The bio-ecology of the blue tick (*Boophilus decoloratus*) in the central Free State

By

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Declaration

I declare that the thesis hereby submitted for the Master of Science degree at the University of the Free State is an original work by the author under the supervision of Professor Leon J. Fourie. The thesis has not been submitted in any form to another University. I therefore cede copyright of this dissertation infavour of the University of the Free State.

Moeketsi Solomon Phalatsi

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Chapter One

General Introduction

Background

Ixodid ticks are economically important ectoparasites of livestock (Howell, Walker & Nevill, 1978; Masina & Broady, 1999; Regassa, 2001). They also act as vectors of major animal diseases such as, theileriosis, babesiosis, cowdriosis and anaplasmosis (Norval, 1994). An estimated 600 million cattle worldwide are at risk of babesia and anaplasmosis (Angus, 1996). Heavy tick infestation can also cause low meat and milk production (Regassa, 2001). Pegram, Tatchell, de Castro, Chizynka, Snick, McCosker, Moran & Nigarura (1993) estimated that 80% of the world's cattle population of 1281 million is at risk from ticks and tick borne diseases. About US$7000 million is lost annually as productive losses and costs to control ticks (Pegram *et al.*, 1993). The tick problem in Africa has been described by Pegram, James, Oosterwijk & Chizyuka (1991) as ambiguous since countries with no large scale intensive tick control programmes were keen to establish it whereas those where it was practised were looking at ways to stop it. In Kenya alone the annual costs for acarides was estimated at US$ 10M (Tatchell, Chimwani, Chirchir, Ong'are, Mwangi, Rinkanya & Whittington, 1986).

With specific reference to *Boophilus decoloratus* which is the subject of this study, it is a one host tick, which can complete the parasitic phase of its life cycle within three weeks on the same host (Baker & Ducasse, 1967). It also passes through several generations in one year (Norval, 1994; Dreyer, Fourie & Kok, 1998). The tick is primarily a parasite of larger domestic and wild ungulates. Cattle are, however, considered to be its main domestic hosts and heavy infestations may also occur on horses (Theiler, 1959; Walker, 1991). Other domestic animals appear to be much less important as hosts (Baker & Ducasse, 1967). The tick may also occasionally infest dogs (Theiler, 1959).

B. decoloratus is frequently implicated in the transmission of *Babesia bigemina* causing African redwater,b and *Anaplasma marginale* and *A. centrale*, causing gallsickness. In addition the tick can also transmit *Borrelia theileri*, the cause of spirochaetosis in various domestic animals (Walker, 1991). Heavy infestation on cattle can also cause skin damage (Dreyer *et al.*, 1998) and other deleterious effects on the host, such as irritation with subsequent anorexia and loss of body condition (Amoo & Dipeolu, 1992). The direct effect of *Boophilus* infestations is proportional to the number of ticks engorging successfully on the host (Uilenberg, 1992). A loss of 0.6-1.5g body mass per engorgd female *Boophilus microplus* was recorded (Surtherst, Maywald, Kerr & Stegena, 1983). *B. decoloratus* and *B. microplus* are closely related species and it has been shown that the body mass loss in calves as a result of *B. decoloratus* infestations may even be higher (0.8-9.0g) for each engorged female (Scholtz, Spickett, Lombard & Enslin, 1991). In the central Free State *B. decoloratus* is considered to be the most important tick species that should be controlled (Dreyer *et al.*, 1998)

The geographical distribution (Fig. 1.1) of *B. decoloratus* and *B. microplus* have been given by Howell *et al.* (1978). *B. decoloratus* is widely distributed in Gauteng, Mpumalanga, the Northern Province, Kwazulu Natal, Northern and Eastern Free State, Eastern Cape and the southern and western coastal belts of the Western Cape. Theiler (1964) suggested that *B. decoloratus* can occur anywhere regardless of altitude and frost, type of vegetation, summer or winter rainfall, provided the mean annual rainfall is adequate (>380mm/year).

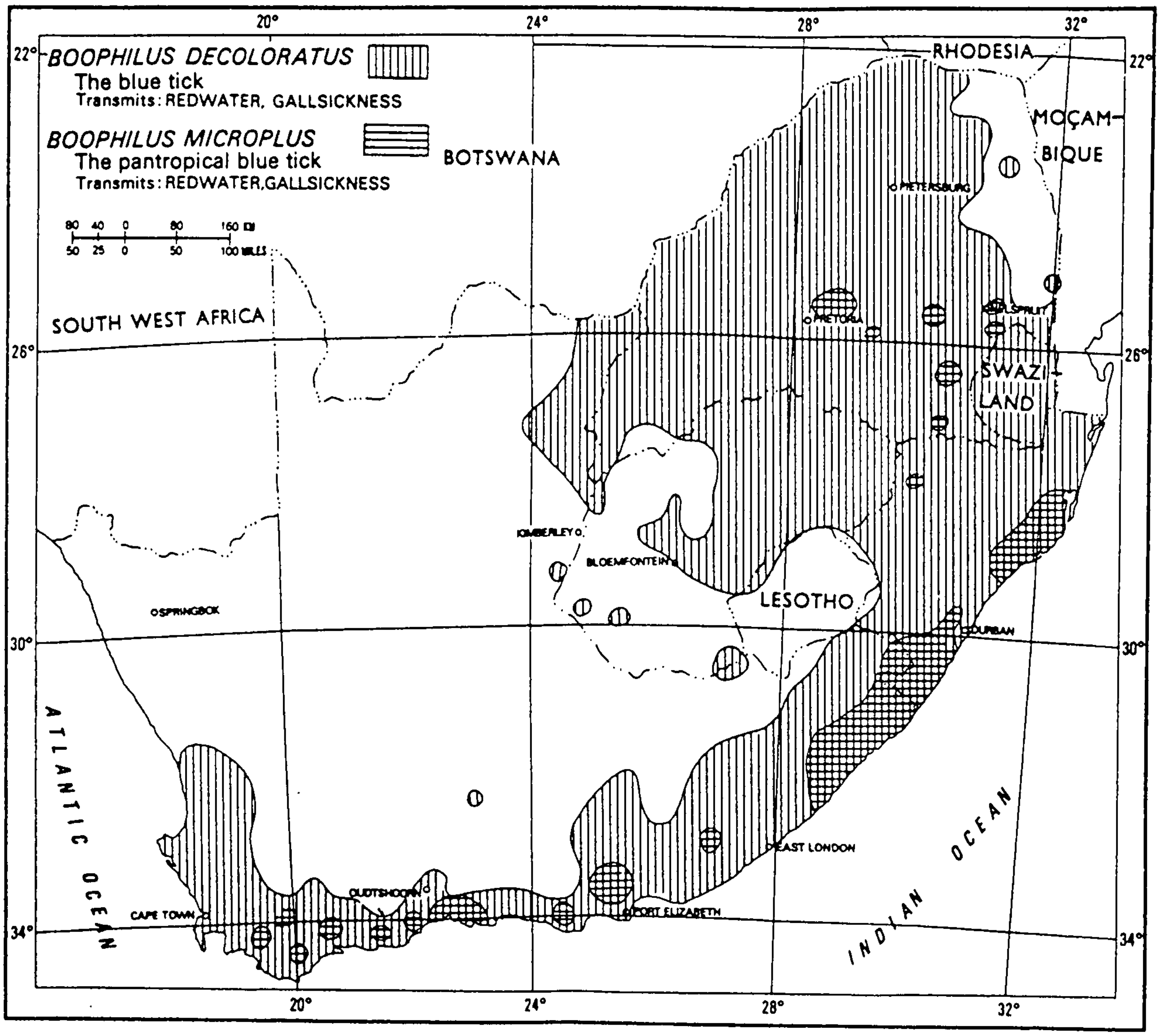

Figure 1.1 A map showing the distribution of *Boophilus decoloratus* and *B. microplus* in South Africa. (from Howell, Walker & Nevill, 1978).

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Chapter Two

The Study Objectives

The status of *B. decoloratus* as an economically important tick in South Africa, has caused it to be the target of intensive chemical control practices, and this has led to the development of resistance to almost all the currently available active ingredients (Fourie; unpublished data 2001). In the absence of efficient tick control programmes, production losses and mortalities may become significant. As such either new active ingredients should be made available for use or other methods of control, for example, vegetation manipulation, sterile male technique, use of pathogenic fungi, resistant hosts, etc. should be further explored and combined into integrated control strategy.

In order to formulate an integrated control programme a fundamental knowledge on the bio-ecology of the target pest species is required. Relatively little research work on the parasitic stages of *B. decoloratus* and also the bio-ecology of non-parasitic larvae and eggs have been conducted in South Africa. Rechav (1982) and Robertson (1981) reported on the seasonal abundance of *B. decoloratus* on farms in the Eastern Cape and Baker & Ducasse (1967) on the seasonal abundance in Natal. A study was also performed on the pre-hatch period and larval survival of *B. decoloratus* under natural conditions in the former Transvaal, South Africa

(Spickett & Heyne, 1990). Londt (1974) studied the pre-oviposition period of the blue tick under laboratory and field conditions and Londt (1977) the oviposition and incubation of *B. decoloratus*. Londt & Whitehead (1972) studied the ecology of larval ticks including the blue tick in South Africa. Spickett, Horak, Van Niekerk & Braack (1992) did investigations on the effect of veld burning on seasonal abundance of free living ixodid ticks in the Kruger National Park. A study on the cold resistance of *B. decoloratus* eggs and larvae was also done by Gothe (1967). An analysis on the relative resistance of six cattle breeds to *B. decoloratus* in South Africa was done by Rechav & Kostrzewski (1991).

Various other studies on the morphology (Gothe, 1967; Arthur & Londt, 1973), gonad development and gametogenesis (Londt & Spickett, 1976) and geographical distribution (Theiler, 1949), amongst others, were also conducted on *B. decoloratus*. The only study performed on *B. decoloratus* in the central Free State was by Dreyer, Fourie & Kok (1998) on the abundance, distribution and seasonal dynamics of the tick on cattle in the resource poor communities of Botshabelo and Thaba Nchu. The authors reported that *B. decoloratus* was the most abundant species constituting about 87% of the total number of ticks removed from cattle in the area.

The specific objectives of this study were:

1. To investigate aspects of the oviposition and reproduction of *B. decoloratus* with specific reference to:
    a. The effect of temperature and relative humidity on pre-oviposition, oviposition and incubation periods under laboratory conditions.

b. The effect of different combinations of temperature and relative humidity on daily patterns of egg laying under laboratory conditions.

c. The relationship between female engorgement mass and egg production.

d. The conversion efficiency and nutrient indices of engorged females.

e. The effect of field conditions on pre-oviposition, oviposition, incubation and survival of larvae.

2. To investigate tolerance of engorged *B. decoloratus* females to subzero temperatures.

3. To determine microhabitat selection of engorged female *B. decoloratus* ticks.

4. To investigate the bio-ecology of free living larvae with specific reference to:

   a. Survival of larvae exposed to different temperature and relative humidity regimes in the laboratory.

   b. Vertical migration patterns and height preference of larvae.

5. To investigate the seasonal dynamics of *B. decoloratus* parasitic on cattle with special reference to:

   a. Seasonal changes in the sex ratios and the ratios of immature and adult ticks.

   b. Seasonal occurrence of the free living larvae in the environment.

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Chapter Three

Study Area

Experimental Locations

The collection of ticks from cattle was carried out from March 1998 to August 1999 in Botshabelo (29°12'–29°18'S; 26°40'–26°45'E), a peri-urban area situated in the central Free State, 55km east of Bloemfontein. The investigation on the effect of natural fluctuating environmental conditions on the development and survival of engorged *Boophilus decoloratus* was carried out on the campus of the University of the Free State (28°50'S; 26°12'E) in Bloemfontein. Botshabelo and Bloemfontein are situated more to the central part of the Free State Province (Fig. 3.1). The topography of the Central Free State highveld is flat at an altitude of between 1200 and 1500 m above see level (Dreyer, 1997). The region also comprises of small hills and ridges, as well as rivers and streams flowing from east to west. The eastern area is situated at a higher altitude between 1500 and 1800 m above sea level (Mostert, Roberts, Heslinga & Coetzee, 1971).

Climate

The central Free State is situated in a grassland biome characterized by summer rainfall. The grassland biome receives an annual rainfall of between 400 and 2000mm (Rutherford & Westfall, 1986) (Fig 3.2). About 85% of the rain occurs in summer from October to March, with the maximum fall normally recorded in January (Fig. 3.3). Winter months (April to September) are normally dry. The area is also characterized by the highest frequency of hailstorms in South Africa. In general more than five hailstorms are recorded per year (Rutherford & Westfall, 1986,1994). The average daily maximum temperature ranges from about 27°C in January to 17°C in July whilst the average daily minimum temperature ranges from about 13°C in January to less than +1°C in July (Rutherford & Westfall, 1986,1994). Mean monthly atmospheric temperatures vary between 21°C recorded during January and 6°C recorded during July (Fig. 3.4). Frost occurs regularly during the winter months. The highest absolute temperature recorded at the station in Tweespuit was 39.4°C during January (1981-1998) and lowest was –12°C recorded during June (1981-1998).

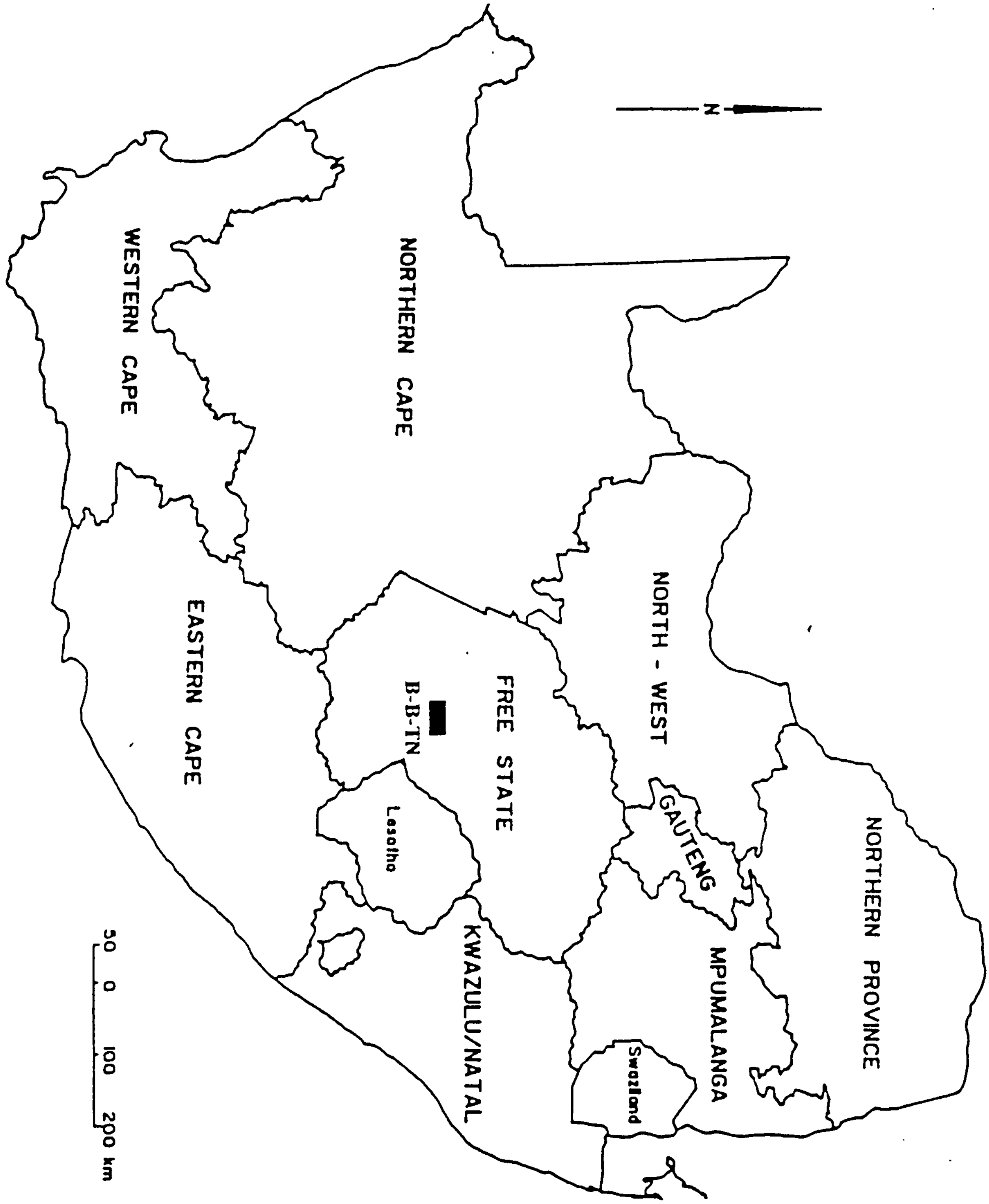
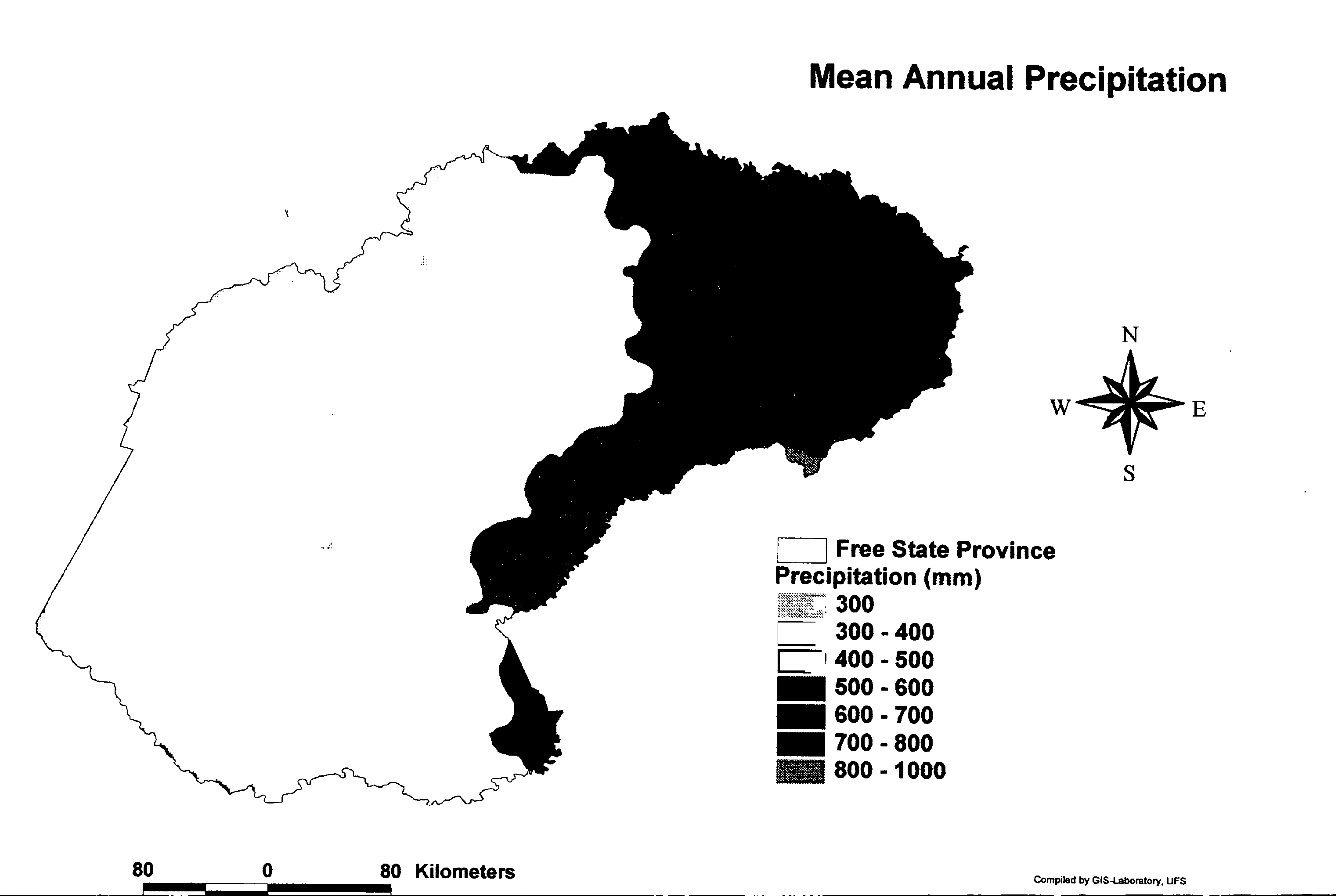


