

**Species of questing ixodid ticks on the vegetation of sable antelope
(*Hippotragus niger*) enclosures and a surrounding multi-herbivore
enclosure**

by

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Declaration

I declare that with the exception of assistance with the statistical analyses, this is my own original work and that it has not been presented for any other degree to this or another university.

Signed:



André Charles Uys

Date 21 January 2014

Acknowledgements

I wish to express my sincere appreciation and gratitude to Waterberg Holdings, the owners of the farm Hoopdal KQ96 for allowing me to conduct this research on their property, as well as to the students and staff members of Waterberg Holdings who assisted with the tick collections.

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ABSTRACT

Species of questing ixodid ticks on the vegetation of sable antelope (*Hippotragus niger*) enclosures and a surrounding multi-herbivore enclosure

The aim of this study was to determine the species composition of questing ixodid ticks on the vegetation in intensive breeding enclosures for sable antelopes (*Hippotragus niger*), on which strategic tick control is practiced, and to compare it with that of questing ixodid ticks in a multi-species herbivore enclosure surrounding the breeding enclosures, and where no tick control is practiced. A total of eight ixodid tick species were collected namely, *Amblyomma hebraeum*, *Amblyomma marmoreum*, *Haemaphysalis elliptica*, *Rhipicephalus appendiculatus*, *Rhipicephalus decoloratus*, *Rhipicephalus evertsi evertsi*, *Rhipicephalus simus* and *Rhipicephalus zambeziensis*. *A. marmoreum* was only collected in the intensive breeding enclosures and *H. elliptica* only in the multiple herbivore species enclosure, whilst the remaining tick species were collected in both enclosures. The study was also designed to determine the abundance, seasonal abundance and proportion of ixodid tick larvae collected in the sable antelope breeding enclosures as well as in the multi-species herbivore enclosure and to compare the population dynamics of tick species in the respective enclosures with particular emphasis on *R. decoloratus*. *R. decoloratus* accounted for 65.4% of the total number ticks collected in the sable enclosures, whilst it represented only 24.3% of the total number of ticks collected in the multi-species herbivore camp. *R. decoloratus* was more abundant than *A. hebraeum* and *R. appendiculatus* in the woodlands of the sable antelope enclosures whilst *R. decoloratus* and *R. evertsi evertsi* were more abundant in grassland habitats of the sable antelope enclosures than both *A. hebraeum* and *R. appendiculatus*. *R. decoloratus* larvae were collected throughout the year with peak collections in November 2012 and between October and December 2013 in the sable breeding enclosures, and in April and May 2012 and February and April 2013 in the multi-species herbivore enclosure. The numbers of *R. decoloratus* larvae collected in the sable breeding enclosures increased significantly during the study period ($p \leq 0.020$).

Table of Contents

| | |
|---|----|
| Declaration | 2 |
| Acknowledgements | 3 |
| Abstract | 4 |
| Table of Contents | 5 |
| List of Figures | 6 |
| List of Tables | 9 |
| 1.1 INTRODUCTION | 10 |
| 1.2 OBJECTIVES AND HYPOTHESES | 12 |
| 1.3 MATERIALS AND METHODS | 12 |
| 1.3.1 <i>Study area</i> | 12 |
| 1.3.2 <i>Drag-sampling</i> | 13 |
| 1.3.3 <i>Tick identification</i> | 14 |
| 1.3.4 <i>Statistical methods</i> | 14 |
| 1.4 RESULTS | 15 |
| 1.4.1 <i>Total ticks</i> | 15 |
| 1.4.2 <i>Amblyomma hebraeum</i> | 22 |
| 1.4.3 <i>Rhipicephalus appendiculatus</i> | 27 |
| 1.4.4 <i>Rhipicephalus decoloratus</i> | 31 |
| 1.4.5 <i>Rhipicephalus evertsi evertsi</i> | 35 |
| 1.4.6 <i>Less commonly collected ticks</i> | 40 |
| 1.5 DISCUSSION | 40 |
| 1.5.1 <i>Rhipicephalus decoloratus</i> | 40 |
| 1.5.2 <i>Other commonly collected ticks</i> | 42 |
| 1.6 CONCLUSION | 45 |
| 1.7 REFERENCES | 46 |

