% R.H., 70% R.H. and 50% R.H. Three engorged females were studied at each relative humidity level at each of the three highest temperatures while four were studied at the three lower levels. The results of weighing daily collections of eggs from all the females studied are shown in Fig. 29a-e. No oviposition took place at 10°C.

The form of the graphs seen in Fig. 29a-e obtained at the three

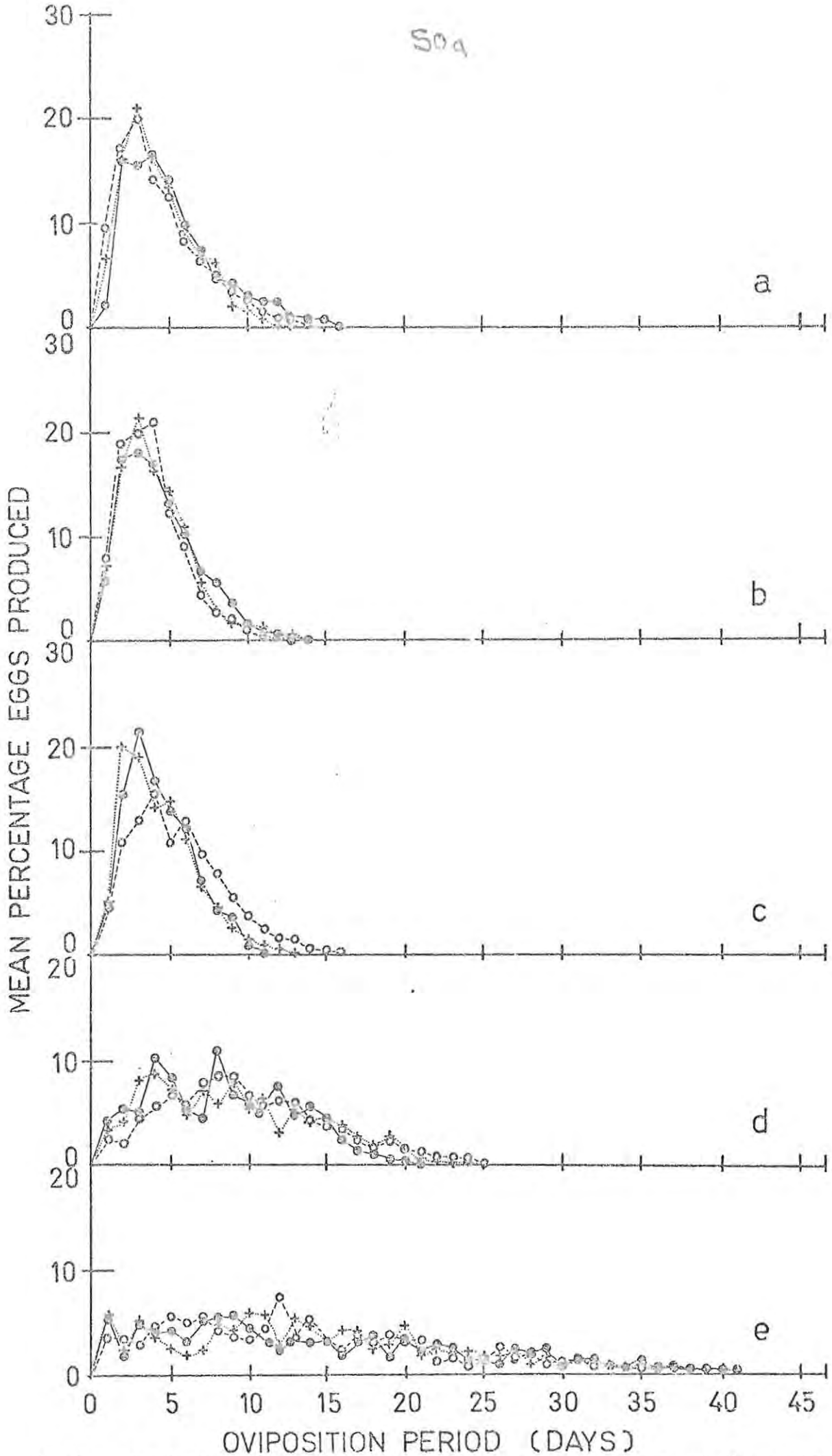


Fig. 29. (a-e). The influence of constant temperature and humidity on the oviposition pattern of Boophilus decoloratus. (a) 38°C (b) 32°C (c) 26°C (d) 20°C (e) 15°C. Data collected at 90% R.H. (●—●), 70% R.H. (o-o), 50% R.H. (+·····+).

different relative humidity levels were similar at each temperature. The conclusions drawn from these data indicate that relative humidity, at any of the temperatures studied had no effect in lengthening or shortening the period or form of the oviposition pattern.

Fig. 30, a contour diagram showing the relationships existing between temperature, saturation deficit and the duration of the oviposition period support this suggestion in that the lines of equal oviposition period duration lie almost parallel to the saturation deficit axis. The complicated curves shown between 26° and 38°C are a result of small variations in the slight differences which occur in the duration of the oviposition period between these temperatures. Temperature is the dominant factor in Fig. 30 as is evident in Fig. 31 where humidity effects are ignored. If the relationship between saturation deficit and the duration of the oviposition period are examined ignoring temperature effects (Fig. 32), an erroneous saturation deficit effect is seen (as in the case of the preoviposition period work reported in section 3.3). The overriding effects of temperature give this apparent positive correlation (see discussion in section 4.4).

The occurrence of definite peaks in daily egg production (on the third day usually) is a feature of the oviposition patterns at 26°C, 32°C and 38°C, but with the extension of the oviposition period that takes place at lower temperatures this peak becomes less defined. At 20°C the peak in egg production probably occurs somewhere between the fifth and tenth day of the oviposition period while at 15°C the curves are so 'flattened' that it is difficult to suggest where the peak occurs although it is probably in the region of the tenth day.

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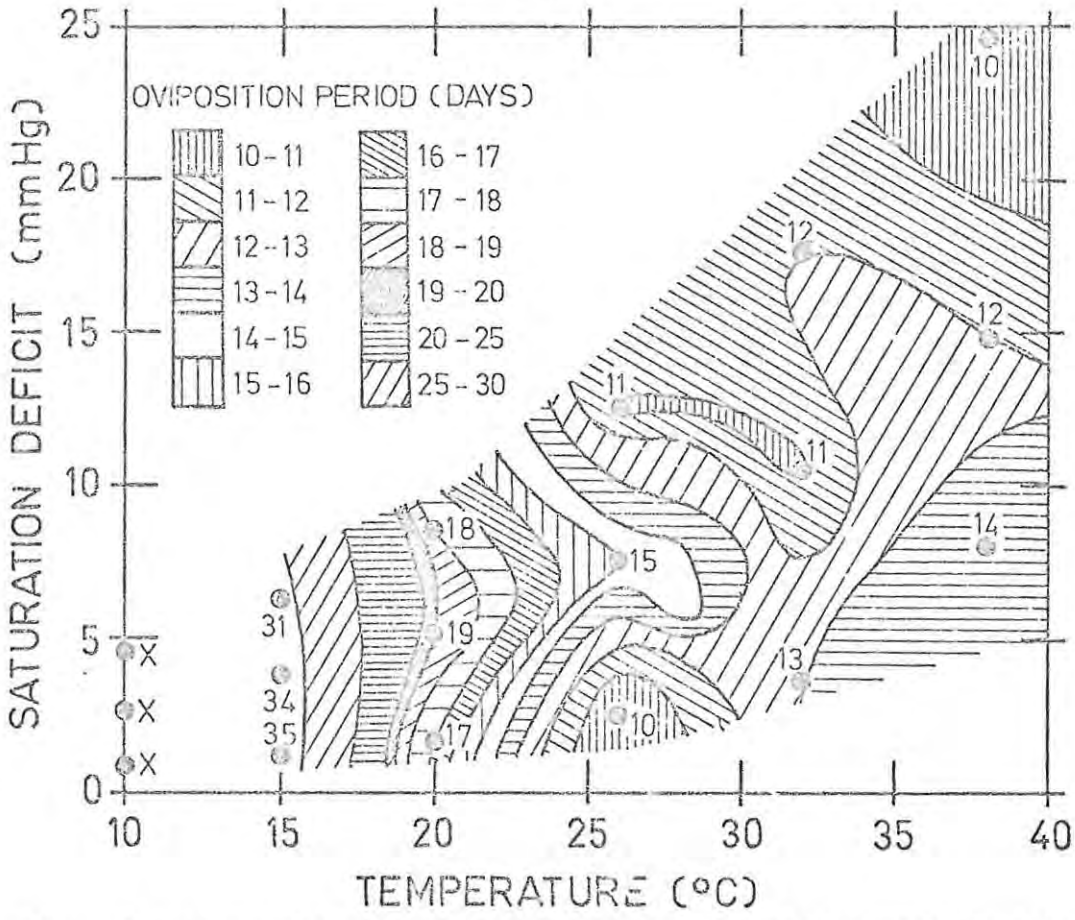

Fig. 30. A contour diagram showing the relationships between temperature, saturation deficit and the duration of the oviposition period of Boophilus decoloratus. Actual data points from which the contours were constructed are shown (⊛). X = no oviposition.

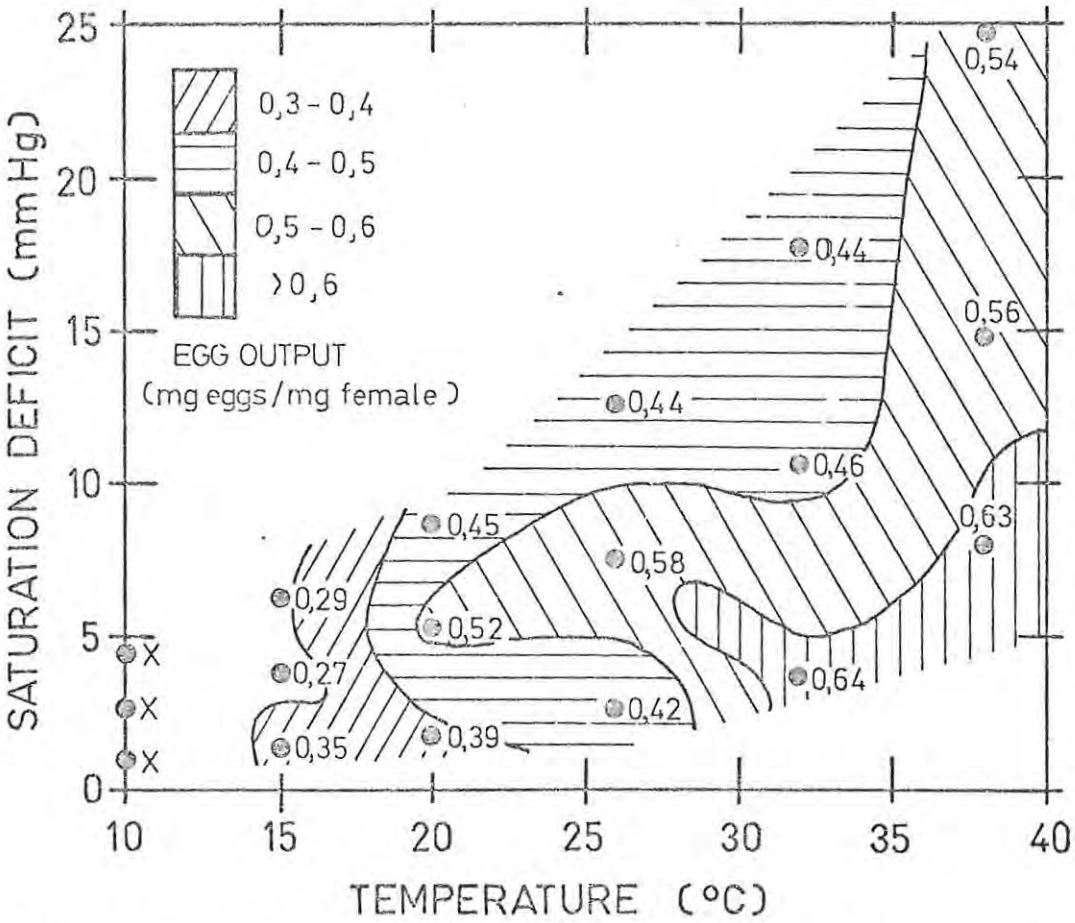

Fig. 33. A contour diagram showing the interactions of temperature, saturation deficit and the output of eggs by Boophilus decoloratus females. Actual data points from which the contours were constructed are shown (⊛). X = no oviposition.

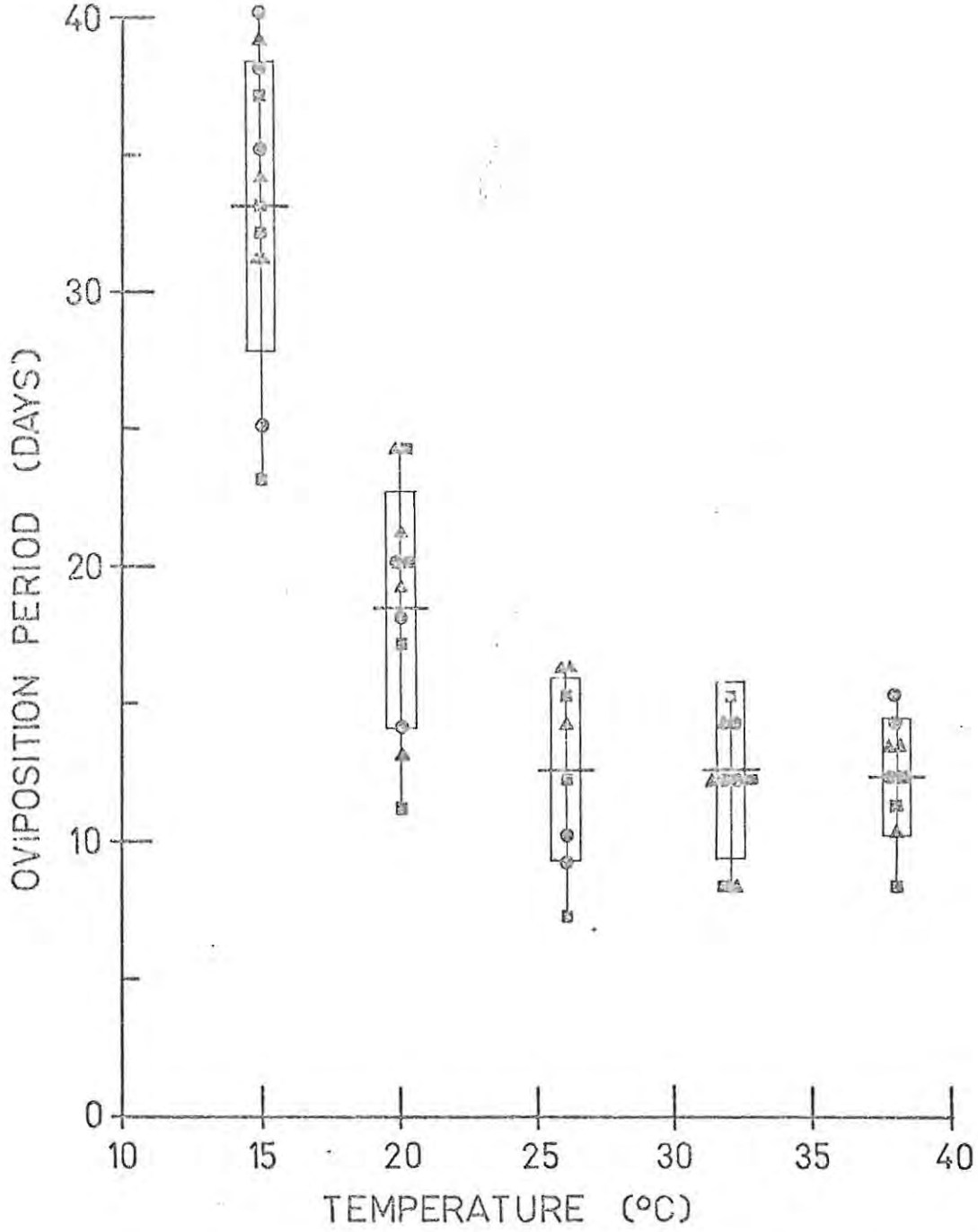

Fig. 31. The relationship between temperature and the duration of the oviposition period of Boophilus decoloratus ignoring any humidity effects. ● = data collected at 90% R.H., ▲ = data collected at 70% R.H., ■ = data collected at 50% R.H. The horizontal lines indicate the means, the open boxes, one standard deviation about the mean and the vertical lines the range at each temperature level.

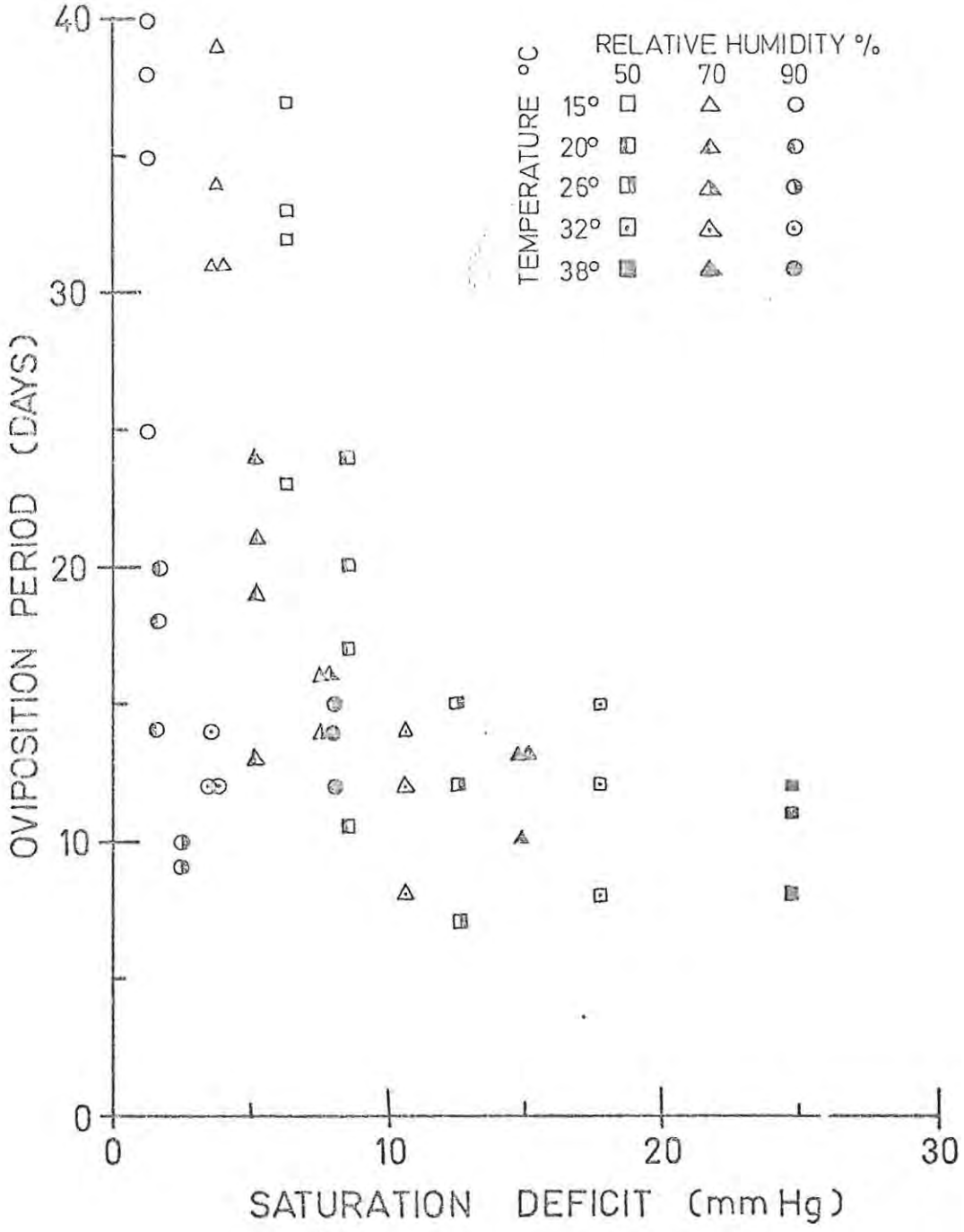

Fig. 32. The relationship between saturation deficit and the duration of the oviposition period of Boophilus decoloratus ignoring any effects of temperature.

In order to assess the total egg output of females at the six different temperature and three different relative humidity levels, the weight of eggs per milligramme of initial female weight was calculated for each tick so as to eliminate any effects of female size or degree of engorgement. A positive correlation between temperature and egg output (i.e. mg eggs/mg female weight) is shown in Fig. 33 (placed below Fig. 30) and although the lines of equal egg output undulate considerably it is suggested that there is little effect of humidity on egg output. In Fig. 34 the temperature correlation when humidity effects are ignored are shown and, although there are no statistically significant differences between the mean egg outputs of females held at each of the following temperature pairs, 15° + 20°C; 20° + 26°C; 26° + 32°C; 32° + 38°C, a significant difference was found when the means at 15°C and 38°C were examined statistically. This means that the tendency which is apparent in Fig. 34 is real. The relationship between egg output and humidity (Fig. 35), is difficult to interpret, but it is suggested that the apparent, although not very obvious, relationship is a result of an overriding temperature effect. Thus although the data in Figs 33-35 are 'spread' it is suggested that increased temperature has a direct effect on egg output by increasing the number of eggs produced. Humidity however does not have any marked effect on egg output.

4.33 The influence of naturally fluctuating temperatures and humidities on oviposition

Two batches of fully fed B.decoloratus females were placed in open ended plastic tubes (6, 5 cm long; 3,0 cms diam.) in a Stephenson's screen standing on the ground in a cattle camp on the farm Upper Gletwyn near Grahamstown. B.decoloratus larvae had

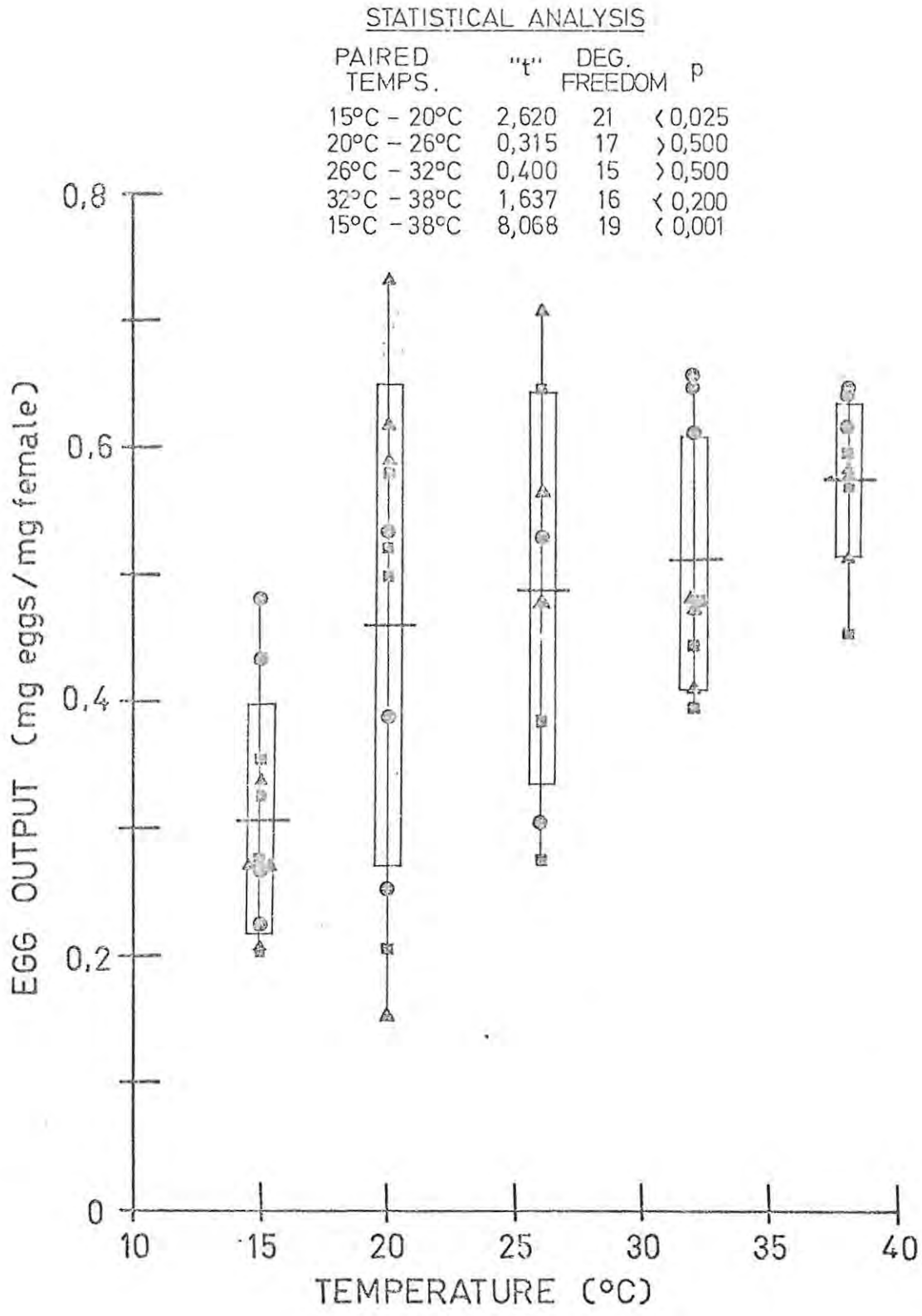

Fig. 34. The relationship between temperature and egg output by Boophilus decoloratus ignoring any effects of humidity. ● = data collected at 90% R.H., ▲ = data collected at 70% R.H., ■ = data collected at 50% R.H. The horizontal lines indicate the means, the open boxes, one standard deviation about the mean and the vertical lines the range at each temperature level.

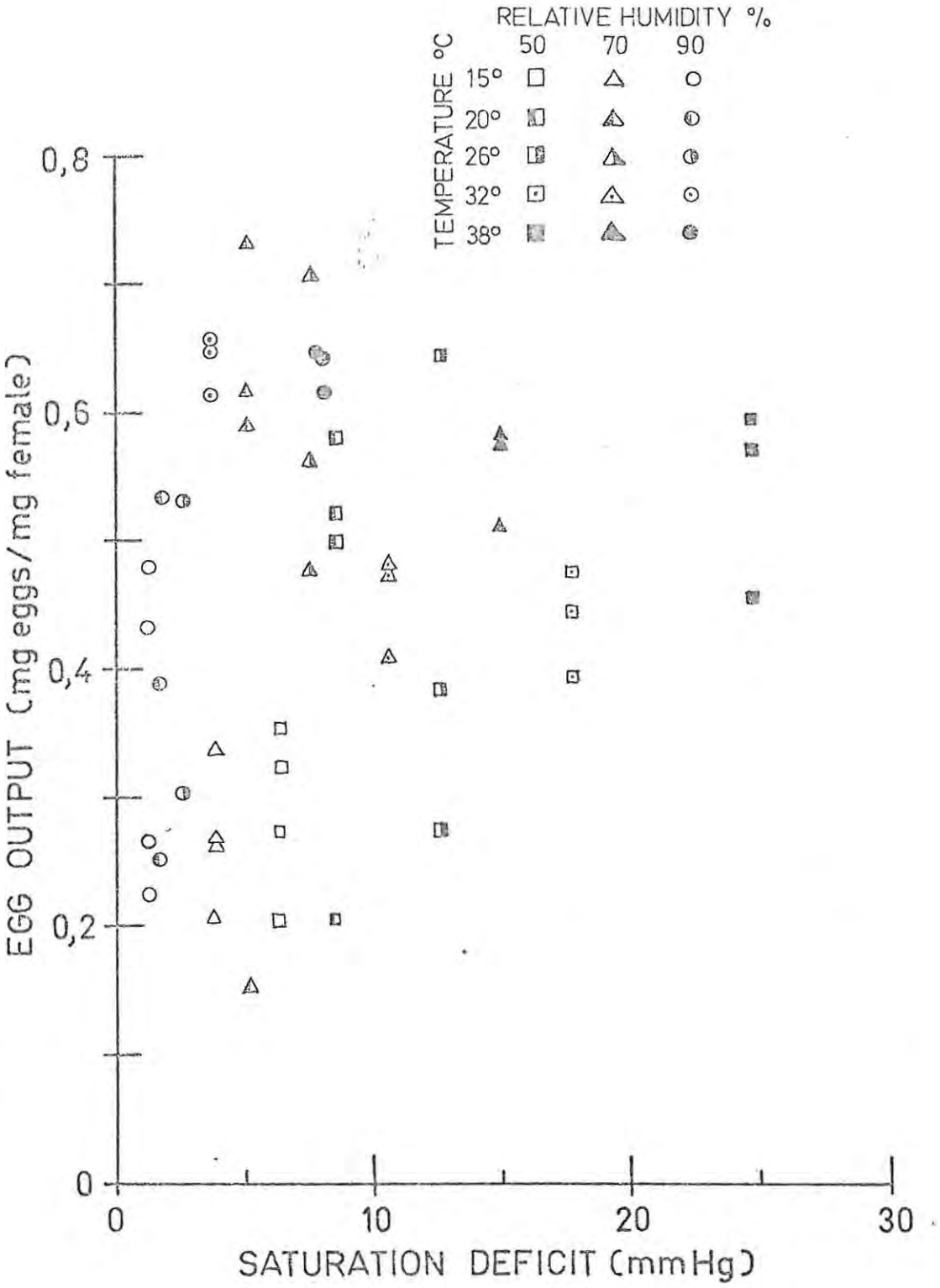

Fig. 35. The relationship between saturation deficit and egg output by Boophilus decoloratus ignoring any effects of temperature.

previously been collected in the camp using the conventional dragging technique (Londt and Whitehead, 1972). The female ticks were examined daily and all eggs laid were removed and weighed. The daily fluctuations in the mean weight of eggs laid per female, of both batches, over the oviposition periods are presented in Fig. 36. In one batch oviposition commenced on 29 August 1972 and persisted until 8 October 1972. Egg laying in the second batch began on 16 September 1972 and ceased on 28 October 1972. The duration for oviposition in each batch was 40 and 43 days respectively. Details of the oviposition period of each female and the mean length of the egg-laying periods are given in Table 6.