Figure 3.1 Location of the Free State Province with the Bloemfontein-Botshabelo-Thaba Nchu (B-B-TN), region indicated by ▬

Figure 3.2. Map of the mean annual precipitation (mm) of the Free State Province


Mean Annual Precipitation
N
W
E
S
Free State Province
Precipitation (mm)
300
300 - 400
400 - 500
500 - 600
600 - 700
700 - 800
800 - 1000
80
0
80 Kilometers
Compiled by GIS-Laboratory, UFS

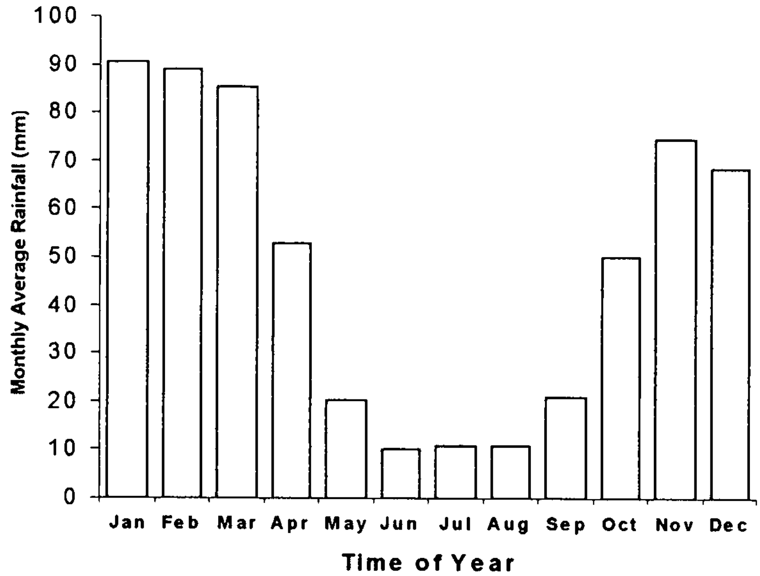


Figure 3.3. Histogram indicating the mean monthly rainfall from 1903 to 1998 recorded at Thaba Nchu, 8km from Botshabelo (from Institute of Soil, Climate & Weather, Pretoria).

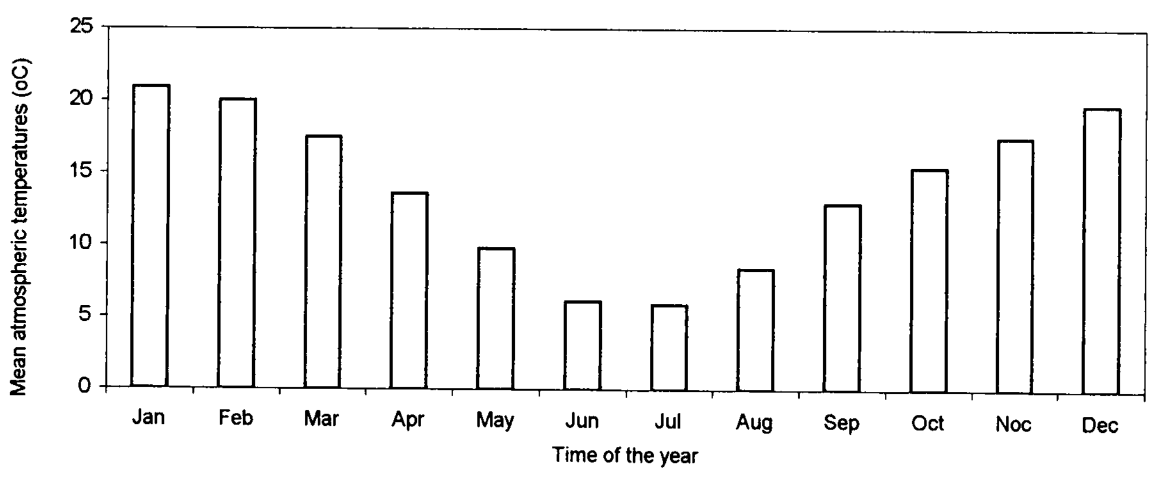


Figure 3.4. The mean monthly atmospheric temperatures (°C) from 1981 to 1998 recorded at Tweespruit (about 25 km from Botshabelo) (from Institute of Soil, Climate & Weather, Pretoria).

Vegetation

The Botshabelo area falls within the grassland biome of southern Africa (Acocks, 1975) (Fig. 3.5). The natural vegetation in the area is grassland classified as Themeda-Cymbopogon-veld (Acocks, 1975; 1988) (Fig. 3.6) or more recently as the moist cool highveld grassland (Bredenkamp & Van Rooyen, 1996). The area around Botshabelo is mixed veld consisting of both sweat and sour grasses (Van Oudtshoorn, 1999). Sweet grasses, in contrast to sour grasses, usually have a lower fibre content, maintain a higher above ground nutrient level during winter and tend to be more palatable to stock (Rutherford & Westfall, 1994). Typical grasses that do occur in the area are summarized in Table 3.1.

Table 3.1 Grasses that often occur in the Botshabelo area (Mostert *et al.*,1971: Van Oudtshoorn, 1999)

Grass species	**Nutritive Status**	**Common name**
Themeda triandra	High palatability	Themeda/ rooigras
Setaria sphacelata	High palatability	Creeping bristle grass
Microchloa caffra	Moderate palatability	Pincushion grass
Elionurus muticus	In palatable	Wire grass
Eragrostis chloromelas	High palatability	Curly leaf
E. racemosa	Moderate palatability	Narrow-heart love grass
E. capensis	Moderate palatability	Heart-seed love grass
E. plana	Inpalatable	Tough love grass
Cymbopogon plurinodis	Inpalatable	Turpentine Grass
Digitaria spp.	Varies according to species	Finger grasses
Tristachya leucothrix	Inpalatable	Hairy trident grass

Figure 3.5. A map showing divisions of vegetation types in the Free State Province.

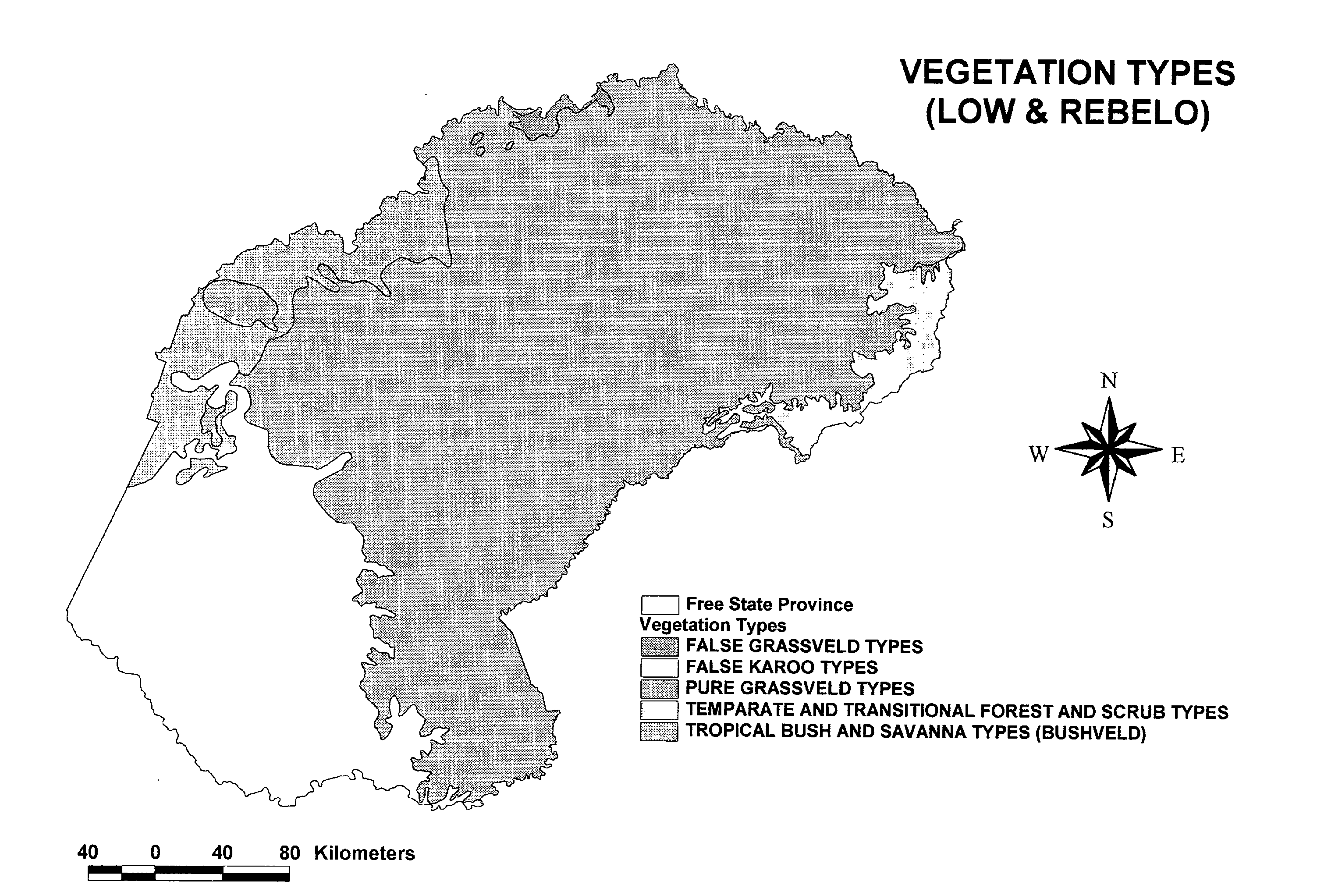
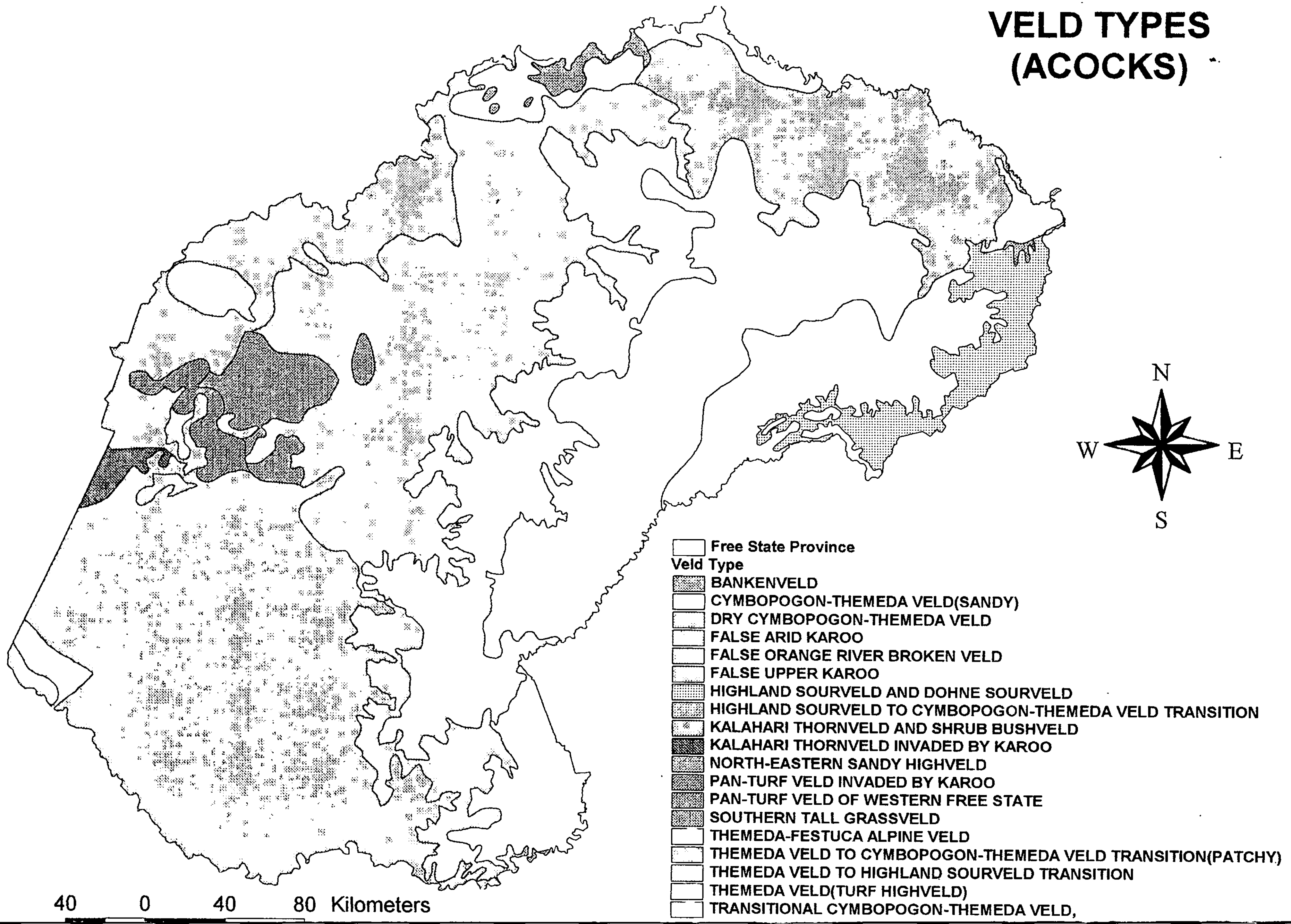

VEGETATION TYPES
(LOW & REBELO)
N
W
E
S
Free State Province
Vegetation Types
FALSE GRASSVELD TYPES
FALSE KAROO TYPES
PURE GRASSVELD TYPES
TEMPARATE AND TRANSITIONAL FOREST AND SCRUB TYPES
TROPICAL BUSH AND SAVANNA TYPES (BUSHVELD)
40 0 40 80 Kilometers

Figure 3.6. The distribution of veld types in the Free State Province.



VELD TYPES
(ACOCKS)
N
W
E
S
Free State Province
Veld Type
BANKENVELD
CYMBOPOGON-THEMEDA VELD(SANDY)
DRY CYMBOPOGON-THEMEDA VELD
FALSE ARID KAROO
FALSE ORANGE RIVER BROKEN VELD
FALSE UPPER KAROO
HIGHLAND SOURVELD AND DOHNE SOURVELD
HIGHLAND SOURVELD TO CYMBOPOGON-THEMEDA VELD TRANSITION
KALAHARI THORNVELD AND SHRUB BUSHVELD
KALAHARI THORNVELD INVADED BY KAROO
NORTH-EASTERN SANDY HIGHVELD
PAN-TURF VELD INVADED BY KAROO
PAN-TURF VELD OF WESTERN FREE STATE
SOUTHERN TALL GRASSVELD
THEMEDA-FESTUCA ALPINE VELD
THEMEDA VELD TO CYMBOPOGON-THEMEDA VELD TRANSITION(PATCHY)
THEMEDA VELD TO HIGHLAND SOURVELD TRANSITION
THEMEDA VELD(TURF HIGHVELD)
TRANSITIONAL CYMBOPOGON-THEMEDA VELD,
40 0 40 80 Kilometers

Soils

The most common soil group in the Grassland Biome is the red-yellow-grey latosol plinthic. This soil group is characterized by good internal drainage but has an impermeable underlying layer at about 1200mm causing some degree of periodic subsoil saturation (Rutherford & Westfall, 1994; Bredenkamp & Van Rooyen, 1996). Soils in the area also comprise a combination of black and red clays, solonetzic types and black clay (Rutherford & Westfall, 1994).

Cattle Management

Animal grazing in Botshabelo is predominantly communal. Either cattle owners or herd-boys herd animals into grazing areas during the day. Cattle are mostly kept for milk production, a reason for the domination of Friesian-crosses in the area (Dreyer, 1997). Animals are kept in kraals at night in the backyards. The kraals are made of a combination of iron fence, stones and/or wooden materials. Backyard kraals facilitate evening and morning milking of cattle and also protect them against theft. Animals are taken to grazing in the mornings and return home during late afternoon. Cattle in Botshabelo drink from gravel dams, when nearer to home, or from the "Klein Modder river." Unweaned cattle and the immatures are left at home grazing on the commonage around the settlement. Communal grazing is highly practiced in this area owing to the overall low income of the cattle owners. Animal farming in Botshabelo is mostly managed traditionally and mainly practiced as a part-time occupation. Cattle from different households mix whilst grazing. Other domesticated livestock such as sheep and goats also mix with cattle in kraals and in the pastures whilst grazing. The grazelands are unfenced and there are practically no pasture management practices done in the area.

The cattle are of poor quality and also poorly managed. Cattle production, management and the breeding of quality stock is virtually impossible. The maintenance of the animals is also difficult due to the fact that most livestock owners are subsistence and part-time farmers of a low-income group.

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Chapter Four

Engorged Female *B. decoloratus*

Introduction

Ticks, like insects are poikilothermic animals (Saunders, 1977). Their body temperature tends to change along with that of the ambient temperature. In poikilotherms, the ambient temperature markedly affects metabolic rate and other physiological processes (Oliver, 1989). This, however, does not mean that body temperature is always similar to ambient temperature since physiological processes and behavioural attributes can both affect body temperature. Ixodid ticks are estimated to spend about 94 to 98% of their lives off the host (Bennett, 1974; Howell, Walker & Nevill, 1978). It is under these situations that the ambient conditions act unavoidably on the ticks (Bennett, 1974). The survival of ticks as poikilotherms depends on their ability to complete their life cycle. Although tick survival may be greatly influenced by ambient air temperature, microclimatic conditions in the leaf litter may be the most important limiting factor (Lindsay, Mathison, Barker, McEwen, Gillespie & Surgeoner, 1999).

A number of factors are known to affect tick survival and their reproductive ability. These include temperature, relative humidity, quantity of blood meal, quality of blood meal and photoperiodism (Lancaster & McMillan, 1955; Londt, 1974; Diehl, Aeschlimann & Obenchain, 1982).

Temperature, for example, determines the rate at which pre-oviposition, oviposition or embryogenesis take place. Due to the direct correlation between the reproductive and the digestive system, ticks are able to convert a larger proportion of their blood meal directly into eggs in comparison to other arthropods (Diehl *et al.*, 1982; Oliver, 1989; Chilton & Bull, 1993).

Ixodid and argasid ticks display different reproductive strategies. Adults of all ixodids, except species of *Ixodes* (Prostriate ticks) require a blood meal to initiate the gonotrophic cycle. Metastriata ticks mate exclusively on the host, i.e. while feeding (Sonenshine, 1991). Argasid ticks on the other hand feed rapidly, and the females feed and oviposit frequently (i.e. multiple gonotrophic cycles) (Oliver, 1989). The mated argasid females deposit small (<500 eggs/cycle) egg masses. The capability of many gonotrophic cycles ensures extended survival of these ticks (Sonenshine, 1991). The percentage body mass (engorged female) converted to egg mass in ixodid ticks is normally over 50% (Van der Lingen, Fourie, Kok & Van Zyl, 1999) and can be as high as 74% (Hagras & Khalil, 1988). In the natural environment the females select a suitable microhabitat in which to oviposit. Walker (1970) and Howell *et al.* (1978) mentioned that these microhabitats may include cracks in the ground, areas under tufts of grass or similar hiding places. The prolonged existence of ticks in the environment has, however, resulted in the development of special adaptations to fluctuating and sometimes adverse environmental conditions (Bennett, 1974; Howell *et al.*, 1978).