List of Figures

- Figure 1:** Total numbers of *Amblyomma hebraeum* and *Rhipicephalus appendiculatus* larvae collected between July 2011 and July 2013 by monthly drag-sampling of the vegetation on the farm Hoopdal KQ96. 15
- Figure 2:** Total numbers of *Rhipicephalus decoloratus* and *Rhipicephalus evertsi evertsi* larvae collected between July 2011 and July 2013 by monthly drag-sampling of the vegetation on the farm Hoopdal KQ96. 16
- Figure 3:** Boxplot depicting the significant difference in abundance between individual tick species in the woodland of the sable antelope breeding enclosures on the Farm Hoopdal KQ96. The middle bar, the box and the whiskers refer to the median, the interquartile range, and the interoctile (80%) range, the dots are extremes or outliers 17
- Figure 4:** Boxplot depicting the significant difference in abundance between individual tick species in the grassland of the sable antelope breeding enclosures on the Farm Hoopdal KQ96. The middle bar, the box and the whiskers refer to the median, the interquartile range, and the interoctile (80%) range, the dots are extremes or outliers. 18
- Figure 5:** Seasonal abundance of *Amblyomma hebraeum* larvae collected monthly by drag-sampling the vegetation in the multi-species herbivore enclosure on the farm Hoopdal KQ96 between July 2011 and July 2013. 20
- Figure 6:** Seasonal abundance of *Amblyomma hebraeum* larvae collected monthly by drag-sampling the vegetation in the sable antelope breeding enclosures on the farm Hoopdal KQ96 between July 2011 and July 2013. 21
- Figure 7:** Boxplot depicting the number of *Amblyomma hebraeum* larvae collected from the vegetation in the woodland in the multi-species enclosure versus the sable antelope enclosures on the farm Hoopdal KQ96. The middle bar, the box and the whiskers refer

to the median, the interquartile range, and the interoctile (80%) range, the dots are extremes or outliers. 22

Figure 8: Boxplot depicting the number of *Amblyomma hebraeum* larvae collected from the vegetation in the grassland in the multi-species enclosure versus the sable antelope enclosures on the farm Hoopdal KQ96. The middle bar, the box and the whiskers refer to the median, the interquartile range, and the interoctile (80%) range, the dots are extremes or outliers. 23

Figure 9: Boxplot depicting the number of *Rhipicephalus appendiculatus* larvae collected from the vegetation in the woodland in the multi-species enclosure versus the sable antelope enclosures on the farm Hoopdal KQ96, The middle bar, the box and the whiskers refer to the median, the interquartile range, and the interoctile (80%) range, the dots are extremes or outliers. 25

Figure 10: Boxplot depicting the number of *Rhipicephalus appendiculatus* larvae collected from the vegetation in the grassland in the multi-species enclosure versus the sable antelope enclosures on the farm Hoopdal KQ96. The middle bar, the box and the whiskers refer to the median, the interquartile range, and the interoctile (80%) range, the dots are extremes or outliers. 26

Figure 11: Seasonal abundance of *Rhipicephalus appendiculatus* larvae collected on the farm Hoopdal KQ96 in the multi-species herbivore enclosure between July 2011 and July 2013. 27

Figure 12: Seasonal abundance of *Rhipicephalus appendiculatus* larvae collected on the farm Hoopdal KQ96 in the multi-species herbivore enclosure between July 2011 and July 2013. 28

Figure 13: Seasonal abundance of *Rhipicephalus decoloratus* larvae collected in the sable antelope breeding enclosures on the farm Hoopdal KQ96 between July 2011 and July 2013. 30

Figure 14: Seasonal abundance of *Rhipicephalus decoloratus* larvae collected in the multi-species herbivore enclosure on the farm Hoopdal KQ96 between July 2011 and July 2013. 31

Figure 15: Seasonal abundance of *Rhipicephalus evertsi evertsi* larvae collected monthly by drag-sampling the vegetation in the multi-species herbivore enclosure on the farm Hoopdal KQ96 between July 2011 and July 2013. 33

Figure 16: Seasonal abundance of *Rhipicephalus evertsi evertsi* larvae collected monthly by drag-sampling the vegetation in the sable antelope breeding enclosures on the farm Hoopdal KQ96 between July 2011 and July 2013. 34

Figure 17: Boxplot depicting the number of *R. evertsi evertsi* larvae collected in grassland versus woodland in the multi-species enclosure on the farm Hoopdal KQ96. The middle bar, the box and the whiskers refer to the median, the interquartile range, and the interoctile (80%) range, the dots are extremes or outliers. 35

Figure 18: Boxplot depicting the number of *Rhipicephalus evertsi evertsi* larvae collected in grassland in the multi-species enclosure versus the sable antelope enclosures on the farm Hoopdal KQ96. The middle bar, the box and the whiskers refer to the median, the interquartile range, and the interoctile (80%) range, the dots are extremes or outliers. 36

List of Tables

Table 1.1: Total numbers of ticks collected from the vegetation on the farm Hoopdal KQ96 between July 2011 and July 2013. 13

Table 1.2: Total numbers of ticks collected in the multi-species herbivore enclosure and sable antelope breeding enclosures on the farm Hoopdal KQ96 between July 2011 and July 2013. 14