TABLE 6.

The oviposition periods, in days, of females of Boophilus decoloratus in each of two batches laying under fluctuating macroclimatic conditions.

	Duration of oviposition period (days)	
No. of female.	Batch 1	Batch 2
1	38	37
2	36	25
3	34	37
4	—	41
5	—	41
6	—	29
7	—	37
8	—	32
9	—	36
Mean	36	35

The means of both batches approximate closely to one another. The patterns of oviposition demonstrated by field studied females fluctuated from day to day throughout the oviposition periods (Fig. 36). The type of pattern which was seen under constant temperature conditions in the laboratory appeared to be completely masked by these fluctuations. It is however evident that the fluctuations in egg production followed, very closely, the fluctuations in daily hour

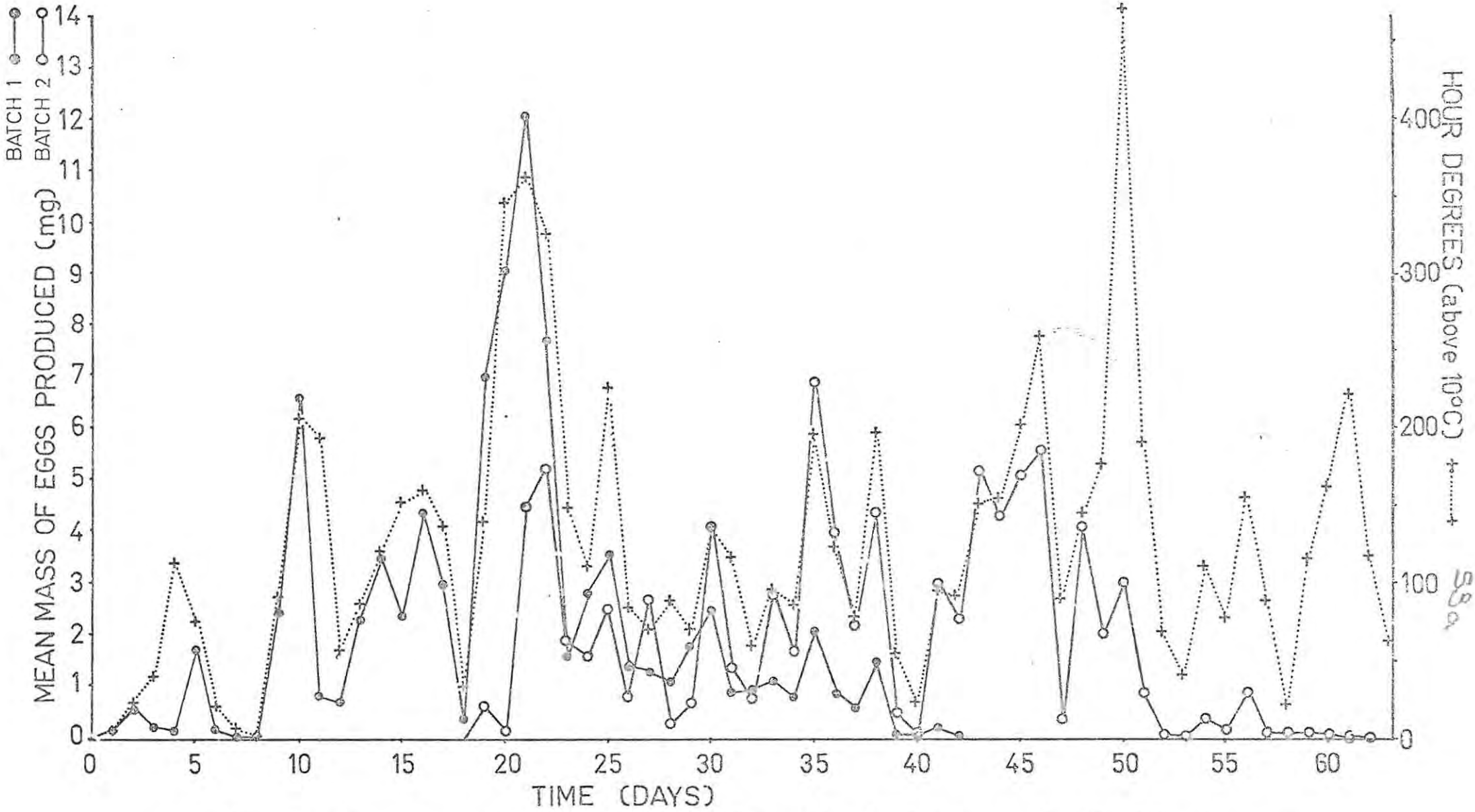

Fig. 36. The oviposition patterns of two batches of Boophilus decoloratus females (Batch 1 = ●——●, batch 2 = ○——○), placed in a Stephenson's screen, in relation to daily fluctuations in total hour degrees (above 10°C) (+·····+).

degrees (calculated above 10°C). In order to assess the possible effects of relative humidity and saturation deficit fluctuations, which took place concomitantly with those of temperature, the numbers of hour % R.H. (above 50% R.H.) and hour mmHg[1] (above zero) were calculated and plotted together with the fluctuations in daily hour degrees (Fig. 37). 10°C was selected as the baseline for the calculation of hour °C as it was found that no oviposition took place at 10°C in laboratory experiments. 50% R.H. was selected in the calculation of hour % R.H. as the thermohygrograph did not frequently register levels lower than this. All three physical parametres followed the same pattern of fluctuations (Fig. 37) except that those of relative humidity were converse to those of temperature and saturation deficit as would be expected. Oviposition studies undertaken in the laboratory (section 4.32) demonstrated that the duration of the oviposition period and the total mass of eggs produced are independent of humidity. It is therefore suggested that the fluctuations in egg production observed in Fig. 36 are a direct consequence of temperature fluctuations and remain uninfluenced by fluctuations in relative humidity or saturation deficit, within the limits necessary for the survival of the female ticks.

If the mean number of temperature units (hour degrees above 10°C) are calculated for the entire period during which females in the second batch produced eggs, a mean value of approximately 145 h°C would result for each day. This value would be equivalent to a constant temperature, or mean daily temperature, of approximately 16°C (see Fig. 21). This means that it was generally cold over the

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1. i.e. Saturation deficit units (S.D.U's).

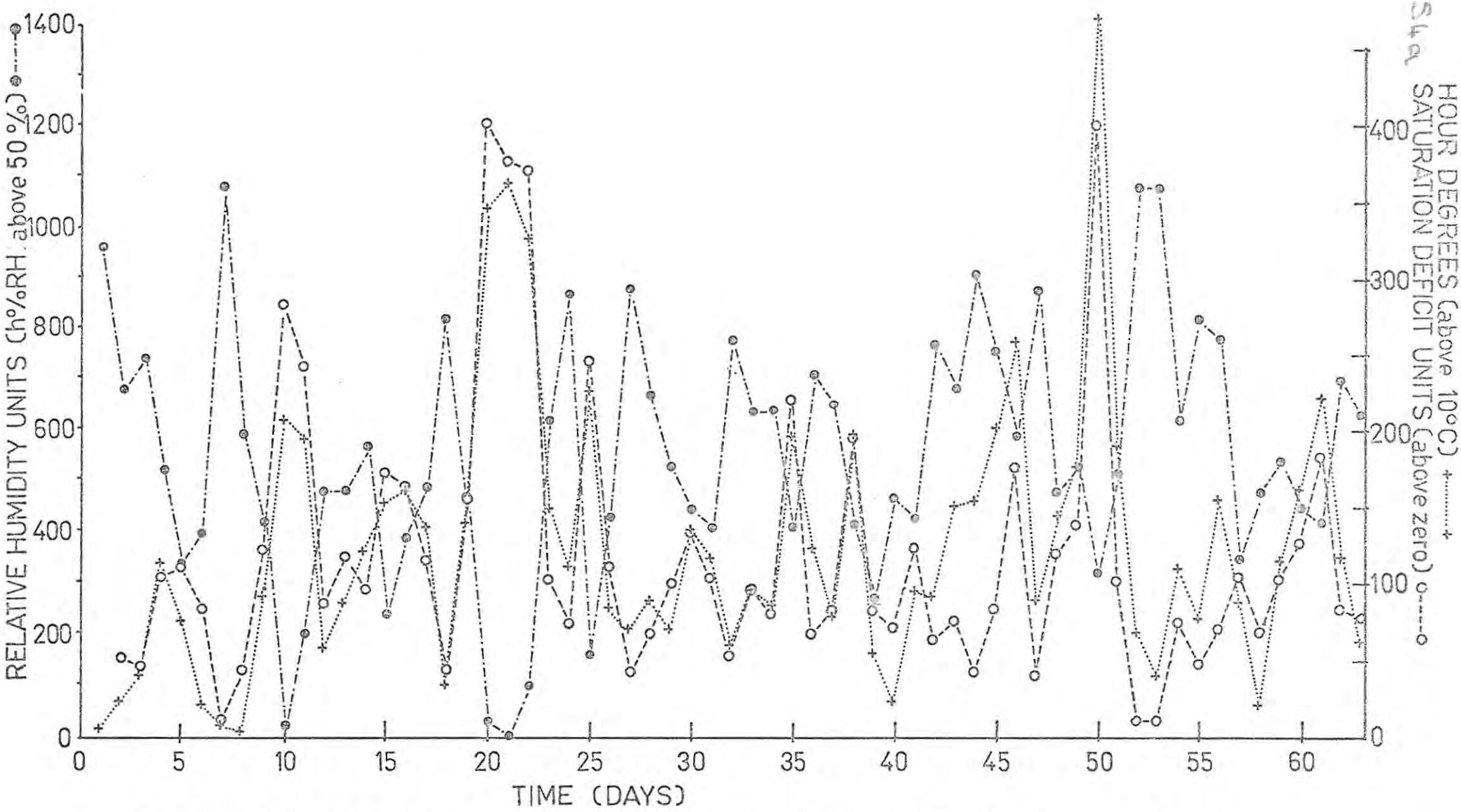

Fig. 37. The interactions between temperature (h °C above 10°C, +·····+), relative humidity (h % R.H. above 50%, ●----●) and saturation deficit units (h mmHg above zero, o----o) throughout the duration of studies on oviposition by Boophilus decoloratus females in a Stephenson's screen.

period of the investigation and that it would be expected that the general shape of the oviposition curve would most closely resemble that found at 15°C in the laboratory (i.e. Fig. 29e). If a visual comparison of the two curves is made it reveals that both curves are 'long and flat' which suggests that the field laying females respond in a very similar way to temperature as do the laboratory ones.

4.4 DISCUSSION

The present investigation has been primarily concerned with the effects of temperature and moisture differences and their influence on the duration of the oviposition period and the output of eggs during this period. All previous workers (Table 3) have shown that there is a positive correlation between the length of the oviposition period and temperature. The only possible exception was Sweatman (1968) working on H.aegyptium. The results of Sweatman's (1968) investigations demonstrated a tendency for the oviposition period to become shorter with increase in temperature but this was not a statistically significant relationship. Sweatman (1968) however, combined the results of females fed on three different hosts and there is a possibility that this had an effect on his results. Norval (pers.commun.) has shown that A.hebraeum has different feeding patterns on different hosts. Sweatman (1968) also stated that there was "some unrecognized factor causing mortality which occurs at all temperatures and humidities" as he also suggested that this mortality occurred during the oviposition period Sweatman's results pertaining to oviposition must be viewed with caution. Apart from Sweatman's (1968) results there is unanimous agreement that the length of the oviposition period of ixodid ticks is decreased with an increase in temperature within the lethal limits set by this parameter. A

characteristic of this temperature relationship which appears to have been overlooked by previous workers, but is evident from the results reported in this investigation (Fig. 31) and partly from the results of both Snow and Arthur (1966, Fig. 4), working on Hyalomma anatolicum (Koch), and Sweatman (1968, Fig. 12), working on H.aegyptium, is that the oviposition period appears to reach a minimum length at a certain temperature value (approximately 25°C in both B.decoloratus and H.aegyptium and about 37°C in H.anatolicum). Little deviation from this minimum oviposition period duration takes place at higher, but sublethal, temperatures. The reasons for this characteristic are not known but several suggestions could be offered. One is that once this specific temperature is reached it becomes physically or physiologically impossible for the female ticks to further increase the rate at which eggs are produced. Since the amount of imbibed food, as implied by engorged weight, is one of the most important factors responsible for determining the potential egg output (Table 4) and the length of the oviposition period (Fig. 26) it stands to reason that, if the above suggestion is valid, the oviposition periods of ticks producing eggs at the greatest rates possible, would largely be determined by the quantity of food imbibed by them. The results of the present investigation concerned with the effects of temperature on the total egg output by B.decoloratus (Fig. 34) indicate that the potential egg production of females is directly dependent on temperature in that more eggs are produced per milligram of female weight with increase in temperature. The mean weight of eggs produced per milligram of female weight at 38°C was almost double that at 15°C. This evidence implies that the rate of egg production in B.decoloratus does not reach a maximum as suggested above, but that it continues to increase above 25°C and that, due to the increased egg output potential of females at higher

temperatures, more eggs are being produced which results in similar oviposition period durations above 25°C (Fig. 31).

The situation regarding the effects of atmospheric humidity on oviposition is less clear. A number of workers including Lancaster and MacMillan (1955), Snow and Arthur (1966) and Sweatman (1967) all show positive correlations between the duration of the oviposition period and humidity. Lancaster and MacMillan's (1955) results need verification (Sweatman 1967). The only comment made by Snow and Arthur (1966) was "In our experiments at relative humidities below 50% the only difference observed is that with increased relative humidity, at constant temperature, egglaying is extended from about 16 days at 25% R.H. to 20-22 days at 40-50% R.H.". As relative humidities of 25% R.H. are not frequently encountered in the microclimate of most tick species it is possible that this very dry condition caused H.anatolicum to lose a great deal of its water reserves and this could have seriously limited the oviposition period. More detailed work is necessary on H.anatolicum over a wide range of humidities before a realistic appraisal of the effects of humidity on the duration of the oviposition period can be made.

Sweatman (1967), working on R.sanguineus, states that "an increased saturation deficit shortened the oviposition period at temperatures between 20° and 30°C and at 35°C, but not at 15° or at 40°C" (His Fig. 12 - repeated here as Fig. 38a). This statement is partly unjustified as Sweatman (1967) does not take into account the effects of temperature between 20° and 30°C. If his data at any single temperature level is examined only a slight correlation may be likely at 20°C but certainly not at 25°C and 30°C. On the other hand he does show a slight correlation at 35°C, but with so

57a

TEMP. (°C)	15	20, 25, 30	35	40
LINE	a	b	c	d
EQUATION	Y = 28.46 - 0.02X	Y = 15.33 - 0.16 X	Y = 13.21 - 0.12 X	Y = 6.06 + 0.05 X
N	9	27	13	8
r	-0.02	-0.43	-0.60	0.42
P	>0.1	<0.05	<0.05	>0.1

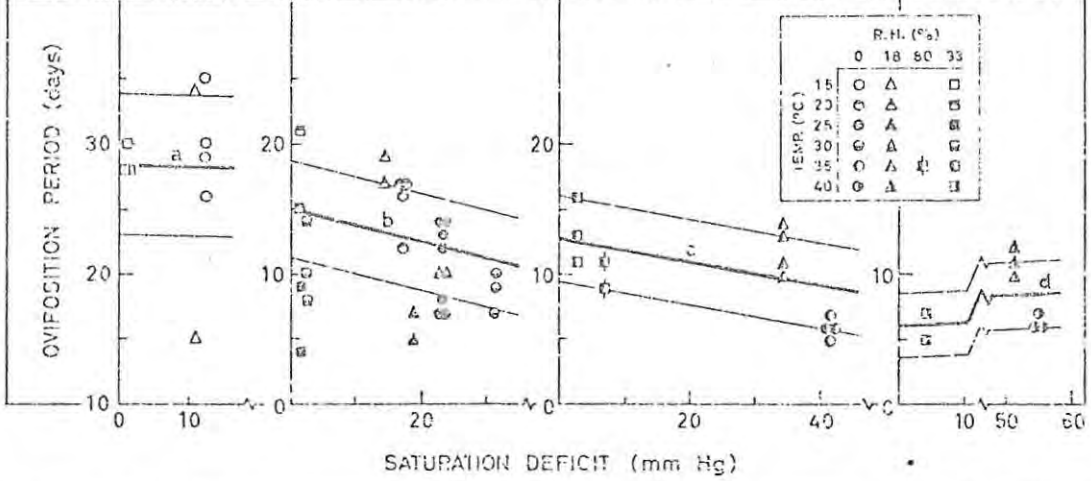

FIGURE 12. Oviposition periods of female *R. sanguineus* at different saturation deficits for different temperatures. Abbreviations as in Figure 4.

TEMP. (°C)	15	20, 25, 30	35	40	45
LINE	a	b	c	d	e
EQUATION	Y = 53.12 + 0.27 X	Y = 26.09 - 0.37 X	Y = 18.40 - 0.11 X	Y = 13.93 + 0.04 X	Y = 4.51 + 0.04 X
N	9	28	13	10	9
r	0.34	-0.61	-0.46	0.26	0.49
P	>0.1	<0.001	>0.1	>0.1	>0.1

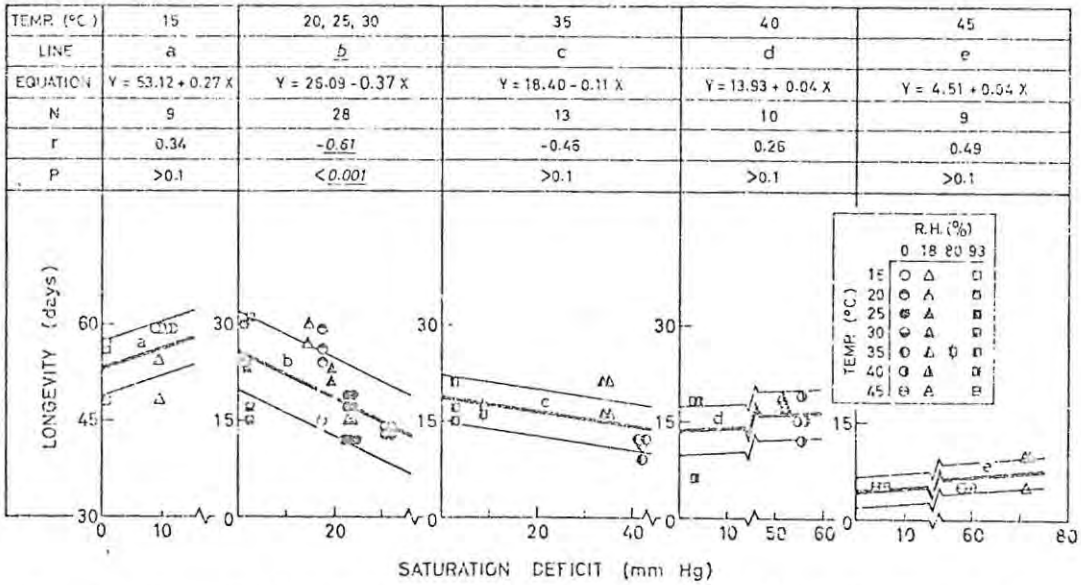

FIGURE 5. Longevity of female *R. sanguineus* at different saturation deficits for different temperatures. Abbreviations as in Figure 4.

Fig. 38: (a). A copy of Sweatman's (1967) figure 12 with accompanying legend. (b). A copy of Sweatman's (1967) figure 5 with accompanying legend.

few replications this may be coincidence. If the data given in the present investigation are studied (Fig. 32) it is possible to suggest that there may be a very slight correlation between saturation deficit and oviposition period duration at 38°C but this relationship does not appear at any other temperature level. Another explanation for Sweatman's (1967) results is that at the very low R.H. values used by him (0% and 18% R.H.) the female ticks lost a lot of water which could have seriously reduced their egg producing potential by reducing their effective oviposition period duration or longevity. This suggestion is supported by the information given by Sweatman (1967) in his Fig. 5 (repeated here as Fig. 38b). Sweatman shows that there is a positive correlation between saturation deficit and female longevity between 20° and 30°C ($P = <0,001$). Apart from the fact that Sweatman (1967) disregards any effect of temperature his data taken at 0% R.H. appear to be the reason for this correlation. It is suggested that if he had used relative humidity values less 'damaging' the positive correlation may not have resulted. Even if Sweatman (1967) is correct in suggesting that saturation deficit does influence the duration of the oviposition period in R.sanguineus the effects are so insignificant when compared to the 'powerful' influence of temperature that for all practical purposes it can be ignored.

An important consideration becomes evident from the present study and the work of Sweatman (1967). It is clearly quite unrealistic to attempt to assess the influence of saturation deficit, when these are calculated from different temperature conditions, on the duration of any developmental period in the life cycle of a tick species as the overriding effects of temperature makes any statistical analysis meaningless.

Two main factors appear to be of prime importance in determining the output of eggs by female B.decoloratus (Table 4). These are temperature and the amount of food ingested by the female tick (as implied by the initial engorged weight of the female). Other factors such as disturbance (or handling) of the female tick during oviposition and immersion in water are unlikely to be of any significance under natural conditions. Humidity, on the other hand, does not appear to be at all important in determining the total egg output in B.decoloratus.

Sweatman (1967) demonstrated that by summing the total number of eggs produced and the engorged weights of females there was a direct relationship between temperature and egg production in R.sanguineus (his Fig. 7). Sweatman (1968), Arthur (1951) and Macleod (1935), however, reported negative correlations for the species they studied. The work of Sweatman (1968) on H.aegyptium may be suspect as he states that there was some factor which caused mortality in some of his ticks during the oviposition period. Both Arthur (1951) and Macleod (1935) neglected to take the engorged weights of their females into account and therefore erroneous results may have been reported. Both Hitchcock (1955b), working on B.microplus and Sweatman (1967), working on R.sanguineus, demonstrated a temperature relationship with egg output in that, at a particular temperature value, females would produce a maximum number of eggs. Above and below this temperature value total egg production decreased. Although Hitchcock (1955b) also ignored the effects of female weight his results support those of Sweatman (1967) who took this into account. This type of relationship between temperature and total egg output was not seen in B.decoloratus which appears to produce more eggs with increase in temperature in

a near linear response. This aspect needs further examination.

As regards the effects of atmospheric humidity on total egg output there is almost complete agreement that there is no correlation between these parametres (Table 4). Macleod (1935) and Arthur (1951) are, however, exceptions in that they report positive correlations. Both these workers show that in the Ixodes species studied by them more eggs are produced with increase in relative humidity. This is a seemingly sensible relationship as egg output is directly related to the amount of ingested food and this is largely composed of water. In a dry atmosphere the female ticks would probably lose water and this might be expected to reduce the total egg output. Sweatman (1967) suggests that as female ticks are usually large (implying copious supplies of water) and are probably able to take up water vapour from damp atmospheres, that dry air, even when present for long periods, does not significantly effect them. Sweatman's (1967) suggestion appears to be reasonable but it may not necessarily apply to the genus Ixodes the species of which are generally smaller than those of other genera, produce fewer eggs and appear to be limited to habitats experiencing almost continuous high humidity.

5. THE INCUBATION PERIOD

Synopsis

During preliminary experiments it was found that the viability of B.decoloratus eggs laid during the first half of the oviposition period was greater than for eggs laid in the second half of the period. The critical temperature of eggs was found to be 42°C while the critical humidity (at 26°C) was approximately 70% R.H. (i.e. 7,53 mmHg). The development of eggs, traced throughout the incubation period by monitoring the build-up of nitrogenous excretory product (guanine) spectrophotometrically, was studied under different humidity conditions. It was found that the successful development of eggs was dependent on the water content of the eggs at the time of laying. This suggestion was supported when it was found that eggs could not take up water vapour from damp atmospheres.
From work at constant, alternating and naturally fluctuating temperatures and humidities it was found that the duration of the incubation period was temperature dependent and humidity independent within the tolerance limits set by these parameters. Egg viability was however humidity dependent and temperature independent. The implications of these findings are discussed.

5.1 INTRODUCTION

Two main aspects of the egg stage have been studied by past workers (Tables 7 and 8); the first being concerned with the effects of various factors, including temperature and humidity, on the duration of the incubation period, the second being concerned with the effects of similar factors on the percentage hatch, or 'viability'[1], of tick eggs. The results show that the incubation period duration is related directly to temperature, whereby increases in this parameter shortens the period. Humidity does not appear to affect the duration of the incubation period of

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1. The term 'viability' has been used as a 'synonym' of 'percentage hatch'.

the female within the limits of survival imposed by this parameter. Both temperature and humidity do however influence the hatch of tick eggs (Table 8).

The present investigation has been divided into four main sections and for convenience materials and methods have been outlined in each section.

TABLE 7.

Factors influencing the duration of the incubation period in ixodid ticks.

Species	Factors investigated			Reference
	H	T	TOL	
Amblyomma hebraeum Koch		+		Lounsbury (1899)
Amblyomma maculatum Koch			+	Drummond and Whetstone (1970)
Boophilus annulatus (Say)	-	+ +		Hunter and Hooker (1907) Graybill (1911)
Boophilus decoloratus (Koch)	-	+		This study
Boophilus microplus (Canestrini)	-	+ +	-	Hitchcock (1955b) Legg (1930)
Dermacentor variabilis (Say)	-			Smith et al (1946)
Ixodes hexagonus Leach	-	+		Arthur (1951)

Abbreviations: + = positive correlation; - = negative correlation; H = humidity; T = temperature; TOL = time of laying.

TABLE 8.

Factors influencing the hatch of ixodid tick eggs.

Species	Factors investigated									References
	H	T	OH	OT	I	DM	C	TL	D	
Amblyomma americanum Koch	+						+			Lancaster and McMillan (1955)
	+						+			Gladney and Drummond (1970)
										Sonenshine and Tigner (1969)
Amblyomma hebraeum Koch	+	+								Lounsbury (1899)
Boophilus annulatus (Say)		+			+					Hunter and Hooker (1907)
	+	+							-	Graybill (1911)
Boophilus decoloratus (Koch)		+								Gothe (1967b)
	+	+								This study
Boophilus microplus (Can.)	+	+			+					Legg (1930)
	+	+						+		Hitchcock (1955b)
		+								Gothe (1967b)
					+					Sutherst (1971)
Dermacentor variabilis (Say)	+	+								Smith et al (1946)
						-				Sonnenshine (1967)
	+						+			Sonenshine and Tigner (1969)
Ixodes hexagonus Leach	+	+					+		+	Arthur (1951)
Ixodes ricinus L.	+	+	+	-			+		+	Macleod (1935)
Margaropus winthemi Karsch		+								Gothe (1967b)

Abbreviations: + = positive correlation; - = negative correlation; H = humidity; T = temperature; OH = humidity conditions during oviposition period; OT = temperature conditions during oviposition period; I = immersion in water; DM = delay in mating; C = clustering of eggs; TL = time of laying; D = disturbance.

5.2 PRELIMINARY EXPERIMENTS

Preliminary experiments were chiefly undertaken to provide information for the design of experiments to ascertain the effects of humidity and temperature on the incubation period and viability of B.decoloratus eggs.

5.21 The viability of eggs produced on different days of the oviposition period

B.microplus eggs laid towards the end of the oviposition period yielded a lower percentage hatch than those produced during the first few days (Hitchcock, 1955b). Accordingly it was considered expedient to determine the viability of eggs laid on successive days throughout the egg laying period of B.decoloratus. Eggs were collected daily from ten fully engorged, and naturally detached, females and ten semi-engorged females removed by hand a few hours prior to natural drop-off. These eggs were placed in separate glass tubes, sealed with fine nylon mesh, which were in turn placed in a container in which a relative humidity of 95% R.H. was maintained. All eggs were kept at 26°C in an incubator until hatching ceased; the numbers of hatched larvae and unhatched eggs being then determined.

Of the eggs laid by ticks dropping naturally from the host those produced during the first thirteen days of the oviposition period gave a percentage hatch of approximately 95% (Fig. 39), after which there was a rapid decrease to 10% hatch over the succeeding six days. Eggs produced by females removed prior to natural drop-off (Fig. 39) responded similarly to those produced by females which had fallen naturally. The percentage hatch over the first thirteen days was less constant and lower than eggs produced by naturally fallen females and the decrease after this period was less marked and more complete. These results support the findings of Hitchcock (1955b) working on B.microplus; accordingly only eggs collected from a single day (2nd, 3rd or 4th day of oviposition) were used in subsequent experimental work.