The broad objectives of this study were to investigate, (a) oviposition and egg development in *Boophilus decoloratus* both under laboratory and natural environmental conditions, (b) microhabitat selection of detached engorged females, and (c) tolerance of engorged females to sub-zero temperatures.

Materials and Methods

A. Oviposition and egg development

The specific objectives of this section of the study were to determine:

- The effect of different combinations of temperature and relative humidity on pre-oviposition, oviposition and incubation period of *B. decoloratus.*
- The effect of different combinations of temperature and relative humidity on the daily pattern of egg laying of *B. decoloratus* females.
- The relationship between female engorgement mass and egg production.
- The conversion efficiency and nutrient indices of engorged females.
- The effect of field conditions on pre-oviposition, oviposition, incubation and survival of larvae.

(i). <u>The effect of temperature and relative humidity on pre-oviposition, oviposition and incubation period.</u>

Engorged female *B. decoloratus* ticks were removed from cattle at Botshabelo with the aid of a pair of forceps. The ticks were subsequently placed in perforated plastic containers and transported to the laboratory for experimental investigations. The engorged female ticks were weighed to the nearest 0.01mg. Each tick was assigned a reference number. Ticks were subsequently placed individually in numbered cylindrical (15 x 30mm) Perspex containers. The open ends of the containers were sealed with nybolt gauze (bolting cloth with pores of 200μm). Each container consisted of two equally sized sections that were screwed together. The small Perspex containers with ticks were placed in larger containers in which the relative humidity (RH) could be controlled through the use of saturated salt solutions. Temperature controlled incubation cabinets were used to expose females to temperatures in the range of 10 - 30°C (5°C intervals) and RH of 35% and 75% respectively (Table 4.1). Five ticks were used for each temperature and RH combination respectively, in order to determine pre-oviposition and oviposition periods and the pattern of oviposition.

Temperature °C	**Relative Humidity %**	
10	35	75
15	35	75
20	35	75
25	35	75
30	35	75

Table 4.1. Temperature and relative humidity regimes to which *B. decoloratus* females were exposed.

In order to determine pre-oviposition and oviposition periods, ticks were observed on a daily basis. Pre-oviposition period was taken as the period (days) from removal of the tick from the cattle until the deposition of the first egg. Oviposition period was taken as the period between deposition of the first and the last egg.

The effect of temperature and relative humidity on the incubation period of *B. decoloratus* eggs was also determined. The same sets of conditions, as mentioned above (Table 4.1) were used. The incubation period was taken as the time (days) from the laying of the first egg until hatching of the first larva. In order to determine this period the ticks and eggs were monitored on a daily basis. A linear graph depicting the relationship between the inverse of the incubation period against temperature was constructed.

(ii). <u>The effect of different combinations of temperature and relative humidity on daily pattern of egg laying</u>

In order to determine the daily pattern of egg laying engorged ticks were collected as before. Ticks were placed in individual containers, as described in (i) above, and exposed to different combinations of temperature and relative humidity (Table 4.1). Three ticks were exposed to each set of conditions. The eggs were counted on a daily basis and subsequently discarded. Eggs were counted under a stereomicroscope with the aid of a counter.

(iii). <u>The relationship between female engorgement mass and egg production</u>

An experiment was conducted to investigate the relationship between female engorgement mass and the number of eggs produced. Engorged *B. decoloratus* (n=16) of varying masses were collected from cattle in Botshabelo. Ticks (n=16) were cleaned and weighed to the nearest 0.01mg and placed individually in labeled plastic vials. They were exposed to a 75% RH and a temperature of 25°C. The onset of oviposition was determined and eggs were counted on a daily basis and recorded. The overall number of eggs produced by individual females was recorded against the mass of the tick and a linear regression graph constructed.

(iv). Conversion efficiency and nutrient indices of engorged female *B. decoloratus*

In order to determine the relationship between engorgement mass, egg mass and conversion efficiency and nutrient indices of engorged *B. decoloratus*, ticks of varying engorgement masses were collected from cattle as in (i) above and transported to the laboratory in Bloemfontein. In the laboratory, the ticks were cleaned and weighed to the nearest 0.01mg. Twenty-five ticks of varying engorgement masses were placed individually in plastic pill vials (10ml) with holes drilled in the lids to allow for air circulation. The containers were placed in airtight one-liter bottles with a saturated sodium chloride solution to provide a relative humidity of 75±3%. The one-liter bottles were placed in photographic black plastic bags and placed in incubators at a constant temperature of 25±2°C.

Ticks were monitored daily to determine the onset of oviposition. After completion of oviposition, the residual tick masses and egg batches were weighed. In order to determine the conversion efficiency index (CEI), which gives the percentage of the engorged female converted into eggs, the following formula from Bennett (1974) was used:

$$CEI = \frac{\textit{Mass of eggs}}{\textit{Initial mass of engorged female}} \times 100$$

The nutrient index (NI) given as a percentage, measures the amount of eggs produced from the blood meal ingested by the engorged female and is given by the following formula:

$$NI\ (\%) = \frac{\textit{Mass of eggs}}{\textit{Initial mass of female} - \textit{residual female mass}} \times 100$$

(v). Ovipositon and survival of *B. decoloratus* females under field conditions

In order to gain insight into the seasonal occurrence of free living and parasitic *B. decoloratus* larvae a study was designed whereby oviposition of engorged females, hatching of eggs and survival of larvae in the field were studied. An attempt was made to collect about six fully engorged female *B. decoloratus* on a monthly basis from March 1998 to August 1999. The mass of each tick was greater than 150mg. Each tick was placed in a specially designed cylindrical Perspex container (15x30mm). The open ends were sealed with nybolt bolting cloth with apertures of 250µm. Each container consisted of two equal-sized parts, which were screwed together. The containers were placed approximately 2cm deep in the ground and covered with a mixture of grass litter and sand. The placement site of the containers was on the campus of the University of the Free State. In order to prevent rodents from disturbing the containers a 1m square wire mesh cage was placed over the placement site for protection. The containers were monitored daily and the onset of oviposition, eclosion of eggs and survival of larvae recorded. Larval survival was recorded as the period between the date of egg eclosion and date all larvae were considered dead.

Soil temperature was monitored by placing a temperature probe 2cm deep in the soil at the tick placement site. Average daily Soil temperature was recorded on a MCS data logger. Atmospheric relative humidity for Bloemfontein was obtained from the weather bureau. Rainfall was measured on the campus of the University of the Free State.

B. Microhabitat selection

Engorged *B. decoloratus* females were collected from cattle in Botshabelo, taken to the laboratory, cleaned and weighed as mentioned in the previous section. Only ticks with masses ranging between 150 and 250mg were used for this investigation. The study was performed in an environmental room where temperature was maintained at 24±3°C with a light cycle of 14L:10D. An arena which contained four different soil textures, was constructed (Fig. 4.1). The soil textures simulated four ground microhabitats the tick may potentially encounter after detachment from the host. The textures were loose soil, loose soil covered with debris, gravel and compacted soil. Loose soil was taken from the field and sieved to remove stones. Debris (dried grass) was placed in patches on the loose soil. The gravel used consisted of 5-8mm sized particles. Hard soil was prepared by wetting clay soil, compacting it and letting it dry. Cracks formed on the surface as the clay soil dried.

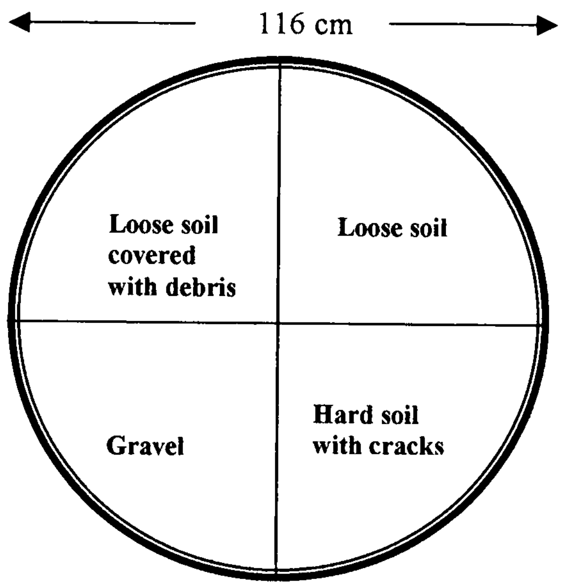

Figure 4.1. The composition and shape of the structure used for the microhabitat selection experiment performed in an environmental room.

The different soil textures were arranged in such a way as to form four equal sectors in a ring. The circular arena was made of a hard plastic material with a diameter of 116cm and a height of 35cm (Fig. 4.1). The soils were divided by pieces of cardboard, which did not prevent ticks from moving from one sector to the other. Prior to releasing the ticks into the arena moisture was sprayed over all the sectors.

Fifty engorged female *B. decoloratus* ticks were released within 24hr of removal from cattle. Ticks were released in the center of the arena by placing them on a piece of filter paper. The paper was removed immediately after all ticks had moved from the paper. Ticks were left unhindered for

48 hours before their position in the arena was determined and recorded. Typical recordings included the location of the ticks in the different sectors, and whether the ticks remained exposed on the surface or whether they occurred underneath the soil surface or debris. The study was repeated thrice and the data were pooled and an average calculated for the final analysis.

C. Tolerance to sub-zero temperatures

Engorged female *B. decoloratus* ticks were collected in Botshabelo, placed in perforated plastic bottles and transported to the laboratory. Only engorged female ticks with masses ranging between 150mg and 250mg were selected for the study. Ten engorged female ticks were used for each temperature and time exposure. The ticks were exposed to a range of sub-zero temperatures (-2, -4, -6, -8, -10°C). For each sub-zero temperature the exposure time was one to eight hours with an hourly increment. To serve as a control 10 engorged females were placed at 25±2°C and 75±2%RH. In order to obtain sub-zero temperatures Haake F3 circulating coolers were used. Conical flasks were place in the water of the circulating cooler, set at a specific temperature, two hours before the commencement of the actual experiment in order to obtain the required temperature in the flasks. The mouths of the flasks were initially opened for about one hour after immersion to let out the warm air inside the flasks, but were subsequently closed in order for the temperature inside the flask to equilibrate with that of the water in the coolers. The ticks were placed in nylon gauze for maximum exposure to the environment on the inside of the conical flasks. At the end of each exposure time, the ticks were removed from the flasks and placed individually in 10ml pill vials with perforated lids. The pill vials with ticks were maintained at 75±2% RH and 25±2°C. The transfer of ticks from the cooled flasks was done rapidly in order to allow the temperature in the flasks to re-stabilize rapidly for the following repeat or time exposure. The ticks were observed on a daily basis for egg laying and they were also categorized as dead or alive. In those cases where ticks were able to oviposit the viability (hatching) of the eggs was also monitored and recorded.

Results

A. Oviposition and egg development

(i) The effect of different combinations of temperature and relative humidity on pre-oviposition, oviposition and incubation

The mean, minimum and maximum pre-oviposition and oviposition periods of engorged *B. decoloratus* exposed to different temperature and RH regimes are summarized in Table 4.2. No eggs were laid by *B. decoloratus* females exposed to 10°C. On the other hand ticks exposed to 15, 20, 25, and 30°C, respectively, laid eggs. Females exposed to 10°C turned black and hardened after several weeks of exposure. The shortest mean pre-oviposition period (3.5days) was recorded at 30°C and 75% RH. The longest mean pre-oviposition period (13.2 days) was recorded at 15°C and a RH of 35%. A decrease in temperature resulted in an increase in the mean pre-oviposition period.

Table 4.2. The length of the pre-oviposition and oviposition periods (days) of engorged *Boophilus decoloratus* ticks exposed to different combinations of temperature and relative humidity.

Parameters		Mean Female mass (mg)	Pre-oviposition (days)			Oviposition (days)			No. of ticks
Temp °C	R.H. %		mean	min	max	mean	min	max	
10	75	185.744	-	-	-	-	-	-	5
	35	160.70	-	-	-	-	-	-	5
15	75	196.52	11.4	10	13	16.8	15	19	5
	35	186.93	13.2	12	14	17.2	15	20	5
20	75	220.52	9	8	10	17.6	17	19	5
	35	186.48	6.4	5	8	14.8	13	16	5
25	75	194.26	5.2	5	6	11.4	10	12	5
	35	172.36	5.6	5	6	11.2	9	14	5
30	75	215.61	3.5	3	4	10.6	8	12	5
	35	208.33	4	3	5	8	7	9	5

The relationship (R^2= 0.894) between temperature and the reciprocal of the pre-oviposition period (Fig 4.2) is described by the following equation:

$y=0.0123x - 0.1124$, where x= temperature (°C) and y= reciprocal of the pre-oviposition period (days). The regression line intersects the x-axis at 9.138°C which represents the developmental zero temperature for pre-oviposition (Fig. 4.2).

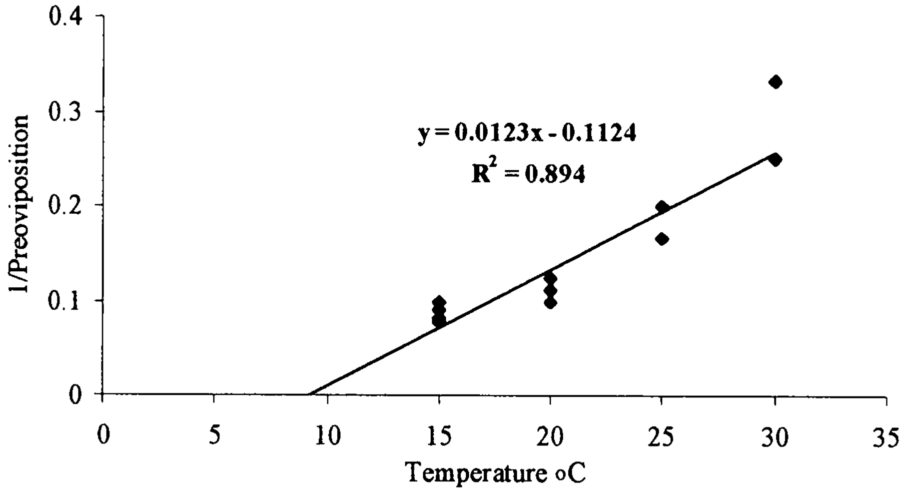

Figure 4.2. Linear regression illustrating the relationship between the 1/pre-oviposition period and temperature in *Boophilus decoloratus* females exposed to various temperatures at 75%RH.

Temperature also affected oviposition periods. The shortest mean oviposition period of eight days was recorded at 30°C and 35% RH whilst the longest mean oviposition period of 17.6 days was recorded at 20°C and 75% RH. In general the oviposition period was more extended at the lower temperatures. No fixed pattern in terms of the effect of relative humidity on the duration of the oviposition period was discernable (Table 4.2).

Incubation period was also affected by temperature. The shortest mean incubation period of 26.2 days was recorded at 30°C and 75% RH whilst the longest mean incubation period (73.4 days) was recorded at 15°C and 75%RH. No eclosion was observed at 10°C. Table 4.3 gives a summary of the results on the effect of different temperatures at a 75% RH on the incubation period of *B. decoloratus* eggs.

Table 4.3. The length of the incubation periods (days) of *Boophilus decoloratus* eggs exposed to different combinations of temperature at a 75% relative humidity.

Temperature (°C)	Incubation Period (days)			S.D.	Sample size
	Mean	Min	Max		
10	-	-	-	-	5
15	73.4	72	76	1.95	5
20	37.6	37	40	1.34	5
25	39.4	27	44	7.02	5
30	26.2	23	28	2.05	5

The relationship (R^2=0.7916) between temperature and the reciprocal of the incubation period is given by the following equation:

y=0.0015x - 0.0071, where x=temperature (°C) and y=reciprocal of incubation period (days). The x-intercept of the line which is the developmental zero temperature for incubation was calculated as 4.73°C (Fig. 4.3).

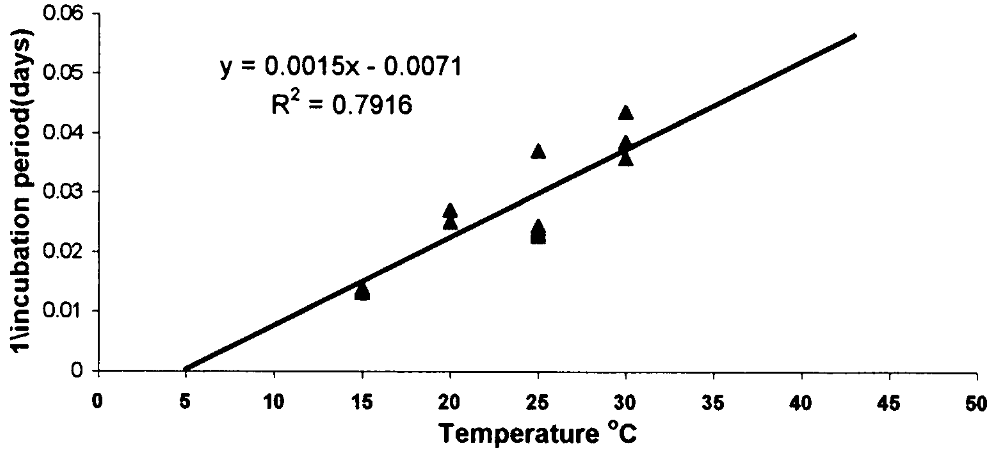

Figure 4.3. Linear regression illustrating the relationship between the 1/incubation period and temperature in *Boophilus decoloratus* eggs exposed to various temperatures at 75%RH.

(ii). The effect of different combinations of temperature and relative humidity on the daily pattern of egg laying

Figures 4.4 and 4.5 graphically represent the total daily number of eggs produced by *B. decoloratus* females when exposed to different combinations of temperature and relative humidity regimes. At 30°C and a RH of 75%, the mean number of eggs deposited rose steadily to reach a peak production of 533 eggs per day on the fifth day after which it decreased gradually to the 17th day. Females maintained at 25°C and a RH of 75% displayed a similar pattern with a peak mean daily egg production of 396 laid on the ninth day. In the case of the ticks maintained at 20°C and 15°C, respectively, and a RH of 75%, the increase in egg production was very gradual with peaks being reached at 14 and 17 days, respectively. Oviposition was also greatly extended and continued until day 25 and 28, respectively (Fig. 4.4). The oviposition patterns of females exposed to the various temperatures at RH=35% were basically similar to those recorded at RH=75%. Daily egg production decreased at the lower temperatures (15°C and 20°C) and oviposition took place over more extended periods of time (Fig. 4.5).