1.1 INTRODUCTION

Commercial wildlife ranching has become increasingly popular in the past two decades and has to a large extent displaced commercial cattle farming in the bushveld regions of Limpopo Province, South Africa (Schroder, Uys & Reilly 2006). Moreover, commercial game ranching in South Africa has adopted the practice of ranching rare and endangered species such as sable antelopes (*Hippotragus niger*) and roan antelopes (*Hippotragus equinus*) in intensive breeding enclosures to stimulate reproduction and minimise neonatal mortality, which, due to tick-borne diseases, is high in these species (Nijhof, Pillay, Steyl, Prozesky, Stoltsz, Lawrence, Penzhorn & Jongejan 2005). Neonatal and calf mortalities have not been eliminated by the confinement and the intensification of breeding of these species and in some cases have even been exacerbated.

Roan and sable antelopes have a low tolerance to competition from other herbivore species, and are usually accommodated in mono-species intensive breeding enclosures ranging in size from 10 to 150 hectares. Breeding enclosures are fenced to exclude predators and other herbivorous species. In addition, animals in the enclosures are subject to strategic tick control and are maintained through dry periods by supplementary feeding (Schroder *et al.* 2006). Commercial ranching of these species has resulted in an increase in the numbers of animals translocated within South Africa, and this in turn has led to the introduction of hosts and/ or ticks into non-endemic areas and has resulted in mortalities from tick-borne diseases and losses due to tick toxicosis, anaemia and tick worry (McInnes, Meltzer, Stewart & Penzhorn 1991; Nijhof *et al.* 2005). Mortalities due to tick-borne disease have recently been reported in a number of wildlife species, including sable and roan antelopes, but little is known of the tick vectors that transmit these diseases (Grootenhuis, Morrison, Karstad, Sayer, Young, Murray & Haller 1980; Nijhof, Penzhorn, Lynen, Mollé, Morkel, Bekker & Jongejan 2003; Nijhof *et al.* 2005; Oosthuizen, Zwegarth, Collins, Troskie & Penzhorn 2008; Uys 2010, unpublished data).

Rhipicephalus appendiculatus and *Rhipicephalus evertsi evertsi* have recently been proposed as potential vectors of *Theileria* sp. (sable), the causative organism of mortalities in both roan and sable antelopes, whilst the role of *Rhipicephalus decoloratus* in its transmission is unclear (Benade 2010; Steyl, Prozesky, Stoltsz & Lawrence 2012). *R. decoloratus* and *R. evertsi evertsi* were the only tick species

recovered from sable antelopes that died during an outbreak of clinical babesiosis in an intensive breeding enclosure in which the animals were under nutritional stress (Uys 2006 unpublished data). *R. appendiculatus*, *R. decoloratus* and *R. evertsi evertsi* were the most common tick species collected from confirmed cases of fatal theileriosis in roan and sable antelopes (Wilson & Hirst 1977). Determining the epidemiology of important tick-borne diseases in wildlife and identifying the tick vectors responsible for the transmission of these diseases will require information on the preferred host status and seasonality of the potential tick vectors as well as their distribution in relation to disease outbreaks (Oosthuizen, Allsopp, Troskie, Collins & Penzhorn 2009).

A non-destructive, non-invasive method of determining the species composition and seasonality of tick populations questing for hosts from the vegetation is by drag-sampling the vegetation with flannel strips. Drag-sampling has been carried out in numerous wildlife reserves in southern Africa as well as on commercial game ranches (Spickett, Horak, Braack & Van Ark 1991; Spickett, Horak, Van Niekerk & Braack 1992; Zieger, Horak & Cauldwell 1998a; Uys & Horak 2005; Schroder *et al.* 2006). No such surveys have been performed comparing free-living ixodid tick populations in intensive mono-species wildlife breeding systems with those found on surrounding multiple herbivore species ranches.

The population dynamics of ticks are influenced by complex relationships and interactions between ticks, their hosts and the environment (Spickett, Gallivan & Horak 2011). Long-term surveys of free-living ixodid ticks in the Kruger National Park have detected significant, erratic and periodic declines and increases in localised tick populations in response to climatic factors, host density, host species composition and host resistance (Horak, Gallivan & Spickett 2011). Drag-sampling of the vegetation over a 4-year period on a commercial game ranch in Limpopo Province, on which intensive breeding and strategic tick control is practiced, has revealed changes in the species composition of free-living ixodid ticks in single species breeding camps (Uys 2012 unpublished data). Regular acaricidal application on cattle on a mixed wildlife and cattle ranch in Zambia reduced populations of free-living ticks on the vegetation and parasitic ticks on sympatric impalas (*Aepyceros melampus*) (Zieger, Horak, Cauldwell, Uys & Bothma 1998c). However, *R. decoloratus* appeared to be unaffected by the acaricide, possibly because of acaricidal resistance (Zieger *et al.* 1998c).