Reasons for the decrease in viability of eggs produced during the latter part of the oviposition period have not been established, but

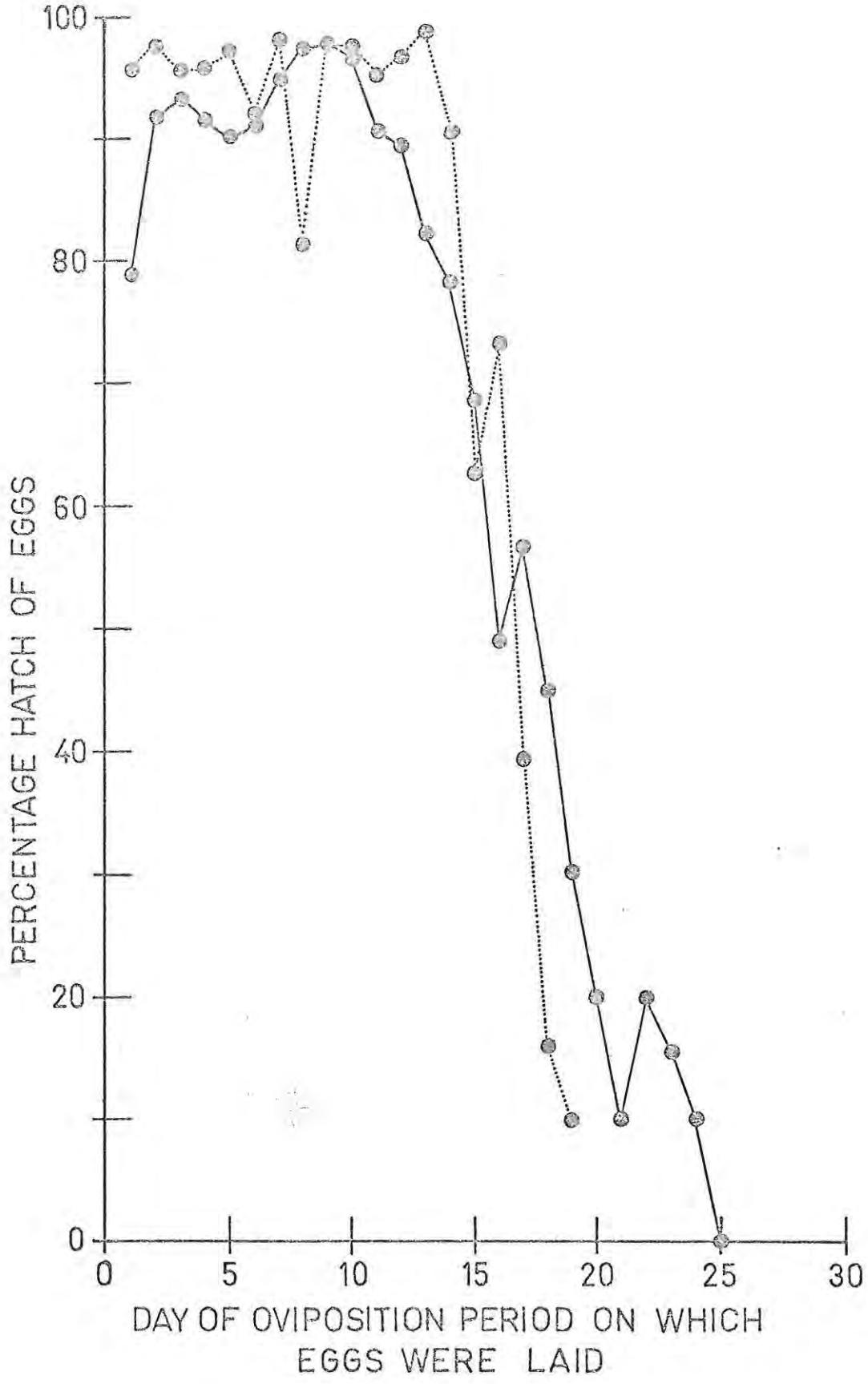

Fig. 39. The percentage hatch of Boophilus decoloratus eggs in relation to the particular day of the oviposition period on which they were laid. ⊙·····⊙ = eggs laid by naturally fallen females, ⊙——⊙ = eggs laid by females hand picked off the host.

may be contributed to by the following possibilities.

i. A decrease in the supply of spermatozoa for fertilization of eggs.

ii. A decrease in the ability of the female tick to supply each egg with spermatozoa.

iii. A decrease in the quality of quantity of nutrients supplied to the eggs during their formation in the ovaries and oviducts.

iv. A decrease in the efficiency of waterproofing eggs by Gene's organ.

The majority of eggs which did not hatch showed no signs of embryological development and in surface characters were similar to those which did hatch and were not desiccated at the end of the experimental period. Thus it is likely that the causal factor here may be an inadequacy or breakdown of the fertilization mechanism.

5.22 Critical temperature of cuticular 'wax' layer

The only work which has been seen on the critical temperature of tick eggs was that of Lees and Beament (1948). They demonstrated that at a certain temperature level, the so called critical temperature, the wax molecules of the protective covering of the cuticle are reorientated with consequent loss of water from the tick. This temperature may vary from one species to another but for any one species is constant. They report that for I.ricinus this critical temperature is 35°C, for Ixodes canisuga 42,5°C, Dermacentor andersoni 43°C, Hyalomma savignyi 44°C, O.moubata 45°C and Ornithodorus delanoei acinus 46°C.

The critical temperature of B.decoloratus was determined as follows. Batches of B.decoloratus eggs, collected on the third day of egg laying, were placed in small glass vials sealed at both ends with nylon mesh secured with nylon thread: nylon was used as it did not absorb water-vapour (Londt and Whitehead 1972). Separate vials of eggs were placed in individual test tubes, exposed to waterbath temperatures of 35°, 40°, 45°, 50°, and 55°C for one hour in each instance. Each vial containing

eggs was weighed before and after exposure to ascertain the amount of weight lost by the eggs, and this was repeated four times at each temperature level. After primary evaluation of these results further intermediate values of 40°, 42°, 43° and 44°C were investigated. The results are depicted in Fig. 40 and show a critical temperature of 42°C for B.decoloratus eggs.

The biological significance of the critical temperature is questionable as temperatures of the order of 42°C probably never occur in the microhabitat occupied by B.decoloratus eggs. Londt and Whitehead (1972) have measured microclimatic temperatures and humidities in a range of microhabitats and have shown that in some habitats, namely situations where vegetation cover is limited and protection afforded by neighbouring trees and bushes was minimal, microhabitats could have midday temperatures of over 42°C. No larvae of B.decoloratus[1] were collected from such areas and it is likely that eggs in the event of being deposited in such situations would not survive.

5.23 Critical humidity affecting egg survival

The term 'critical humidity' as used in the present context is defined as that humidity, at a particular temperature, which will allow a hatch of fifty percent of the eggs.

For the purpose of examining this parameter, batches of B.decoloratus eggs in small glass vials sealed with nylon mesh were placed in six different relative humidity levels (50%, 60%, 70%, 80%, 90% and 100% R.H.) at 26°C. The relative humidity values were obtained with the appropriate potassium hydroxide solutions (Peterson 1953). The vials (4 in each of the six humidities) were weighed initially and subsequently at five day intervals until hatching occurred. The percentage hatch was

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1. No larvae of any of the species collected at Barville Park were found in these situations (Londt and Whitehead, 1972).

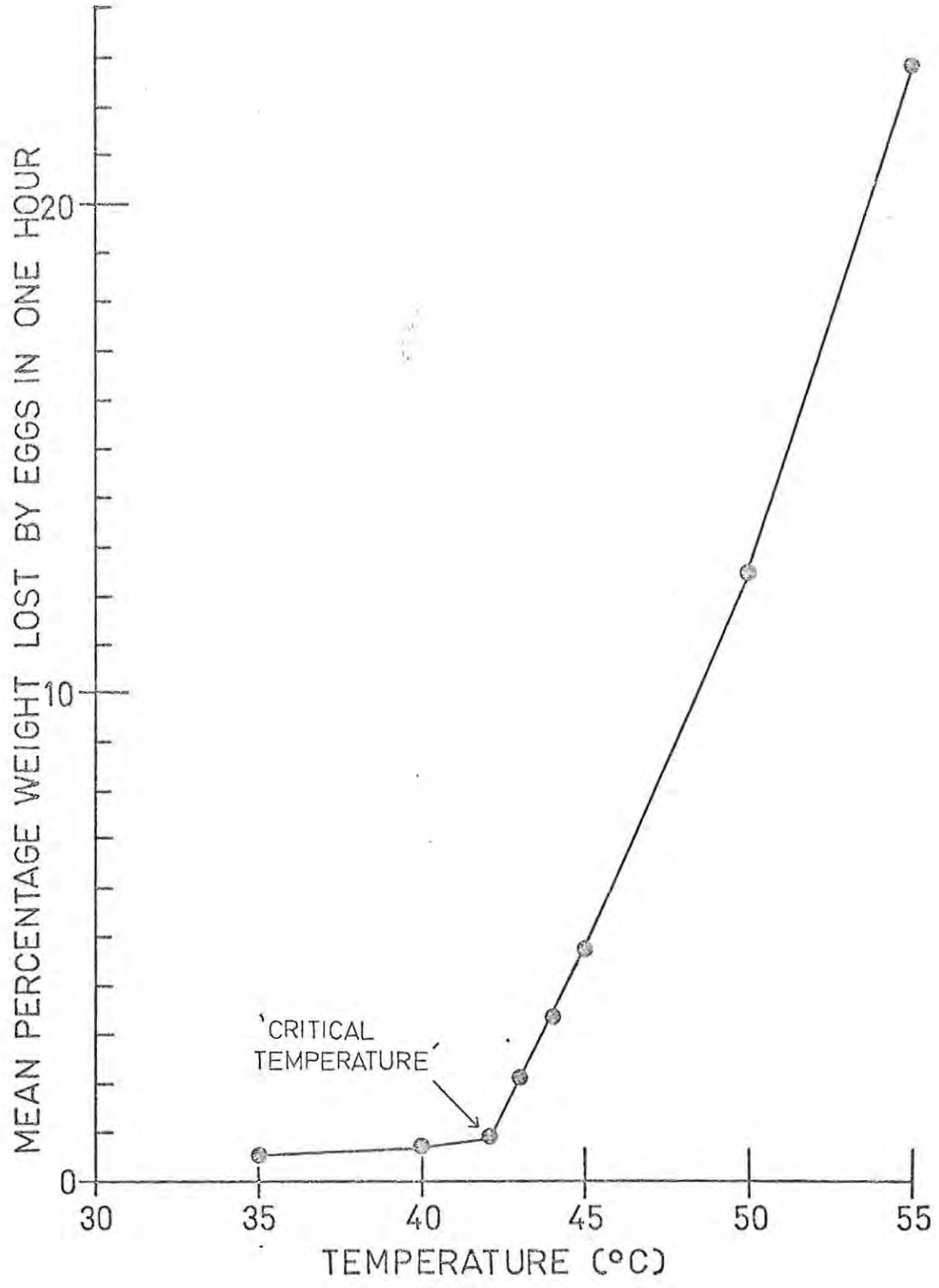


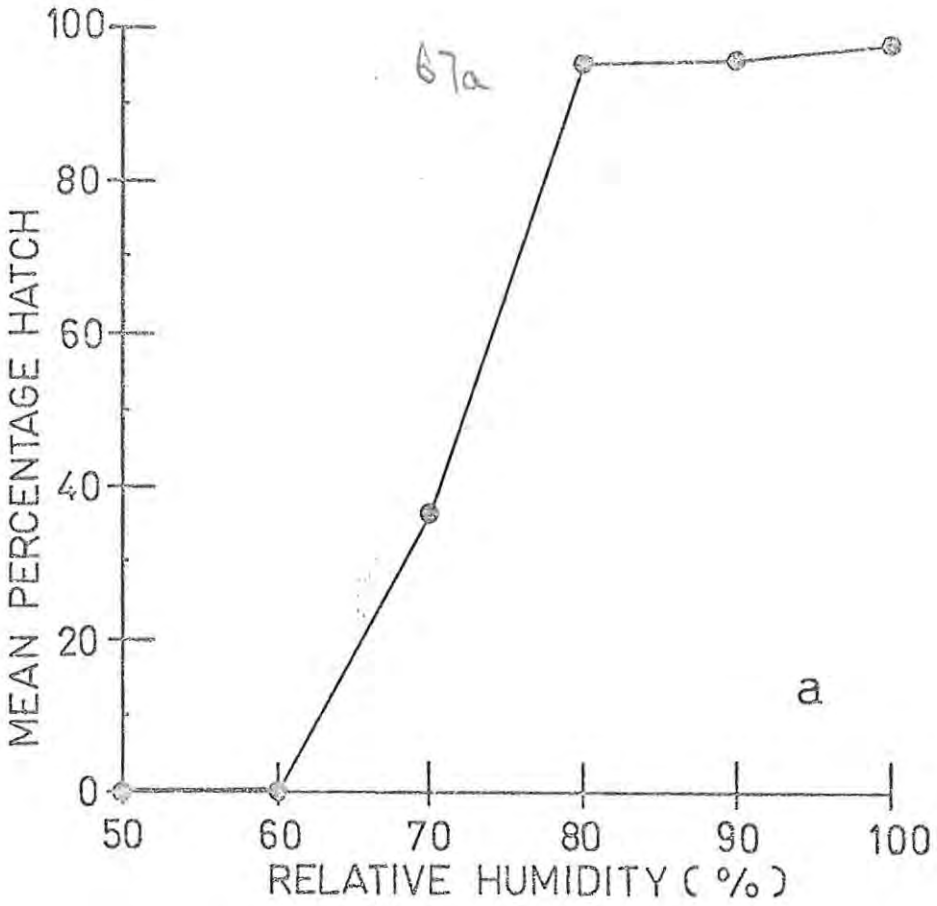
Fig. 40. The mean percentage weight lost by Boophilus decoloratus eggs when exposed to constant temperature values between 35°C and 55°C for one hour. Critical temperature of 42°C is indicated.

determined two weeks after hatching was observed so that an adequate time was allowed for completion of hatching. The critical humidity at 26°C and at 10% R.H. intervals (Fig. 41a) was found to be approximately 70% R.H. (i.e. 7,53 mmHg - saturation deficit). In order to obtain a more quantitative estimate of this value an experiment was conducted in which 5% R.H. intervals in the region of 70% R.H. were used. The values selected were 60%, 65%, 70%, 75% and 80% respectively. The results of this experiment (Fig. 41b) were slightly different from those obtained earlier (Fig. 41a) in that the relative humidity value resulting in a fifty percent hatch of eggs was approximately 69% R.H. whereas it was approximately 72% R.H. when 10% R.H. intervals were used. This difference is due to experimental variation and although an exact value of the critical humidity can not be given this value probably lies between 69% and 72% R.H. (i.e. approximately 70% R.H. or 7,53 mmHg saturation deficit at 26°C).

The changes in weight of egg batches held at 10% R.H. intervals at constant temperature (26°C) are shown in Fig. 42, and suggest that B.decoloratus eggs are able to lose up to approximately 35% of their initial weight before showing decreased viability. This loss in weight is probably due to loss of water through evaporation and therefore the actual percentage loss of water is likely to be in excess of 35%. It must be stressed that the critical humidity alters with temperature as temperature effects the rate at which eggs develop (see section 5.3). The critical humidity can be expressed either in terms of saturation deficit or relative humidity but in each instance the temperature at which the work is done must be stated.

5.24 Water loss and its effect on larval development

During the investigation into the critical humidity of B.decoloratus, eggs held at relative humidities of 70%, and below, showed the rectal



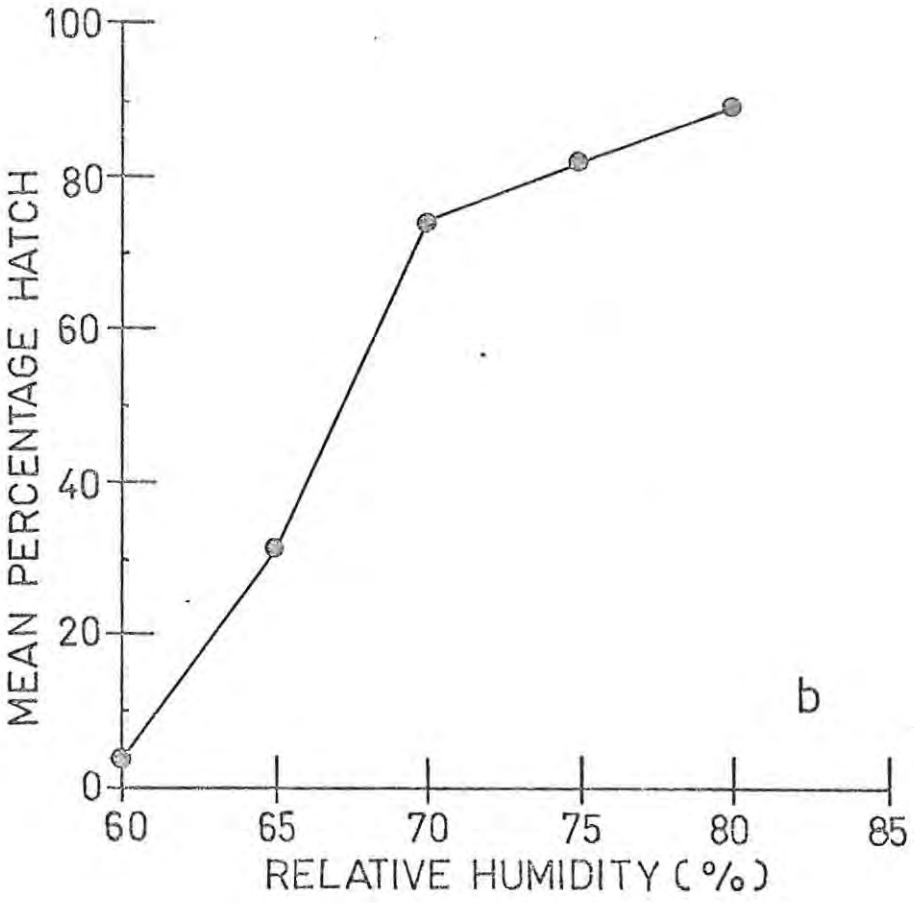


Fig. 41. (a-b). The percentage hatch of Boophilus decoloratus eggs held at constant temperature (26°C) and various constant relative humidity levels. (a). R.H. intervals of 10%, (b) R.H. intervals of 5%.

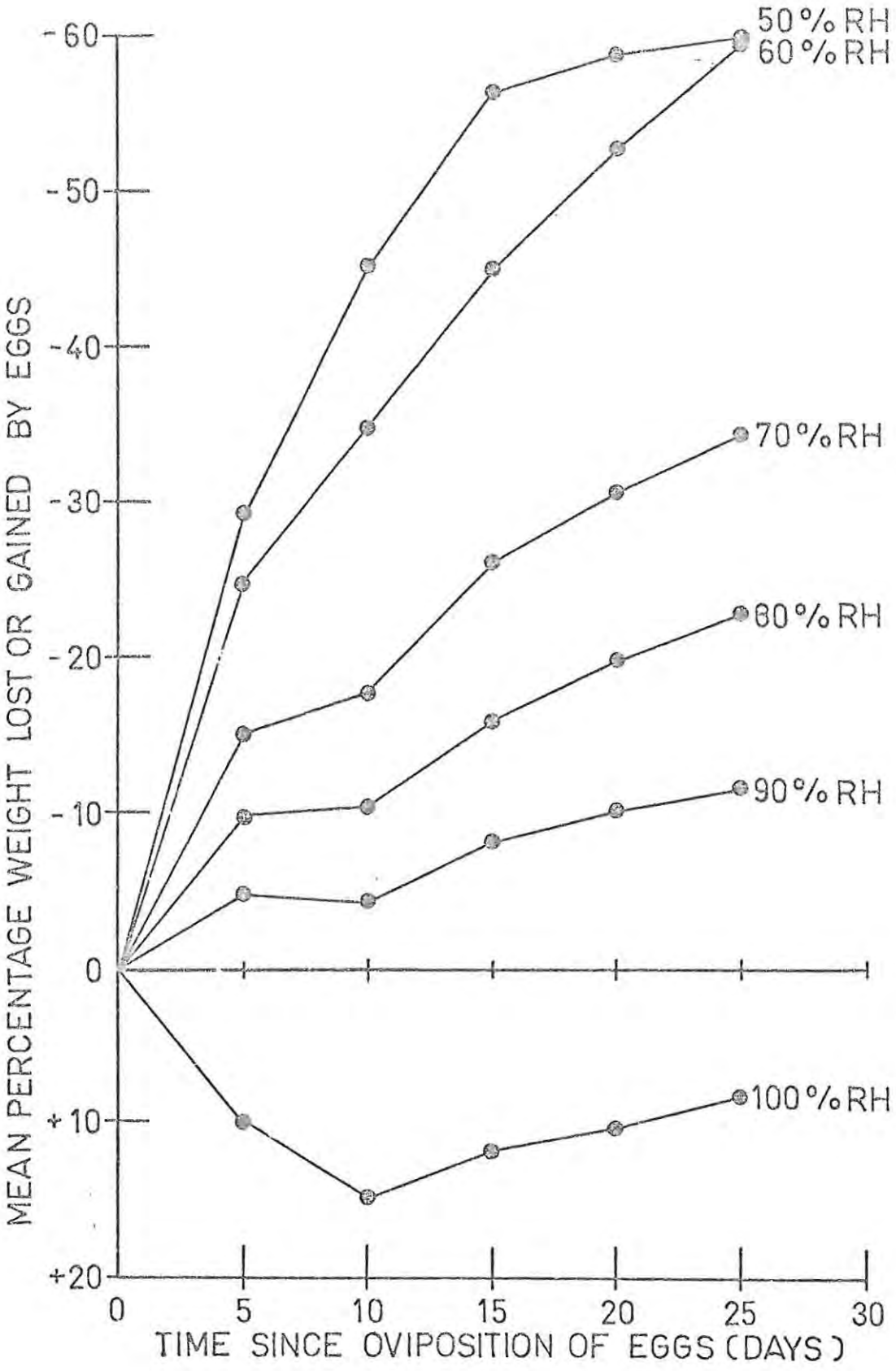


Fig. 42. The mean percentage weight lost or gained by Boophilus decoloratus eggs held at various constant relative humidities (as indicated) and constant temperature (26°C) throughout their incubation periods.

sac filled with excretory matter and, in some instances, the fully developed larvae were visible through the egg shells. These eggs frequently failed to hatch. A day-by-day monitoring of the development of larvae was needed for comparing changes in eggs placed in various humidity conditions. As the nitrogenous end product of metabolism is slowly built-up in the rectal sac of the developing larva, it was thought that a method of evaluating its accumulation might provide an assessment of egg development under different environmental conditions.

Guanine, a purine base, is the principal nitrogenous component of the excreta of certain spiders, scorpions, amblypygids, uropygids and solifugids (Schmidt et al, 1955; Rao and Gopalakrishnareddy, 1962; Horne, 1965). A chilopod, however, possessed non-detectable amounts of guanine (Horne, 1965). Arthur (1962, pers.commun.) and Balashov (1967) have reported that Guanine is also the chief component of tick excrement.

To ascertain whether this is so in B.decoloratus, a sample of pure guanine, a sample of deposited larval excretory matter and a sample of egg[1] homogenate were studied using paper chromatography. In each instance guanine, larval excreta and eggs were homogenized in a 0,1 N solution of H_2SO_4 (Visher and Chargaff (1948) showed that guanine is only slightly soluble at neutrality). Using a solvent system of 95% ethanol: 0,4 N NaOH (3:1), the RF values for the two possible sources of guanine (larval excreta and egg homogenate) and of the pure guanine standard were determined (Table 9). Paper chromatographs studied under UV light show guanine as a black spot (Oser, 1965). All three RF values were similar which suggests that larval excreta and egg homogenate contain guanine. Centrifuged solutions of pure guanine, larval excreta and egg homogenate were also studied spectrophotometrically, using a Beckmans spectrophotometer, between the wave-lengths of 220-320 nanometres

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1. Eggs which had almost completed the incubation period were used as these would possess the greatest amounts of stored excretory product.

(i.e. in the UV range). The curves, recorded on a 10 inch (25,4 cms) pen recorder coupled with the spectrophotometer are shown in Fig. 43. There is a general similarity in the shape of the curves between 246 µm and 320 µm, this being highly suggestive of compatibility between samples as regards their purine content. The possibilities of greater precision on a comparative basis for estimating guanine in developing eggs followed.

TABLE 9.

RF values obtained chromatographically for pure guanine, larval excreta and egg homogenate using an ethanol:sodium hydroxide solvent system.

	RF value		
Replicate no.	Pure guanine	Larval excreta	Egg homogenate
1	0,46	0,49	0,44
2	0,49	0,46	0,44
3	0,48	0,46	0,43
4	0,46	0,47	0,43
5	0,48	0,46	0,43
6	0,47	0,48	0,44
Means:	0,47	0,47	0,44

Visher and Chargaff (1948) developed a technique whereby minute amounts of purines and pyrimidines could be separated and quantitatively examined; this incorporated the use of chromatography and ultra-violet spectrophotometry. As the technique described by Visher and Chargaff (1948) proved to be time consuming in practical terms, no attempt was made to extract the guanine from tick eggs prior to examination spectrophotometrically. As guanine has a very specific absorption wavelength the possibility of detecting guanine proved feasible, though its limitations in terms of critical quantitative work is recognised.

Large numbers of freshly laid B.decoloratus eggs were placed in six chambers containing atmospheres of 50%, 60%, 70%, 80%, 90% and 100% R.H. respectively, and all six chambers placed in an incubator at

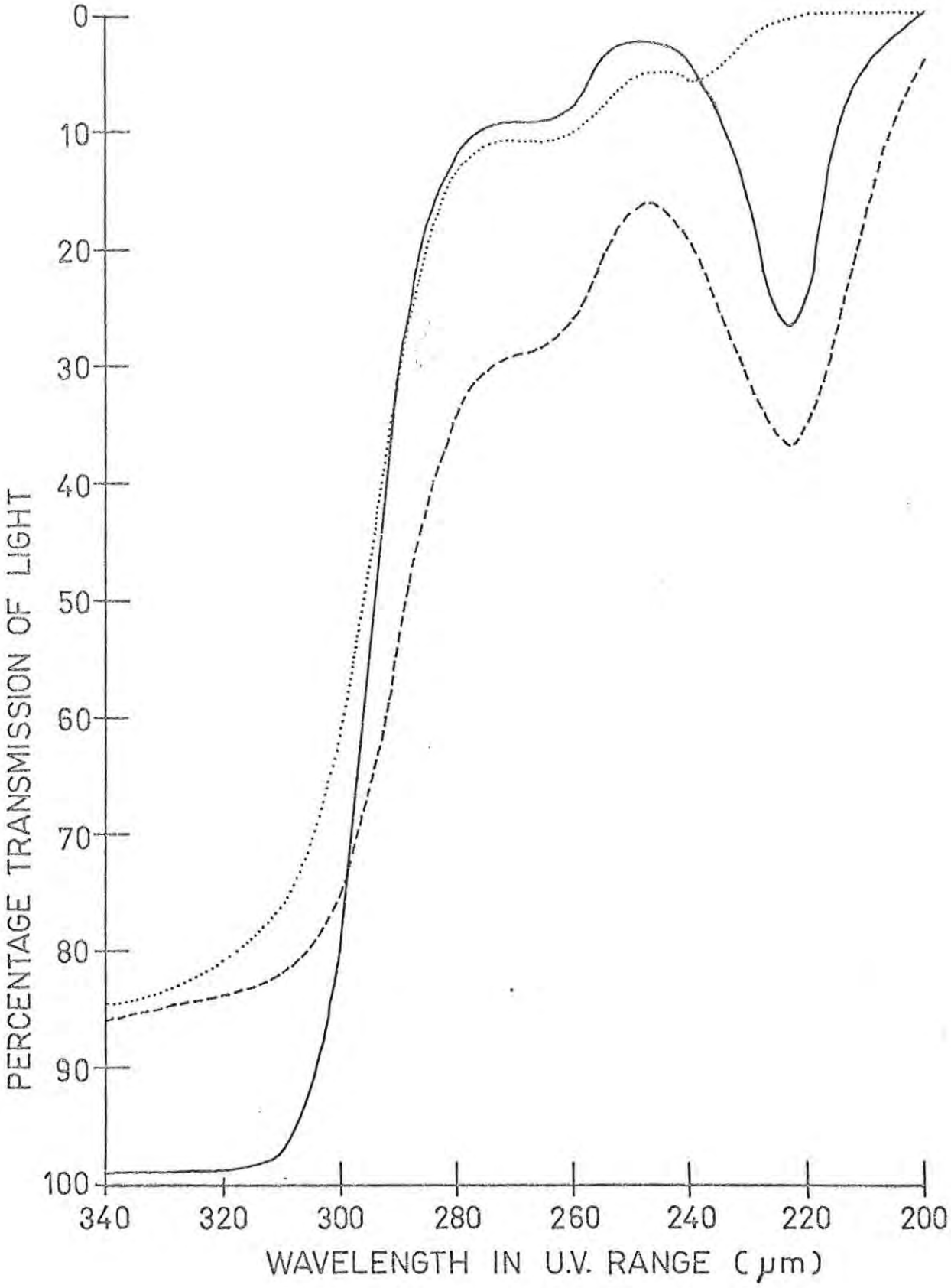


Fig. 43. The form of spectrophotometric transmission curves obtained for samples of pure guanine (——), larval excrement (----) and Boophilus decoloratus egg homogenate (.....). The concentrations of guanine in each instance was unknown.

26°C. Three batches, each of 100 eggs, were removed from each humidity chamber, at three day intervals, homogenized in 5 ml of 0,1 N H_2SO_4, centrifuged and examined spectrophotometrically against a 0,1 N H_2SO_4 reference solution. Curves of the percentage light transmission were recorded by means of the pen recorder and, to compare successive curves, the differences between the percentage transmission at 246 µm and 320 µm were calculated. A wavelength of 246 µm was selected as one of the reference points as this appeared to be at the absorption maximum for guanine. Visher and Chargaff (1948) gave this value as 249 µm. The other reference point (320 µm) was selected for convenience. The transitional changes of the transmission curves as development of eggs proceeded towards hatching is shown in Figs 44, 45 and 46. In these figures only the curves of eggs held at 100% R.H., 70% R.H. and 50% R.H. are given as these adequately illustrate the nature of the changes observed in all six humidity conditions. Eggs held at 100% R.H. displayed a more or less continuous increase in the difference between percentage light transmission at 246 µm and 320 µm. Eggs held at 70% R.H. demonstrated a similar but less marked increase while eggs at 50% R.H. did not show any marked increase after approximately the sixth day of development. The relationships between the mean change in percentage transmission and development of eggs (in days) at the six different humidities (Fig. 47) shows that eggs developed at similar rates for the first six days of the incubation period irrespective of the humidity values in which they were placed. After the sixth day the rate of development remained almost constant for the eggs held at 90% and 100% R.H. while at lower humidity values the rates gradually decreased. At 50% R.H. the rate of development declined to almost zero after the sixth day and there was little further accumulation of guanine.