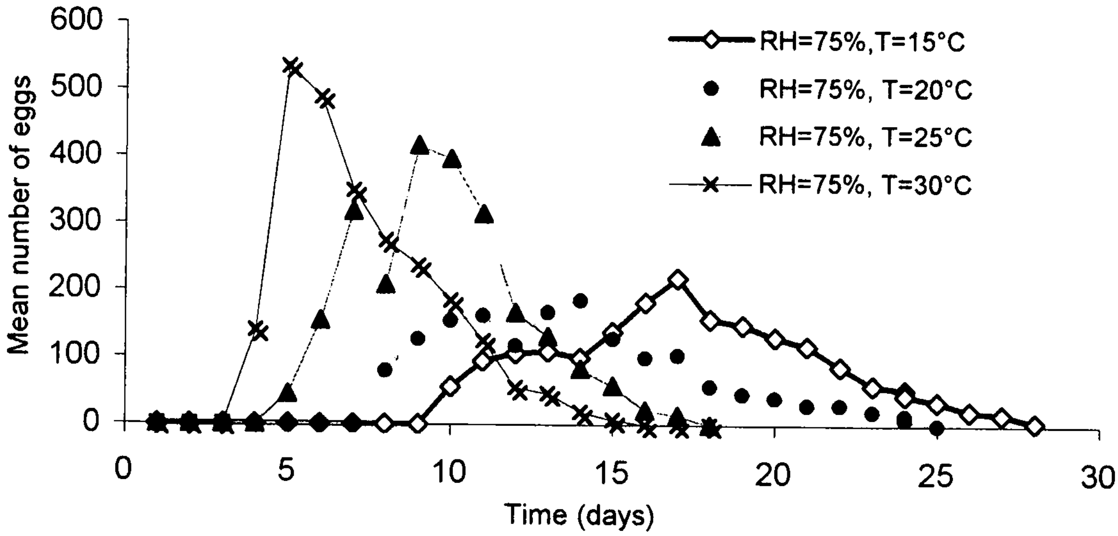

Figure 4.4. Mean number of eggs laid per day by *Boophilus decoloratus* ticks exposed to different temperatures at RH of 75%.

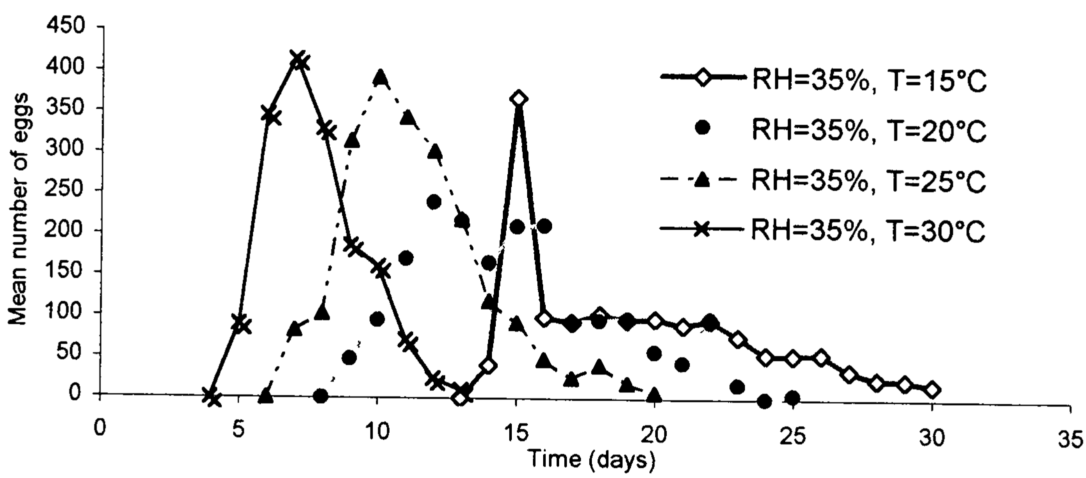

Figure 4.5. Mean number of eggs laid per day by *Boophilus decoloratus* ticks exposed to different temperatures at a RH of 35%.

(iii) <u>The relationship between female engorgement mass and egg production</u>

The number of eggs laid by *B. decoloratus* females of various engorgement masses are summarized in Table 4.4. A maximum number of 2968 eggs laid by a female weighing 240.91 mg was recorded. The least number of eggs (1264) was laid by a tick weighing 168.82. The general trend regarding female engorgement mass and egg production was that an increase in engorgement mass resulted in an increase in the number of eggs produced.

Table 4.4. The number of eggs produced by *Boophilus decoloratus* females of varying engorgement mass at 25°C and a 75% RH.

Tick No.	Female Mass (mg)	Number of Eggs Produced
1	158.72	1400
2	163.06	1385
3	165.74	1691
4	166.29	1550
5	168.82	1264
6	170.47	1797
7	172.8	2064
8	174.42	1824
9	197.43	1963
10	197.43	2120
11	203.99	2400
12	207.9	1941
13	208.53	2729
14	211.93	2155
15	223.88	2624
16	240.91	2968

The relationship between the number of eggs produced by the ticks and their engorgement mass is represented by a linear regression with a correlation coefficient of 0.799 (Fig. 4.6).

The regression equation is as follows:

y =17.759x-1373.6

Where

x = female engorgement mass (mg)

y= number of eggs

(Correlation coefficient) R^2= 0.7991

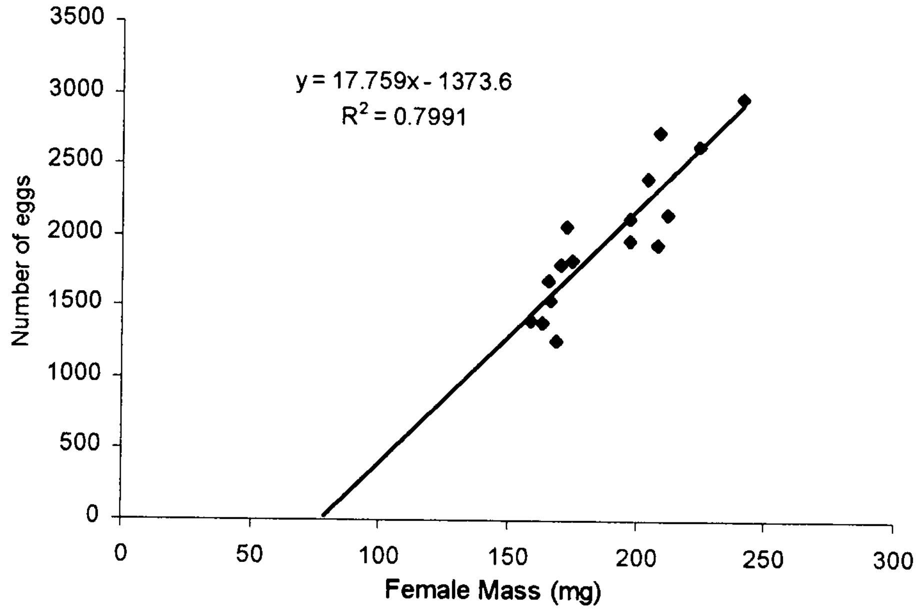

Figure 4.6. Linear regression indicating the relationship between female *Boophilus decoloratus* engorgement mass and the number of eggs laid at 25°C and a 75% RH.

(iv). <u>Conversion efficiency and nutrient indices of engorged female *B. decoloratus*</u>

Data on the weights of engorged female *B. decoloratus* used in this part of the study as well as the conversion efficiency and nutrient indices values are summarized in Table 4.5. Female tick masses ranged from 8.89 to 311.28mg. Ticks with engorgement masses of less than 44.36mg all failed to oviposit. Three heavier ticks also failed to oviposit due to damaged mouthparts, these were thus excluded from the analysis.

The general trend was for both the nutrient and conversion indices to increase with an increase in female engorgement mass. The highest CEI (63.41%) was recorded for a female weighing 252.93mg and the lowest (31.85 %) for a female weighing 50.01mg. The highest (74.36%) and the lowest (43.68%) NI values were recorded for females weighing 252.93mg and 75.18mg, respectively (Table 4.5)

The relationship between female engorgement mass CEI and NI values are graphically presented in Fig. 4.7. The relationships are best described by a logarithmic equation with correlation coefficients of R^2=0.8935 and R^2=0.8259 for the CEI and NI respectively. The relationship between the initial female engorgement mass and mass of the egg batch is represented by a linear regression with a correlation coefficient of 0.9918 (Fig. 4.8).

Table 4.5. Initial tick mass, the nutrient and conversion efficiency indices of *Boophilus decoloratus* females.

Tick Number	Initial tick mass (mg)	Conversion efficiency index (CEI)(%)	Nutrient index (NI) (%)
1	8.89	No oviposition	No oviposition
2	9.42		
3	14.44		
4	18.97		
5	19.45		
6	35.65		
7	44.36	34.31	49.29
8	48.42	36.78	53.47
9	50.01	31.85	44.50
10	75.18	31.88	43.68
11	77.68	41.34	57.05
12	87.41	40.01	51.13
13	90.72	45.62	63.17
14	135.22	46.95	62.17
15	146.79	58.74	70.09
16	189.36	57.25	69.14
17	218.70	57.64	68.34
18	229.80	59.77	73.31
19	252.93	63.41	74.36
20	262.27	60.02	73.76
21	311.28	59.75	72.46
Mean		49.35	61.73

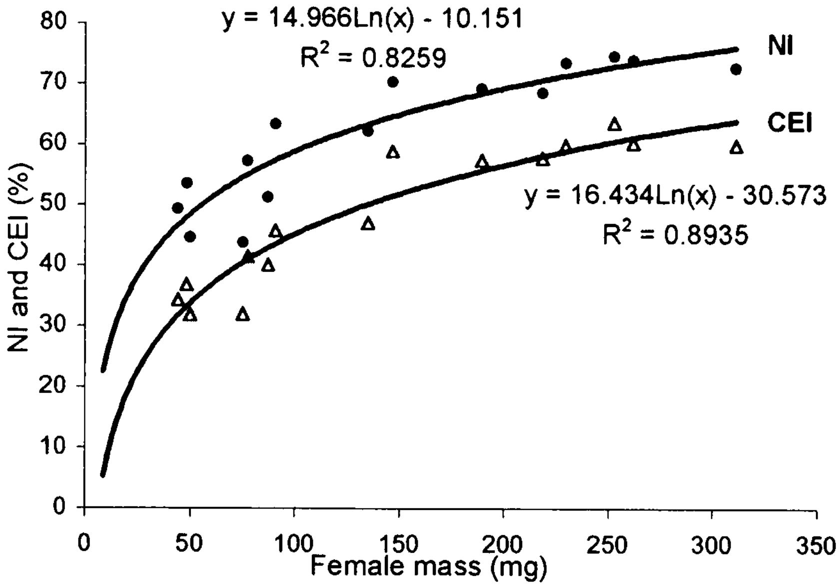

Figure 4.7. Relationship between conversion efficiency and nutrient indices and engorgement mass of individual *Boophilus decoloratus* females at 25°C and 75%RH (NI= Nutrient index and CEI= conversion efficiency index).

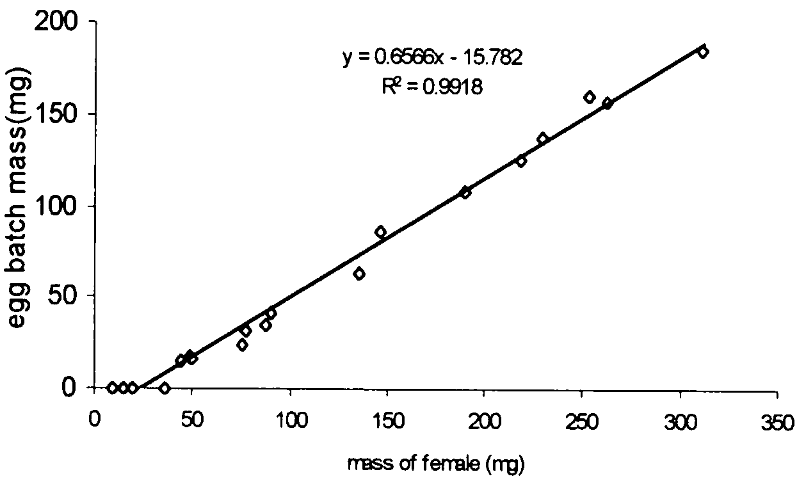

Figure 4.8. The relationship between egg batch mass and female *Boophilus decoloratus* engorgement mass (25°C and 75%RH).

(v). <u>Ovipositon and survival of *B. decoloratus* females under field conditions</u>

Engorged *B. decoloratus* females were exposed to ambient conditions from March 1998 to August 1999. The pre-oviposition period of the engorged ticks was very variable ranging from seven days for those ticks collected and exposed during January 1999 to 89 days for those ticks collected and exposed during May 1999 (Table 4.6). Ticks exposed during the warmer months (October- March) with mean daily temperatures varying between 18°C and 24°C in general had lower (<21 days) pre-oviposition periods compared to those ticks exposed during the colder months. Females exposed during May 1998 died before laying eggs. During September 1998 and July 1999 no engorged females were collected from the cattle.

Table 4.6. Summary of the pre-oviposition period, oviposition period and larval survival of *Boophilus decoloratus* exposed to naturally fluctuating conditions between March 1998 to August 1999.

Tick Placement month	Pre-oviposition period (days)	Oviposition Period (days)	Date of first egg eclosion	Larval Survival Period (days)
March 1998	11	30	Eggs dried out before eclosion	
April	31	31		
May	No eggs laid			
June	74	8	21/11/1998	56
July	33	58	20/11/1998	44
August	17	70	23/11/1998	38
September	No engorged ticks collected			
October	20	22	22/12/1998	31
November	8	23	12/01/1999	30
December	8	19	19/01/1999	24
January 1999	7	24	04/03/1999	26
February	21	27	05/04/1999	84
March	7	29	02/05/1999	56
April	36	21	20/07/1999	42
May	89	13	Eggs dried out, no larvae was produced	
June	67	15		
July	No engorged ticks were collected			
August	20	53	19/11/1999	28

As far as the incubation period is concerned the eggs laid by females, which were exposed during March and April 1998, and May and June of 1999, dried out and did not hatch. Rainfall for the period between April and September was low (Fig. 4.10) and may have contributed towards prolonged developmental periods for ticks exposed from June to August. As was the case with the pre-oviposition period, incubation periods were also strongly influenced by soil temperature. For females exposed during June 1998 the first eggs hatched during late September 1998.

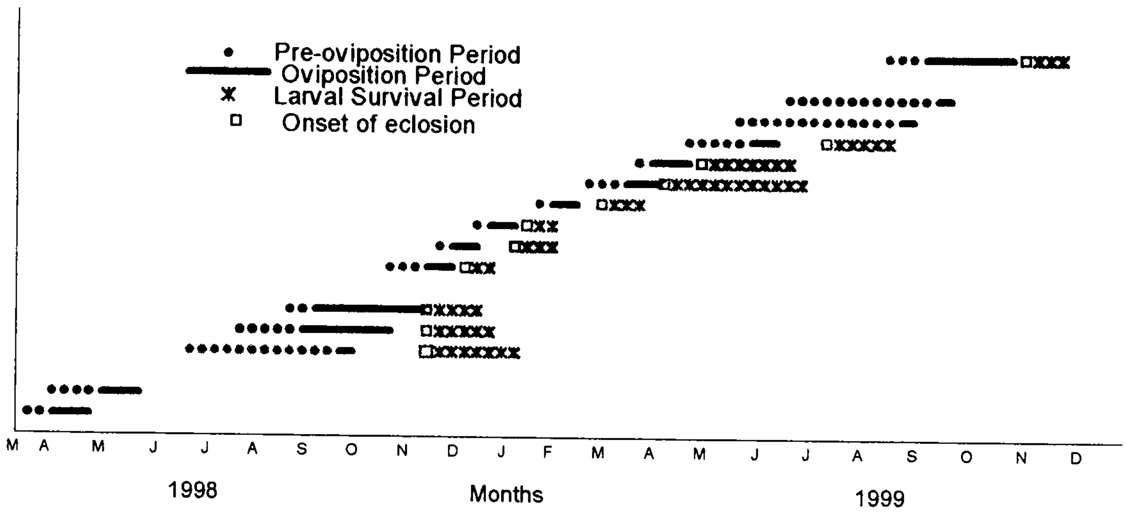

Figure 4.9. Monthly placements (March 1998 to August 1999) of engorged female *Boophilus decoloratus* in a natural environment showing the duration of the pre-oviposition, oviposition, onset of eclosion and larval survival periods

The total monthly rainfall and average temperatures for the period January 1998 to December 1999 as measured on the campus of the University of the Free State are presented graphically in Figs. 4.10 and 4.11, respectively.

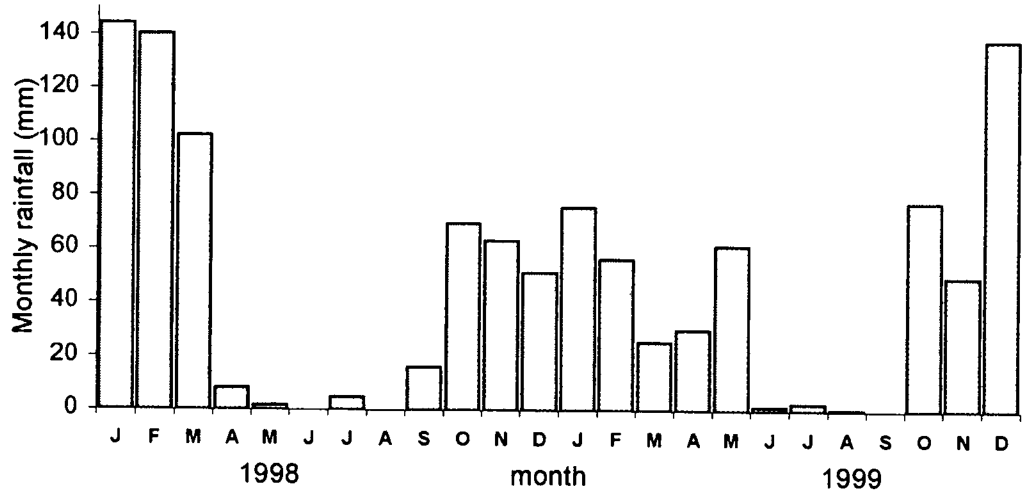

Figure 4.10. The monthly total rainfall for the period 1998-1999 as measured on the campus of the University of the Free State (Obtained from Department of Agrometeorology, University of the Free State).

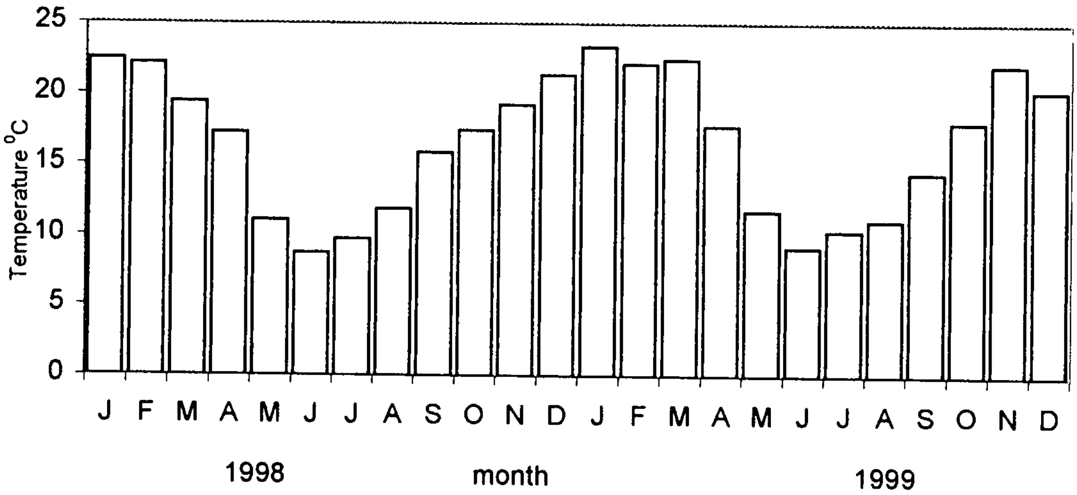

Figure 4.11. Average monthly atmospheric temperatures recorded on the campus of the University of the Free State from January 1998 to December 1999.