A comparison between the questing tick populations in intensive breeding enclosures for sable antelopes, where strategic tick control is practiced, and similar tick populations on a commercial game ranch with multiple herbivore species surrounding the sable enclosures will assist in determining the species composition and the population dynamics of tick species recognized as potential vectors of fatal tick-borne diseases in sable antelopes.

1.2 OBJECTIVES AND HYPOTHESES

1.2.1 To determine the species composition of questing ixodid ticks on the vegetation in intensive breeding enclosures for sable antelopes, on which strategic tick control is practiced, and to compare it to the species composition of questing ixodid tick populations on the vegetation of a multiple herbivore species enclosure surrounding the sable enclosures and where no strategic tick control is practiced.

1.2.2 To determine the prevalence of *R. decoloratus* in intensive sable breeding enclosures and compare this with the prevalence of this species on the game ranch with multiple species of herbivores.

1.2.3 To determine the abundance and seasonal abundance of *R. decoloratus* in intensive sable antelope breeding enclosures and on the game ranch with multiple herbivore species.

1.3 MATERIALS AND METHODS

1.3.1 Study area

The survey was conducted on the commercial game ranch, Hoopdal KQ96 (S24°17.993 E027°29.365), Thabazimbi district, Limpopo Province. The farm comprises approximately 1900 hectares, and is subdivided into an enclosure of approximately 1070 hectares in which several herbivore species are contained, as well as three sable antelope breeding enclosures of approximately 50 hectares each. One adult male and 10-15 adult female sable antelopes and their offspring are kept in each of the three breeding enclosures. Regular strategic tick control is practiced in these enclosures by treating the sables every two to three weeks with a synthetic pyrethroid. The synthetic

pyrethroid is applied by means of a specially designed square feeding trough which is framed with a gutter into which the acaricide is poured. The acaricide-filled gutter contains a steel rolling pin, which becomes coated in acaricide and rubs against the animal's neck when it leans over the pin to feed. Species in the multiple herbivore area include, 63 plains zebras (*Equus quagga*), unknown numbers of warthogs (*Phacochoerus africanus*), 15 giraffes (*Giraffa camelopardalis*), 46 greater kudu (*Tragelaphus strepsiceros*), five elands (*Tragelaphus oryx*), five blue wildebeest (*Connochaetes taurinus*), 12 gemsbok (*Oryx gazella*), 27 waterbuck (*Kobus ellipsiprimnus*), an unknown number of common duikers (*Sylvicapra grimmia*), an unknown number of steenbok (*Raphicerus campestris*), and 93 impalas (*Aepyceros melampus*). Schroder *et al.* (2006) conducted a survey of questing ixodid ticks on the same property between September 2003 and August 2004. Since then all of the African buffaloes (*Syncerus caffer*) have been removed and the property has been buffalo-free since 2008. Furthermore the intensive breeding enclosures have also stood empty since 2008 and were only restocked in 2011. The numbers of plains wildlife were also reduced in 2011 when 13 zebras, 11 kudu, 46 elands, ten waterbuck and 53 impalas were removed from the property for live sale. No tick control is practiced in the multi-species herbivore enclosure.

The vegetation on the farm is classified as Western Sandy Bushveld of the Savanna Biome with erratic rainfall varying between 450 mm and 750 mm per annum (Mucina & Rutherford 2006).

1.3.2 Drag-sampling

With the exception of January 2013, when no collections were made, drag-sampling of the vegetation in the sable antelope breeding enclosures and the multiple herbivore species enclosure, was conducted in the third week of each month starting in July 2011 and continuing until July 2013. Drag-sampling, which favours the collection of questing ixodid tick larvae (Spickett *et al.* 1991), was accomplished by dragging flannel strips as described by Petney & Horak (1987) over the vegetation. Each drag-sampling event included three 250m long drags of woodland and three of grassland habitats in the multiple herbivore species area, and a woodland and grassland in each of the three intensive sable antelope breeding enclosures. A sharp pointed forceps was used to remove the ticks from the flannel strips after each drag, after which they were stored in

glass, screw-top vials filled with 70% ethanol and internally labelled for later identification and counting.