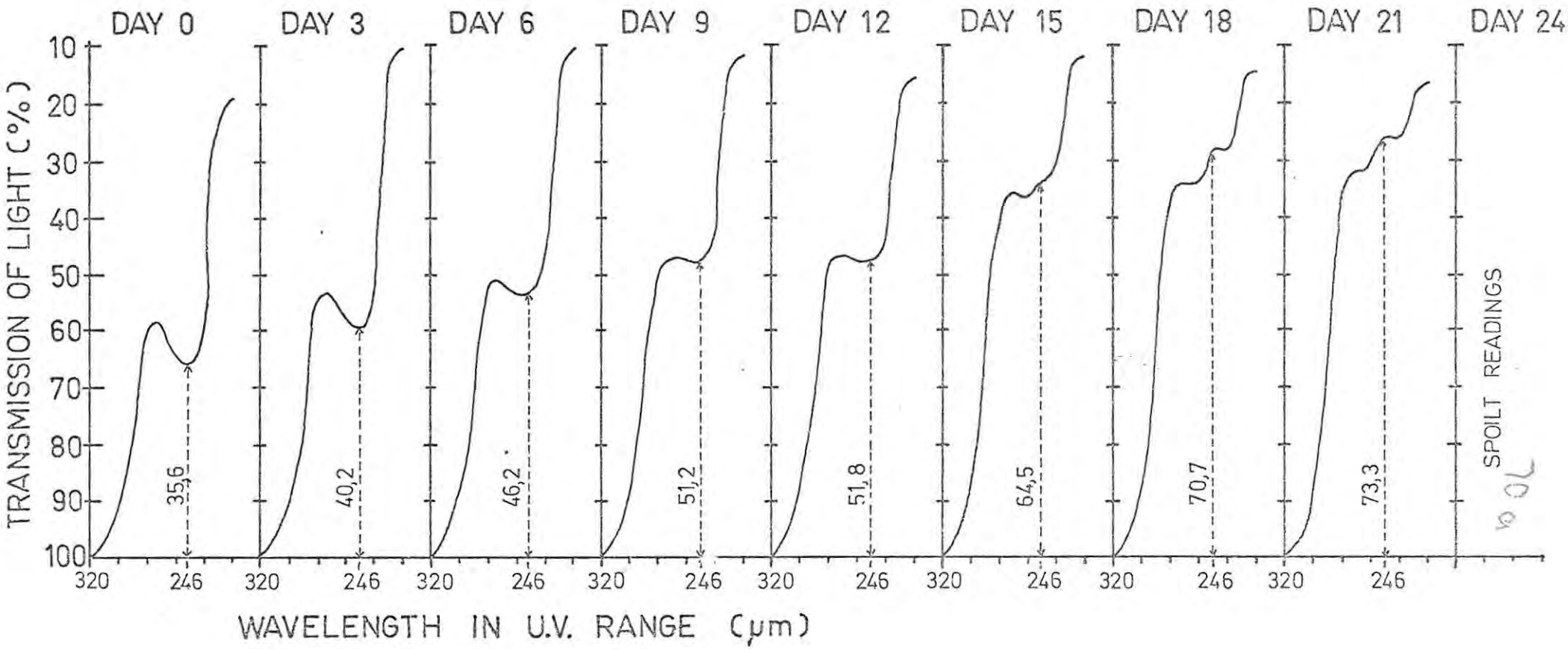


Fig. 44. The change in form of spectrophotometric transmission curves produced, at three day intervals, by egg homogenates during the incubation of Boophilus decoloratus eggs held at 26°C and 100% relative humidity. All the curves have been adjusted such that transmission at 320 µm equalled 100% for comparative purposes.

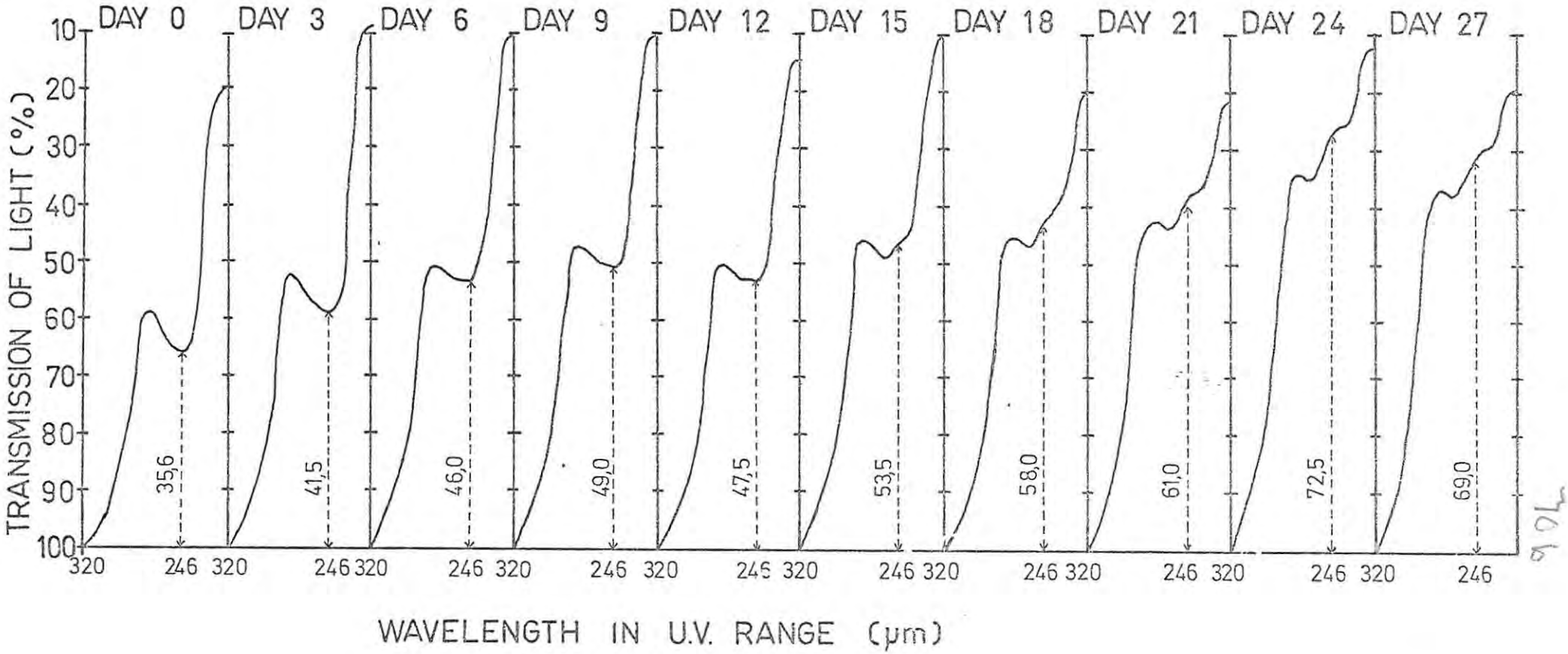


Fig. 45. The change in form of spectrophotometric transmission curves produced, at three day intervals, by egg homogenates during the incubation of Boophilus decoloratus eggs held at 26°C and 70% relative humidity. All the curves have been adjusted such that transmission at 320 µm equalled 100% for comparative purposes.

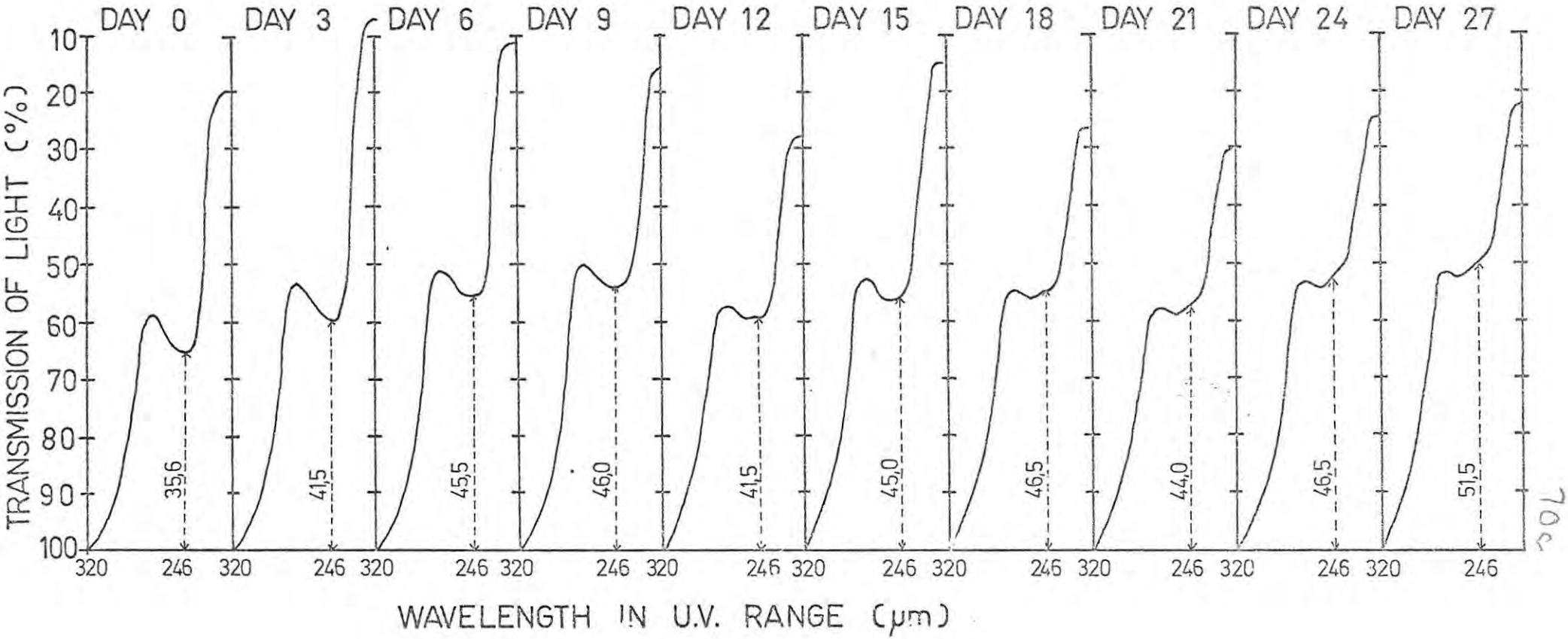


Fig. 46. The changes in form of spectrophotometric transmission curves produced, at three day intervals, by egg homogenates during the incubation of Boophilus decoloratus eggs held at 26°C and 50% relative humidity. All curves have been adjusted such that transmission at 320 μm equalled 100% for comparative purposes.

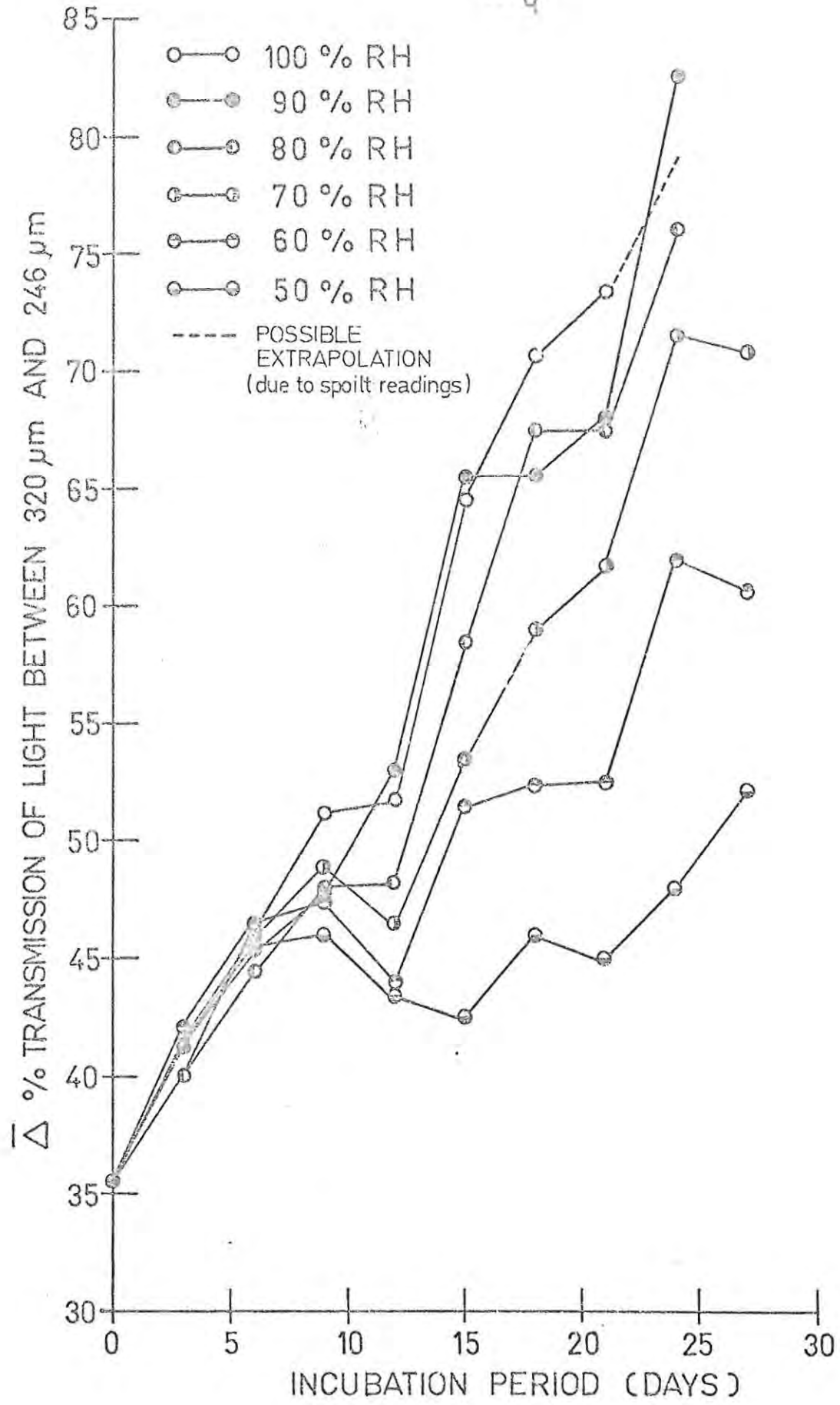


Fig. 47. The relationship between the mean difference ($\bar{\Delta}$) between the transmission of light at 320 µm and 246 µm against time for the eggs of Boophilus decoloratus held at various constant relative humidity levels (as indicated) and constant temperature (26°C) throughout their incubation periods.

Eggs held at 50% R.H. and 26°C (i.e. 12,51 mmHg saturation deficit) (Fig. 42) lost almost 35% of their initial weight by the sixth day; this is in excess of the critical amount which may be lost in order that a hatch of at least 50% may result. The implication is that development ceases once 35% of the initial weight of the eggs has been lost through evaporation of water. The important fact that emerges is that the rate of embryonic development is humidity independant but that eggs losing a critical quantity of water in the early stages of development fail to hatch. Eggs held at 70% R.H. and 26°C (i.e. 7,53 mmHg saturation deficit) give a hatch of approximately 50% as they will just have lost the critical 35% of their initial weight by the time development is complete.

5.25 Water uptake by eggs from a damp atmosphere

Since tick eggs lose weight in dry air at 26°C and only give 50% hatch at 70% R.H. (i.e. 7,53 mmHg saturation deficit) it is important to consider the possibility of eggs taking up water from damp atmospheres in the same way shown by Londt and Whitehead (1972) for larvae of a number of southern African tick species including B.decoloratus. If water cannot be taken up by the eggs then the water content of the eggs on being laid is very important to their survival. If water vapour can be actively taken up through the egg shell then clearly the supply of water originally built into the egg can be added to during the course of development provided favourable conditions for water uptake are available.

To assess the ability of water vapour uptake from damp atmospheres by B.decoloratus eggs, four batches of eggs (3 vials in each batch) were weighed and placed in four different humidity chambers held at 26°C and 95% (1,30 mmHg), 80% (5,04 mmHg), 60% (10,02 mmHg) and 40% (15,00 mmHg) respectively. After 24 hours under these conditions all

the tubes were again weighed and placed into the 95% R.H. chamber. The tubes were then weighed at 24 hour intervals up until 96 hours after the start of the experiment. The entire experiment was performed at 26°C. The results (Fig. 48) show that during the first 24 hour period eggs lost weight in accordance with the humidity conditions into which they had been placed. Eggs at 40% R.H. lost more weight than eggs at 60% R.H., those at 60% R.H. lost more than those at 80% R.H. The eggs held at 95% R.H. throughout actually demonstrated a small weight increase after the first 24 hour period. On being placed into the atmosphere of 95% R.H. eggs held at 40% and 60% R.H. increased slightly in weight; the weights of eggs held at 80% R.H. did not alter appreciably after being placed in the damper atmosphere. In subsequent weighings the eggs did not show any marked increase in weight as is known to occur in larvae similarly treated (Londt and Whitehead, 1972). The implication here is that eggs do not have the capacity to take up water vapour to any marked degree from the atmosphere. It would thus seem that the successful development of eggs depends on the water content of the eggs at the time of laying and that, in the event of a loss of 35% or more of the initial weight of the eggs, development is inhibited and at best will yield a 50% hatch. The unpublished work of Norval (1971 and pers.commun.) has shown that the habit of laying eggs in clusters is of survival value in that the outer layer of eggs is more prone to desiccation than the inner layers. This factor may also be important in the survival of eggs during adverse microclimatic conditions.

5.3 THE EFFECTS OF DIFFERENT CONSTANT HUMIDITY AND TEMPERATURE CONDITIONS ON EGG DEVELOPMENT AND HATCH

To assess the influence of different combinations of temperature

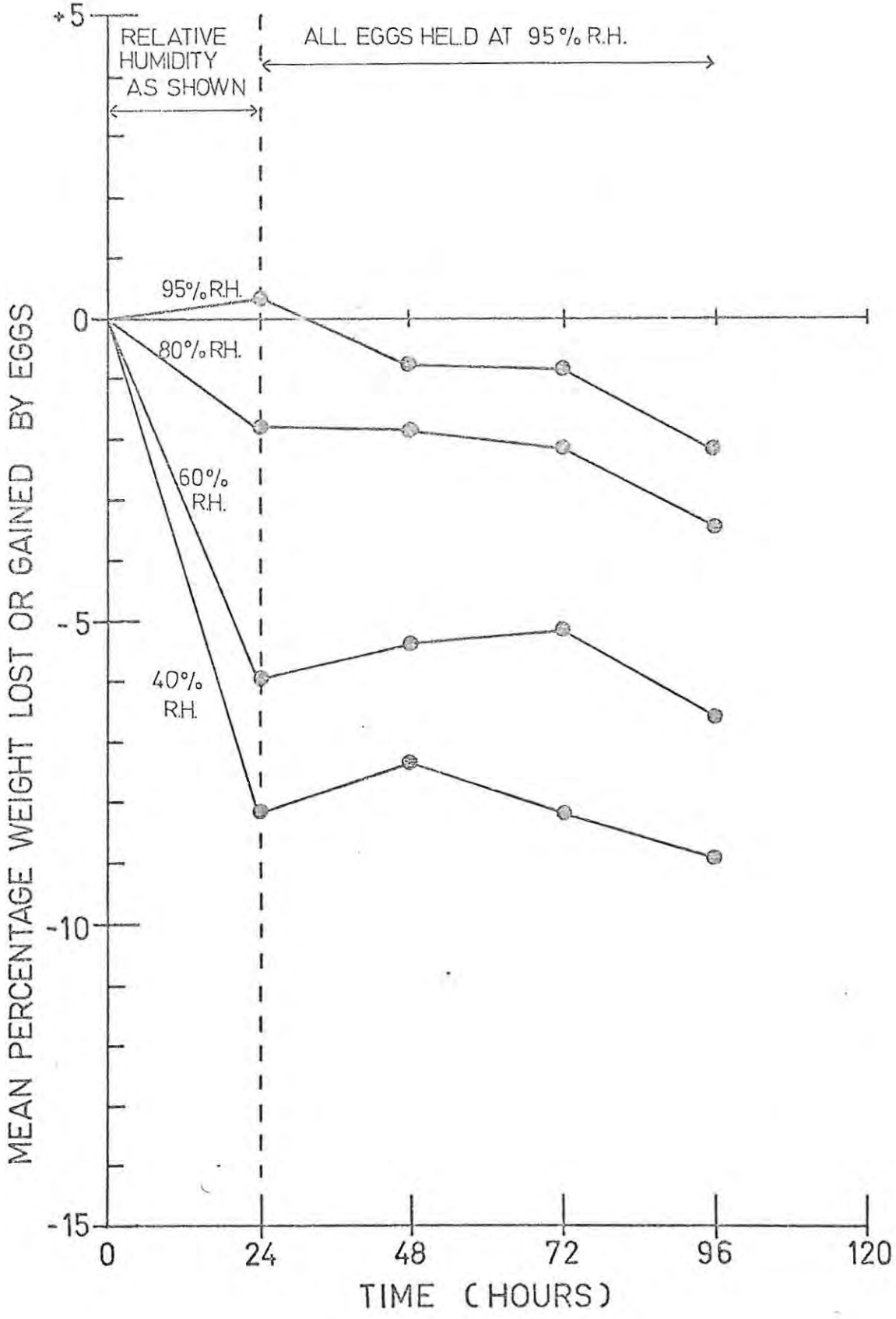


Fig. 48. The effects of placing Boophilus decoloratus eggs in a damp atmosphere (95% R.H.) after a 24 hour period of 'desiccation' in one of three different relative humidity levels (as indicated). The temperature was held at 26°C throughout.

and humidity on the incubation period and larval yield of B.decoloratus eggs, batches of eggs were placed in glass vials and exposed to 18 different combinations of temperature, relative humidity and saturation deficit (Table 2). Three separate batches of eggs were placed in each of the 18 different environmental situations. All vials were examined daily and the length of the incubation period recorded when the first larvae hatched. Individual vials were removed two weeks after the hatch of the first larvae in each batch was observed and the percentage hatch established.

The relationship between temperature, saturation deficit and the duration of the incubation period of batches of B.decoloratus eggs is given in Fig. 49a while Fig. 49b is a contour diagram showing the interactions of temperature, saturation deficit and egg viability of the same batches of eggs. As eggs placed in 13 of the 18 different combinations of environmental conditions failed to hatch the contour diagrams (Figs 49a and b) give only a generalized visual impression of the relationships involved. Both figures show that hatch took place at three temperatures only, 20°C, 26°C and 32°C respectively. At 20°C only the eggs held at 90% R.H. (i.e. 1,73 mmHg saturation deficit) hatched while at 26°C and 32°C eggs hatched at both 70% and 90% R.H. In no instance did eggs hatch when held at 50% R.H. The duration of the incubation period is temperature dependent (Fig. 50) within the tolerance limits set by both temperature and saturation deficit.

From the information contained in Fig. 51 it could be suggested that saturation deficit has a direct effect on the duration of the incubation period of B.decoloratus eggs but on close examination it becomes evident that the data points are grouped according to the temperatures at which the particular data were obtained. This

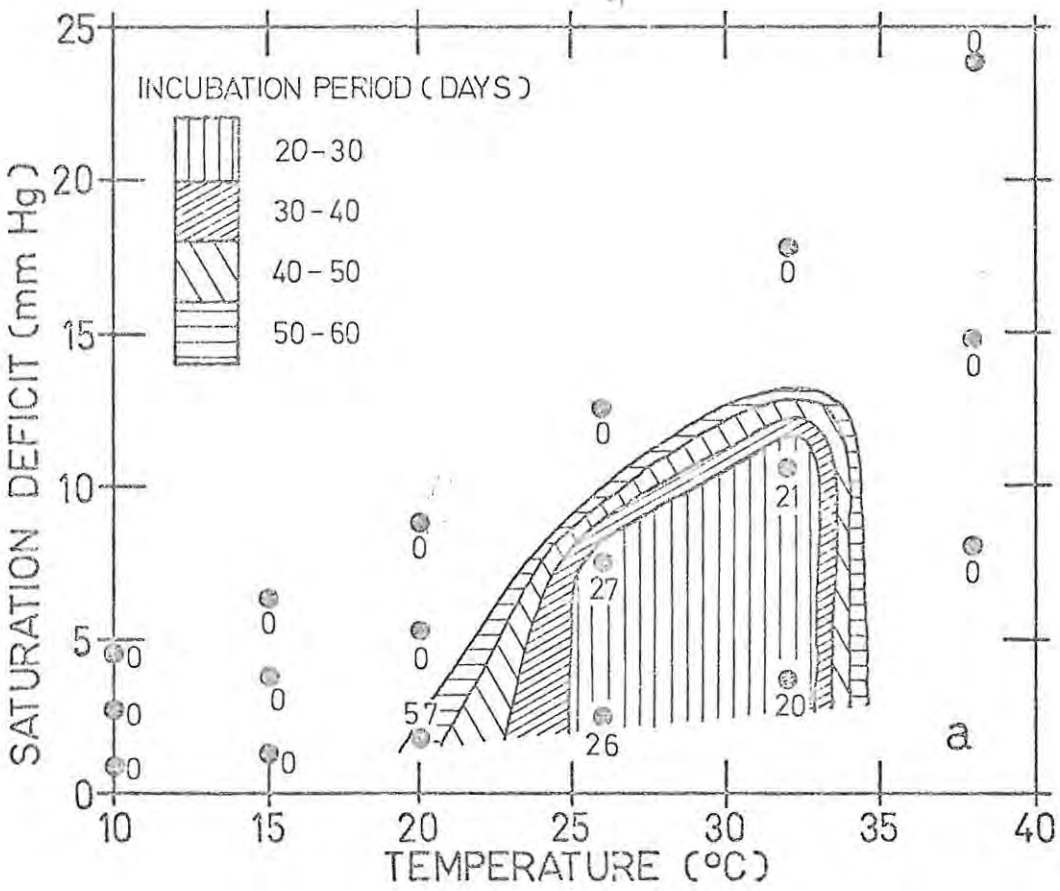
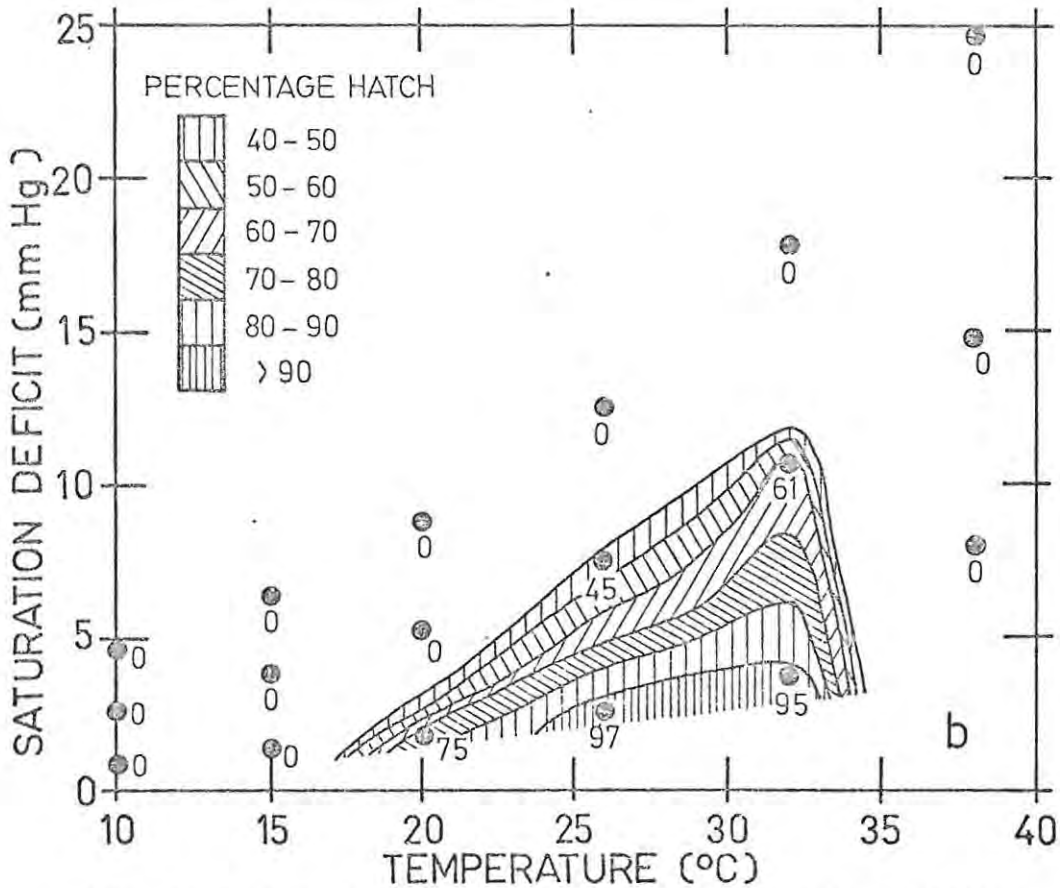


Fig. 49. (a-b). (a). A contour diagram showing the relationships existing between temperature, saturation deficit and the incubation period duration of Boophilus decoloratus. 0 = eggs did not hatch.



(b). A contour diagram showing the relationships existing between temperature, saturation deficit and percentage hatch of Boophilus decoloratus eggs. Actual data points from which the contours were constructed are indicated (●).