B. Microhabitat selection

Ticks were found both beneath and on the surface of the soil in the different sectors. The distribution of female ticks within the different sectors is presented graphically in Fig. 4.12. An average of 46% of all the recovered ticks were from the hard soil of which an average of 72.15% of the female ticks occurred in the cracks formed in the hard soil. The rest (27.85%) were found on top of the surface of the hard soil. Most of those that were found on the surface were recovered on the periphery of the arena. Female ticks recovered from the gravel constituted an average of 27% of which most (59.68%) occurred underneath the surface. Ticks beneath the surface were recovered by carefully removing layers of gravel and checking them for ticks. An average of 17% of the ticks was recovered from the loose soil. Most of these ticks (54.34%) occurred underneath the debris whereas the rest occurred on the periphery of the arena on top of the soil. None of the ticks were found beneath the soil surface. Most of the ticks found on the soil surfaces were still moving but relatively slower when compared to the time of release.

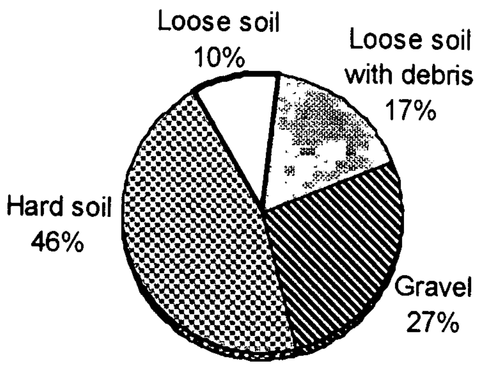

Figure 4.12. The average percentage of engorged female *Boophilus decoloratus* ticks recovered from different soil textures within an arena 48 hours after release in an environmental room.

C. Tolerance to sub-zero temperatures.

The percentage mortality recorded for engorged *B. decoloratus* maintained at different sub-zero temperatures for periods ranging from 60 to 480 minutes are summarized in Table 4.7. At a 60 minute exposure period no mortality was recorded except for 10% of the females which died at –10°C. At a 120 minute exposure period mortalities were also recorded at –8°C and the percentage mortality recorded at -10°C was also higher (40%). The general trend was for the mortality to increase at the different maintenance temperatures with an increase in exposure time. The highest percentage mortality (60%) was recorded for females exposed to –10°C for 420 and 480 minutes respectively (Table 4.7). All the females which survived exposure to the low temperatures laid eggs and the eggs hatched normally.

The results summarized in Table 4.7 were subjected to probit analysis (only exposures >240 minutes) in order to determine the LT_{50} values (temperature at which 50% of the females will die when exposed for certain fixed periods). For an exposure period of 300 minutes 50% of the females will die when exposed to –21.9°C. In the case of an exposure period of 480 minutes 50% of the females will die at –9.3°C (Table 4.8). The relationship between exposure time and LT_{50} values are presented graphically in Fig. 4.13.

Table 4.7. Percentage mortality recorded for *Boophilus decoloratus* females exposed for different time periods to sub-zero temperatures.

Temperature °C	Time periods (minutes)							
	60	120	180	240	300	360	420	480
-2	0	0	0	0	10	10	0	10
-4	0	0	10	0	40	10	20	20
-6	0	0	0	0	10	20	20	30
-8	0	20	20	40	20	30	30	40
-10	10	40	30	40	50	40	60	60

Table 4.8. Summary of probit analysis results indicating the LT_{50} values for different exposure temperatures.

Time	**LT_{50} (°C)**
300	-21.94263
360	-16.99901
420	-10.38562
480	-9.32353

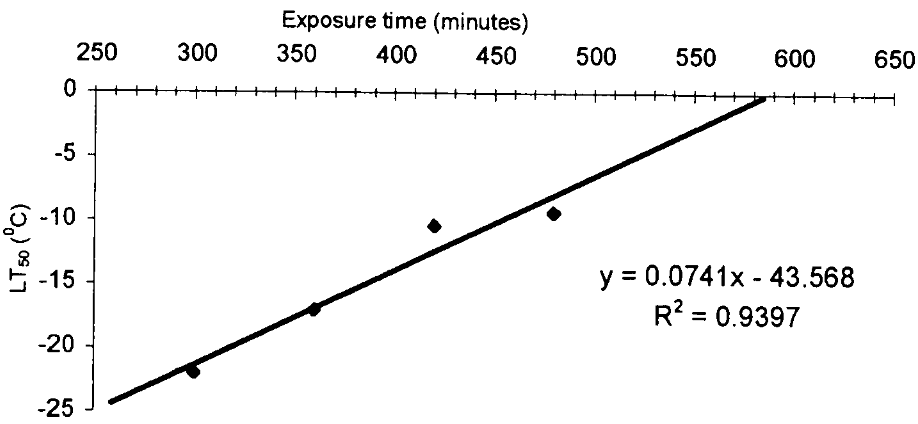

Figure 4.13. The relationship between exposure time and LT_{50} values for engorged female *Boophilus decoloratus*. Each point shows the temperature required for 50% of the females to die at certain fixed time exposures.

Discussion

<u>The effect of different combinations of temperature and relative humidity on pre-oviposition, oviposition and incubation periods</u>

The lengths of the pre-oviposition and oviposition periods are influenced by temperature (Fujimoto, 1989; Despins, 1992; Pereira, 1998; Van der Lingen *et al.*, 1999). Most studies on the factors involved in the control of egg production focused on cause-and-effect relationships (Diehl *et al.*, 1982). This was also evident in the present study, which showed that low temperature resulted in longer pre-oviposition periods. The shortest mean pre-oviposition period recorded in the present study was 3.5 days (T=30°C and RH=75%) and the longest 13.2 (T=15°C and RH=35%). From the results presented in this study it is evident that relative humidity has little or no effect on the pre-oviposition period. Similar observations have been made by Hichcock (1955) on *B. microplus* and Londt (1974; 1977) on *B. decoloratus*. Results of a study conducted by Short, Floyd, Norval & Sutherst (1989) showed that the pre-oviposition period of *B. decoloratus* and *B. microplus* ranged from 3-6 days (median 4.2 days) and 3-4 days (median 3.2 days), respectively. Recordings were made at 25°C and 85% RH. The corresponding values recorded for *B. decoloratus* at 25°C and 75% RH in this study were 5-6 days. This is fairly similar to the results of Short *et al.* (1989) and also to the mean value of 5.7 days (at 25°C) recorded by Londt (1974) for *B. decoloratus*.

In this study the constructed linear regression line between the reciprocal of the pre-oviposition period and temperature intersected the x-axis at 9.138 °C. The value may be regarded as the developmental zero (or lower critical temperature), below which no oogenesis or oviposition is expected to take place. In his study on *B. decoloratus,* Londt (1974; 1977) determined that the lower critical temperature is between 10 and 15°C. Estimates on the lower critical temperature for *B. decoloratus* by Short *et al.* (1989) and Spickett & Heyne (1990) confirm the above mentioned temperatures. Fujimoto (1989) and Van der Lingen *et al.* (1999) determined that the developmental zero temperatures for oviposition are 8.2°C and 9.2°C for *Ixodes ovatus* and *I. rubicundus* respectively. For *I. nipponensis* and *I. persulcatus* values of 5.6 and 2.9°C respectively, were recorded (Fujimoto, 1992). Ticks like other poikilotherms have a range of permissible developmental temperatures (Diehl *et al.* 1982). The available data suggest that the developmental zero temperature is a reflection of the environment in which the tick occurs. Lower values are adaptations to cool conditions.

Temperature does not only affect the pre-oviposition periods but also the length of the oviposition period. This has been shown to be the case for many tick species (Fujimoto, 1992; Chilton & Bull, 1993; Zehler & Gothe, 1995; Pereira, 1998; Van der Lingen *et al.*, 1999). Oviposition periods are much reduced at higher temperatures. Temperature influences the efficiency of blood meal utilisation and subsequent development rates. As far as incubation periods are concerned the trend in terms of the influence of temperature on the incubation period is similar to that of pre-oviposition period. The incubation period (39.4 days) recorded during this study at 25°C is slightly longer than the 33.8 days and 32.0 days recorded (Short *et al.* 1989) for *B. decoloratus* and *B. microplus* respectively. The above mentioned authors also recorded a developmental zero temperature for incubation at 10 and 8°C for *B. decoloratus* and *B. microplus* respectively. The 4.73°C recorded for *B. decoloratus* in this study is lower than expected and is probably due to a small sample size or lack of values for eggs incubated between 10 and 15°C. With reference to developmental temperatures for incubation, the value is normally close to that recorded for oviposition (Fujimoto, 1989; 1992).

The effect of different combinations of temperature and relative humidity on the daily pattern of egg laying

Female ixodid ticks have a strict gonotrophic cycle which is initiated by a single extended period of adult engorgement, followed by the maturation of one large egg batch and female senescence and death at the end of oviposition (Diehl *et al.*, 1982). Unlike the situation in argasid ticks, ixodid ticks have a single gonotrophic cycle (Sonenshine, 1991). The pattern of daily egg production is similar for many ticks in that peak egg laying occurs soon after the initiation of oviposition (Fujimoto, 1989; Van der Lingen *et al.*, 1999). The peak is most pronounced at higher temperatures. At lower temperatures the peak in egg laying is reached later and it is also not as pronounced as at higher temperatures. The maximum mean egg production (533) recorded in this study on day five at 30°C corresponds well with the 510 recorded on day four for *B. microplus* (Pereira, 1998). As mentioned in the previous section relative humidity did not affect the oviposition period or the pattern of daily egg production. This was also confirmed during a previous study conducted on *B. decoloratus* (Londt, 1977).

The relationship between female engorgement mass and egg production

Ticks are documented as some of the most prolific animals known because of the high conversion ratios of their life mass into eggs (Deihl *et al.*, 1982). Several workers have studied the relationship between the weight of fully engorged ixodid ticks and number of eggs oviposited (Despins, 1992; Pereira, 1998). The result of this present study is similar to that of previous studies, which also showed a linear relationship between engorged female mass and the number of eggs oviposited (Honzakova, Olejnjcek, Cerny, Daniel & Dusbabek, 1975). Diehl *et al.* (1982) suggested that at optimum conditions, the size of the blood meal is positively correlated with the number of eggs produced while at critical sub-optimum levels oogenesis may not be initiated or, if commenced, it may not be sustained. According to Howell, Walker & Nevill (1978; 1983) fully engorged female *B. decoloratus* ticks lay about 2500 eggs and *B. microplus* about 3000 eggs. In this study *B. decoloratus* weighing between 212 and 241 mg produced between 2155 and 2968 eggs. These values are in line with that reported in the literature. The average weight of a single *B. decoloratus* egg was determined at 50.22µg (Fourie, personal communication). One gram will thus contain 19 912 eggs which is similar to the 20 000 eggs/g reported by Rohr (1909) but less than the 24,750 eggs/g reported by Roulston & Wilson (1965) for *B. microplus*. Pereira (1998) also confirmed a value of 20 000 eggs per gram for *B. microplus*. The author, however, reported that the weight of eggs produced decreased from the 20th to 30th day of oviposition. Should these egg weights be used to determine eggs/gram, the value of 20 000 will no longer be reliable.

The results of this study have also shown that for those ticks weighing more than 200mg a mean of 11.4 eggs are produced per mg body mass. This value is markedly higher than the 5.6 and 2.5 eggs per mg body mass recorded for *Amblyomma limbatum* and *A. hydrozari* respectively (Chilton

& Bull, 1993). The value recorded for *B. decoloratus* is, however, similar to that (12.61) recorded for *Hyalomma truncatum* (Lithicum, Logan, Kondig, Godon, & Bailey, 1991) but in general lower than the values (15.0 – 17.7) recorded for *Ixodes rubicundus* (Van der Lingen *et al.*,1999). It is thus evident that *B. decoloratus* lay average sized eggs.

Conversion efficiency and nutrient indices of engorged female *Boophilus decoloratus*

The off host physical environmental factors and quantity and quality of the blood meal ticks imbibe from their hosts are the major determinants of the overall reproductive ability in ticks. In ixodid ticks the eggs are laid in a single gonotrophic cycle after which the tick dies (Diehl *et al.*, 1982). In this study ticks weighing between 8.89 and 35.65mg did not lay eggs while those weighing more than 44.36 mg laid eggs. It is thus evident that ticks must attain a threshold engorgement mass before oviposition can take place (Diehl *et al.*, 1982). The threshold oviposition mass may be regarded as the minimal engorgement mass the tick needs to undergo oogenesis. Kitaoka & Yajima (1958) also observed that lighter body weights of *Boophilus caudatus* failed to lay eggs. The engorgement stage attained at the time of tick detachment would then determine the females' ultimate reproductive success, a situation Kaufman & Lomas (1996) referred to as part of the ticks' "reproductive strategy." When ticks are removed from the host, they will re-attach to a host only if they are below their critical engorgement mass. If ticks attain a body mass above the threshold mass they will not reattach but proceed to produce a batch of eggs.

According to Balashov (1972) ixodid ticks generally lay eggs after reaching about 10% of their normal engorgement mass. The theoretically calculated threshold engorgement mass of *B. decoloratus* in this study was 18.04 mg which is similar to the value of 17mg reported for *B. microplus* (Bennett, 1974) and 20.5mg reported for *B. decoloratus* (Londt, 1977). The theoretically calculated value for *B. decoloratus* is, however, less than the actual recorded value of 44.36mg. It should, however, be mentioned that of the six ticks that did not lay egg in this part of the study, one weighed 35.65mg and the rest of the ticks less than 20mg.

In this study there was a definite trend for the CEI values to increase with tick weight. The average value of 49.36% recorded in this study is close to the 53.3% recorded for *B. annulatus* (Davey, 1993). As far as the NI values are concerned a similar pattern was observed, the values were in general 10 – 15% higher compared to the CEI. The data thus suggest that lighter ticks (>100mg) are not as efficient in converting the blood meal to eggs. The recorded NI values in this study are about 10 –15°C less than the values recorded for specific weight classes of *B. microplus* females (Bennett, 1974).

Oviposition and survival of *B. decoloratus* under field conditions

Engorged *B. decoloratus females* in this study were exposed to fluctuating natural conditions monthly from March 1998 to August 1999. The ticks' developmental stages were closely monitored to record their survival periods and seasonal behaviour. Temperature is regarded as a major determinant of the rate at which ticks develop (Londt & Whitehead, 1972; Londt, 1974; Fujimoto, 1989; Short *et al.*, 1989; Spickett & Heyne, 1990; Davey, Pound & Cooksey, 1994;

Fourie & Horak, 1994; Van der Lingen *et al.*, 1999). Short *et al.* (1989) and Spickett & Heyne (1990) showed that climatic changes have a marked effect on survival of detached females and unfed stages of *B. decoloratus*. In this study females exposed during May 1998 died during late winter (August 1998) before they laid eggs. Death was probably due to a prolonged exposure to low temperatures. Londt (1974) suggested that if oviposition is inhibited long enough death subsequently results. On the other hand ticks placed out during May and June 1999 managed to oviposit during spring. This seemingly contradictory result was due to difference in mean monthly temperatures with those for 1999 being higher compared to 1998. Long pre-oviposition periods and oviposition during colder seasons may be expected due to the influence of temperature on these processes (Londt, 1974; Spickett & Heyne, 1990). A similar study on *B. decoloratus* under natural conditions in the former Transvaal illustrated that eggs failed to develop into larvae during mid-winter months where mean temperatures were below 10°C (Spickett & Heyne, 1990). In the present study eggs laid during April and May 1998 did not accumulate sufficient temperature for eclosion to take place. It was also evident that the extended periods of low temperature also affected fecundity. Females exposed during June of 1998 laid a small batch of eggs over a period of eight days after a pre-oviposition of 76 days.

High initial temperatures and rapid temperature accumulation following tick detachment during summer months resulted into short pre-oviposition periods. High temperatures increase metabolic rate in poikilothermic organisms, which in turn accelerates physiological processes involved in egg production resulting in shortened pre-oviposition, oviposition and prehatch periods (Despins, 1992). Pre-oviposition periods in this study were shortest in January 1999 (7 days) and longest (89 days) for ticks placed out during May 1999. Eggs that were laid during the earlier part of winter dried out and failed to produce larvae. Temperatures in winter were below 10°C, which is close to critical minimum temperature below which hatching does not take place. This is consistent with results of Londt (1974; 1977) and Spickett & Heyne (1990) where no egg development was observed below 10°C.

Larval survival periods were inversely dependent on increased temperature accumulation (Spickett & Heyne, 1990). Larvae that hatched during cool months survived for longer periods (12 weeks, hatched in April 1999) whilst those that hatched during warmer months (January 1999) survived for just over three weeks. Spickett & Heyne (1990) showed much longer survival periods (10-35 weeks) compared to the results of this study. A possible explanation is the differences in prevailing microclimatic conditions.

Microhabitat selection of adult *B. decoloratus*

Detached engorged female ticks face a number of threats in the natural environment including desiccation, freezing and predation (Short *et al.*, 1989; Chilton & Bull, 1993). Though fully engorged female ticks are usually impeded by their enlarged bodies against extensive movement after detachment (Patrick & Hair, 1979), they execute interesting behaviour in the choice of the final microhabitat in which they would eventually oviposit their eggs. From the results of this study it is evident that engorged female *B. decoloratus* ticks do not dig into the soil. The ticks seek refuge in cracks and openings in between gravel particles, or underneath debris. Similar observation were made on engorged *Amblyomma americanum* ticks, where 34 out of 42 released ticks, were found aggregated at a protective site at the base of a clump of grass or crevice in the ground within the experimental arena (Patrick & Hair, 1979).

The hiding behaviour displayed by ticks is most probably an adaptation which enables the tick to protect itself against unfavourable environmental conditions for egg development and also to protect itself from its potential predators. The ability of a tick to select a favourable microhabitat for its survival, development and one that provides an opportunity for larvae to contact a potential host is associated with the evolution of a species in quest of conditions favourable for its survival and development (Oliver, 1989).

Tolerance to sub-zero temperatures

Survival of poikilothermic animals at subzero temperatures depends on physiological and biochemical characteristics that can be described as cold hardiness. There are two general strategies which are adopted by animals. An animal can be freeze-intolerant and die when there is internal ice-formation or it can be freeze tolerant and survive extensive freezing of tissues (Schmidt-Nielsen, 1991)

Studies on the cold hardiness of insects have basically indicated three different scenarios. Firstly, direct chilling injury where death is induced in response to chilling episodes of less than two hours, secondly indirect chilling injury occurs when longer exposures (days or weeks) are required to induce death. Thirdly innoculative freezing occurs due to contact with external body fluids (Burks, Stewart, Needham & Lee, 1996). Animals differ in the range of temperatures they can tolerate. Some have a very narrow tolerance range, others have a wide tolerance range. Temperature may change with time and a degree of adaptation is possible so that continued exposure close to the limit of tolerance extends the limit (Schmidt-Nielsen, 1991).

In this study the engorged ticks were not gradually exposed to lower temperatures or acclimatized to the low temperatures in order to allow for adaptation but rather the ability of the ticks to withstand an acute exposure (chilling injury) to subzero temperatures was assessed. From the results it is evident that *B. decoloratus* are able to withstand subzero temperatures. Mortality, however, increased with an increase in exposure time. At an exposure temperature of -10°C and an exposure time of eight hours , 40% of the females still managed to survive and lay eggs which hatched normally. Dautel & Knulle (1997) suggested that freezing in arthropods is probably a function of both temperature and time. At a temperature slightly above the freezing point, crystallization may be expected to occur in some ticks with increasing time of exposure.

Laboratory studies on the cold hardiness of ticks have shown that in the Antarctic tick, *Ixodes uriae* crystallization temperatures (synonymous with the supercooling point) ranged from –21 to –7°C in the post embryonic stages, with no indication of freeze tolerance or increases in cold tolerance following acclimation to low temperatures (Lee & Baust, 1987). A similar observation was made on *Amblyomma americanum* where acclimation at 5°C for seven days had no influence on supercooling points for unfed males and females, engorged nymphs and larvae, and eggs (Needham, Jaworski, Chen & Lee, 1996). Supercooling points for *Dermacentor variabilis, Amblyomma americanum* and *Ixodes scapularis* ranged from <-22 to –18°C for nymphs, and –22 to –8°C for adults (Burks *et al.*, 1996).