1.3.3 Tick identification

The ticks that had been collected were identified and counted in a Perspex tray with a grid pattern on its base under an Olympus VMZ 1-4x stereoscopic dissecting microscope. The larvae of *Amblyomma hebraeum* and *Amblyomma marmoreum* were identified using descriptions by Arthur (1973, 1975) and Voltzit and Keirans (2003). The adults of *H. elliptica* were identified using the descriptions of Apanaskevich, Horak & Camicas (2007). The larvae and nymphs of *R. appendiculatus*, the larvae of *R. evertsi evertsi*, the larvae and nymph of *Rhipicephalus simus*, and the larvae of *Rhipicephalus zambeziensis* were identified by comparison with the descriptions and scanning electron micrographs of Walker, Keirans & Horak (2000). The larvae of *R. decoloratus* were identified using the descriptions of Gothe (1967). Moreover, I have several years of personal experience in identifying ticks collected by drag-sampling.

1.3.4 Statistical methods

To examine the effects of habitat type, the total number of ticks collected in woodland transects were compared to that of grassland transects for each individual tick species within each enclosure type using a Wilcoxon signed rank test paired by the month of collection.

The same statistical approach was used to examine the effect of enclosure type where the total number of ticks collected in multi-species enclosures was compared to that of mono-species enclosures for both woodland and grassland habitats for each tick species.

A Friedman test with cases paired by month was used to determine if there were any overall differences in the relative abundance of tick species in each habitat/enclosure combination (woodland/multi-species, grassland/multi-species, woodland/mono-species, grassland/mono-species). Friedman tests that gave significant results were examined further with pairwise Wilcoxon signed rank tests, again paired by month but with Bonferroni correction for multiple comparisons, thus identifying where significant differences between tick species occurred. Boxplots of data yielding significant differences between groups are presented.

Ticks differ in their host-finding strategies, only larvae of *R. decoloratus* quest from the vegetation for hosts as this is a one-host tick and its remaining parasitic life stages are completed on a wide range of ungulates. The larvae of the two-host tick, *R. evertsi evertsi*, quest for hosts from the vegetation and the adults probably from the soil surface. Larvae of *A. hebraeum*, a three-host tick, quest for hosts from the vegetation, whilst the nymphs and adults actively hunt for hosts from the ground (Horak *et al.* 2011; Spickett *et al.* 2011; Gallivan, Spickett, Heyne, Spickett & Horak 2011). Since drag-sampling mostly collects ticks and particularly larvae questing from the vegetation, the statistical comparisons of the relative abundance of tick species only took larvae into account.

1.4 RESULTS

1.4.1 Total Ticks

During the 24 month sampling period a total of 10399 ticks were collected from the vegetation (Table 1.1). More ticks (66.45%) were collected in the multi-species herbivore enclosure than were collected in the intensive sable antelope breeding enclosures (33.55%), and more ticks (56.72%) were collected in the grasslands than in the woodlands. *R. decoloratus* was the most commonly collected tick and accounted for 38.05% of all ticks collected, followed by *R. evertsi evertsi* (27.56%), *R. appendiculatus* (24.19%) and *A. hebraeum* (9.51%).

Table 1.1: Total numbers of ticks collected from the vegetation on the farm Hoopdal KQ96 between July 2011 and July 2013

| Tick species | Total ticks collected in woodland | Total ticks collected in grassland | Total ticks collected |
|--------------------------------------|-----------------------------------|------------------------------------|-----------------------|
| <i>Amblyomma hebraeum</i> | 534 | 455 | 989 |
| <i>Amblyomma marmoreum</i> | 2 | 0 | 2 |
| <i>Haemaphysalis elliptica</i> | 0 | 2 | 2 |
| <i>Rhipicephalus appendiculatus</i> | 1611 | 904 | 2515 |
| <i>Rhipicephalus decoloratus</i> | 1455 | 2502 | 3957 |
| <i>Rhipicephalus evertsi evertsi</i> | 847 | 2019 | 2866 |
| <i>Rhipicephalus simus</i> | 5 | 2 | 7 |

| | | | |
|-----------------------------------|-------------|-------------|--------------|
| <i>Rhipicephalus zambeziensis</i> | 47 | 14 | 61 |
| | 4501 | 5898 | 10399 |

The total numbers of ticks collected in the respective enclosures are summarized in Table 1.2. *R. decoloratus*, which was collected throughout the year, was the most abundant tick in the intensive sable antelope breeding enclosures accounting for 65.38% of all ticks collected in these enclosures. *R. appendiculatus* was the most abundant tick collected in the multi-species herbivore enclosure (33.2%), followed by *R. evertsi evertsi* (29.62%) and *R. decoloratus* (24.25%). The total numbers of *A. hebraeum* and *R. appendiculatus* larvae collected are depicted graphically in Figure 1 and the total numbers of *R. decoloratus* and *R. evertsi evertsi* larvae collected are graphically depicted in Figure 2.