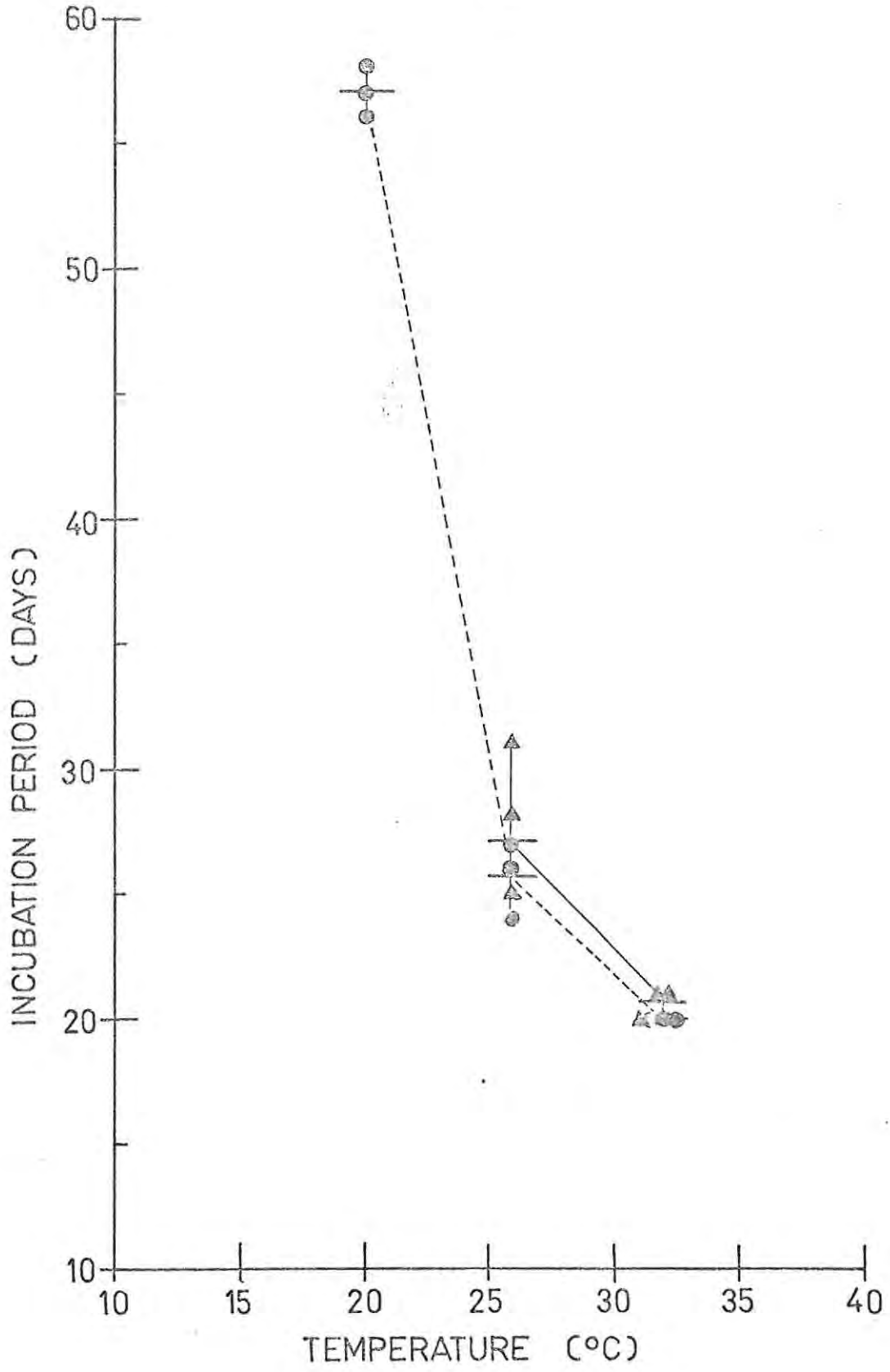


Fig. 50. The relationship between temperature and the duration of the incubation period of Boophilus decoloratus eggs, ignoring any effects of humidity. ● = data collected at 90% R.H., ▲ = data collected at 70% R.H. Eggs held at 50% R.H. failed to hatch. For convenience the means at each temperature level have been joined. (– – –) joins means of data collected at 90% R.H., (——) joins means of data collected at 70% R.H.

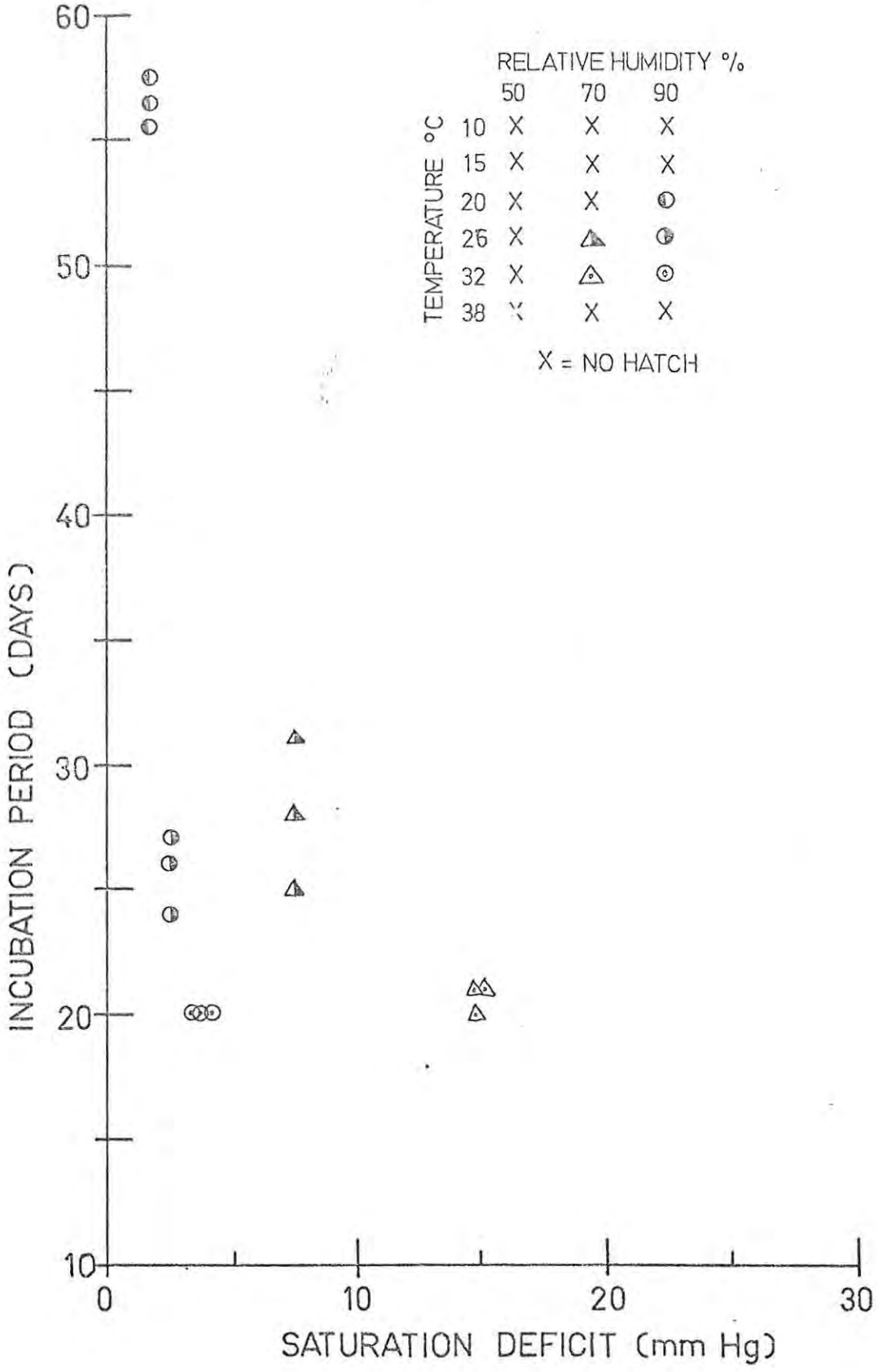


Fig. 51. The relationship between saturation deficit and the duration of the incubation period of Boophilus decoloratus eggs, ignoring any effects of temperature.

therefore suggests an overriding effect of temperature. If the data obtained at 32°C (Fig. 51) are examined it can be seen that, over a wide range of saturation deficit, virtually no difference in incubation period duration was registered. The same is true at 26°C. It is therefore evident that temperature is the most important parameter influencing the length of the incubation period and that saturation deficit does not have any direct effect.

While considering the effects of temperature and saturation deficit on the viability of eggs (Figs 52 and 53) it is evident that there is a definite saturation deficit dependence and an apparent temperature dependence. It was suspected, however, that as there was a greater 'degree of viability' with increase in temperature and saturation deficit between 20°C and 32°C, the relationship is a rather 'indirect' one. As temperature influences the duration of the incubation period eggs held at low temperatures will have longer periods during which to lose water through evaporation. Therefore at similar saturation deficit levels eggs which would give rise to larvae at 32°C may not necessarily be able to complete development at 20°C due to the difference in the lengths of the incubation periods at these two temperatures.. It is quite possible that the results shown in Figs 50-53 are reflecting, to some extent, a limiting factor such as the water content of the eggs.

5.4 THE EFFECTS OF ALTERNATING CONSTANT HUMIDITY AND TEMPERATURE CONDITIONS ON EGG DEVELOPMENT AND HATCH

The assessment of biological phenomena at constant environmental conditions is unrealistic in terms of explaining what happens under natural conditions apart from establishing possible tolerance levels. Accordingly experiments were designed to determine the effects of

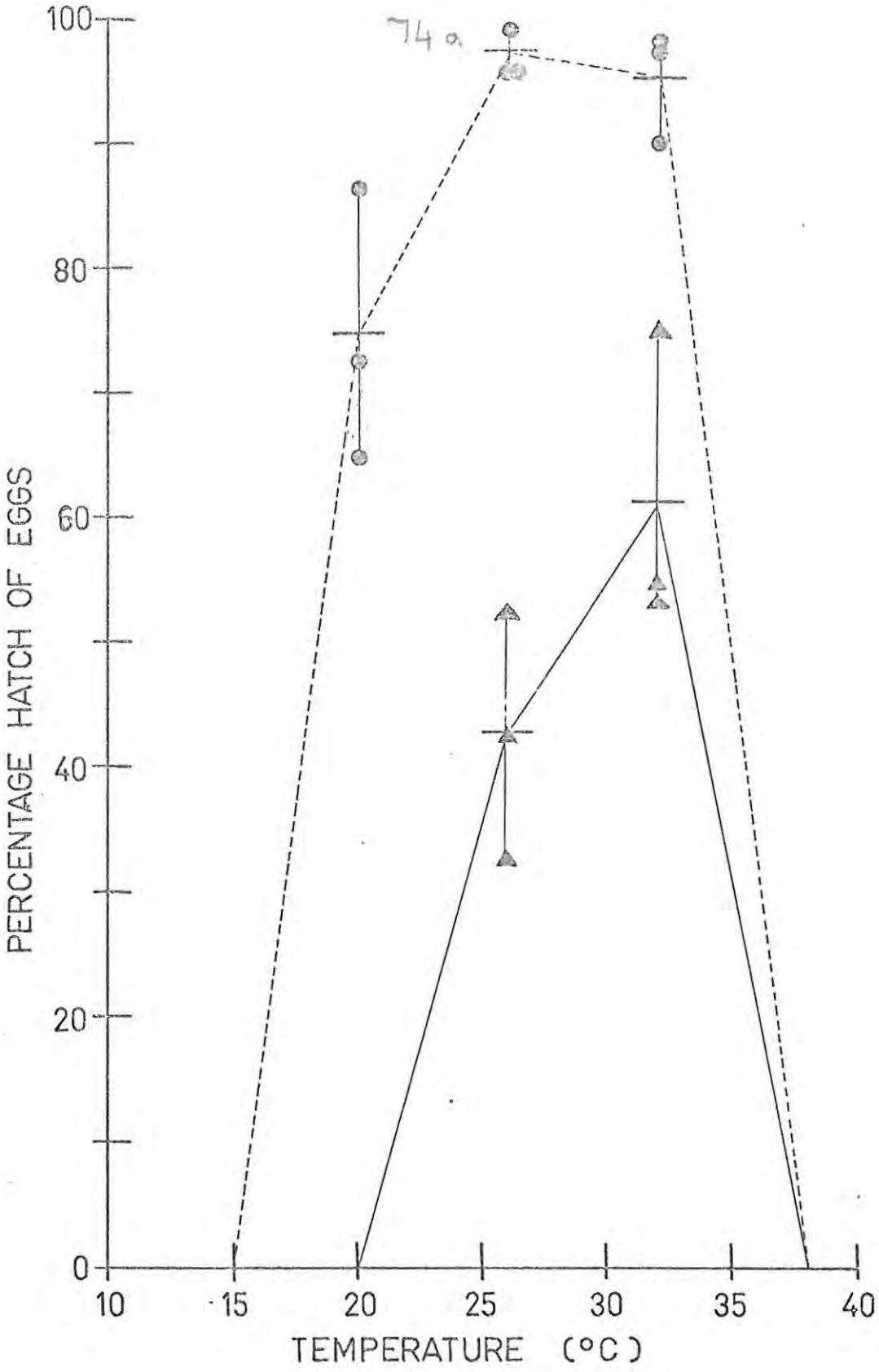


Fig. 52. The relationship between temperature and the percentage hatch of Boophilus decoloratus eggs. ● = data collected at 90% R.H., ▲ = data collected at 70% R.H. For convenience means have been joined.

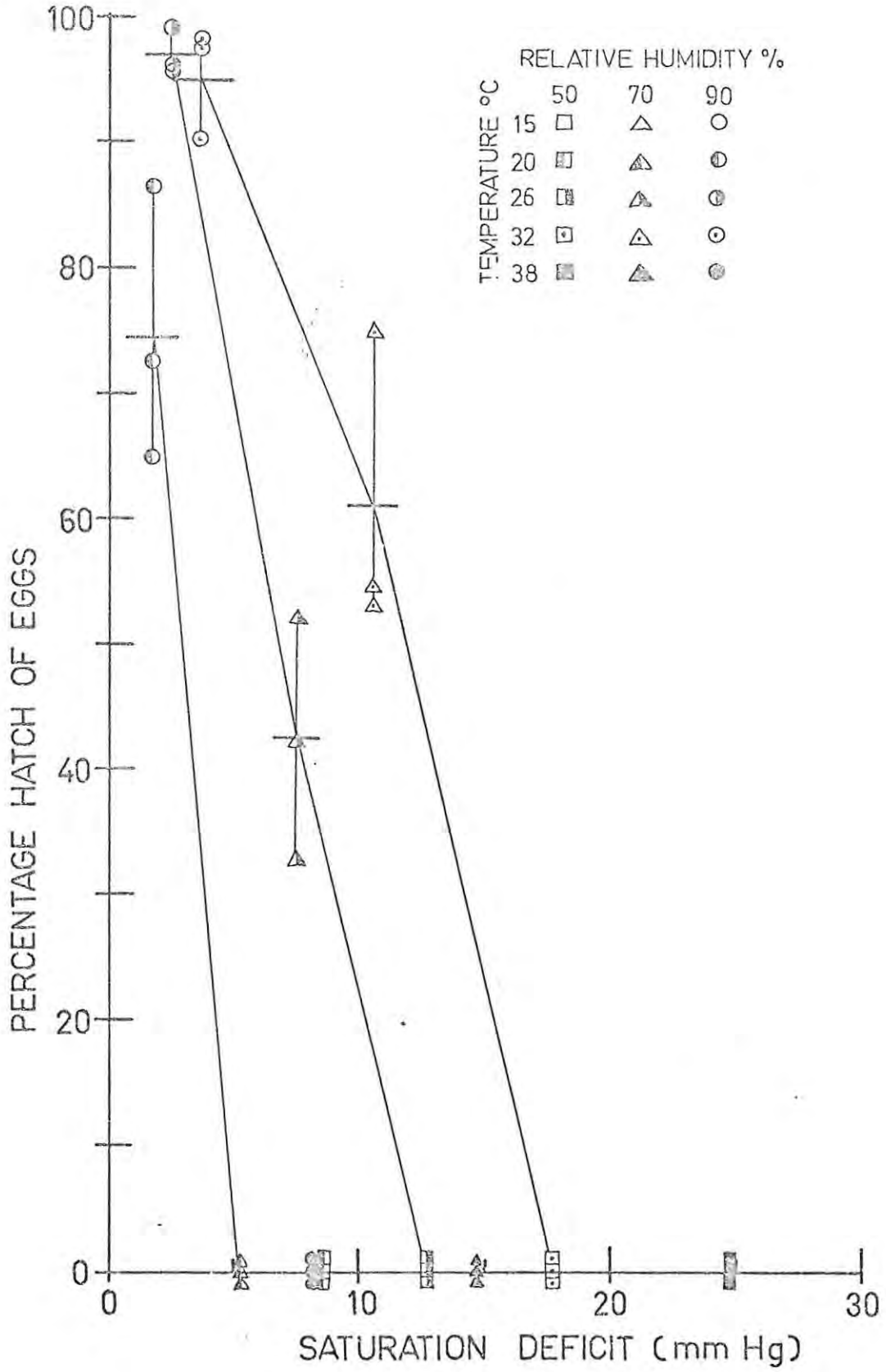


Fig. 53. The relationship between saturation deficit and the percentage hatch of Boophilus decoloratus eggs held at various different temperature levels (as indicated).

alternating conditions of temperature and saturation deficit on the duration of the incubation period and viability of B.decoloratus eggs. For these purposes a constant environment room was employed, wherein the conditions were adjusted so as to give twelve hours of light ('day') and twelve hours of darkness ('night'). The light was produced by eight, six foot long, neon tubes. The temperature conditions in the room were adjusted to give a higher temperature during the day than during the night. Three different temperature regimes were used during the investigation and, as there was only a single environmental room available, this necessitated the running of three separate tests in which eggs from different batches of females were used. At each of the three temperature regimes two different humidity values were used during hours of darkness while five different levels were used during 'day' periods in two of the three regimes and six in one. The various combinations of temperature, relative humidity and saturation deficit to which batches of eggs were exposed are presented in Table 10. Two batches of eggs were placed in each environmental combination in the first temperature regime while three batches were used in the second and third regimes.

To assess the effects of both temperature and saturation deficit on the duration of the incubation period and viability of B.decoloratus eggs under alternating environmental conditions temperature and saturation deficit are expressed in terms of hour degrees centigrade (hour degrees or $h^{o}C$) and hour millimetres of mercury (here referred to as Saturation deficit units or SDU's). The total number of hour degrees and SDU's daily experienced by egg batches held under the various temperature and saturation deficit combinations (Table 10) are shown in Table 11.

Hour degrees were calculated above a base-line of $15^{o}C$ as no

TABLE 10.

Combinations of temperature, relative humidity and saturation deficit used in the study of the effects of changing physical factors on the duration of the incubation period and viability of Boophilus decoloratus eggs.

Regime 1. Temperature (°C)			Regime 2. Temperature (°C)			Regime 3. Temperature (°C)		
D	N	T.h°C	D	N	T.h°C	D	N	T.h°C
26	15	160,6	30	20	238,4	30	15	195,3

% R.H.		Sat.def.		% R.H.		Sat.def.		% R.H.		Sat.def.	
D	N	D	N	D	N	D	N	D	N	D	N
90	90	2,55	1,26	90	90	3,15	1,73	90	90	3,15	1,26
80	90	5,04	1,26	80	90	6,30	1,73	80	90	6,30	1,26
70	90	7,53	1,26	70	90	9,45	1,73	70	90	9,45	1,26
60	90	10,00	1,26	60	90	12,60	1,73	60	90	12,60	1,26
50	90	12,51	1,26	50	90	15,75	1,73	50	90	15,75	1,26
80	70	5,04	3,80	80	70	6,30	5,21	40	90	18,91	1,26
70	70	7,53	3,80	70	70	9,45	5,21	90	70	3,15	3,80
60	70	10,00	3,80	60	70	12,60	5,21	80	70	6,30	3,80
50	70	12,51	3,80	50	70	15,75	5,21	70	70	9,45	3,80
—	—	———	———	—	—	———	———	60	70	12,60	3,80
—	—	———	———	—	—	———	———	50	70	15,75	3,80
—	—	———	———	—	—	———	———	40	70	18,91	3,80

Abbreviations: D = Day; N = Night; T.h°C = Total hour degrees centigrade every twenty-four hours; % R.H. = Percentage relative humidity; Sat.def. = Saturation deficit.

TABLE 11.

The total number of hour degrees and SDU's daily experienced by Boophilus decoloratus egg batches held under various temperature and saturation deficit combinations (Table 10).

Regime 1. Temperature (°C)			Regime 2. Temperature (°C)			Regime 3. Temperature (°C)		
D	N	T.h°C	D	N	T.h°C	D	N	T.h°C
26	15	160,6	30	20	238,4	30	15	195,3

Saturation deficit (mmHg)			Saturation deficit (mmHg)			Saturation deficit (mmHg)		
D	N	Total SDU's	D	N	Total SDU's	L	N	Total SDU's
2,55	1,26	47,1	3,15	1,73	58,9	3,15	1,26	56,6
5,04	1,26	77,1	6,30	1,73	95,0	6,30	1,26	95,9
7,53	1,26	107,8	9,45	1,73	131,4	9,45	1,26	136,7
10,00	1,26	138,0	12,60	1,73	168,9	12,60	1,26	175,7
12,51	1,26	170,6	15,75	1,73	206,5	15,75	1,26	217,3
5,04	3,80	109,2	6,30	5,21	139,9	18,91	1,26	256,9
7,53	3,80	140,0	9,45	5,21	177,5	3,15	3,80	87,8
10,00	3,80	169,1	12,60	5,21	215,4	6,30	3,80	127,4
12,51	3,80	200,2	15,75	5,21	253,1	9,45	3,80	167,9
———	———	———	———	———	———	12,60	3,80	207,5
———	———	———	———	———	———	15,75	3,80	247,7
———	———	———	———	———	———	18,91	3,80	288,3

Abbreviations: D = Day; N = Night; T.h°C = Total hour degrees centigrade above 15°C every 24 hours; Total SDU's = Total hour millimetres of mercury above zero every 24 hours.

hatch was reported at this or lower temperatures (see section 5.3), and no temperature lower than 15°C was used in any of the combinations employed in this experiment. SDU's were calculated above zero. The calculation of the total hour degrees and SDU's was done by graphically recording the hour by hour changes in temperature and saturation deficit for each environmental combination (Table 11); these were cut out with a pair of scissors and weighed together with square pieces of the same graph paper (each representing 10 hour degrees or 10 SDU's). This method proved to be rapid and reliable in gaining an assessment of the total number of hour degrees and SDU's experienced daily by tick eggs in each of the temperature and saturation deficit combinations.

The relationships between the total number of hour degrees and the total number of SDU's experienced within each 24 hour period, in relation to the duration of the incubation period of B.decoloratus eggs are shown in Fig. 54. The lines of equal incubation period duration lie parallel to the saturation deficit axis and therefore suggests that humidity has no effect on the incubation period. Temperature, on the other hand, has a very definite influence in that the duration of the incubation period is shortened with temperature increase.

An assessment of the effects of alternating temperatures and saturation deficits on the viability of eggs was attempted by drawing another contour diagram (Fig. 55) in which these three parameters are considered. Here the relationship between the total number of hour degrees and SDU's experienced by eggs throughout their incubation periods were used so that the direct effect of temperature on the shortening of the incubation period was considered. The data shown in Fig. 55 was used to produce a smoothed out contour

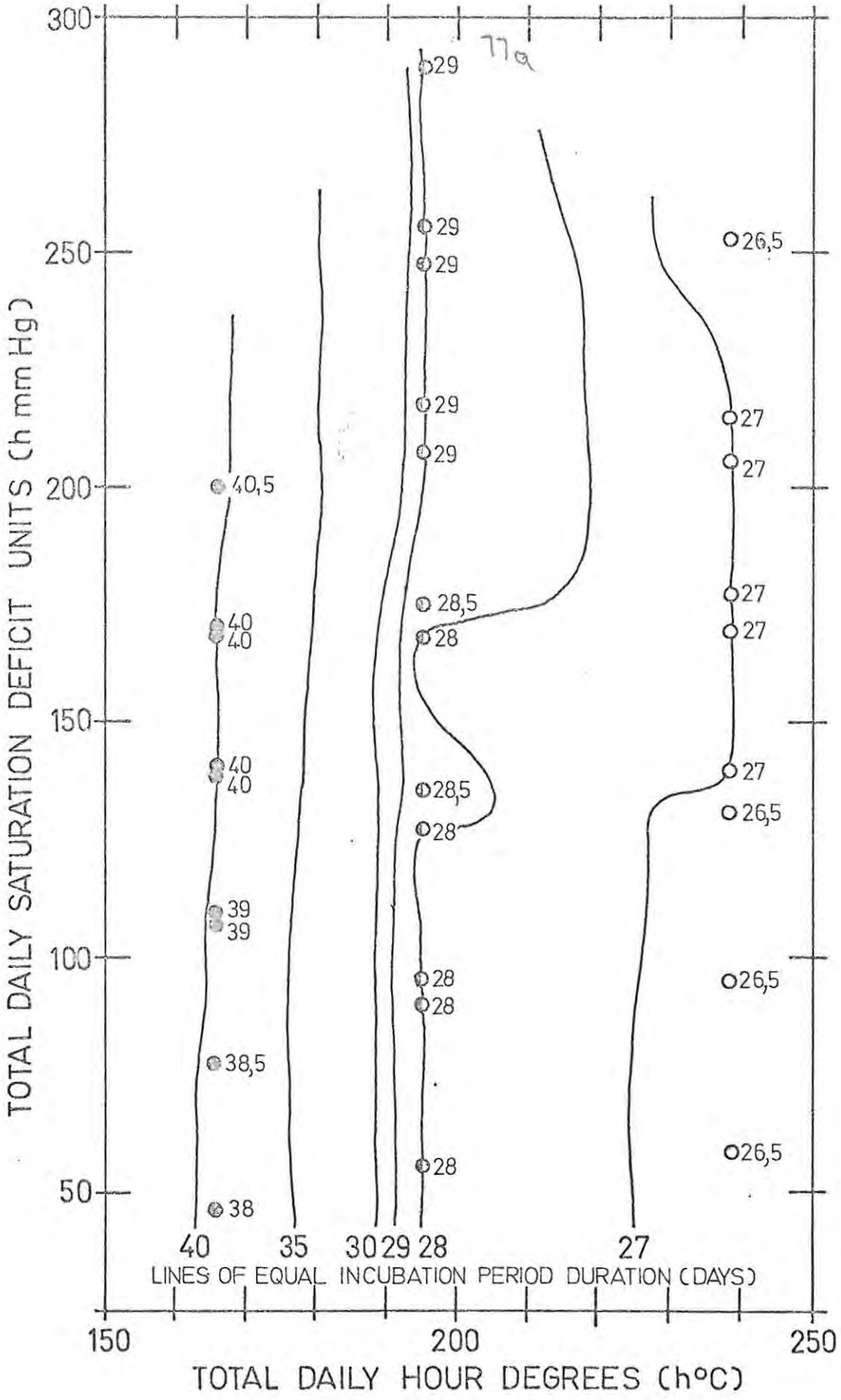


Fig. 54. A contour diagram showing the interactions of temperature (total hour degrees per 24 hours), humidity (total SDU's per 24 hours) and the duration of the incubation period of Boophilus decoloratus eggs held under various alternating temperature and humidity conditions (Table 10).

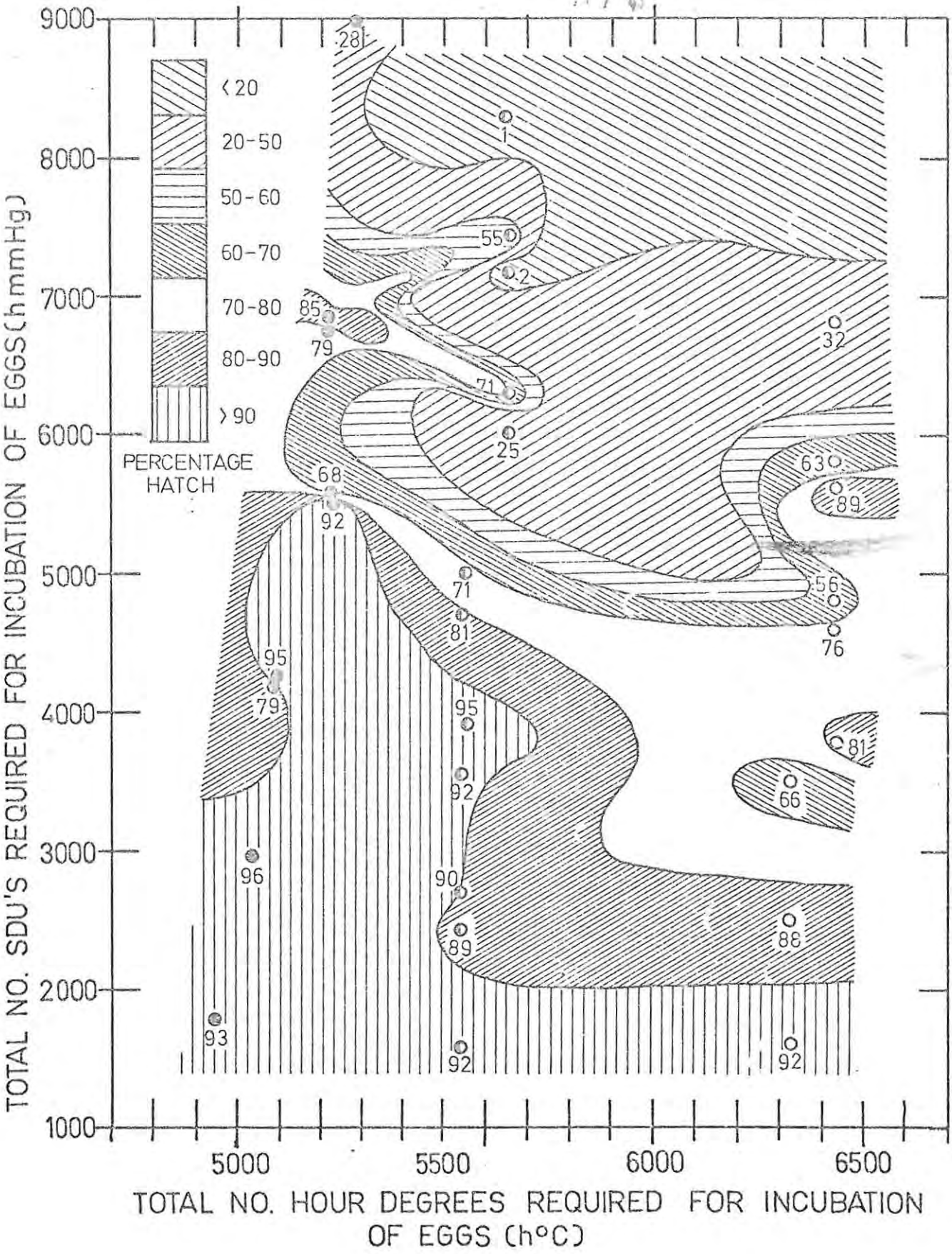


Fig. 55. Contour diagram of percentage hatch against total number of saturation deficit units and hour degrees experienced by incubating Boophilus decoloratus eggs. ◍ = data from first environmental regime, O = data from second environmental room regime, ◐ = data from third environmental room regime. Contour curves before being smoothed out.

diagram (Fig. 56) in the manner described by Shelford (1930). Eggs give a greater percentage hatch when the number of SDU's experienced by the developing eggs is low (Fig. 56): that is the viability of eggs is saturation deficit dependent. There is also an indication that percentage hatch of eggs is greatest when the number of hour degrees was in the region of about 5700 $h^{\circ}C$ (Fig. 56). Above and below this value the percentage hatch, or viability, decreased. The fairly rapid decrease at values lower than 5700 $h^{\circ}C$ is probably attributable to the longer incubation periods of eggs at low temperatures, with their consequent longer period in which water is lost by evaporation. The decrease in viability was therefore probably due to the water content of the eggs becoming a limiting factor. The gradual decrease in viability at hour degrees greater than 5700 $h^{\circ}C$ was probably due to the first signs of temperature becoming limiting. It is suggested that percentage hatch is independent of a direct temperature influence but that the direct temperature dependence of the period of incubation modifies the percentage hatch of eggs by indirectly affecting the amount of water lost from the eggs during their development. From Fig. 56 it can be postulated that the optimal conditions for egg viability are approximately 5700 $h^{\circ}C$ and as few SDU's as possible (i.e. high humidity) during the incubation period.