In this study the supercooling points for engorged *B. decoloratus* females were not determined. Based on the results obtained when ticks were exposed to subzero temperatures for two hours, it is evident that direct chilling injury can occur at temperatures <-8 °C. This may also be indicative of the supercooling point for engorged *B. decoloratus.* Temperatures in the central Free State may very occasionally drop below -8°C and this will induce mortality as a result of inoculative freezing. Freezing of water between soil particles may bring the ticks into direct contact with external ice and as such causes crystallization of body fluids.

Those ticks that did survive exposure to subzero temperatures oviposited and their eggs hatched normally. In an investigation into the cold hardiness of the eggs and larvae of *B. decoloratus, B. microplus* and *Margoropus winthemi* it was shown that *M. winthemi* is the most cold hardy followed by *B. decoloratus* and lastly *B. microplus* (Gothe, 1967). Mortality was directly related to temperature and exposure time. It was, however, shown that in the case of *B. decoloratus* the percentage hatch for eggs exposed to temperatures of –15 to –5°C for periods of 24 to 120 hours was <20%. The above mentioned author also concluded that cold is not a limiting factor in the spread of *M. winthemi,* that it exerts a slight influence on *B. decoloratus,* by limiting its numbers, but it plays a major role in restricting the spread and survival of *B. microplus.*

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Chapter 5

B. decoloratus Larvae

Introduction

Studies on the ecology and behaviour of larval ticks are important in order to gain information that can possibly be used in tick control programmes (Londt & Whitehead, 1972). Such studies may be of major importance to small scale farmers living in resource poor areas that utilize communal pastures, since they may result in cost-effective tick control methods being developed (McCosker, 1979). The ability of tick larvae to survive and find a suitable host is important in order to complete the life cycle and sustain the population. Many tick species migrate vertically on the vegetation up to a vantage point in order to engage a host (Short, Floyd, Norval & Sutherst, 1989). Some ticks, for example *Ixodes rubicundus* (Snyman, Fourie, Kok & Horak, 1994) may stay for extended periods on the vegetation whereas other tick species such as *Boophilus microplus* may show a diurnal vertical migration pattern (Waladde & Rice, 1982). The ability of ticks to survive in the environment will also contribute towards the success of finding a suitable host. Survival of the tick in the environment is enhanced by selection of a specific microhabitat in which the transpiration rate of the tick is low (Patrick & Hair, 1979) and also specific behavioural traits such as aggregation and synchronization of the tick activity with seasons which are favourable for tick survival (Belozerov, 1982).

The off-host biology of *B. decoloratus* larvae in the Free State has not been studied at all. In order to gain information on the off-host survival and vertical migration of *B. decoloratus* larvae a study was conducted with the following objectives: (1) to study the survival of larvae exposed to different combinations of temperature and relative humidity, (2) to study the vertical migration pattern of the larvae and to determine possible selection for vertical migration substrates.

Materials and Methods

A. Survival of Larvae under laboratory conditions.

In order to determine the survival of larvae under different sets of conditions engorged *B. decoloratus* females were collected from cattle in Botshabelo. The ticks were transported to the laboratory and exposed to 25± 2 °C and a relative humidity (RH) of 75% in order to lay eggs. From the onset of oviposition each daily egg batch was isolated. Isolation of eggs was done to obtain ticks of the same age. The eggs laid were subsequently kept at 25± 2 °C and RH=75% and monitored daily for eclosion. Seven-day-old larvae were then transferred to different temperature (10-30°C; 5°C increments) and relative humidity (35% and 75%) regimes as set out in Table 5.1. Ten larval ticks were used for each treatment. The ticks were kept in cylindrical Perspex containers similar to those that have previously been described (Chapter 4). The larvae were monitored daily in order to determine the time it took for all 10 ticks to die. The larva was presumed dead when no movement was observed and when the tick did not respond to being breathed on. Survival periods were recorded for each set of conditions.

B. Observations on vertical migration

In order to determine if *B. decoloratus* larvae have any preference for the length of questing substrates, a study was designed in which larvae were exposed to three different lengths (30, 60 and 90cm) of copper rods (diameter 1.5mm). Copper rods were used in preference to glass, wood or grass because they were straight, of even thickness and could be cleaned properly after each experiment. Equal numbers of the different lengths of copper rods were equally spaced in an alternating sequence, in a square metal arena (16x16x6cm) with a wooden base. The base of the square was covered with moist vermiculite and the test system was placed in a room in which the temperature was controlled at 24 ± 2^{0}C and the photoperiod at 14 hours light and 10 hours dark.

A mass of 50mg of seven-day old *B. decoloratus* larvae was released into the center of the square. The ticks were prevented from escaping by the use of double-sided tape placed on the edges of the metal square. The ticks were left undisturbed and after 48 hours the larvae on each length of rod were collected and counted. The counts for similar rod lengths were pooled.

In a follow-on study a similar approach was adopted with rod lengths of 5, 25 and 45cm, respectively. The larvae (50mg) were again released into the center of the metal square and observations were made at 24, 48 and 72 hours after release of the larvae. The number of larvae on the different rod lengths was categorized on a subjective scale (x = <10 larvae; xx=>10<50 larvae; xxx =>50 larvae). After each assessment the larvae were left undisturbed since the objective was to observe the dynamics of increment between different rod lengths.

Results

A. Larval survival under laboratory conditions

The survival of unfed larvae was determined from the first day of exposure up to the day all larvae were confirmed dead. The data are summarized in Table 5.1 and presented graphically in Fig. 5.1. There was a marked difference between 35% and 75% RH. The time it took for all larvae to die was the longest (54 days) at 10^{0}C and RH = 75% and the shortest (12 days) at 30^{0}C and RH = 35%. At 10^{0}C and RH=35% all larvae were dead after 24 days. The difference in larval survival between 35% and 75% RH became less with an increase in temperature to the point (30^{0}C) where larval survival was similar.

Table 5.1. Larval survival periods (days) of larvae exposed to different combinations of temperature and relative humidity.

Temperature °C	Larval survival (days ±SD)	
	RH=75%	RH=35%
10	54 ± 10.61	24 ± 0
15	34 ± 4.24	14 ± 1.41
20	23 ± 5.67	13 ± 0
25	20 ± 1.41	12 ± 1.41
30	12 ± 2.83	11 ± 2.83

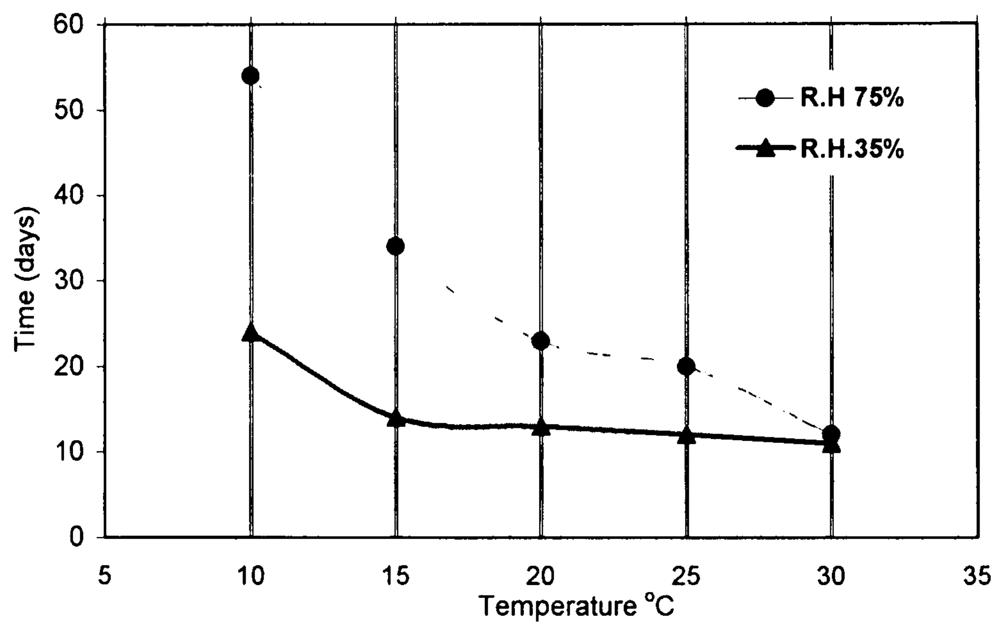

Figure 5.1. Survival periods (days) of *B. decoloratus* larvae exposed to different sets of temperature and relative humidity regimes.

B. Observations on vertical migration

The results of this study are presented graphically in Fig. 5.2. After 48 hours only 10.1% (n=52) of the total number of larvae occurred on the 30cm rods, 32.38% (n=165) on 60cm rods and 57.52% (n=294) were collected on the 90cm rods. Observations have also shown that the larvae occurred in clusters at the tips of the rods.

A similar pattern was observed in the study where assessments were made on a daily basis over a 72-hour period. After 24 hours most of the larvae occurred on the 45cm rod lengths and the least number on the 5cm rod lengths (Table 5.2). After 48 hours the situation changed and no larvae were observed on the 5cm rods. After 72 hours still no larvae were observed on the 5cm rod lengths and fewer larvae were also observed on the 25cm rod lengths.

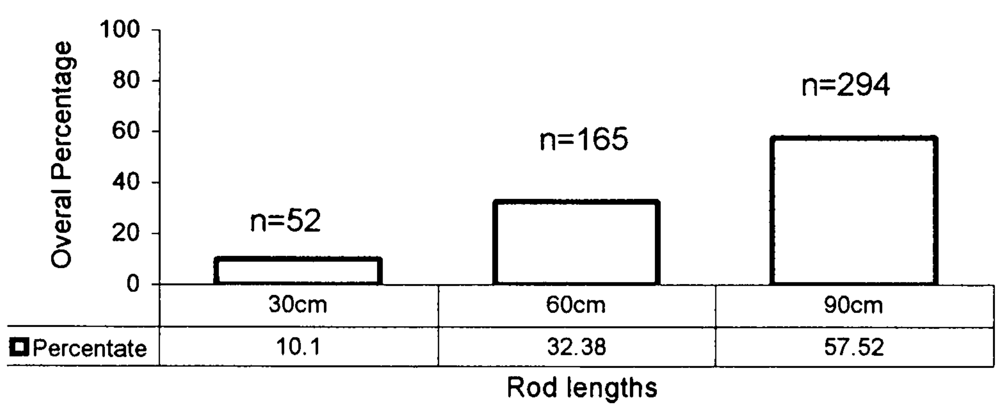

Figure 5.2. Percentage of the larvae occurring on different lengths of rods 48 hours after release.

Table 5.2 The relative number of larvae on rods of different lengths as assessed daily over a 72 hours period.

Assessments after 24 hours.

Rod Length	5	25	45
Number of larvae	x	xx	xxx

Assessments after 48 hours.

Rod Length	5	25	45
Number of larvae	0	xx	xxx

Assessments after 72 hours.

Rod Length	5	25	45
Number of larvae	0	x	xxx

Subjective scale (x = <10, xx = <50, xxx = >50)

Discussion

Survival of larvae under laboratory conditions

One of the major factors that can affect the longevity of ticks is their ability to conserve their body water (Knülle & Rudolph, 1982). Ticks have a relatively large surface to volume ratio but have developed many mechanisms with which they can maintain their body water balance (Needham & Teel, 1991). Among these are their ability to absorb water from unsaturated atmospheres, ability to limit transpiration through waterproofing of the cuticle (Sonenshine, 1991), and behavioural attributes (Knülle & Rudolph, 1982). Temperature, however, directly affects passive transpiration by increasing the energy of water molecules in the body of the animal. Direct sorption of atmospheric moisture by unengorged ticks only occurs above a certain relative humidity. The lowest relative humidity at which active uptake can occur for a particular tick species or life stage at a given temperature is the critical equilibrium activity (CEA) (relative humidity divided by 100; Sonenshine, 1991). In order for a tick to actively absorb atmospheric moisture it must be maintained at or above its CEA.

In this study it was evident that temperature to a great extent affected the longevity of larvae as manifested by the relatively short survival period (12 days) of larvae kept at 30°C and 75%RH. Larvae that hatched during January in the field in this study also displayed a limited survival period (25 days; Chapter 4). Hazari & Misra (1993) also reported a limited survival period for *B. microplus* larvae during the summer season. Similar observations have also been made for *Dermacentor reticulatus* (Zehler & Gothe, 1995) and *Ixodes rubicundus* (Van der Lingen, Fourie, Kok & Van Zyl, 1999). In the latter case larvae exposed to 30°C and RH = 75% survived for a period of 15 days indicating that these ticks are more tolerant to desiccation when compared to *B. decoloratus.* The fairly short survival period of larvae kept at 20°C and 75% RH suggests that the relative humidity to which the larvae were exposed may have been below the CEA. Studies should, however, be performed to confirm the CEA for *B. decoloratus*. Other studies on the survival and behaviour of unfed stages of *B. decoloratus* and *B. microplus* have shown that larvae survived longer during the rainy seasons compared to the cooler seasons (Short *et al.*, 1989). These authors have also shown that larvae exposed to long grass survived longer compared to those exposed to short grass. The long grass provided the ticks with better protection against direct sunlight and desiccation compared to short grass, resulting in longer survival periods. In the present study larvae exposed to 10°C and 30°C, both at high relative humidity of 75% RH survived for 12 and 54 days respectively. Patrick & Hair (1979) illustrated in *Amblyomma americanum* that lower temperature and high humidity and above average precipitation in the field resulted in longer survival periods compared to warmer and dryer conditions. Except for the fact that high temperatures can increase the transpiration rate in ticks (Garris & Popham, 1990) it also increases metabolic rate and activity. Increase in locomotor activity can result in the depletion of energy reserves (Lees, 1969) which can also affect active water uptake (Knülle & Rudolph, 1982). Spickett and Heyne (1990) observed that *B. decoloratus* larvae survived for 10 to 35 weeks during periods of high and low seasonal temperatures. This is significantly longer compared to observations made during this study. Possible explanation for these differences is that in the present study only 10 larvae were used which may not have formed a cluster. It has previously been shown that in the American dust mite, clustering of larvae reduced overall body water loss and enhanced survival (Glass, Yoder & Needham, 1998). This study was also conducted under controlled laboratory conditions compared to the fluctuating natural conditions which prevailed during the study conducted by Spickett and Heyne (1990). The maximum RH used in this study was 75%. The shorter than expected survival periods observed in this study thus strongly suggests that this relative humidity is below the CEA for *B. decoloratus*. From the literature it is evident that the CEA values for larvae in general varies between 0.8 and 0.9. The values given for *B. microplus* was >0.7, ≤ 0.95 (Knülle & Rudolph, 1982).

Observation on vertical migration

Ticks may display appetence by active hunting of hosts or by preparing an ambush (Wallade & Rice, 1982). From the results presented in this study it is evident that *B. decoloratus* display appetence by preparing an ambush for their hosts. It was also evident from the results that larvae showed a preference for longer (45 cm) questing substrates compared to the shorter (5 and 25 cm) questing substrates. Vertical migration to the tips of questing substrates will thus place the ticks at a vantage point to engage a host. In this study the maximum lengths of the rods that were used was 45cm. If longer rods were used the *B. decoloratus* larvae would most probably have moved to greater heights. Research on *B. microplus* has shown that larvae migrate vertically and form small aggregations between 35 and 121cm. Some larvae have also been recorded a height of 2.9m (Wilkinson, 1953). In another study the larvae aggregated in small groups (2 - 250 larvae) between 15 and 85.1cm (Garris & Popham, 1990). The questing heights of ticks have been shown to correlate with the size of preferred hosts (Lees, 1969; Garris & Popham, 1990; Goddard, 1992; Snyman *et al.*, 1994). The height at which *B. decoloratus* larvae occur on questing substrates correlates with the size of their main hosts, thus maximizing tick-host contact. *B. decoloratus* commonly parasitize larger domestic and wild ungulates (Artiodactyla, Bovidae, Perissodactyla and Equidae). Cattle are its main domestic host (Walker, 1991). A study performed by Londt & Whitehead (1972), however, indicated that the optimal vegetation height for *B. decoloratus* larvae was 20cm. This is substantially less than the value recorded in this study.

The results of this study further demonstrated that there was a gradual shift in the number of larvae occurring on shorter questing substrates, towards those occurring in the longer questing substrates. The results suggest that the initial reaction of the larvae is to migrate vertically on any questing substrate with which they make contact, but to subsequently change their position in order to seek questing substrates that comply with their specific height preferences. Similar observations have been made for *B. microplus* where larvae ascended objects whether the objects were plants or nylon cloth (Garris & Popham, 1990). In a study on *Ixodes rubicundus* it has been shown that the survival of ticks exposed to sub-optimal length of questing substrates was three times less compared to ticks exposed to optimal length questing substrates (Fourie, Kok, Krugel, Snyman & Van der Lingen, 1996). The above mentioned authors hypothesized that sub-optimal questing substrates may induce ticks to move more in search of optimal length substrates on which to quest, thereby depleting their energy reserves more rapidly, resulting in increased mortality. By manipulating the grass lengths, through for example heavy grazing, it should theoretically be possible to reduce the probability of *B. decoloratus* larvae making contact with their hosts. Such sub-optimal length questing substrates may also induce greater movement of the larvae resulting in increased mortalities. This contention remains to be verified.

In this study the pattern of vertical migration of *B. decoloratus* larvae was not studied. Many ticks display a diurnal vertical migration pattern. Vertical migration has also been correlated with the intensity of solar radiation, and temperature and humidity changes (Belozerov, 1982). Wilkinson (1953) reported that the vertical movement of *B. microplus* is a response to light intensity. The results of the study conducted by Garris & Popham (1990) on *B. microplus* showed that the vertical distribution of larvae in cylinders changed over time, but was not correlated with changes in relative humidity and temperature. In *Ixodes rubicundus* it has been shown that the extent of vertical migration was more pronounced in females compared to males and was also more pronounced in older than younger ticks (Snyman *et al.*, 1994). The periodicity in vertical migration of *B. decoloratus* larvae and regulating factors should be elucidated in order to contribute towards the formulation of possible integrated control practices.

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Chapter 6

Seasonal Dynamics

Introduction

Traditional communal farming in southern Africa has often been described as unproductive and directly responsible for regional poverty and vegetation degradation (Boonzaier, Hoffman, Archer & Smith, 1990). This type of farming predominates in low-income (poor resource) communities such as occupied by blacks in rural areas of South Africa. The history of agricultural subsidies in South Africa has been such that black small-scale farmers have always been marginallized (Dreyer, Fourie & Kok, 1999) and services were directed at commercial farmers. Subsistence farming operations in terms of herd size, are usually small and as such cannot generate sufficient surplus for the market or generate the finances for ranch development. This is the reason why small-scale farmers are dependent upon low cost grazing systems such as the commonage.

Livestock farmers in Botshabelo still keep and manage their animals in a traditional way. Farm animals provide a special protection to families, acting as a buffer between the family and an uncertain food supply (Norton & Alwany, 1993). Animals are kept in kraals in the backyards and are herded into the pastures in the morning and returned home in the evening. Cattle are mainly reared for milk, and are usually milked twice a day, in the morning before they are lead into pastures and in the evenings after grazing. Botshabelo's pastures are communal, and herds are grazed at will. There are no fences and since the stocking rates are not controlled on the commonage, overgrazing is a common phenomenon. There are practically no range and pasture management practices undertaken in the area and this renders livestock management difficult for these small-scale farmers.