Table 1.2: Total numbers of ticks collected in the multi-species herbivore enclosure and sable antelope breeding enclosures on the farm Hoopdal KQ96 between July 2011 and July 2013

| Tick species | Total Ticks collected in multi-species herbivore enclosure | Total ticks collected in sable antelope enclosures | Total Ticks collected |
|--------------------------------------|--|--|--------------------------|
| <i>Amblyomma hebraeum</i> | 862 | 127 | 989 |
| <i>Amblyomma marmoreum</i> | 0 | 2 | 2 |
| <i>Haemaphysalis elliptica</i> | 2 | 0 | 2 |
| <i>Rhipicephalus appendiculatus</i> | 2291 | 224 | 2515 |
| <i>Rhipicephalus decoloratus</i> | 1676 | 2281 | 3957 |
| <i>Rhipicephalus evertsi evertsi</i> | 2047 | 819 | 2866 |
| <i>Rhipicephalus simus</i> | 2 | 5 | 7 |
| <i>Rhipicephalus zambeziensis</i> | 30 | 31 | 61 |
| | 6910 | 3489 | 10399 |

There was no significant difference between the abundance of each tick species in the woodland habitat in the multi-species enclosure (Friedman rank sum test paired by month, $\chi^2=6.65$, $df= 3$, $p=0.08$). Overall there was a significant difference in the abundance of individual tick species in the woodland habitat in the mono-species enclosure ($\chi^2=13.42$, $df= 3$, $p<0.01$) and post-hoc analysis (pairwise Wilcoxon signed

rank tests with Bonferroni correction) demonstrated *R. decoloratus* was more abundant than both *R. appendiculatus* ($p < 0.05$) and *A. hebraeum* ($p < 0.05$) (Fig. 3). For grassland in the mono-species enclosure, again there was an overall difference in the abundance of tick species ($\chi^2 = 23.06$, $df = 3$, $p < 0.001$). Post-hoc analysis showed that *R. decoloratus* and *R. evertsi evertsi* were more abundant than *R. appendiculatus* (both $p < 0.05$) and *A. hebraeum* ($p < 0.001$ and $p < 0.01$, respectively) (Fig. 4).