5.5 THE EFFECTS OF NATURALLY FLUCTUATING MACROCLIMATIC TEMPERATURES, RELATIVE HUMIDITIES AND SATURATION DEFICITS ON EGG DEVELOPMENT AND HATCH

B.decoloratus eggs placed in a Stephenson's screen on Upper Gletwyn farm, near Grahamstown, during the months of August and September 1972 did not hatch and became desiccated within a few days. As has already been shown (section 4.33) the mean daily

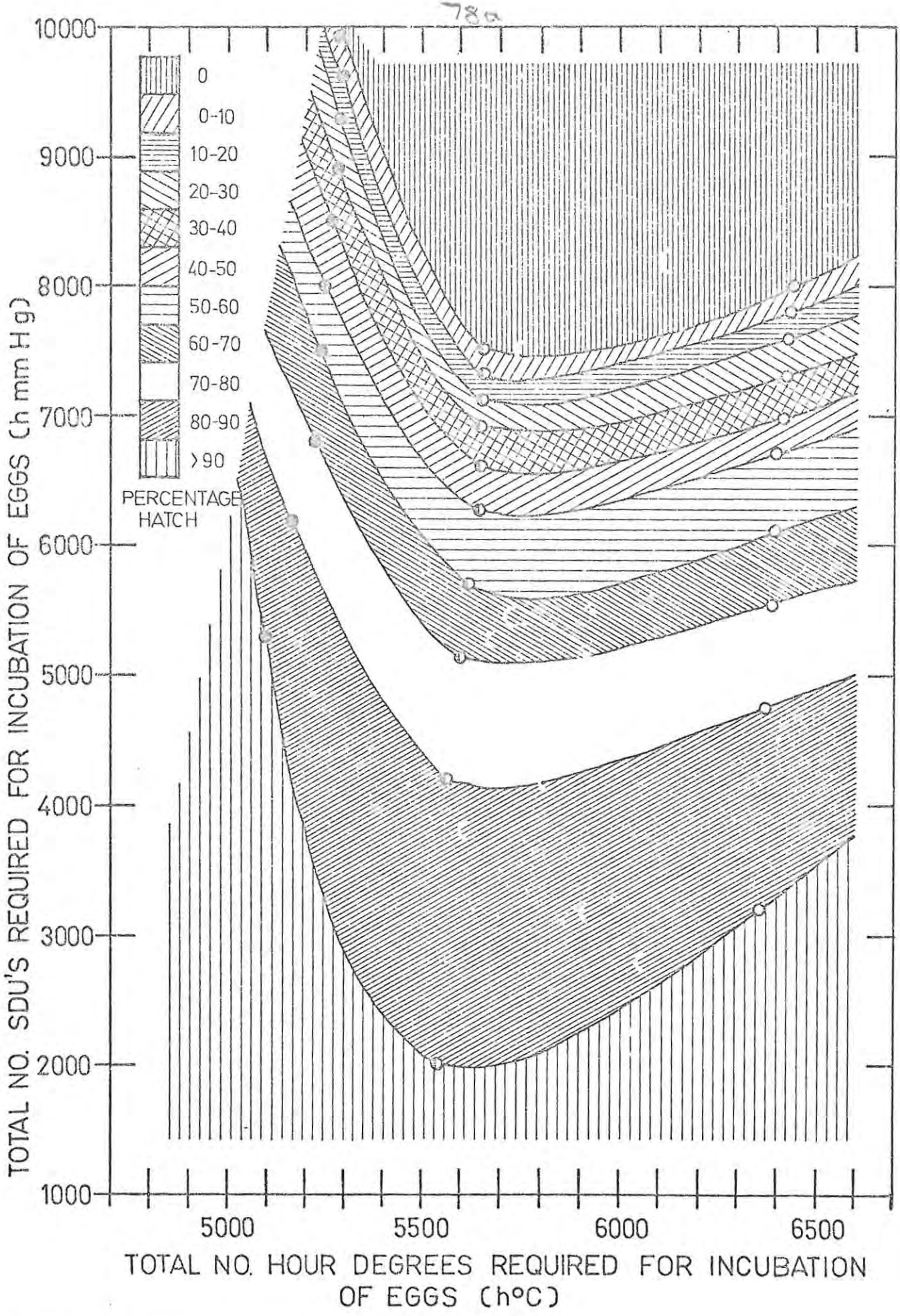


Fig. 56. Smoothed contour diagram of percentage hatch against total number of saturation deficit units and hour degrees experienced by incubating Boophilus decoloratus eggs. ● = data from first environmental room regime, ○ = data from second environmental room regime, ◐ = data from third environmental room regime.

temperature over this period was approximately 16°C. As eggs gave only a low percentage hatch at a constant temperature of 20°C (section 5.3) and no hatch at all at 15°C the results obtained in the Stephenson's screen were to be expected. As the conditions recorded by the thermohygrograph in the screen are likely to have been very different from the microclimatic conditions in the vegetation next to the screen little can be said concerning the possible responses of eggs which may have been present in these microclimates. As B.decoloratus larvae were found in the vicinity of the Stephenson's screen it must be concluded that microclimatic conditions in the vegetation were favourable for egg development and hatch.

5.6 DISCUSSION

The effects of temperature and moisture on the duration of the incubation period reported in this chapter support the previous findings (Table 7). The direct influence of temperature increases on shortening the length of the incubation period, within the tolerance limits of this parameter, probably operates by accelerating the overall metabolic rate of the developing embryo. The incubation period is not successfully completed at constant temperatures lower than 20°C or higher than 32°C. As 20°C is higher than the minimum temperature for egg production by female ticks (i.e. approximately 15°C) it implies that eggs which may be produced by ticks during cold weather may not necessarily hatch if the cold weather persists. This contention is supported by the results obtained in the Stephenson's screen (Section 5.5). Females held under these macroclimatic conditions produced eggs over long oviposition periods but eggs held under the same conditions failed to hatch. The implications of this observation are fully discussed in section 6.3.

The highest temperature at which successful embryological development takes place is difficult to assess. Under constant temperature conditions, 42°C is the critical temperature of the 'wax' layer covering the eggs. At constant low saturation deficit (i.e. high humidity) however eggs held at 38°C failed to hatch. A high egg viability was recorded at 32°C when saturation deficits were 3,64 and 10,68 mmHg respectively. As weight loss, probably due to water loss, was only slightly higher at 40°C than at 35°C it was not to be expected that the degree of egg viability of eggs held at 32°C and 38°C should be so different. As this was, however, the case, prolonged temperatures of 38°C must be considered as 'unfavourable' for egg development. As temperatures under natural conditions are never constant and are hardly ever as high as 38°C it is unlikely that temperature would ever become a limiting factor in the field.

Saturation deficit does not have any direct influence on the duration of the incubation period of B.decoloratus eggs although eggs held at high saturation deficit levels (i.e. dry conditions) do not hatch or give poor hatches due to desiccation effects. The percentage hatch is therefore directly dependent on the conditions of saturation deficit. It is impossible to state what the tolerance limits are for the effects of saturation deficit on egg viability as temperature does have an indirect effect on hatch by its controlling influence over the duration of the incubation period. As the water content of the egg appears to be a very important limiting factor, long incubation periods, experienced when temperatures are low, allow eggs a longer period in which to lose water and this may result in a low egg viability. It therefore becomes clear that it is unrealistic, and quite impossible, to separate the parameter of 'egg viability' from the parameter of 'incubation period duration' and that it is necessary to examine both these factors simultaneously.

6. DISCUSSION

Synopsis

Some of the problems associated with the assessment of climatic effects on ticks are discussed with special reference to the use of saturation deficit and relative humidity as indicators of atmospheric humidity. The problems associated with assessing the effects of three different variables (e.g. temperature, saturation deficit and oviposition period duration) are also discussed. A model is presented which shows the relationships between the main physical factors of the environment and the various biological phenomena studied during the course of this investigation. Finally the effects of climatic conditions on the life cycle and distribution of B.decoloratus are discussed.

6.1 INTRODUCTION

The results of work on the parasitic cycle, preoviposition period, oviposition period and incubation period have been fully discussed at the ends of the relevant sections dealing with these phases of the life cycle of B.decoloratus. This discussion is therefore confined to some of the more fundamental aspects which have emerged from the work as a whole. There are three main aspects which need consideration. Firstly the difficulties encountered when a realistic assessment of the effects of climatic conditions, such as temperature and humidity, on tick populations is made. Secondly it is necessary to bring the relationships established during the work on the preoviposition, oviposition and incubation periods together in some way so as to put the whole 'prehatch period'[1] into perspective. Although it has been convenient to deal with the prehatch period in three parts, the successful competion of each of these phases is directly dependent on the

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1. The prehatch period is the period between female fall and the emergence of larvae from the eggs produced by the females i.e. it includes the preoviposition, oviposition and incubation periods.

successful completion of the preceeding phase, and therefore these three phases in the non-parasitic life cycle of B.decoloratus must not be considered as distinct within themselves. A model of the interactions of the two main abiotic factors (temperature and humidity) and the various factors of the prehatch period dealt with during this investigation is therefore presented. The third aspect requiring attention is the way in which the findings reported in this study have contributed to our knowledge of the life cycle and distribution of B.decoloratus in South Africa.

6.2 ASSESSMENT OF THE EFFECTS OF CLIMATIC CONDITIONS ON TICKS

The physical, or abiotic, factors of the environment in the present context have been limited to temperature and humidity. When attempting to assess the effects of such parameters on any stage in the life cycle of a tick, or for that matter any terrestrial poikilothermic animal, it is important to distinguish between tolerance ranges and preferenda. The tolerance range of any species may be established fairly simply but the ecological significance of this range must be viewed with caution. As most animals display certain preferences, they will not necessarily be encountered in some environments in which the prevailing conditions are within the tolerance range. Thus, although all stages of B.decoloratus are probably able to survive temperatures of 30°C they are unlikely to be commonly found where microclimatic temperatures of this order frequently occur. The establishment of preferenda is highly desirable and experimentally can only be determined by continuous gradient experiments in which each physical factor is not treated in isolation. Further complicating such a study is the fact that the resistance on animals is likely to vary with change in any particular environmental factor or change in the combination of all the important

factors involved. Factors such as 'physiological state', 'behavioural state' and the recent history of the animals are all likely to cause variability in response to physical factors of the environment. As Macfadyen (1957) states "Acclimatization is the rule rather than the exception" and "ignorance of such effects have caused much confusion among not very physiologically minded ecologists and even among physiologists who should have known better". It is therefore clear that the assessment of the effects of climatic conditions on ticks is highly complex.

The survival of the various stages of B.decoloratus has been shown to be primarily dependant on water balance. Whilst ticks have evolved efficient behavioural responses, feeding techniques and various other physiological adaptations these developments have taken place within the framework set by the physical environment. Of these physical factors temperature and humidity are undoubtedly the two most important. Other factors such as wind, rainfall, degree and type of vegetational cover are undoubtedly significant, but only in the way in which they influence or modify temperature or humidity at the microclimatic level.

In many ecological studies workers have dealt with relative humidity as the parameter indicative of atmospheric humidity. This has probably been due to a lack of understanding of the interactions between animals and their environments. Relative humidity, simply defined as the amount of water vapour in a definite volume of air at a specified temperature, is expressed as a percentage of the total amount of moisture that the same volume of air is able to carry at the same specified temperature. If the volume of air is kept constant and the temperature altered the number of water molecules required to fully saturate the air is altered. Thus if we consider

the meaning of such a parameter in the biology of a tick species very little reliance can be placed on it. If, for example, two batches of ticks were to be placed in two different environments, A and B (Table 12), where the relative humidity levels are identical (i.e. 70% R.H.) but the temperature values different (i.e. 20°C and 40°C respectively) their water relations are likely to be different due to temperature effects.

TABLE 12.

The values of various abiotic factors in two different environments used as an example in illustrating the relative merits of using relative humidity and saturation deficit as indicators of atmospheric humidity in biological studies.

Physical factor	Environment A	Environment B
Temperature (°C)	20	40
Relative humidity (%)	70	70
Absolute humidity (mg H_2O/litre dry air+)	13	43
Vapour pressure (mmHg)	12,2	38,4
Saturation vapour pressure (mmHg)	17,4	54,9
Saturation deficit (mmHg)	5,2	16,5

+ = readings obtained from Platt and Griffitsh (1964).

Environment B would possess an atmosphere which actually carries more water vapour (i.e. absolute humidity) than A; this is reflected by the higher value of vapour pressure in environment B. With increase in temperature the saturation vapour pressure and vapour pressure increase exponentially (Fig. 57) and are higher at 40°C than at 20°C. Saturation deficit (i.e. the difference between the saturation vapour pressure and the vapour pressure) is therefore relatively greater in environment B than in A due to the fact that the air is not fully saturated and therefore the difference between

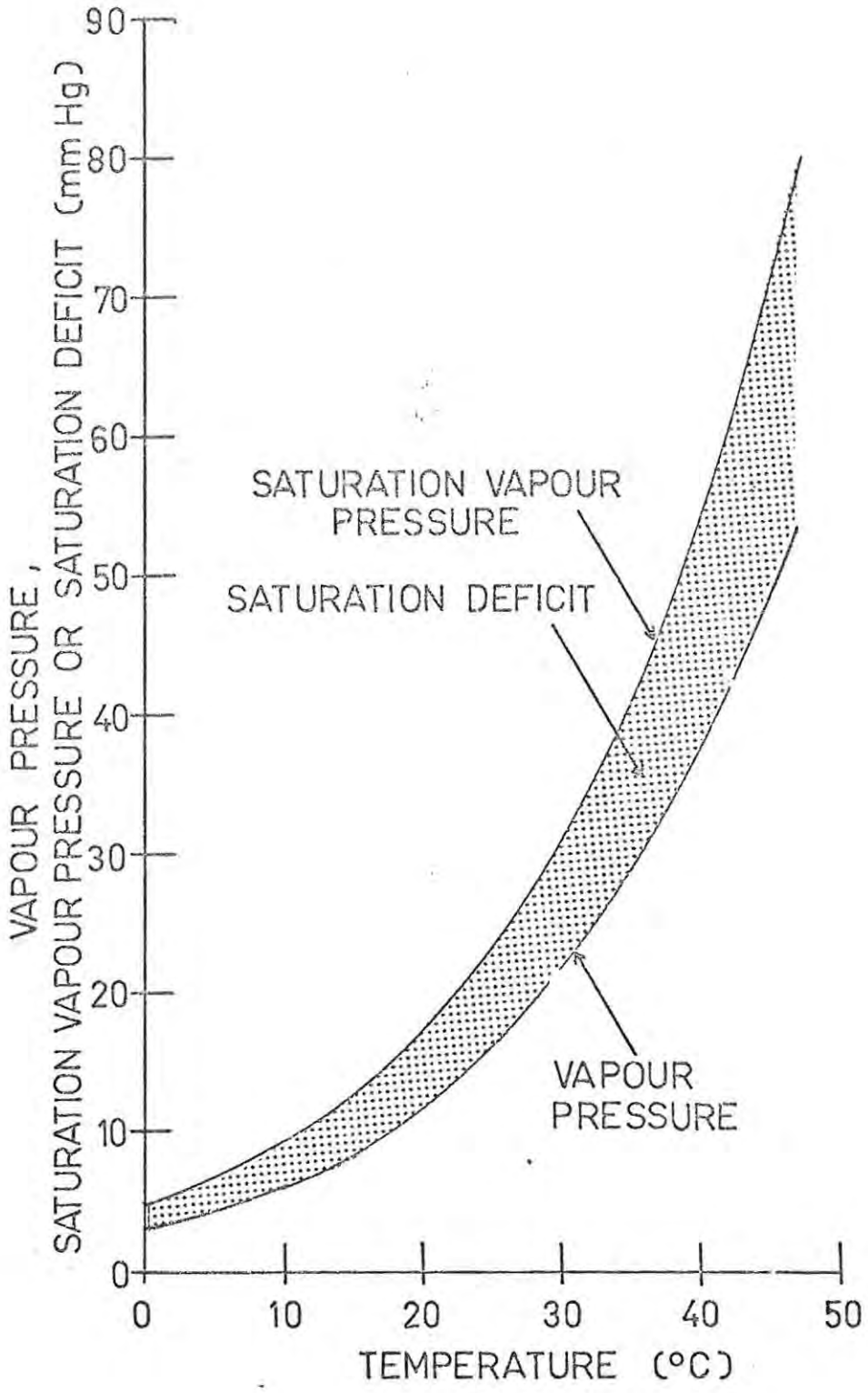

Fig. 57. The relationship between saturation vapour pressure, vapour pressure and saturation deficit and temperature. The example applies only to an environmental humidity of 70% relative humidity (see text for full explanation of figure).

the saturation vapour pressure and vapour pressure increases with increase in temperature. It might be expected therefore, that the ticks held in environment B will lose more water to the environment through evaporation than the ticks in environment A. For this reason it is believed that saturation deficit is a better indicator of the effects of atmospheric humidity when changes in temperature occur. In laboratory experiments at constant temperature it is immaterial whether relative humidity or saturation deficit is used as a simple conversion is possible. For this reason saturation deficit values have, in many instances, been given in parentheses after the relative humidity values selected in the present study, and saturation deficit used in all instances where different, or fluctuating, temperature conditions were involved.

In the assessment of biological responses in terrestrial animals, including ticks, to different or fluctuating climatic conditions there is the problem of having at least three variables (e.g. period of incubation; temperature; saturation deficit). The problems of analysis of such a situation appear to have been handled in three different ways. Some workers have chosen to be blind to the problem and have attempted to assess the biological responses of animals to their physical environment by visually studying curves of the changes in temperature and humidity and the changes observed in the animals concerned. These curves are often simplified by taking maximum and minimum values on a daily, weekly or even monthly basis, or by the calculation of mean conditions. This type of analysis is clearly subjective and it is debatable whether it serves any useful purpose at all. Only the most generalized relationships emerge. Another way of tackling the problem is to attempt to assess the effects of each physical factor separately. This is unrealistic. The work of Sweatman (1967, 1968)

and the present study shows that temperature may have an overriding effect and any analysis of the effects of saturation deficit on ticks may be meaningless without taking temperature into account. The point often missed by workers is that, although the parameter 'saturation deficit' takes into account the effects of temperature on the moisture content of the air, as can be seen from the general formula used in its calculation[1], temperature also has a 'heating' effect which, through its effects on the metabolic rate of the animals, may have a profound effect on biological responses. It may be suggested that a multi-variant analysis would prove to be the answer to the problem of having three, or more, variables. This is however not true in this case as a multi-variant analysis can only be employed when each variable is quite uninfluenced by the others. Humidity is very dependent on temperature. The third approach to the problem is the one proposed in this investigation. Shelford (1930) outlined the use of 'contour diagrams' in which lines of human mortality were drawn against temperature and relative humidity in the example discussed by him[2]. By plotting, for example, lines of equal incubation period duration against temperature and saturation deficit, it was possible to obtain a 'three dimensional' picture of the relationships involved. Although these contour diagrams are difficult to analyse and to interpret and require much data, their use makes a more objective analysis possible. When naturally occurring fluctuations in temperature and humidity occur a means of simplifying the information is necessary. In the present investigation this was done by calculating total daily hour degrees

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1. $SD = \frac{VP \times 100}{\%\ R.H.} - VP$ where SD = saturation deficit; VP = Vapour pressure (dependent on temperature): R.H. = relative humidity.

2. The work of Huntington (1919).

centigrade and saturation deficit units[1]. Saturation deficit units(SDU's) have not been used in previous work but the concept is identical to that involving temperature.

6.3 THE EFFECTS OF TEMPERATURE AND HUMIDITY ON THE PREOVIPOSITION, OVIPOSITION AND INCUBATION PERIODS OF B.DECOLORATUS

The effects of temperature and humidity on the various aspects of the non-parasitic life cycle of B.decoloratus studied during the present investigation may be simply and conveniently summarized in a model (Fig. 58). This model takes a similar form to that described by Macleod (1962) for the dynamic community relations in which I.ricinus participates. Each disc represents a measurable parameter. A clockwise rotation of any disc means an increase in the effect of the parameter involved. It is important to note that the main drive for the model is through the physical factors of the environment i.e. the discs labelled temperature and saturation deficit. This means that an increase in temperature increases the metabolic rate of the ticks but that the reverse is not possible i.e. an increase in the metabolic rate cannot increase the temperature. Discs in direct contact indicate direct relationships established in the present study while discs linked by belts (straight lines joining the discs) indicate suggested relationships assumed from the results gained during the investigations. Thus it can be seen that with temperature increase, relative humidity decreases and saturation deficit increases. This causes both the metabolic rate of the ticks and the amount of water lost to the environment to increase. An increase in water loss causes a decrease in the percentage hatch of

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1. Defined earlier (section 5.4).

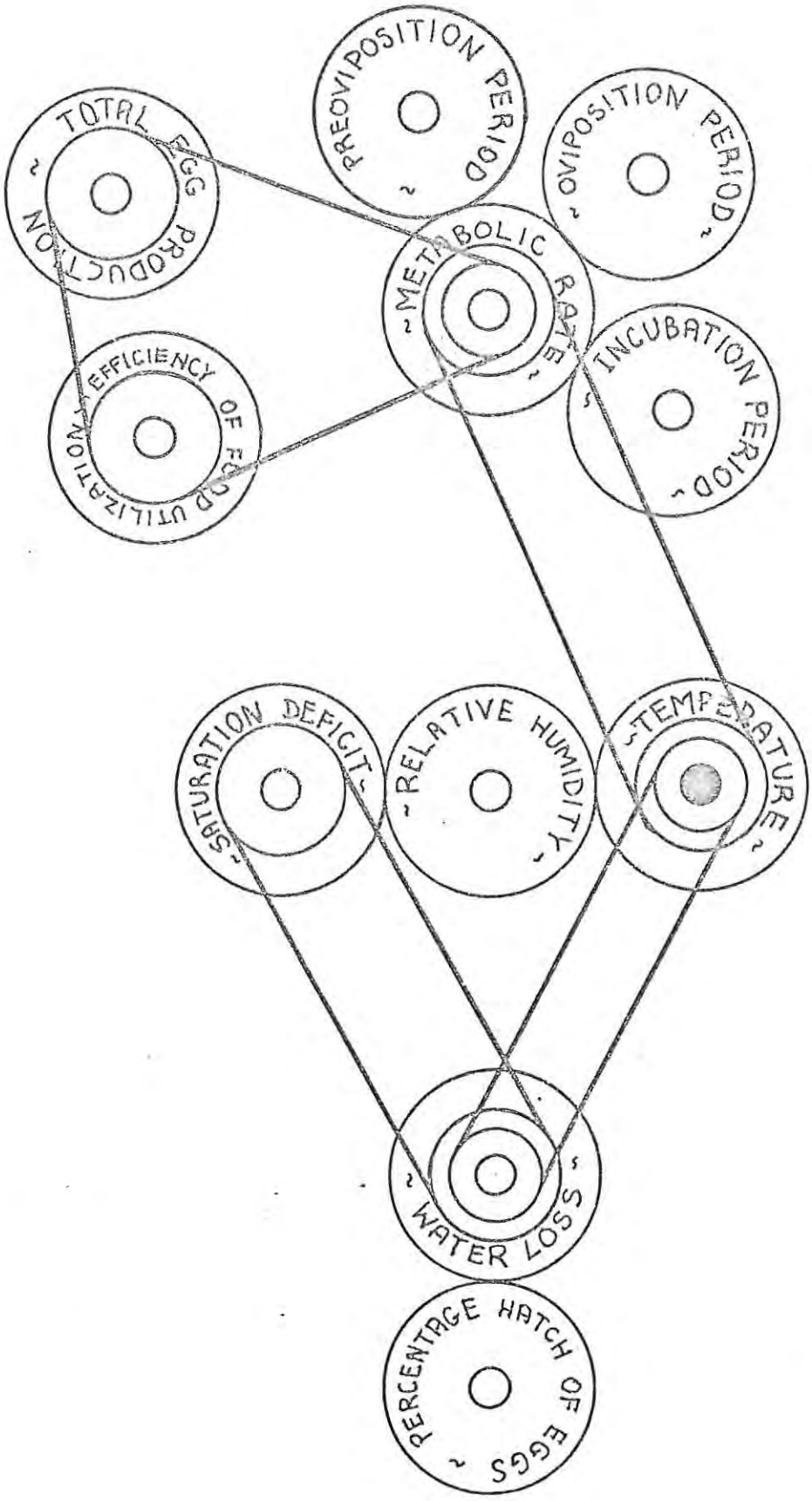

Fig. 58. A model of the interactions between the physical factors of the environment (temperature, relative humidity and saturation deficit) and a number of parameters investigated during a study of the biology of Boophilus decoloratus. (See text for explanation).

eggs but does not have any direct effect on any of the other parameters studied. Increase in metabolic rate causes a shortening of the preoviposition, oviposition and incubation periods as well as indirectly causing an increase in total egg production. This increase in total egg production is probably due to an increase in the efficiency of food utilization during the formation of eggs.

The most important thing to note about the model is that changes in the physical factors of the environment work through the effects of water loss and metabolic rate on the various parameters studied in the present investigation.

6.4 THE EFFECTS OF CLIMATIC CONDITIONS ON THE LIFE CYCLE AND DISTRIBUTION OF B.DECOLORATUS

It has been shown that temperature is the most important physical factor regulating the durations of the various developmental periods in the life cycle of B.decoloratus. The parasitic phase has been shown to be primarily dependent on the hosts surface temperature and accordingly B.decoloratus proceeds through its parasitic cycle at the same rate throughout the year. This was shown to be the case between March and October 1972. This parasitic phase is usually completed within approximately 25 days (i.e. from larval infestation to the peak period of drop-off of the engorged females). Once the engorged female ticks have fallen to the ground the non-parasitic cycle commences. The duration of this becomes more variable in response to the variability of microclimatic temperatures. Using the results of this investigation it may be postulated that during cold weather (i.e. mean daily temperatures in the region of 15^{o}-20^{o}C) the prehatch period may be as long as 120 days when temperatures remain consistently low. The prehatch period shortens during warmer weather. At mean daily temperatures

of 25°C larvae may appear some 35-40 days after female detachment from the host. The implications of this seasonal difference in the rate at which the non-parasitic cycle is completed probably has a direct effect on the 'success' of ticks in different regions of South Africa. In the warmer parts of the country where humidities are sufficiently high to provide eggs and non-parasitic larvae with 'favourable' microclimates, more generations are likely to occur annually, which probably also causes a greater summer peak of abundance in these regions. Such areas might be the coastal regions of the eastern Cape Province, Transkei, Natal and the eastern regions of the Transvaal[1]. Theiler (1949) has shown that B.decoloratus has its highest population density in these areas and has suggested that the distribution is related to annual rainfall. The apparent initiation and rapid development of acaricidal resistance associated with some of these regions, notably the eastern Cape Province (Whitnall, 1969), is probably directly attributable to this temperature relationship.