Effective tick and tick-borne disease control is also difficult to manage in the area. Lack of funds and inaccessibility to veterinary services in pastoral farming systems are major constraints to tick control. Tick control methods used by farmers in Botshabelo include the use of commercial acaricides (though to a lesser extent) as pour-ons, hand sprays and tick grease, application of used engine oil, hand deticking and use of chickens as predators of cattle ticks (Dreyer, 1997; Dreyer, Fourie & Kok, 1997; Dreyer *et al.*, 1999). In order, however, to optimize tick control practices and to formulate integrated control practices a sound knowledge of the seasonal dynamics and biology of target tick species is required.

The objectives of this study were; (1) to obtain information on the seasonal dynamics of immature and adult *B. decoloratus* ticks on cattle in the peri-urban, resource-poor environment of Botshabelo; (2) to investigate the seasonal occurrence of free living *B. decoloratus* larvae in the communal pastures in Botshabelo.

Study Area

The study was carried out from March 1998 to August 1999 at Botshabelo (29°12'-29°18' S; 26°40'-26°45' E), a peri-urban area 55km east of Bloemfontein in the central Free State Province. The area is characterized by small-scale cattle farmers grazing their herds on a communal pasture system. The study was carried out in the block-W section situated on the western periphery of the city. The area is situated along the Modder River that runs through the pastures from east to west.

Materials and Methods

1. On-host seasonal dynamics

The study was initiated on the 25th of March 1998. A sample size of 10 cattle was used. The cattle were of mixed-breed origin, with Friesian crosses being dominant. The cattle were older than one year. Each animal was provided with a numbered tag for individual identification. The same animals were used throughout the study except when animals were sold or died, in which case another animal of similar age and from the same kraal was used as a replacement. Cattle from only one farmer were used in this study and no acaricides were used on the animals during the survey period (March 1998 to August 1999)

On an approximately four weekly basis the ticks were collected from all 10 cattle. To facilitate the collection of the ticks each animal was restrained in a crush pen equipped with a neck clamp. Ticks were removed from 20 x 20cm squares on three body areas of the animal, namely the neck, abdomen, and inguineum. Selection of these sites was based on the fact that these body areas were predilection attachment sites for *B. decoloratus* (Dreyer, 1997). The left and the right hand sides of the cattle were sampled on alternate months in order to allow the attached larvae to develop into adults before the next collection.

In order to delineate the area from which the ticks were collected a square (20x20cm) made of wire was used. The square was placed on the applicable body area and the area was first examined visually. Visible ticks were removed and placed in labeled bottles containing 70% ethanol. This was followed by palpation of the area using the fingers. The located ticks were again removed using a pair of forceps and placed in the same bottle. In order to remove immature ticks a flea comb and a serrated knife were used. The area was firstly combed thoroughly by stroking the comb in the direction of the lie of the hair. Hair and dislodged ticks were removed from the teeth of the comb and placed in labeled bottles. Aggressive scraping of the demarcated area with the serrated knife then followed. Hair and ticks were again placed in labeled bottles. At the laboratory ticks were separated from hair samples, identified and quantified. For this purpose the reference specimens and tick identification keys in the Department of Zoology at the University of the Free State were used.

2. Off-host seasonal abundance of Larvae

In order to collect free-living larvae from the vegetation a dragging apparatus was constructed. This consisted of a one meter long broomstick on which 10 flannelette strips (10cm x 100cm) were attached with Velcro for easy removal. Pieces of wire (2mm diameter) were inserted into the material at the free ends of the flannelettes in order to weigh them down. A string was attached to both sides of the broomstick to enable dragging of the apparatus over the vegetation.

On a monthly basis (September 1998 to August 1999) drags were made in each of the three areas situated to the west of the 'W section' in Botshabelo. The first sampling area (area 1) was situated along the banks of the Modder River, about 2 kilometers from the perimeter of the city. The vegetation consisted of a mixture of grasses, small, medium and large shrubs. The second sampling area (area 2) was situated approximately 500 meters from the periphery of the city on an east-facing slope. The vegetation consisted mainly of grasses and some small shrubs. The third sampling area (area 3) was situated on the periphery of the city and the vegetation consisted mainly of a mixture of grasses and some small shrubs.

In each of the three sampling areas a 100m drag was made on a monthly basis. A 100m rope was used to indicate the distance of the drag. The investigator walked at a steady pace along the side of the rope pulling the dragging apparatus over the vegetation. Dragging time was standardized to between 10.00am and 12.00am for the different areas. After each drag the flannelettes were carefully investigated by using a set of magnifying glasses. The larvae were carefully removed with the aid of a pair of fine tipped forceps and placed in labeled bottles containing 70% ethanol. Larvae were identified at the department of Veterinary Tropical Diseases, University of Pretoria.

Results

1. On-host seasonal dynamics

The on-host seasonal dynamics of *B. decoloratus* are summarized in the Table 6.1 and presented graphically in Figure 6.1. The mean number of larvae collected from cattle (three body areas pooled) was low (< 100) during the autumn (March-May) of 1998. During June to September no larvae were collected. From October the mean number of larvae increased steadily to reach a peak ($\bar{x}$ =427 larvae) during February 1999. The mean number of larvae subsequently declined sharply and was less than 120 during March to May of 1999.

The mean number of nymphs collected from cattle followed a similar pattern to that of the larvae. Mean numbers were low (<70) during the autumn (March – May) of 1998. Except for a mean of 0.6 nymphs collected during June no nymphs were collected from the cattle during the other months up to September of 1999. From October the mean numbers of nymphs increased steadily to reach a peak of 226 during February 1999. A marked decrease occurred during March 1999. Mean numbers of nymphs, however, increased sharply to reach a mean of 306 during April 1999 after which they decreased sharply to only 25 in June. During July and August a mean of 0.5 and 2 nymphs, respectively, were collected.

During March to May of 1998 and 1999 respectively, more nymphs compared to larvae were collected from the cattle. From October 1998 to February 1999 more larvae compared to nymphs were collected. The ratio of larvae to nymphs steadily increased from October 1998 to February

1999 (Table 6.1). Nymph to adult ratios decreased from March to June 1998. The ratios were in favour of nymphs during summer months. The opposite was true for winter months.

Adult *B. decoloratus* numbers increased from March 1998 to reach a peak ($\bar{x}$ =268.71) during May 1998. Mean adult tick numbers subsequently decreased sharply to reach a low ($\bar{x}$=3.2) during September. From October 1998 onwards, adult tick numbers increased steadily to reach a peak ($\bar{x}$=582) during April 1999. Adult tick numbers declined sharply in May 1999 and in July and August a mean of only 18.3 and 14.0 ticks respectively, was collected form the cattle (Table 6.1).

Table 6.1. Minimum, maximum, mean number and ratios of different stages (larvae, nymphs and adults) of *B. decoloratus* collected from cattle in Botshabelo from March 1998 to August 1999 (L=larvae; N=nymphs; A=adult).

Months	Larvae			Nymphs			Adults			Ratios	
	min	max	mean	min	max	mean	min	max	mean	L:N	N:A
March 1998	10	72	32.5	15	154	66.9	6	144	60.3	0.49	1.11
April	36	106	81.6	52	160	89	67	275	114.5	0.92	0.78
May	15	82	42.9	22	76	44.3	84	614	268.7	0.97	0.16
June	0	1	0.1	0	2	0.6	13	71	38.9	0.17	0.02
July	0	0	0	0	0	0	1	36	10.7	-	-
August	0	0	0	0	0	0	1	13	7	-	-
September	0	0	0	0	0	0	1	9	3.2	-	-
October	0	210	82.2	0	201	64.9	3	177	27.6	1.27	2.35
November	58	411	131.7	12	230	72.8	9	390	87.8	1.81	0.89
December	75	330	179.7	60	160	106	36	246	89.9	1.70	1.18
January 1999	168	515	319.2	38	279	168.8	39	348	160.9	1.89	1.05
February	169	1167	427	64	643	226	222	990	204	1.89	1.11
March	32	143	66	49	156	106	132	1494	290	0.04	0.37
April	154	245	110	77	625	305	135	1766	582	0.36	0.52
May	22	112	54	43	439	140	52	359	139	0.39	1.00
June	1	2	0.4	7	82	25	40	314	148	0.07	0.17
July	0	0	0	0	2	0.5	0	47	18.3	-	0.03
August	0	2	0.4	0	4	2	3	34	14	0.2	0.14

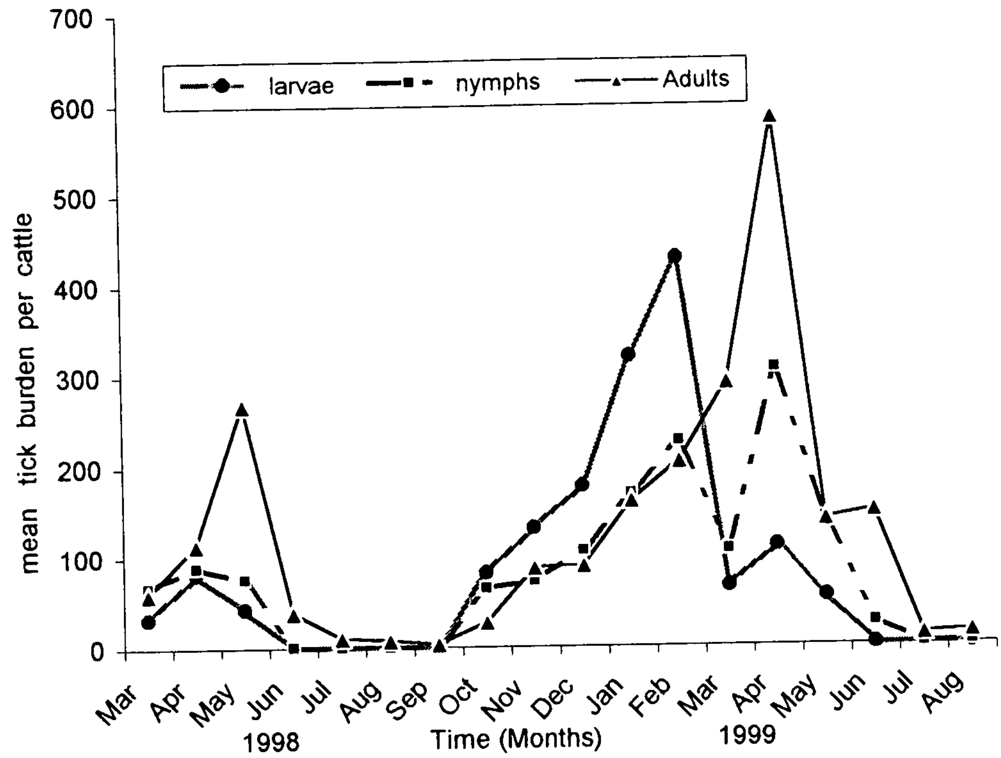

Figure 6.1. Seasonal abundance of parasitic *B. decoloratus* stages, collected from cattle in Botshabelo in the Free State Province from March 1998 to August 1999.

The mean number, as well as sex ratios of male and female *B. decoloratus* ticks collected from the cattle during the various months are summarized in Table 6.2 and represented graphically in Figure 6.2. From the figure it is evident that the pattern of seasonal occurrence for both male and female ticks is very similar.

From March to October of 1998 male numbers exceeded female numbers, except during August when marginally more females compared to males were collected. During November 1998 to January 1999 female numbers dominated with male to female ratios varying between 0.56 to 0.76. From February 1998 to August 1999 male to female ratios were either close to unity or males dominated (Table 6.2). In terms of the collection sites of the ticks most (47.4%) were collected from the inguineum followed by the abdomen 43.4% and lastly the neck area 9.2%.

Table 6.2. Statistics on the numbers of male and female *B. decoloratus* ticks collected from cattle at Botshabelo.

Month	Male			Female			Male:Female Ratio
	min	max	mean	min	max	mean	
March 1998	4	78	34.8	2	66	25.6	1.36
April	51	187	88.29	18	88	43.54	2.03
May	51	389	157.9	33	225	100.8	1.57
June	9	55	26	4	19	12.2	2.13
July	1	28	8	0	7	2.1	3.81
August	0	10	3.7	1	9	4	0.93
September	0	6	2	0	3	1.2	1.67
October	1	85	18.3	2	92	17.1	10.7
November	3	208	31.9	5	182	47.3	0.67
December	21	144	64.3	11	201	115.5	0.56
January 1999	18	184	70.5	21	164	92.4	0.76
February	15	265	100.8	7	321	100.3	1.00
March	68	267	144.2	64	227	146	0.99
April	72	715	270	63	1041	323.18	0.84
May	29	225	83.8	27	124	56.7	1.48
June	13	190	82.1	27	124	66.2	1.24
July	0	35	4.4	0	12	3.7	1.19
August	1	18	7.3	0	20	7	1.04

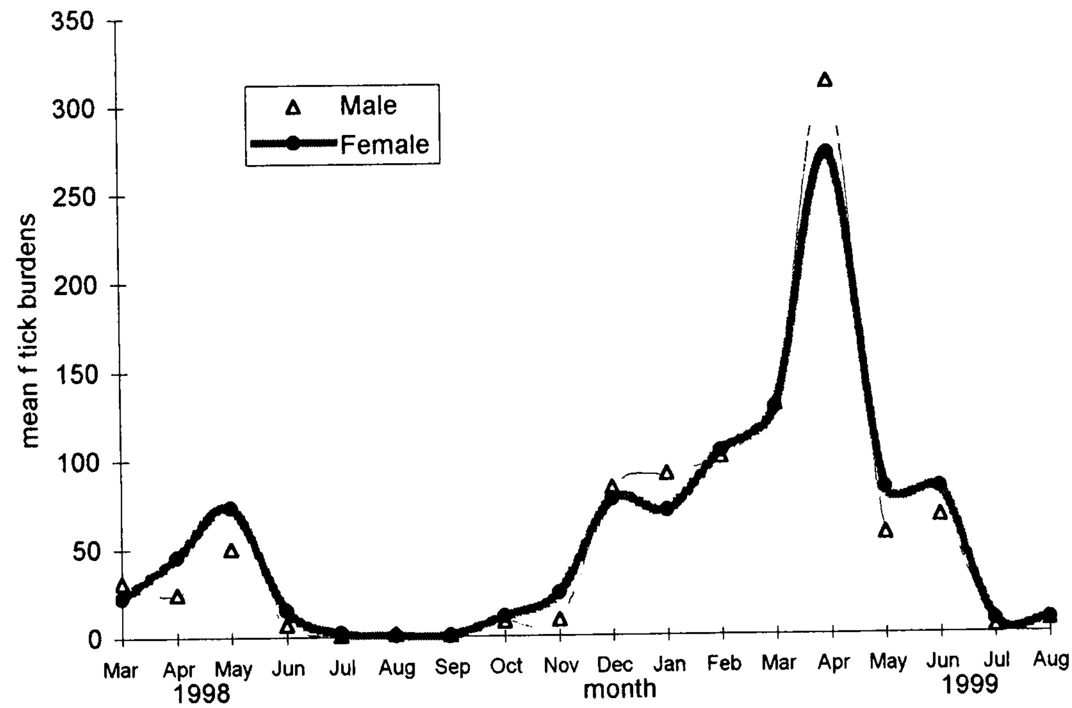

Figure 6.2. The mean monthly numbers of male and female *B. decoloratus* ticks collected from cattle in Botshabelo during March 1998 to August 1999.

2. Off-host Seasonal abundance of larvae

The number of *B. decoloratus* larvae collected from drags in the different sampling areas is presented graphically by Fig. 6.3. From the diagram it is evident that most of the larvae were sampled in area 1, which is alongside the Modder river. The monthly number of *B. decoloratus* larvae collected per 100m drag in area 1 from December 1998 to January 1999, was <100. The numbers showed a dramatic increased to 500 during February, dropped to 98 in March, and increased again to 140 in April. Few or no larvae were sampled from drags from May to August 1999. In area 2 no specific pattern in the seasonal abundance of free-living *B. decoloratus* was discernable and monthly numbers of larvae in general were less than 42. Very few *B. decoloratus* larvae were collected in area 3 with the exception of January and February during which 239 and 58 larvae were collected, respectively (Fig. 6.3).

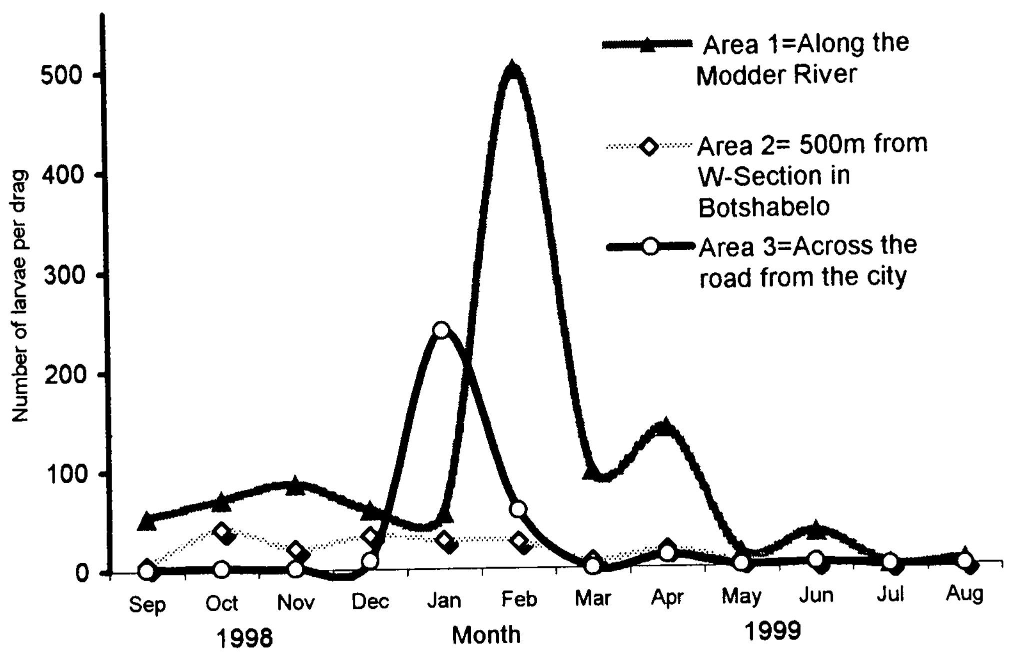

Figure 6.3. The total number of *B. decoloratus* larvae collected from drags in three different collection areas of Botshabelo from September 1998 to August 1999.

The seasonal abundance of free living and parasitic *B. decoloratus* larvae is presented graphically in Fig. 6.4. The pattern of seasonal occurrence was fairly similar with both the free living and parasitic larvae displaying the highest numbers during January and February, respectively.