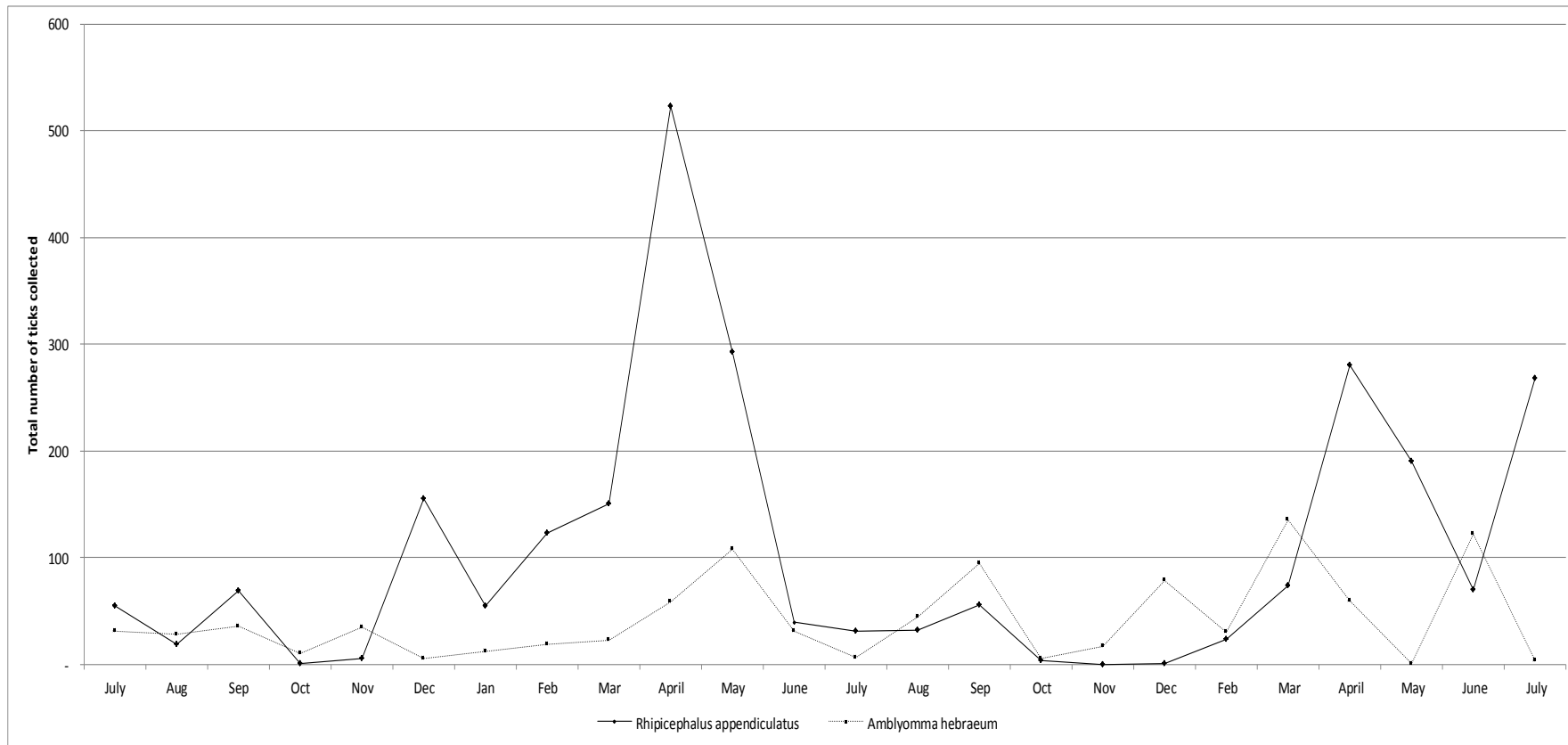


Figure 1: Total numbers of *Amblyomma hebraeum* and *Rhipicephalus appendiculatus* larvae collected between July 2011 and July 2013 by monthly drag-sampling of the vegetation on the farm Hoopdal KQ96