As larval distribution was found to be associated with particular vegetational categories (Londt, 1970; Londt and Whitehead, 1972), and laboratory work suggested thar larvae were able to survive in a wider range of habitats than those in which they were found, one of the original aims of the present investigation was to establish if the egg stage was more susceptible to desiccation than the larval stage. If this were found to be the case then it might explain why larvae are found in some habitats and not in others. As microclimatic work was not undertaken, because of the non-availability of the necessary

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1. As indicated by the maps of mean January and July temperatures and annual rainfall data supplied by Talbot and Talbot (1960).

instrumentation[1], it is only possible to call upon the conclusions derived from laboratory studies. It has been found that both larvae (Londt and Whitehead, 1972) and eggs require approximately the same conditions for survival. Larvae held at a constant humidity of 70% R.H. and temperature of 26°C would lose water to the atmosphere and eventually succumb (Londt and Whitehead, 1972). Similarly eggs held under the same conditions would just give a hatch of 50%. Larvae are, however, able to actively take up water vapour from the atmosphere when the humidity is greater that 70% R.H. and therefore are able to recoup any water lost during periods of unfavourable environmental conditions (Londt and Whitehead, 1972). Eggs, on the other hand, have been shown to not possess this attribute and cannot recoup any water lost through desiccation. Eggs are therefore entirely dependent on the amount of water 'built into' them at the time of their production by the female tick. This important difference between larvae and eggs is probably the only concrete piece of evidence to suggest that it is the effect of microclimatic conditions on the egg stage which determines the subsequent distribution of larvae in the field.

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1. An extensive search for continuous recording equipment to record microclimatic temperatures and humidities prooved unsuccessful. The difficulty appears to be the production of a reliable humidity sensor which is small and does not rely on extensive air movement. Various sensors based on the principle of the wet and dry bulb hygrometer were encountered but there were problems attached to all these sensors. More recently a commercially available instrument has been found. This fairly expensive piece of instrumentation is being investigated and it is hoped that some of the necessary microclimatic work will be undertaken in the future.

7. SUMMARY

1. The external morphology of all stages in the life cycle of B.decoloratus is described with the aid of scanning electron micrographs.

2. The biological activities of B.decoloratus during its parasitic cycle are examined with special reference to the course of feeding of all stages, and to the influence of the pharate nymph and adult conditions in accelerating the completion of the cycle.

3. The pattern of dropping of replete females is considered in relation to their weight and their time of drop-off.

4. The adult male population structure is described. Two distinct weight groups are reported and the taxonomic significance of these is discussed.

5. The preoviposition period duration of B.decoloratus was studied under laboratory conditions and found to be temperature dependent and humidity independent. Female size, as implied by their engorged weights, influenced the duration of the oviposition period: ticks weighing less than 20 mg possessed longer preoviposition periods.

6. The preoviposition period of B.decoloratus females under fluctuating macroclimatic conditions was studied and found to be long in duration. The implications of this are discussed in relation to laboratory findings and the summer build-up of this species in nature.

7. The duration of the oviposition period of B.decoloratus and the number of eggs produced during this period were studied under laboratory and field conditions. Both these parameters were found to be temperature dependent and uninfluenced by humidity. The significance of a temperature dependent oviposition period is discussed.

8. The minimum amount of 'blood' required by female ticks for oviposition of eggs was found to be approximately 16 mg. under laboratory conditions.

9. The oviposition behaviour of B.decoloratus females has been described and the water relations of ovipositing and non-ovipositing females were examined and discussed.

10. The effects of handling female ticks during their oviposition period was studied and females which were handled were found to produce significantly fewer eggs. The significance of this is discussed.

11. During preliminary experiments on the incubation period of B.decoloratus it was found that the viability of eggs laid during the first half of the ovipositing period was greater than for eggs laid in the second half of the period. The critical temperature of the 'wax' coating of eggs was found to be 42°C while the critical humidity was approximately 70% R.H. (7,53 mmHg) at 26°C. The implications of these findings are discussed.

12. The development of eggs, traced throughout the incubation period by monitoring the build-up of guanine spectrophotometrically, was studied under different humidity conditions. Successful development was found to be dependent on the water content of the eggs at the time of laying. As eggs were also found to be unable to take up water vapour from the atmosphere this finding is important evidence to support the suggestion that the survival of the egg stage largely determines the spacial distribution of larvae in the field.

13. Results of work done at constant, alternating and naturally fluctuating temperatures and humidities demonstrated that the duration of the incubation period of B.decoloratus is

temperature dependent and humidity independent. The significance of this is discussed in relation to the findings of previous workers and to the biology of the species.

14. Egg viability (percentage hatch) was found to be humidity dependent and temperature independent. The implications of these findings are discussed.

15. Some of the problems associated with the assessment of the effects of climatic conditions on ticks are discussed. Special reference has been made to the advocation of using saturation deficit instead of relative humidity as the parameter indicative of environmental humidity in any biological study involving fluctuating humidity conditions.

16. The problems associated with having three variables (e.g. temperature, saturation deficit and incubation period duration) are discussed and reasons given for why contour diagrams have been used in this investigation.

17. A model of the interactions between the main abiotic factors of the environment (temperature and humidity) and the biological parameters studied during the second part of this investigation is presented.

18. The effects of climatic conditions on the life cycle and distribution of B.decoloratus are discussed. Special reference has been made to the available evidence explaining the summer build-up in tick numbers known to occur in nature and the way in which the present findings have helped in an understanding of the spacial distribution of B.decoloratus in the field.

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Ecological studies of larval ticks in South Africa (Acarina: Ixodidae)

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The work of Stampa (1959, 1969) on the Karoo Paralysis Tick, *Ixodes rubicundus* Neumann, has shown how an ecological study may lead to more efficient chemical control of a tick species as well as contribute to the possibility of a practical means of biological control. Stampa (1969) suggested that by a process of 'alteration of environment' the density of *I. rubicundus* populations could be suppressed. As a number of ixodid tick species are a matter of concern to cattle, sheep and angora goat farmers in the Eastern Cape Province of South Africa, ecological work on these ticks is of considerable importance. The continual threat of acaricidal resistance and the ever increasing prices of these compounds are further factors which stimulate a concerted effort in the study of tick ecology. The early work of Lounsbury (1899) on the Bont Tick, *Amblyomma hebraeum* Koch, laid the foundations for ecological work on cattle ticks in the Eastern Cape and Stampa (1959, 1969) and Kraft (1961) have further developed this type of research. This paper deals, in part, with a distributional survey of larval ticks on two coastal farms in the Port Alfred district, Cape Province, in relation to the microclimates in various vegetation types. During the course of the investigation it became evident that larvae were confined to certain microhabitats which were classified according to the height of the grass and their situation in relation to neighbouring trees and bushes. A study of the possible importance of grass height in the behaviour of climbing larvae was therefore undertaken and is reported in the second part of this paper. Finally, a laboratory study of the water relations of larvae of some tick species was undertaken in an attempt to correlate larval survival with larval incidence in microclimatically different habitats in the field.

LARVAL DISTRIBUTION

Methods

The work was carried out on two farms, Barville Park and Faithful Fountain, approximately 13 km S.W. of Port Alfred and 4 km from the sea. The vegetation on both farms was divided into eight convenient categories based on the height of the sward and its relationship to adjoining bushes and trees. Thus, short vegetation was defined as being 5–30 cm high, medium 31–50 cm and tall in excess of 51 cm. Positionally open vegetation refers to vegetation not in the immediate vicinity of

trees and bushes; protected refers to vegetation sheltered or incompletely covered by neighbouring trees and bushes; and covered refers to vegetation under a complete canopy of trees and bushes. The vegetation was categorized as follows: (i) short open vegetation, (ii) medium open vegetation, (iii) tall open vegetation, (iv) short protected vegetation, (v) medium protected vegetation, (vi) tall protected vegetation, (vii) short covered vegetation, (viii) medium covered vegetation.

Larvae were collected using a modified version of the apparatus described by Stampa (1959) and Kraft (1961). Ten flannelette strips were attached at equal intervals along a broom handle of 1 m length. The strips were dragged over the vegetation and provided an effective substrate to which larval ticks clung. Samples were usually taken over a distance of 50 m and the results expressed in terms of the number of larvae collected per 50 m. When trees or bushes made sampling difficult, shorter distances were traversed and the results adjusted so that they were expressed in terms of larvae per 50 m to allow comparisons. Samples were taken between 10 a.m. and 3 p.m. over the period 17 March 1969 to 7 March 1970.

Microclimatic temperatures were measured with a 'thermistor thermometer' at three different levels in selected examples of the vegetation categories. These positions corresponded to the bottom, middle and top of each vegetation type. Relative humidities were determined with cobalt thiocyanate paper indicators as described by Solomon (1957) at the same three vertical levels at which the temperature was measured. As an exposure time of 2 h was required for the cobalt thiocyanate papers to equilibrate they were suspended in small metal mesh cylinders attached to wooden rods placed vertically in the vegetation. The same sites were re-used on each occasion when measurements were taken. In order to establish the range of variation within the categories a number of short open, short protected and short covered sites were compared. All microclimatic measurements were converted to units of saturation deficit to facilitate comparisons. As a result of the long time required for cobalt thiocyanate papers to equilibrate (Solomon, 1957), relative humidity measurements were confined to periods when the macroclimatic conditions were reasonably static. Times at which macroclimatic conditions varied least were between 12 noon and 2 p.m. and between 3.30 a.m. and 5.30 a.m. These periods were established by studying daily thermohygrograph records and were found to be convenient as they represented the periods at which maximum and minimum climatic conditions could be expected. Microclimatic air currents were measured with an anemometer (Casella Air Metre).

Results

The macroclimatic conditions over the duration of the field survey appear in Fig. 1. The results of the survey at Barville Park and Faithful Fountain are summarized in Tables 1 and 2 respectively. In the more extensive survey undertaken at Barville Park four tick species were found, *Ixodes pilosus* Koch, *Haemaphysalis silacea* Robinson, *A. hebraeum* and *Boophilus decoloratus* (Koch). The results show that both *I. pilosus* and *H. silacea* were found predominantely in short covered vegetation while *A. hebraeum* was associated with medium and tall protected vegetation. As very few *B. decoloratus* larvae were collected at Barville Park no

meaningful conclusions could be drawn about their distribution. The brief survey undertaken at Faithful Fountain yielded the foregoing four species and *Rhipicephalus evertsi* Neumann. The Faithful Fountain data suggest that *B. decoloratus* may be associated with short and medium protected vegetation. *R. evertsi* was collected in one sample only and consequently could not be associated with any vegetation type. The population fluctuations of the two most common species at Barville Park, *I. pilosus* and *H. silacea*, throughout the sampling period are shown in Fig. 2. Both species have their greatest incidence in the cooler months of the year. *I. pilosus* was found to be most active during the months of May, June and July, after which activity decreased to almost zero between October and March. *H. silacea* activity increased between April and August and was highest in August and the beginning of October. Thereafter activity decreased to almost zero.

The microclimatic conditions recorded at Barville Park are shown in Tables 3 and 4. These results show that conditions of saturation deficit approach zero on most evenings in all vegetation categories throughout the year. At midday open habitats were generally warmer and drier than protected and covered ones. During the warm summer months ground level conditions in open habitats were hotter and drier than those higher in the vegetation. In winter, however, the opposite was the case on a number of occasions. As tick eggs are laid at ground level this observation suggests that winter months might be more suitable for egg development and survival. Microclimatic air current speeds indicated that open habitats are far windier than protected and covered ones. Ground level air speeds were in all cases lower than those measured at the tops of the vegetation. The effects of wind on atmospheric saturation deficits is one of drying (Schütte & King, 1965) and therefore it would be expected that open habitats would usually be drier than protected and covered ones. The microclimatic information together with the survey data suggested that larval ticks are not normally collected in habitats which experience midday saturation deficits in excess of approximately 10 mm Hg. In most instances habitats which did experience values higher than 10 mm Hg, and yielded larvae, possessed at least one level in the vegetation which had a saturation deficit in the region of 10 mm Hg. The implication is that larvae are able to migrate to more suitable microclimatic levels to enhance their chances of survival. Migrations of this type have been reported for *Ixodes ricinus* L. (Lees & Milne, 1951) and this suggestion requires further consideration.

The study of variation in microclimatic conditions in short vegetation sites, the results of which appear in Table 5, show that short open and short covered sites have similar microclimatic conditions. Short protected sites, however, differ greatly, depending on the position of the site with respect to the neighbouring trees and bushes. Sites facing north (i.e. bushes and trees on the south side of the site) were much hotter and drier than other sites. East-facing sites appeared to be similar to west-facing sites but were slightly warmer and drier, probably because dew was often retained for longer periods in sites protected from the morning sun (i.e. west-facing sites). The short protected site used throughout the microclimatic study (reported in Table 3) appeared to be approximately midway between the hottest and coldest sites as regards midday conditions.

Table 1. *The average number of larvae of the four species collected per 50 m at Barville Park, from eight vegetation categories*

(Figures expressed to the nearest whole number. Based on the results of 23 sampling days.)

	Tick species			
Vegetation category	*I. pilosus*	*H. silacea*	*A. hebraeum*	*B. decoloratus*
Short open	0	0	0	0
Medium open	0	0	1	0
Tall open	0	0	0	0
Short protected	8	10	0	0
Medium protected	10	0	15	0
Tall protected	6	1	10	0
Short covered	118	57	2	1
Medium covered	7	7	0	0
Total	149	75	27	1

Table 2. *The average number of larvae of the five species collected per 50 m at Faithful Fountain, from eight vegetation categories*

(Figures expressed to the nearest whole number. Based on the results of five sampling days.)

	Tick species				
Vegetation category	*I. pilosus*	*H. silacea*	*A. hebraeum*	*B. decoloratus*	*R. evertsi*
Short open	0	0	0	0	0
Medium open	0	0	0	2	0
Tall open	—	—	—	—	—
Short protected	0	1	5	34	1
Medium protected	0	0	2	11	0
Tall protected	—	—	—	—	—
Short covered	7	11	1	0	0
Medium covered	—	—	—	—	—
Total	7	12	7	47	1

—, No samples taken.

VEGETATION HEIGHT, DISTRIBUTION AND BEHAVIOUR OF LARVAL TICKS

Methods

Larvae of *A. hebraeum*, *B. decoloratus*, *I. pilosus*, *R. evertsi* and *Rhipicephalus appendiculatus* Neumann were reared and maintained in an incubator at 26 °C and approximately 95 % relative humidity (R.H.) for use in the experiments reported in this section. The movements of individual *A. hebraeum* larvae presented with glass rods of six different lengths on which to climb, to simulate grass stems, were observed and recorded in the manner described by Lees (1948). The rods used were 10, 15, 30, 40, 50 and 90 cm in length and approximately 0·4 cm in diameter, with one end drawn into a pointed tip approximately 0·5mm in diameter. Each rod was marked at 1 cm intervals to facilitate observations and

Table 3. *Microclimatic conditions at Barville Park, expressed as saturation deficits (mm Hg), measured in eight sites representing eight vegetation categories*

Date	P	SO	MO	TO	SP	MP	TP	SC	MC	T/H
Day conditions (measured between 11 a.m. and 1 p.m.)										
8. v. 69	Top	20·44	–	16·06	9·95	7·44	9·06	7·44	10·57	18·13
	Mid	21·06	12·69	13·09	9·95	7·21	8·52	5·61	9·71	
	Bot.	11·67	1·00	0·00	3·26	1·36	2·42	2·88	3·36	
26. v. 69	Top	15·09	10·35	13·81	7·68	7·45	–	4·91	6·16	10·32
	Mid	14·66	10·06	13·01	6·54	6·54	–	4·75	6·16	
	Bot.	12·02	6·39	7·36	3·26	2·12	–	2·00	1·99	
12. vi. 69	Top	8·16	7·01	5·52	5·79	6·79	7·68	5·97	6·79	7·65
	Mid	8·16	7·01	5·52	5·79	6·79	7·68	5·97	6·79	
	Bot.	8·41	0·77	4·05	1·61	2·56	3·26	2·56	1·99	
18. vi. 69	Top	19·25	15·58	19·04	15·58	15·58	12·31	9·95	12·69	18·74
	Mid	16·03	15·11	18·47	15·58	12·31	12·31	9·95	12·69	
	Bot.	8·40	11·04	0·91	8·16	3·16	0·94	7·92	9·95	
23. vii. 69	Top	20·44	13·91	12·69	8·36	7·68	7·68	5·61	5·61	12·94
	Mid	20·44	13·49	12·69	7·21	6·79	7·21	4·21	5·44	
	Bot.	13·81	7·68	7·92	2·40	3·16	4·21	2·97	4·08	
18. viii. 69	Top	13·41	8·67	9·75	9·95	6·15	6·15	7·92	7·92	9·95
	Mid	11·32	8·67	9·75	9·92	6·15	6·15	7·68	7·68	
	Bot.	11·67	10·57	9·75	6·15	6·15	4·34	7·68	7·92	
16. ix. 69	Top	13·09	9·35	10·88	8·52	6·08	7·62	8·52	8·25	11·57
	Mid	29·45	10·42	10·88	8·52	6·08	7·62	8·52	8·25	
	Bot.	30·26	13·09	10·42	6·78	5·37	5·27	10·55	8·25	
14. x. 69	Top	22·34	20·24	12·25	17·55	12·25	17·36	12·31	16·06	18·50
	Mid	22·23	20·24	20·24	21·69	12·25	11·57	12·31	16·06	
	Bot.	71·44	20·24	20·24	26·50	18·13	17·36	18·47	13·09	
1. xii. 69	Top	18·60	13·13	19·14	14·66	8·40	10·66	10·66	8·92	40·79
	Mid	17·10	15·66	21·41	12·02	9·27	8·40	10·66	8·92	
	Bot.	+	26·43	16·71	12·02	10·66	13·01	10·66	10·08	
6. i. 70	Top	46·42	24·97	14·32	39·84	15·63	22·24	15·22	24·26	·5·15
	Mid	54·45	24·97	22·24	39·84	12·54	22·24	15·22	24·26	
	Bot.	+	24·97	15·33	+	7·68	22·24	22·24	24·26	
Night conditions (measured between 3 a.m. and 5 a.m.)										
31. v. 69	Top	0·13	0·13	0·13	0·13	0·13	0·13	0·55	0·55	0·34
	Mid	0·13	0·13	0·13	0·13	0·13	0·13	0·55	0·55	
	Bot.	0·00	0·00	0·00	0·00	0·00	0·55	0·55	0·55	
18. vi. 69	Top	0·13	0·13	0·13	0·13	0·13	0·13	0·55	0·55	0·34
	Mid	0·13	0·13	0·13	0·13	0·13	0·13	0·55	0·55	
	Bot.	0·00	0·00	0·00	0·00	0·00	0·00	0·55	0·55	
24. i. 70	Top	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·83
	Mid	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	
	Bot.	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	

KEY. P, Position in vegetation (top, middle and bottom); –, spoilt readings; T/H, thermo-hygrograph readings; +, value higher than the limits of the method; SO, short open vegetation; MO, medium open vegetation; TO, tall open vegetation; SP, short protected vegetation; MP, medium protected vegetation; TP, tall protected vegetation; SC, short covered vegetation; MC, medium covered vegetation.

Table 4. *Microclimatic air speeds recorded on two fairly windy days at Barville Park*

Vegetation category	Position in vegetation	Air speed and direction (m/min) 30 June 1969	29 September 1969
Short open	Top of veg.	239·5 W	217·0 W
	Ground level	66·5 W	28·0 W
Medium open	Top of veg.	238·0 W	138·0 W
	Ground level	4·0 W	22·0 W
Tall open	Top of veg.	283·5 W	153·0 W
	Ground level	0·0 —	0·0 —
Short protected	Top of veg.	8·5 W	25·0 W
	Ground level	0·0 —	0·0 —
Medium protected	Top of veg.	60·0 W	40·0 W
	Ground level	0·0 —	0·0 —
Tall protected	Top of veg.	17·5 W	180·0 W
	Ground level	0·0 —	0·0 —
Short covered	Top of veg.	0·5 E	0·0 —
	Ground level	0·0 —	0·0 —
Medium covered	Top of veg.	13·0 W	32·0 W
	Ground level	0·0 —	0·0 —

E = east; W = west; — = no direction indicated.

recording. The procedure used in all experiments was as follows. An individual larva was placed, by means of a fine paintbrush, on to a vertical glass rod at a level of known distance from the tip (i.e. 10, 15, 30, 40, 50 or 90 cm respectively). The larva was then observed as it climbed up the rod. If the larva climbed downwards, below the point of release, it was removed and replaced by another. This happened very infrequently. The movements of the larva were recorded in the manner used by Lees (1948). Once a larva had commenced its first 'trip' up the rod it was left undisturbed until it either passed the point of release or came to rest on the rod for a period of at least 30 min. The larva was then considered to have completed the requirements of the test. Larvae which returned past the point of release were removed as it was apparent that under field conditions such larvae would have the opportunity of selecting another grass stem up which to climb. Those larvae which came to rest were considered to have achieved the position most preferable for encountering a passing host. On completion of the test the larva was destroyed so as to ensure that no larva was used more than once.

A brief study of the behaviour of the other four species mentioned above was also undertaken. In this work only a 40 cm rod was used and the behaviour of the species compared with that of *A. hebraeum* on the same length of rod.

Results

Table 6 summarizes the results obtained for *A. hebraeum* larvae climbing on six different rod lengths. All observations were made in the laboratory at 20–22 °C and approximately 60–65 % R.H. A further analysis of the movements of the individual

Table 5. *Saturation deficits recorded in seven short open, short protected and short covered vegetation sites (mm Hg)*

Site no.	Position of measurement	Vegetation category: Short open	Short protected	Short covered
1	Top	19·04	7·68	10·47
	Middle	19·63	8·67 (NE)	10·47
	Bottom	16·06	9·75	10·74
2	Top	19·04	6·16	9·64
	Middle	19·63	6·16 (E)	9·64
	Bottom	16·06	4·62	9·46
3	Top	19·04	6·19	9·64
	Middle	19·63	5·44 (S)	9·64
	Bottom	16·06	4·08	7·68
4	Top	19·04	9·64	8·62
	Middle	19·63	9·35 (SW)	8·62
	Bottom	19·63	4·35	8·62
5	Top	19·04	19·25	9·64
	Middle	19·63	20·40 (N)	9·64
	Bottom	16·06	40·72	7·68
6	Top	19·04	7·68	8·62
	Middle	19·63	6·75 (NE and SW)	8·62
	Bottom	16·06	5·06	7·68
7	Top	19·04	9·06	9·64
	Middle	19·63	6·06 (SW)	9·64
	Bottom	16·06	5·61	9·64
T/H	—	18·47	16·37	18·12
Date of measurement		2. v. 69	2. x. 69	22. ix. 69

Directions in which short protected sites faced are indicated in parentheses.
Site 6 was situated between two bushes.
Abbreviations: N = north; S = south; E = east; W = west; T/H = thermohygrograph reading; site 7 = site used in microclimatic study (Table 3).

larvae was undertaken in order to get an indication of larval activity in various regions of each rod length. In Fig. 3 a diagrammatic representation of the activity of larvae in relation to the region of rod is shown. This graphic representation was produced in the following way:

(i) Each rod was demarcated into a number of 5 cm lengths (regions) numbered from the lowest to the highest.

(ii) The total number of centimetres through which the larvae moved, in both upward and downward directions, in each region was calculated for each rod length. Means were obtained by dividing by the number of larvae tested. The value obtained represented the mean number of centimetres moved by each larva in each region of each rod length.

(iii) Curves of activity were plotted (i.e. the average number of centimetres traversed by each larva) against the region of rod.

(iv) Finally a line was drawn parallel to the X axis and passing through the

10 cm mark of the Y axis. The significance of this line is that if a larva walked up a rod through a region and then returned downwards through the same region, without any turning movements, it would have moved through a total of 10 cm

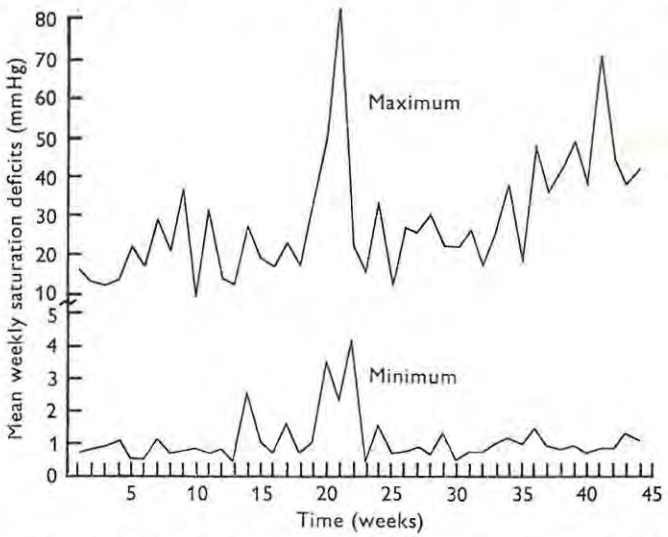

Fig. 1. Mean weekly maximum and minimum saturation deficits recorded at Barville Park between 18 March 1969 and 2 February 1970.

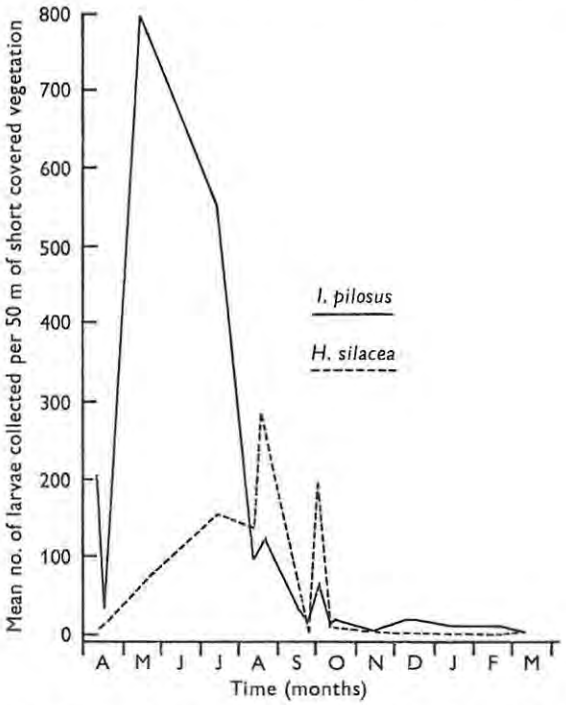

Fig. 2. Average numbers of *Ixodes pilosus* and *Haemaphysalis silacea* larvae collected per 50 m in short covered vegetation at Barville Park over the period of the distributional survey. ——, *I. pilosus*; – –, *H. silacea*.

Table 6. *An analysis of the movements of* Amblyomma hebraeum *larvae climbing on six different lengths of glass rod*

	Rod height (cm)					
	10	15	30	40	50	90
Average height of first trip up the rod (cm)	9·8	14·6	26·5	36·4	45·2	45·0
% of maximum height possible on first trip	98·0	97·3	88·3	91·0	90·4	50·0
% larvae reaching tip of rod on first trip	80·0	90·0	60·0	80·0	70·0	0·0
Average maximum height achieved on any trip (cm)	9·8	15·0	29·2	38·4	47·9	58·3
% of maximum height possible on any trip	98·0	100·0	97·3	96·0	95·8	64·8
% larvae reaching tip of rod on any trip	80·0	100·0	90·0	90·0	80·0	0·0
Average duration of movement on rod (to nearest min)	8	13	21	34	58	48
% larvae actually coming to rest at the tip of the rod	10·0	0·0	20·0	35·0	30·0	0·0
Number of larvae tested	10	10	10	20	10	10

Table 7. *An analysis of the movements of five species of ixodid tick larvae climbing on a glass rod* 40 *cm high*

	I. pilosus	*B. decoloratus*	*R. appendiculatus*	*R. evertsi*	*A. hebraeum*
Average height of first trip up the rod (cm)	12·7	19·1	18·6	20·2	36·4
% of maximum height possible on first trip	31·7	47·7	46·5	50·5	91·0
% larvae reaching tip of rod on first trip	20·0	0·0	10·0	20·0	80·0
Average maximum height achieved on any trip (cm)	17·1	20·3	23·3	24·8	38·3
% of maximum height possible on any trip	42·7	50·7	58·2	62·0	96·0
% larvae reaching tip of rod on any trip	20·0	0·0	10·0	20·0	90·0
Average duration of movement on rod (to nearest min)	14	13	14	9	34
% larvae actually coming to rest at the tip of the rod	0·0	0·0	0·0	0·0	35·0
Number of larvae tested	10	10	10	10	20

A. hebraeum data repeated from Table 1 for comparison.

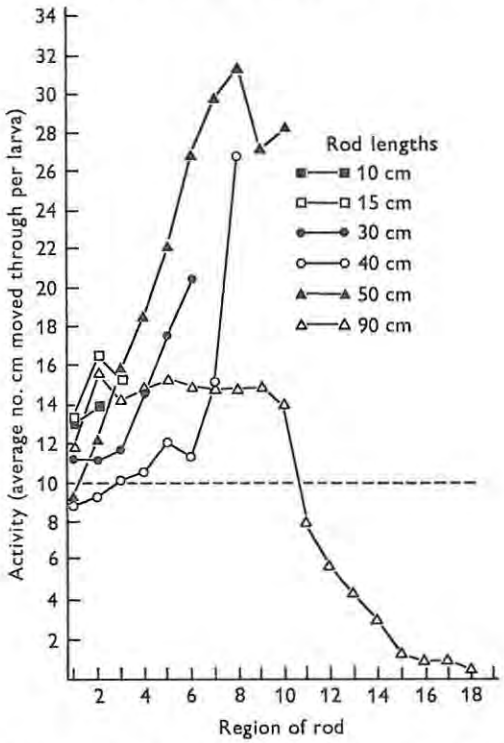

Fig. 3. The activity (average number of centimetres moved through by each larva) of *Amblyomma hebraeum* larvae for each region of six different rod lengths (for full explanation see text).

in that region. This means that should any part of the six curves in Fig. 3 lie below this line, it would be an indication that larvae were, on the average, not passing up and down the region in a simple manner but that larvae were either passing through the region in one direction only or passing through only part of the region. Should any part of a curve lie above the line, it indicates that larvae were either walking up and down a region more than once or moving about within a region with many turning movements.