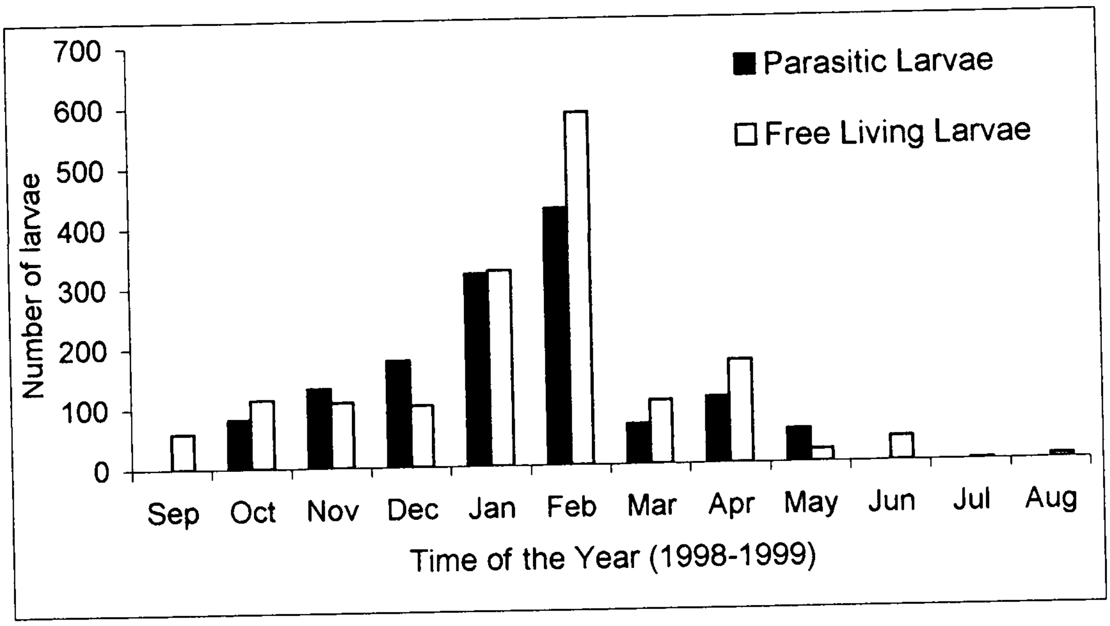

Figure 6.4. Histogram indicating the mean monthly number of *B. decoloratus* larvae parasitic on cattle and total monthly number of larvae collected during drags.

Except for *B. decoloratus* larvae, five other ixodid tick species were also collected (Table 6.3). Most (>90%) of the larvae collected from the drags were *B. decoloratus* followed by *Rhipicephalus evertsi evertsi* (7.6%) and *Hyalomma marginatum rufipes* (1.2%). The other three species (*Haemaphysalis leachi, Rhipicephalus follis* and *R. gertrudae*) each constituted 0.1% of the total number of larvae collected.

Tick species	Month												Total	Relative abundance (%)
	1998				1999									
	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug		
Boophilus decoloratus	59	114	108	102	324	585	105	170	20	39	1	6	1633	90.9
Haemaphysalis leachi	-	-	-	-	2	-	-	-	-	-	-	-	2	0.1
Hyalomma marginatum rufipes	-	-	-	1	6	-	-	2	2	1	4	6	22	1.2
Rhipicephalus evertsi evertsi	1	1	-	1	-	42	6	7	50	7	12	9	136	7.6
R. follis	-	-	-	-	-	-	-	2	-	-	-	-	2	0.1
R. gertrudae	-	-	-	1	1	-	-	-	-	-	-	-	2	0.1
Total													1797	100

Table 6.3. The total monthly number of larvae and relative abundance of the ticks collected from drags (September 1998 to August 1999) in the three sampling areas of Botshabelo.

Discussion

On-host seasonal dynamics

A previous study (Dreyer, Fourie & Kok, 1998a) has shown that the most important tick species parasitizing cattle in Thaba Nchu and Botshabelo is *B. decoloratus*, constituting 87.26% of the total tick burden. Other tick species which also infest cattle in the area are *Rhipicephalus evertsi evertsi, Hyalomma marginatum rufipes, Otobius megnini, R. follis, R. gertrudae, Ixodes rubicundus, H. truncatum* and *Margaropus winthemi* (Dreyer *et al.*, 1998a; Dreyer, Fourie & Kok, 1998b). During this study, adult *B. decoloratus* ticks were present on the cattle all year round. The numbers were, however, low (<15 mean number) during July to September. During 1998 and 1999, peak adult numbers of *B. decoloratus* were collected from the cattle during May and April, respectively. The study by Dreyer *et al.* (1998) has shown that the highest infestation of adults occurs during February to June with a peak in June. It is evident from the results that in the Central Free State *B. decoloratus* adults may reach a peak infestation on cattle during April to June. Annual differences are probably related to temperature, which is considered to be the main regulating factor in the seasonal patterns of the blue tick (Rechav, 1982). In the Eastern Cape Province *B. decoloratus* was also most numerous during February to June with low numbers present on the animals during July to January (Robertson, 1981). A study conducted by Rechav (1982) in the same area has, however, shown that no definite pattern of seasonal occurrence was discernable. The lowest numbers on the hosts were, however, recorded during early spring with peaks in summer and autumn (February to June). Horak, De Vos & De Klerk (1984) reported peak numbers of all stages of *B. decoloratus* on zebra during September in the Kruger National Park. This was attributed to the synchronous hatching of eggs.

Dreyer *et al.* (1998) speculated that in the Central Free State *B. decoloratus* produces three generations per annum, based on peak adult numbers in November/December, March/April and June, respectively. Results from this study seem to confirm this contention although peaks in seasonal abundance, except for April, were not very pronounced. The nymphs, however, displayed two definite peaks during February and April, respectively. Baker & Ducasse (1967) in Natal and MacLeod, Colbo, Madbouly & Mwanaumo (1977) in Zambia also observed a seasonal periodicity with three generations. According to Jooste (1966), *B. decoloratus* in Zimbabwe was present throughout the year without any distinct seasonal peak, indicating a possible overlap in generations. The study by Rechav & Kostrzewski (1991) on *B. decoloratus* conducted in the northern Transvaal showed a distinct pattern of seasonal activity, demonstrating three and sometimes four generations per year with peaks in July, September-October, December-January, and March-April. The low accumulation of temperature for embryogenesis in the field during winter, and long pre-oviposition period of females result in low numbers of ticks during this season (Rechav, 1982). Even though *B. decoloratus* distribution was not directly associated with temperature (Gothe, 1967), low temperatures were, however, found to regulate its seasonal occurrences (Rechav, 1982).

From this study and the one conducted on cattle in Botshabelo by Dreyer *et al.* (1998) it is evident that *R. evertsi evertsi* is the second most important tick which parasitizes cattle in this area. The area. The preferred hosts of all stages of development of this two-host species are large animals such as cattle, horses, zebras and eland (Walker, 1991; Walker, Keirans & Horak, 2000). Although adult and immature *R. evertsi evertsi* are present on host animals throughout the year, immature ticks tend to be more abundant from autumn to spring (MacLeod *et al.*, 1977). The results from the drags in this study confirm this. Certain adult *R. evertsi evertsi* strains produce a toxin resulting in spring lamb paralysis in lambs, kids and calves. It is also reported that severe infestations with the immature stages may result in irritation and secondary bacterial infections of the ear canal (Howell, Walker & Nevill, 1983).

As far as *H. marginatum rufipes* is concerned, the primary domestic hosts of adults are cattle, they also parasitize large wild ungulates (Walker, 1991). Immatures feed commonly on birds and lagomorphs (Howell *et al.*, 1983; Walker, 1991). The long mouthparts of the adults may cause severe hide damage and secondary bacterial infection at the site of attachment. Wounds commonly result in abscess formation. Both the adults of *R. follis* and *R. gertrudae* feed exclusively on large herbivores and the immatures commonly on rodents (Walker, 1991; Walker *et al.*, 2000). In the study conducted by Dreyer *et al.* (1998) the relative abundance of adults of these two tick species on cattle in Botshabelo was less than 1%. This ties in well with the low relative abundance (0.01%) of larvae from drags in the present study.

The overall sex ratio of *B. decoloratus* ticks collected from cattle in this study did not differ from unity. There was, however, a tendency for males to dominate during the colder months of the year (April to September) and for the females to dominate during the warmer months (October to

March). The sex ratio for *Boophilus spp.* collected from their hosts during various studies showed that females dominate. In *B. microplus* 58% of all ticks collected were females (Davey & Cooksey, 1988), in *B. decoloratus* 80% (MacLeod *et al.*, 1977) and in *B. annulatus* 57% (Davey & Cooksey, 1988).

The two groups (prostriata and metastriata) of the Ixodidae differ markedly in terms of the sex ratio of ticks collected from their hosts. Prostriate ticks (genus *Ixodes*) are characterized by a significant dominance of females. In the case of *I. rubicundus* 66% of all ticks collected from host were females (Fourie, Horak & Marais, 1988), in *I. persulcatus* 86% and in *I. ricinus* 77% (Babenko, Arumova, Busch & Skadin'sh, 1977). Metastriate ticks are usually characterized by a definite dominance of males in samples collected from hosts. Data from 45 different tick species indicate a dominance of females in only six tick species (Fourie unpublished data). The reason why females dominate in prostriate ticks is related to specific traits in their reproductive biology. Females belonging to the *Ixodes* genus can, unlike the situation in metastrate ticks, copulate on and off the host (Oliver, 1989). In *Ixodes* ticks, spermatids are produced during the nymphal stage and adults can copulate without a blood meal resulting in the dominance of the females on the host. Metastriate ticks on the other hand require a blood meal during the adult stage before spermatids are produced (Oliver, 1989). Males therefore remain on the body of the host whilst females detach and this results in an accumulation of males. From the result of this and other studies it is evident that *Boophilus* does not conform to the general pattern of male dominance observed for other metastriate ticks. The actual cause for this is unknown. Other one-host ticks such as *M. winthemi* conform (57-58% males) to the general pattern for metastriate ticks.

Off-host seasonal abundance of larvae

Most of the *Boophilus* larvae collected during the drags were from the banks of the Modder River. This is not surprising since it serves as the main grazing area for cattle in Botshabelo. The Modder river also serves as the source of water for grazing cattle. The concentration of cattle in the vicinity of the river may also result in a greater number of detached engorged ticks in this area compared to the other areas in which drags were made. In addition the grasses along the river were greener and denser compared to the other localities in which drags were made. Apart from grasses, the presence of shrubs and bushes in the area may provide shelter to eggs and also the necessary microclimate within which the eggs can develop. Major factors that affect the development and survival of larvae in the environment are protection from high temperatures and desiccation, and the relative humidity in the microhabitat where eggs develop (Walker, 1970; Londt & Whitehead, 1972). Short, Floyd, Norval & Sutherst (1989) have also shown that the survival *of B. decoloratus, B. microplus* and *Rhipicephalus appendiculatus* larvae are greater in long compared to short grass. The off and on host seasonal abundance of *B. decoloratus* larvae correlate well. In both cases a peak in larval numbers was recorded during February. From the results it is evident that drag sampling can give a fairly accurate indication of, not only the seasonal abundance of questing larvae, but also the species composition. In a previous study (Dreyer *et al.*, 1998) on ticks infesting cattle in Botshabelo it was shown that *B. decoloratus* had the highest relative abundance (87.26%) followed by *R. e. evertsi* (6.86%) and *H. marginatum rufipes* (2.42%). These values are closely similar to the relative abundance of larvae collected from drags in this study, namely 90.9 %, 7.6% and 1.2% for *B. decoloratus, R. e. evertsi* and *H. marginatum rufipes,* respectively.

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Abstract

Boophilus decoloratus is the most dominant and economically important tick that infests cattle in the central Free State. Very little research on this tick in the central Free State has, however, been conducted. The broad objectives of this study were to investigate aspects of the oviposition and reproduction, tolerance of engorged females to sub-zero temperatures, and microhabitat selection, survival and seasonal abundance of non-parasitic larvae, and the seasonal dynamics of *B. decoloratus* parasitic on cattle in Botshabelo, central Free State.

Engorged *B. decoloratus* females exposed to 10°C did not lay eggs whereas those kept at 15, 20, 25, and 30°C, respectively laid eggs. The shortest (3.5days) mean pre-oviposition period was recorded at 30°C and 75% RH and the longest (13.2 days) at 15°C and a RH of 35%. The developmental zero temperature for pre-oviposition was calculated at 9.138°C. The oviposition period was more extended at lower temperatures. The shortest mean oviposition period (8 days) was recorded at 30°C and 35% RH. Incubation was also more extended at lower temperatures. The developmental zero temperature for incubation was calculated at 4.73°C. This value was lower than expected and probably due to a smaller sample size or lack of values for eggs incubated between 10 and 15°C. Relative humidity did not affect the oviposition period or the pattern in daily egg production. Peak egg production (533) was reached within five days from the start of oviposition. *B. decoloratus* females lay average sized eggs. Females weighing more than 200mg produced an average of 11.4 eggs per mg of body mass. The average conversion efficiency index (CEI) values recorded in this study

were 49.36% which was close to the values recorded for other *Boophilus* species. The recorded nutrient index (NI) values were 10-15% less than the recorded values for specific weight classes of *B. microplus*.

Females exposed during May 1998 to naturally fluctuating conditions died during late winter (August) without laying eggs. Eggs which were laid during April and May did not accumulate sufficient temperature for eclosion to take place. Larval survival periods were inversely dependent on increased temperature accumulation.

The results of this study have shown that engorged female ticks do not dig into the soil. The ticks seek refuge in cracks or openings in between gravel particles, or underneath debris.

B. decoloratus is able to withstand exposure to sub-zero temperatures. Mortality, however, increased with an increase in exposure time. At an exposure temperature of -10°C and an exposure time of 8 hours, 40% of the females still managed to survive and lay eggs which hatched normally. The results further indicated that direct chilling injury could occur at <-8°C, which may also be indicative of the supercooling point for engorged *B. decoloratus*.

Investigations on the longevity of larvae under laboratory conditions have shown that temperature to a great extent affected survival as manifested by a relatively short survival period (2 days) of larvae kept at 30°C. In general the survival periods for the larvae were less than those reported in the literature. The differences are believed to be related to different methodologies that were used. The results of this study also indicated that *B. decoloratus* displayed an appetence behaviour by preparing an ambush for its hosts. The larvae migrated vertically and showed a preference for longer (45cm) compared to shorter (5 and 25cm) questing substrates. The larvae formed clusters at the tips of the questing substrates.

B. decoloratus ticks were present on cattle all year round. The numbers were, however, low during July to September. Peak adult numbers were collected from cattle during April and May, respectively. Nymphs and larvae displayed peaks during February and April, respectively. It was surmised that *B. decoloratus* can complete three generations per year in the central Free State. The overall sex ratio of the ticks collected from cattle did not differ from unity. This did not conform to the general pattern of male dominance observed for other metastriate ticks.

B. decoloratus was the most abundant larvae collected from the drags. The off and on host seasonal abundance of *B. decoloratus* larvae correlated well. Drag sampling provided a fairly accurate indication of, not only the seasonal abundance of questing larvae, but also the species composition in the Botshabelo area.

Key words: *Boophilus decoloratus,* pre-oviposition period, oviposition period, incubation period, conversion efficiency index, nutrient index, engorgement mass, larval survival period, seasonal dynamics, vertical migration

Opsomming

Boophilus decoloratus is die mees dominante en ekonomies belangrike bosluis wat beeste in die sentrale Vrystaat besmet. Min navorsing is egter in die verlede op hierdie bosluis in die Vrystaat uitgevoer. Die breë doelstellings van hierdie studie was om aspekte van eierlegging en reproduksie tolleransie van volgesuigde wyfies teen sub-zero temprature en mikrohabitat seleksie, oorlewing en seisoenale voorkoms van nie-parasitiese larwes, en die seisoenale dinamika van *B.decoloratus* parasities op beeste in Botshabelo te ondersoek.

Volgesuigde *B.decoloratus* wyfies wat aan 10°C blootgestel was het nie eiers gelê nie, terwyl die wat by 15, 20, 25 en 30°C gehou is, wel eiers gelê het. Die kortste (3.5 dae) gemiddelde voor eierleggings tydperk is by 30°C en 75% RH aangeteken, terwyl die langste (13.2dae) by 15°C en 35% RH aangeteken is. Die ontwikkelings zero temperatuur vir voor eierlegging was 9.138°C. Eierlegging het langer geduur by laer temprature. Die kortste (8dae) gemiddelde eierleggingsperiode was by 30°C en 'n 35% RH aangeteken. Inkubasie het ook oor'n meer verlengde tydperk plaasgevind by laer temprature. Die ontwikkelings zero temperatuur vir inkubasie was 4.73°C. Hierdie waarde is laer as wat verwag is en is waarskynlik te wyte aan 'n klein monstergrootte of 'n tekort aan waardes vir eiers wat tussen 10 en 15°C geinkubeer is. Relatiewe humiditeit het nie eierlegging of die daaglikse patroon in die lê van eiers beinvloed nie. Piek eierproduksie (533) is binne vyf dae vanaf die aanvang van eierlegging bereik. *B. decoloratus* wyfies lê eiers van 'n gemiddelde grootte. Wyfies wat meer as 200mg weeg produseer gemiddeld 11.4 eiers per mg liggaamsmassa.

Die gemiddelde omskakelings doeltreffenheids indeks (CEI) waardes tydens hierdie studie is as 49.36% bereken, wat naby die waardes is wat vir ander *Boophilus* species bereken is. Die voedings indeks (NI) waardes wat tydens hierdie studie bereken is was 10-15% minder as die waardes wat vir *B. microplus* bereken is.

Wyfies wat tydens Mei 1998 aan natuurlike flukterende toestande blootgestel is, het tydens die laat winter (Augustus) gevrek sonder om eiers te lê. Eiers wat tydens April en Mei gelê is het nie genoeg hitte geakkumuleer vir ontwikkeling om plaas te vind nie. Die oorlewing van larwes was omgekeerd afhanklik van 'n toename in tempratuur akkumulasie.

Die resultate van hierdie studie het aangetoon dat wyfies nie in die grond ingrawe nie. Die bosluise soek skuiling in krake in die grond of in openinge tussen gruiskorrels, of onder debrie. *B. decoloratus* kan blootstelling aan sub-zero temprature oorleef. Mortaliteit neem egter toe met 'n toename in die blootstellings tydperk. By 'n blootstellings temperatuur van -10°C en 'n blootstellings tyd van 8 ure, het 40% van die wyfies daarin geslaag om te oorleef en eiers te lê wat normaal uitgebroei het. Die resultate het verder aangedui dat direkte ysingwekkende skade by <-8°C kan voorkom, wat ook moontlike 'n aanduiding van die superverkoelingspunt van volgesuigde *B. decoloratus* is.

Ondersoek na die langlewendheid van larwes onder laboratoruim toestande het aangedui dat temperature in 'n baie groot mate die lewensduurte bepaal. So byvoorbeeld het larwes slegs vir twee dae by 30°C oorleef. Oor die algemeen was die oorlewingstydperk wat vir larwes aangeteken is korter as die waardes wat in die literatuur aangehaal word. Die verskille is waarskynlik te wyte aan verskillende metodes wat gevolg is. Die resultate van hierdie studie het ook aangedui dat larwes na gashere soek deur vertikaal te migreer op substrate en 'n lokval te stel. Die larwes het egter 'n voorkeur getoon om op langer (45cm) vergeleke met korter (5 en 25cm) substrate vertikaal te migreer. Die larwes het in bondels naby die punte van die metaaldrade voorgekom.

B. decoloratus het heeljaar op beeste voorgekom. Die getalle was egter laag tydens Julie en September. Piek volwassene getalle het tydens April en Mei op die beeste voorgekom. Nimfe en larwes het pieke in voorkoms tydens Februarie en April getoon. Die resultate dui daarop dat *B. decoloratus* moontlik drie generasies per jaar in die sentrale Vrystaat kan voltooi. Die geslagsverhouding van die bloubosluise wat op die beeste voorgekom het, het nie van mekaar verskil nie. Dit is teenstrydig met waarnemings op ander metastriaat bosluise waar mannetjies gewoonlik oorheersend in getalle is. *B. decoloratus* is die mees volopste bosluis larwes wat tydens slepe versamel is. Die slepe het 'n redelike akkurate aanduiding gegee van die samestelling en seisonale voorkoms van larwes wat op die grasse voorgekom het.

Sleutelwoorde: *Boophilus decoloratus*, voor eierlegginstydperk, eierleggingstydperk, inkubasieperiode, omskakelings doeltreffendheids indeks, voedings indeks, volsuig massa, larvale lewensduurte, seisoenale dinamika, vertikale migrasie.

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