Table 6 and Fig. 3 show that, on the average, larvae walk to the tip of a rod on the first upward trip, except in the case of the 90 cm rod where larvae only walked approximately half-way up the rod before descending. Larvae usually eventually reached the tip during the time spent on the rod, even if not on the first upward trip; the exception again being the 90 cm rod. The average duration of movement was greatest for the 50 cm rod. The short times spent on the 10, 15 and 30 cm rods were attributed to the fact that larvae frequently passed the point of release after the first trip up the rod. The time spent on the 90 cm rod was less than that on 50 cm rod as larvae would climb fairly high on the rod before returning to the base. The relatively long periods spent on the 40 and 50 cm rods were due to larvae making many short trips to and from the tip. The numbers of larvae coming to rest at the tips of the shorter rods were relatively low, while the number of larvae which came to rest at the tips of the 40 and 50 cm rods was higher. Larval activity appeared to increase with rod length up to approximately 40–50 cm. Larvae

climbing on the 90 cm rod were uniformly active over the first 10 regions but beyond this point activity decreased rapidly. From these results it appears that *A. hebraeum* larvae tend to make successful trips, in which they achieve a position of rest on the rod, on rods 40 and 50 cm high. This is consistent with field observations at Barville Park where this species was found to be associated with medium and tall vegetation (see Table 1).

In Table 7 a summary of findings resulting from a comparison of the behaviour of four other species of ticks, *B. decoloratus*, *I. pilosus*, *R. evertsi* and *R. appendiculatus*, walking on 40 cm rods is shown. The data for *A. hebraeum* larvae on the same rod length have been repeated in Table 7 for comparison. From the data in Table 7 it appears that the 40 cm rod was too tall for the four species studied when compared with *A. hebraeum*. If the average maximum height attained by larvae in any trip up the rod is taken as an indication of the 'optimal vegetation height' of each species, as is apparently indicated for *A. hebraeum*, then the other species may be listed in the following order with respect to their optimal vegetation heights: *I. pilosus* 15–20 cm, *B. decoloratus* 20 cm, *R. appendiculatus* 20–25 cm, *R. evertsi* 25 cm. The values for both *I. pilosus* and *B. decoloratus* fall into the range of vegetation heights in which these species were collected in the field survey reported earlier. The other two species were collected only in small numbers so their optimal vegetation heights could not be correlated with field data.

WATER BALANCE IN LARVAL TICKS

Water loss

Methods

Larvae of *A. hebraeum*, *B. decoloratus*, *R. evertsi*, *R. appendiculatus* and *Rhipicephalus simus* Koch were maintained in an incubator at 26 °C and approximately 95 % R.H. In addition, *I. pilosus* larvae, which were found to be difficult to maintain in an incubator, were brought into the laboratory from the field. Relative humidities of 30, 40, 50, 60, 70, 80, 90 and 100 %, obtained by means of potassium hydroxide solutions of appropriate concentrations (Petersen, 1953), were maintained in glass desiccators. All the work was undertaken at 26 °C unless otherwise stated.

Small glass vials were filled with batches of larvae and stoppered with cotton-wool. Eight vials of larvae, of each species, were placed in the eight different humidities referred to above. A single vial of each species was then removed daily from each desiccator and the number of dead and living larvae counted. Since difficulty was encountered in determining the point of death the following criteria of mortality were adopted. Death was presumed to have taken place when larvae did not move after stimulation by pressure from a needle or when larvae floated (due to air trapped in their limbs and bodies) in the weak soap solution used to facilitate counting. As relatively few larvae of *I. pilosus* were obtained, only a single vial of larvae was placed in each of the eight different humidity conditions. This involved daily counting of larvae without disposing of them. The criterion of death adopted for *I. pilosus* differed from that in other species and here larvae which could not be induced to move by gently warming the vials (by rubbing between the hands) were assumed to be dead.

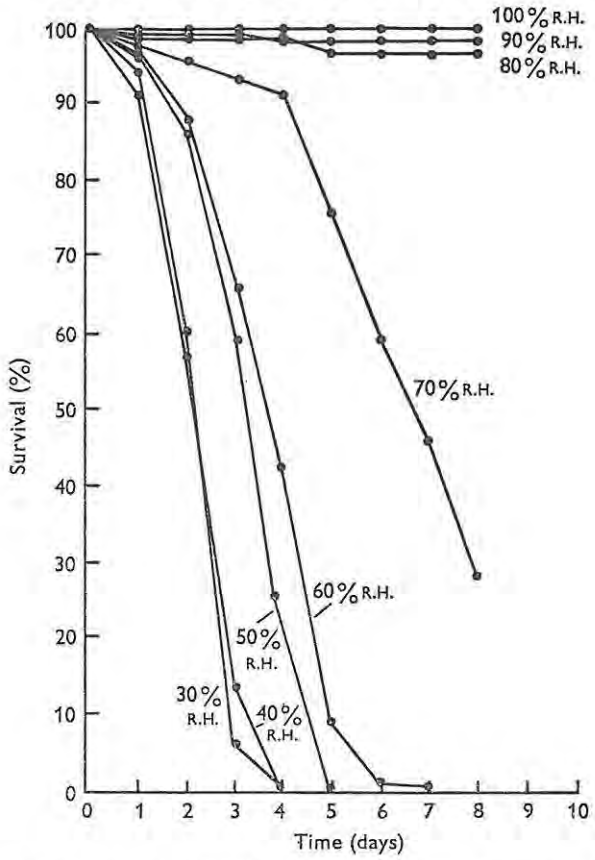

Fig. 4. The survival of *Amblyomma hebraeum* larvae under various conditions of relative humidity at 26 °C.

Results

The results shown graphically in Fig. 4 relate to *A. hebraeum*. All six species studied reacted similarly to the different relative humidities and therefore it is unnecessary to include the individual results obtained for the five other species. Survival was fairly constant when larvae were held at relative humidity levels in excess of 70 % (i.e. 7·53 mm Hg saturation deficit). At 70 % R.H. and less, the survival rate decreased with the lowering of relative humidity. Probits of the percentage survival against time for the larvae held at 70 % R.H. (Fig. 5) show four types of reactions in the six species studied:

(1) An initial resistance to desiccation followed by a gradual increase in mortality, e.g. *A. hebraeum*.

(2) An initial resistance to desiccation followed by a fairly rapid increase in mortality, e.g. *R. evertsi*.

(3) A gradual increase in mortality not preceded by a period of apparent resistance, e.g. *R. simus* and *B. decoloratus*.

(4) A fairly rapid increase in mortality not preceded by a period of apparent resistance, e.g. *I. pilosus* and *R. appendiculatus*.

Comparing the six species with respect to their abilities to resist desiccation was

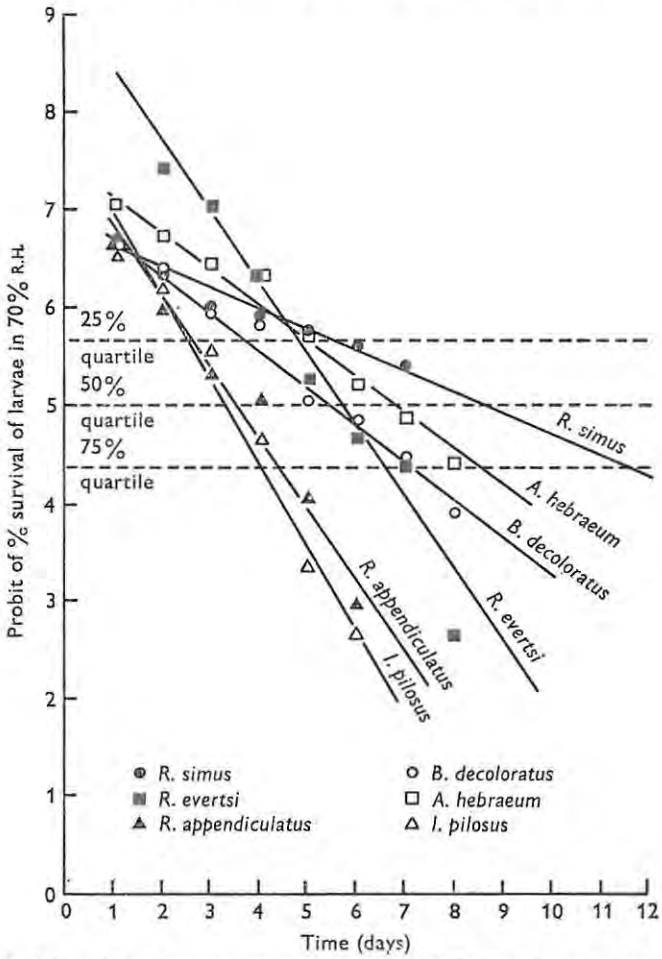

Fig. 5. Probits of the percentage survival of tick larvae in an atmosphere of 70% R.H. and 26 °C, plotted against time in days. Survival at the 25%, 50% and 75% quartiles are indicated.

difficult. At the 50% quartile the species may be listed in the following order of decreasing resistance: *R. simus*, *A. hebraeum*, *R. evertsi*, *B. decoloratus*, *R. appendiculatus*, *I. pilosus*. It should be noted, however, that if the comparison is made at the 75% quartile a slightly different order would result.

To provide quantitative evidence that death at low relative humidities is due to desiccation, large numbers of *R. appendiculatus* larvae were placed in six small glass tubes closed at both ends with fine nylon mesh (which was found not to absorb water vapour) and placed in an atmosphere of 40% R.H. A similar number of tubes was placed in an atmosphere of 95% R.H. All the tubes were weighed initially and at intervals over a period of 9 days so as to assess the weight lost by larvae due to water loss. The results are presented in Fig. 6 and show that death is probably due to water loss. Larvae kept at 40% R.H. also demonstrated visible signs of desiccation in the flattening of the idiosoma and this concurs with the observations on *Boophilus microplus* (Canestrini) (Wilkinson & Wilson, 1959).

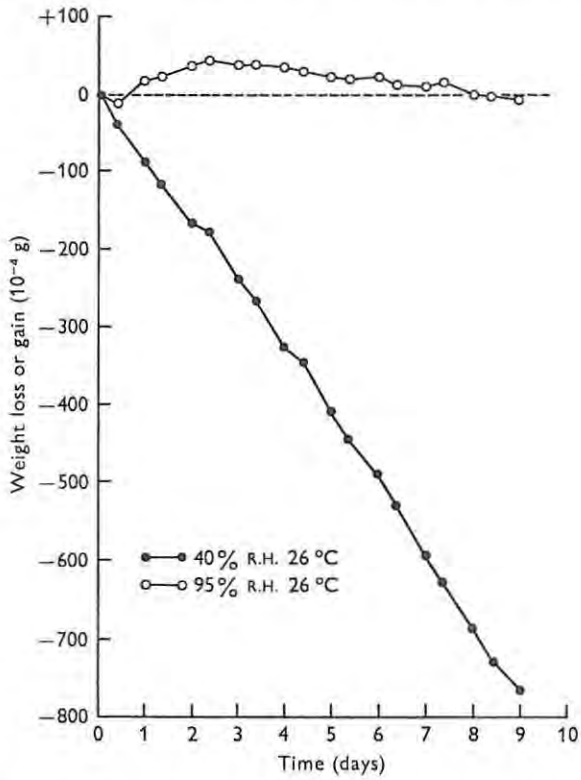

Fig. 6. The effects of low relative humidity on the weight of *Rhipicephalus appendiculatus* larvae.

The effects of temperature on water loss were studied in *R. evertsi*, *B. decoloratus* and *A. hebraeum*. Larvae were placed in small glass tubes similar to those described above, weighed, and enclosed in test tubes which were placed in a water-bath at 20° C. After a period of 30 min the tubes were again weighed and the temperature of the bath increased by 5 °C. This procedure was repeated until there was no further weight loss. The results are shown in Fig. 7. In order to establish the effects of death, which usually occurred at about 50 °C, dead and living *R. evertsi* larvae were compared in the same way. The results of the comparison demonstrated that both dead and living larvae responded similarly. The rate of water loss by evaporation (Fig. 7) was only slightly affected by temperatures below approximately 40 °C. Above this value the rate of water loss increased rapidly. At approximately 55 °C the maximum rate of water loss was recorded and at approximately 65–70 °C the rate decreased to zero. The sudden increase in water loss in the region of 40–45 °C was probably due to reorientation of the wax components of the cuticle as is known to occur in many insects (Wigglesworth, 1965) and other arthropods, including ticks (Beament, 1959).

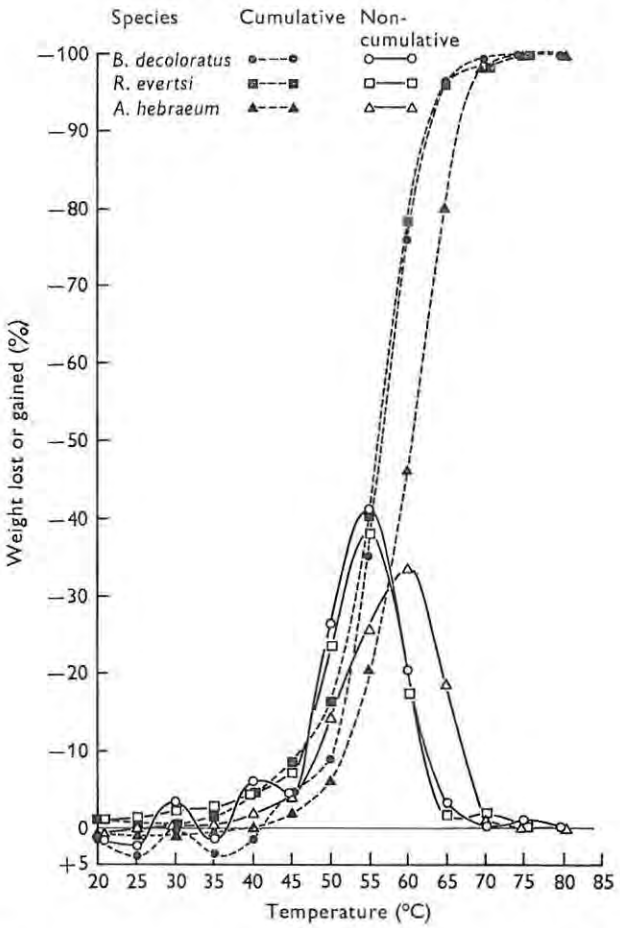

Fig. 7. The percentage weight lost or gained by tick larvae when exposed for $\frac{1}{2}$ h periods to increasing temperature values.

Water uptake

Methods

R. appendiculatus larvae were used to study the uptake of water vapour from the atmosphere. Lees (1946) and Hitchcock (1955) demonstrated that *I. ricinus* and *B. microplus* respectively were able actively to absorb water vapour from the atmosphere through the cuticle. *R. appendiculatus* larvae were placed in 24 glass tubes closed at both ends with nylon mesh. These tubes, after having been placed for an initial period of 12 h in an atmosphere of 95 % R.H. (at 26 °C), were weighed and transferred to a desiccator containing dry air, produced by the use of silica gel, for 24 h. The tubes were then reweighed and distributed equally among eight desiccators containing atmospheres of 30, 40, 50, 60, 70, 80, 90 and 100 % R.H. respectively. The tubes were subsequently weighed at intervals over a period of 96 h.

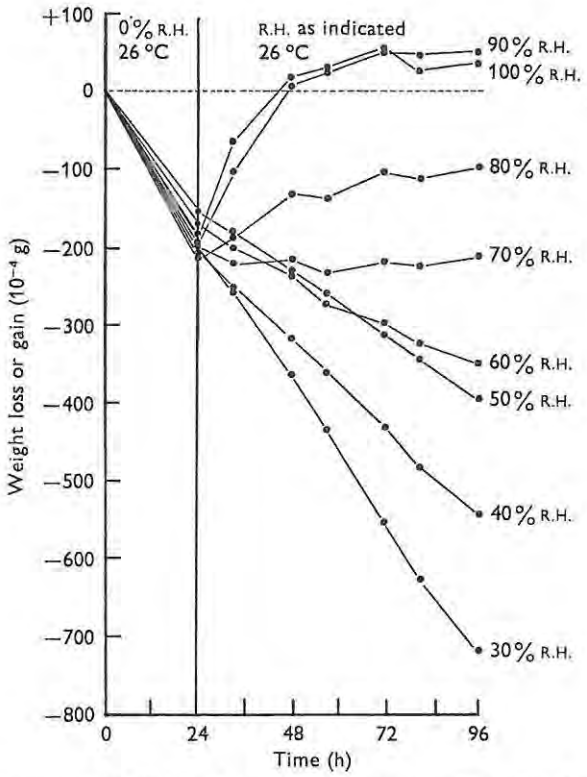

Fig. 8. Water uptake by *Rhipicephalus appendiculatus* larvae from various atmospheric relative humidities at 26 °C.

Results

The results appear in Fig. 8 and show that larvae were able to take up water vapour from atmospheres above 70 % R.H. Below this level water was lost to the atmosphere. This suggests that the 'equilibrium humidity' of *R. appendiculatus* is about 70 % R.H. at 26 °C (i.e. 7·53 mm Hg saturation deficit). As *R. appendiculatus* reacted in a very similar way to the five other species in the survival experiments reported earlier, it seems likely that all have equilibrium humidities in the region of approximately 70 % R.H.

The effect of different periods of desiccation on water vapour uptake were determined using *R. appendiculatus* larvae. Twelve tubes of larvae were placed for an initial period of 12 h in an atmosphere of 95 % R.H. and then weighed. Three tubes were then placed in an atmosphere of 90 % R.H. as a control. The remaining nine tubes were then subjected, in groups of three, for periods of 12, 24 and 36 h respectively, to a dry atmosphere before being weighed and placed together with the control tubes in the atmosphere of 90 % R.H. All 12 tubes were then weighed at 12 h intervals over a period of 10 days. The results appear in Fig. 9 and show that the length of the period of desiccation had no effect on the rate of water vapour uptake from damp air. Fig. 9 shows a gradual but steady decrease in weight in both the control and experimental tubes. The reasons for this are not known.

R. evertsi, *R. appendiculatus*, *B. decoloratus* and *A. hebraeum* larvae were used to

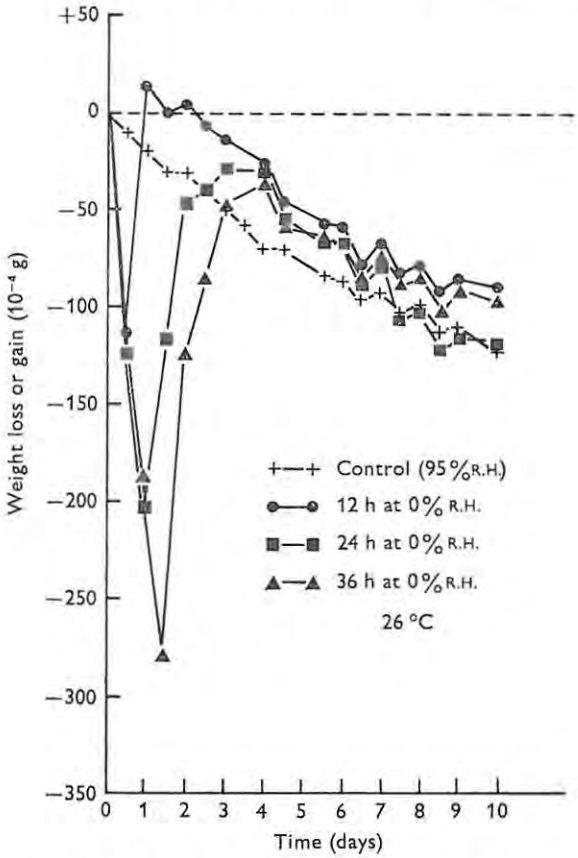

Fig. 9. The effects of different degrees of desiccation on the uptake of atmospheric water during subsequent exposure to high humidity by *Rhipicephalus appendiculatus* larvae at 26 °C.

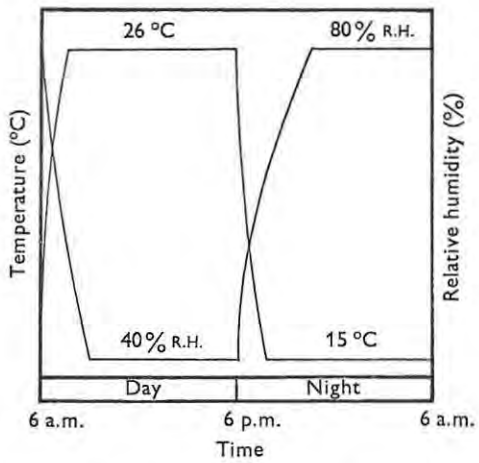

Fig. 10. Environment room régime while studying the survival value of free water on larval ticks.

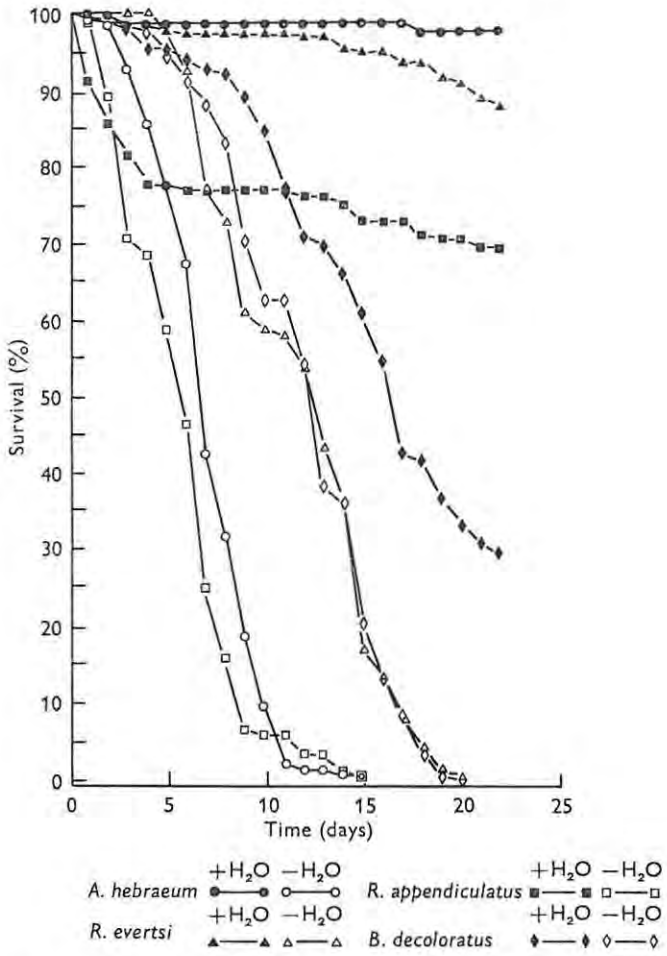

Fig. 11. The importance of free water to the survival of some ixodid tick larvae under environment room conditions.

study the phenomenon of drinking. All these species were observed to imbibe water through the mouthparts. The behaviour pattern was observed in the laboratory when larvae were placed in the centre of a Petri dish and encircled with moist filter paper. Under these circumstances the following sequence of events was observed. A larva would walk in a direct path towards the moist filter paper; on arriving within about 5 mm of the paper the larva showed an 'avoiding' reaction. As the moist filter paper formed a 'barrier' around the tick, radial movement of any distance would have resulted in it coming into contact with the paper. The avoidance reaction was performed on a number of occasions before contact with the paper was established. The larva, on encountering the moist filter paper, thrust the mouthparts into the water at the edge of the filter paper and even between the fibres of the paper. The pedipalps were always splayed outwards during this action in the same way as described by Arthur (1962) in feeding ticks. The larvae then remained motionless, except for active movements of the foregut as observed

through the translucent scutum. Allowing larvae to drink from paper impregnated with methylene-blue water resulted in them having a blue coloration of the gut. Later, the larva would suddenly become active again, remove its mouthparts, and either withdraw from the paper or walk directly on to its surface. Some of the larvae which were observed walking over the surface of the moist filter paper demonstrated a further behaviour pattern in that they would 'squat' on the surface of the moist paper. It is possible that when the ventral surface of the larva rested on the damp filter paper a further means of water uptake was provided. Some larvae demonstrated this 'squatting' behaviour without having previously imbibed water through the mouthparts.

In order to evaluate the role of drinking as a survival mechanism four species of larvae, *R. evertsi*, *R. appendiculatus*, *B. decoloratus* and *A. hebraeum*, were placed in Petri dishes and confined within them by means of a ring of Vaselin applied to the edges of each dish. Four dishes of each species were prepared. Into each dish was placed a piece of filter paper (4 × 4 cm). All the dishes were placed in a constant-environment room which was set to a régime in respect of light, temperature and relative humidity as shown in Fig. 10. Two Petri dishes of each species were supplied with a few drops of water every 12 h. The number of dead and living larvae were counted daily. The results (Fig. 11) show that larve in the presence of free water survive for longer periods than do those deprived of water.

DISCUSSION

Larval ticks are associated with definite microclimatic conditions. Very few larvae were collected from habitats which experienced saturation deficit values in excess of approximately 10 mm Hg over the midday period. Larvae were predominantly collected from habitats protected or covered by neighbouring trees and bushes. These findings support the observations of Kraft (1961) on a coastal farm in the Alexandria district. Survey data show that at least two species, *I. pilosus* and *H. silacea*, have activity peaks during the winter months. Theiler (1969) states that, with the exceptions of *I. rubicundus* and *Margaropus winthemi* Karsch, adult ixodids have their maximum peaks of activity during the summer months. The present findings therefore suggest that each stage in the life-cycle of ixodid ticks in South Africa may exhibit their greatest activity at different times of the year. As the difference in population density relative to the seasons is important in the management of dipping programmes this aspect should be investigated in an attempt to plan more efficient control.

Observations on the behaviour of larval ticks suggest that grass of an optimal height is required in order that they may be 'picked up' by a passing host. This 'optimal vegetation height' in the species studied seems to be species-specific, but the means of detecting such a parameter by larvae is not understood. The optimal vegetation height might, for example, be related to the host size. Theiler (1962) lists numerous hosts of the larval stages of the tick species collected at Barville Park and Faithful Fountain. *I. pilosus*, *H. silacea* and *B. decoloratus* could be 'picked up' by any sized host from the short vegetation with which the larvae are

associated. The larva of *A. hebraeum*, however, would probably be unlikely to attach to hosts much shorter than about 40 cm, because of its tendency to climb to the top of tall vegetation. Theiler (1962), however, lists many small species of birds from which *A. hebraeum* immatures have been collected, but it is not understood how larvae of this species are able to encounter these hosts from their vantage points approximately 40 cm above the ground.

Stampa (1969) has suggested that *I. rubicundus* could be kept at a low population density by altering the environment. The findings reported here indicate that the physical nature of the vegetation in respect to grass height and of concomitant humidity effects has a direct effect on larval distribution in the field by modifying their behaviour in the respective microclimates. It could be suggested that by reducing the vegetation height fewer larvae would successfully attain preferred resting sites at the tips of grass stems. In the case of *A. hebraeum* larvae, mowing or intensive grazing may have a direct effect on the number of larvae successfully encountering host animals and could be a means of reducing the population density.

Observations on water balance indicated that larvae of the species discussed survive in moist habitats. Laboratory tests demonstrated that larvae held at 26 °C were able to take up water vapour from the atmosphere in relative humidities of approximately 70 % R.H. (i.e. 7·53 mm Hg saturation deficit) or higher. Uptake appears to be fairly rapid and larvae in the field which lose water during the day would be able to replenish water in the high night relative humidities. Larvae are also able to imbibe free water and this behaviour is doubtless of survival value as dew is frequently available. As most habitats, with the possible exception of a few open sites, did not experience midday temperatures higher than the critical temperature of between 40 and 45 °C, and as all habitats experienced very high night relative humidities it is probable that microclimatic conditions are not the main delineating factors for larval distribution. It is suggested that one of the more important delimiting factors could be the effects of microclimate on the development of the egg stage. Habitats favouring egg development could be habitats in which larvae would be expected to occur. Lewis (1970) has shown that larval ticks are able to migrate over fairly large distances under the influence of wind. As air-current speeds were found to be negligible in most of the habitats in which larvae were found at Barville Park, it is unlikely that migrations of this type would normally occur in the species studied in the present investigation with the possible exception of *A. hebraeum*.

SUMMARY

The distribution of larval ticks in relation to vegetation cover was studied on two coastal farms in the Port Alfred district of the Cape Province. The following five species were found: *Boophilus decoloratus* (Koch), *Amblyomma hebraeum* Koch, *Ixodes pilosus* Koch, *Haemaphysalis silacea* Robinson and *Rhipicephalus evertsi* Neumann. *B. decoloratus* predominated in short protected vegetation, *I. pilosus* and *H. silacea* in short covered vegetation and *A. hebraeum* in medium-to-tall protected vegetation. *R. evertsi* was collected in too small numbers to allow any

correlation to be established. Both *I. pilosus* and *H. silacea* demonstrated activity peaks during the winter months. Microclimatic measurements indicated that larval ticks were not usually collected in microhabitats which experienced midday saturation deficits in excess of approximately 10 mm Hg. Behavioural studies on larval ticks climbing glass rods demonstrated the possible association of larvae with a definite vegetation height. The optimal vegetation heights were correlated with field data. The water balance of some tick species was studied and it was found that at 26 °C a relative humidity of 70 % or more (i.e. above 7·53 mm Hg saturation deficit) was required by these larvae. Larvae lost water to the atmosphere at humidities lower than this value and took up water vapour from the atmosphere at values higher than 70 % R.H. They were shown to be able to imbibe water through the mouthparts, and this possibly has survival value.

We thank Miss E. R. Norton of Barville Park and Mr G. Reed of Faithful Fountain for their co-operation over the duration of the investigation. Financial help was given by the Sir Percy FitzPatrick Trust (1970) and the Department of Agricultural Technical Services. Thanks are also extended to Professor B. R. Allanson, of the Department of Zoology and Entomology, Rhodes University, for his valuable advice during the course of the work, and to Professor Don R. Arthur of University of London, Kings College, England, for reading and offering constructive criticism of the manuscript.

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