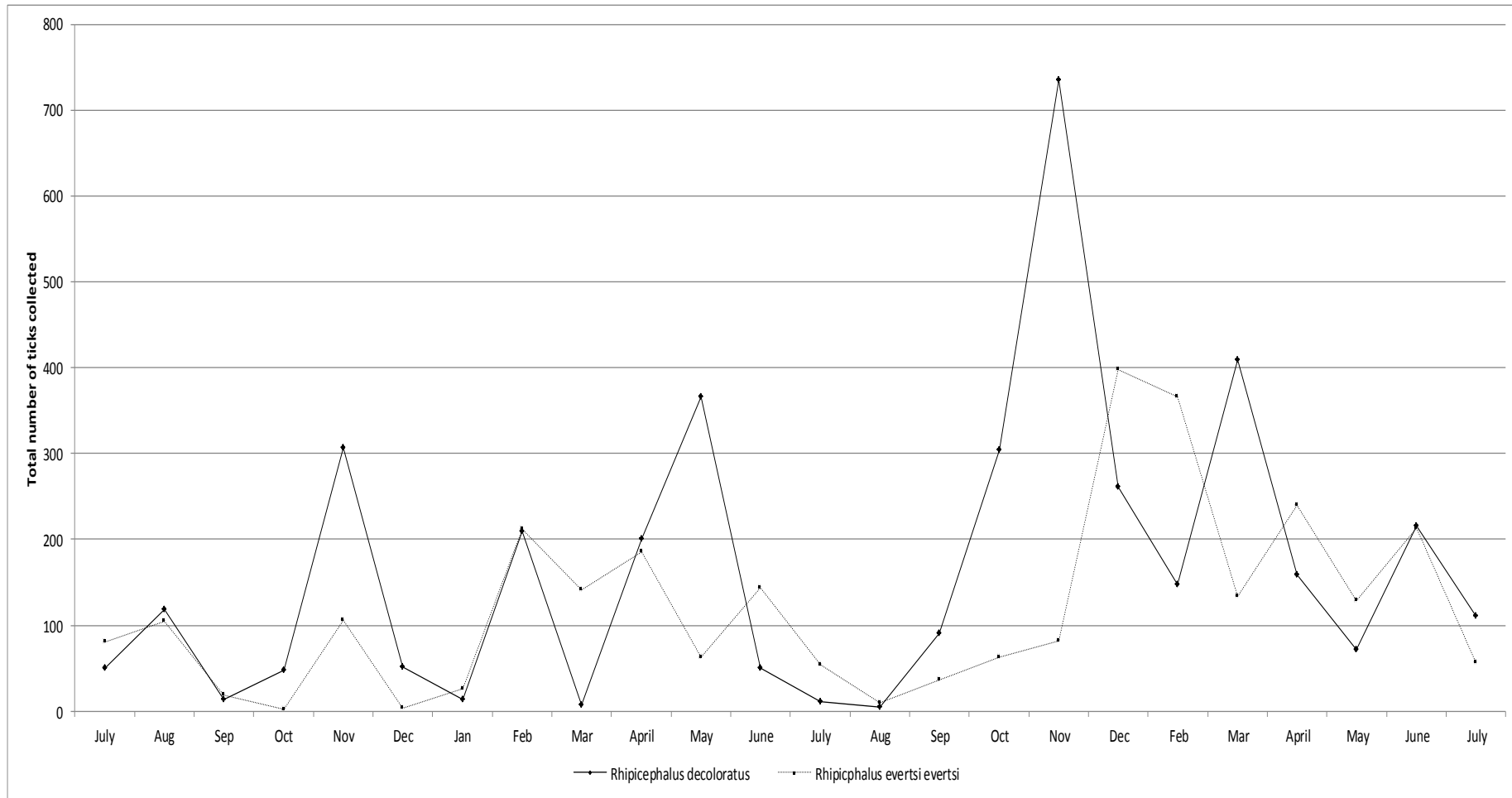


Figure 2: Total numbers of *Rhipicephalus decoloratus* and *Rhipicephalus evertsi evertsi* larvae collected between July 2011 and July 2013 by monthly drag-sampling of the vegetation on the farm Hoopdal KQ96 between July 2011 and July 2013

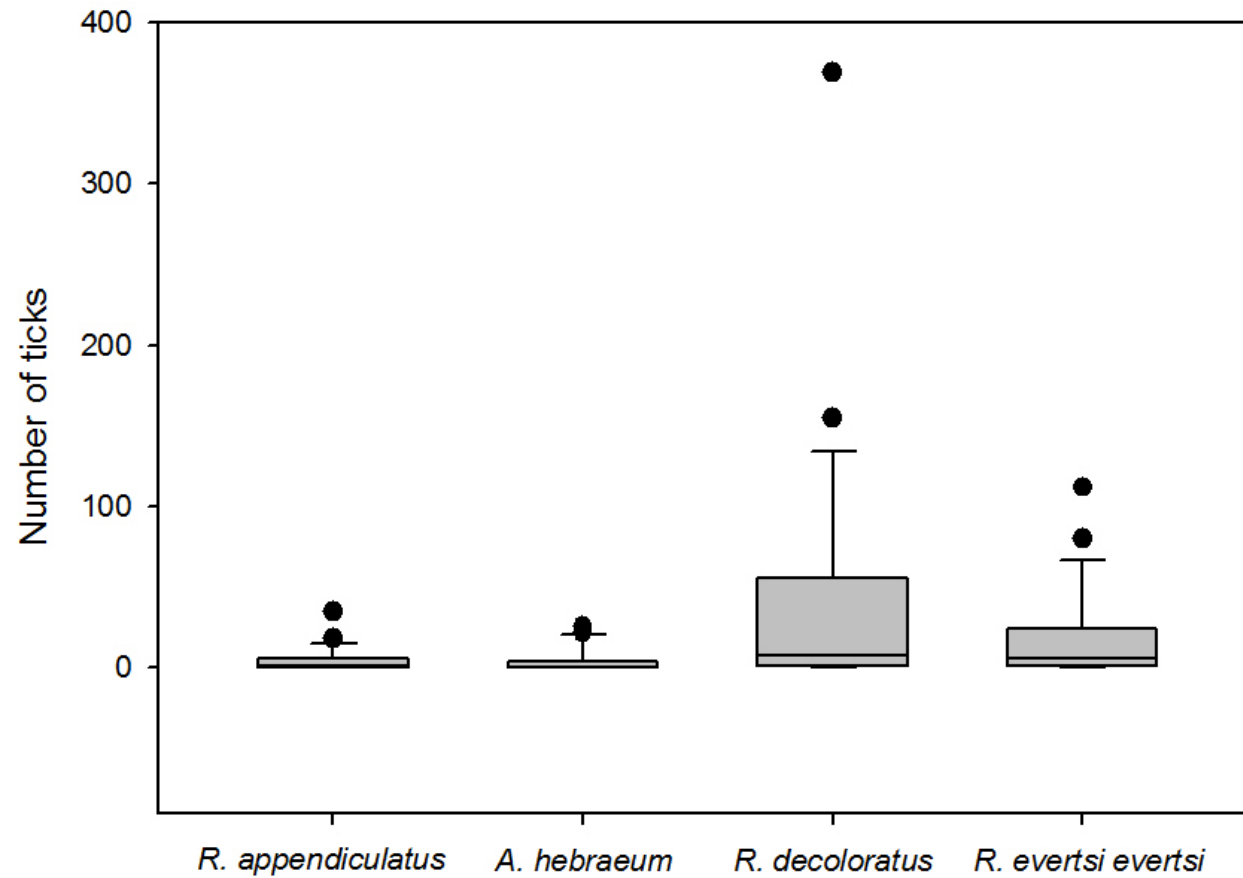


Figure 3: Boxplot depicting the significant difference in abundance between individual tick species in the woodland of the sable antelope breeding enclosures on the Farm Hoopdal KQ96. The middle bar, the box and the whiskers refer to the median, the interquartile range, and the interocile (80%) range, the dots are extremes or outliers.

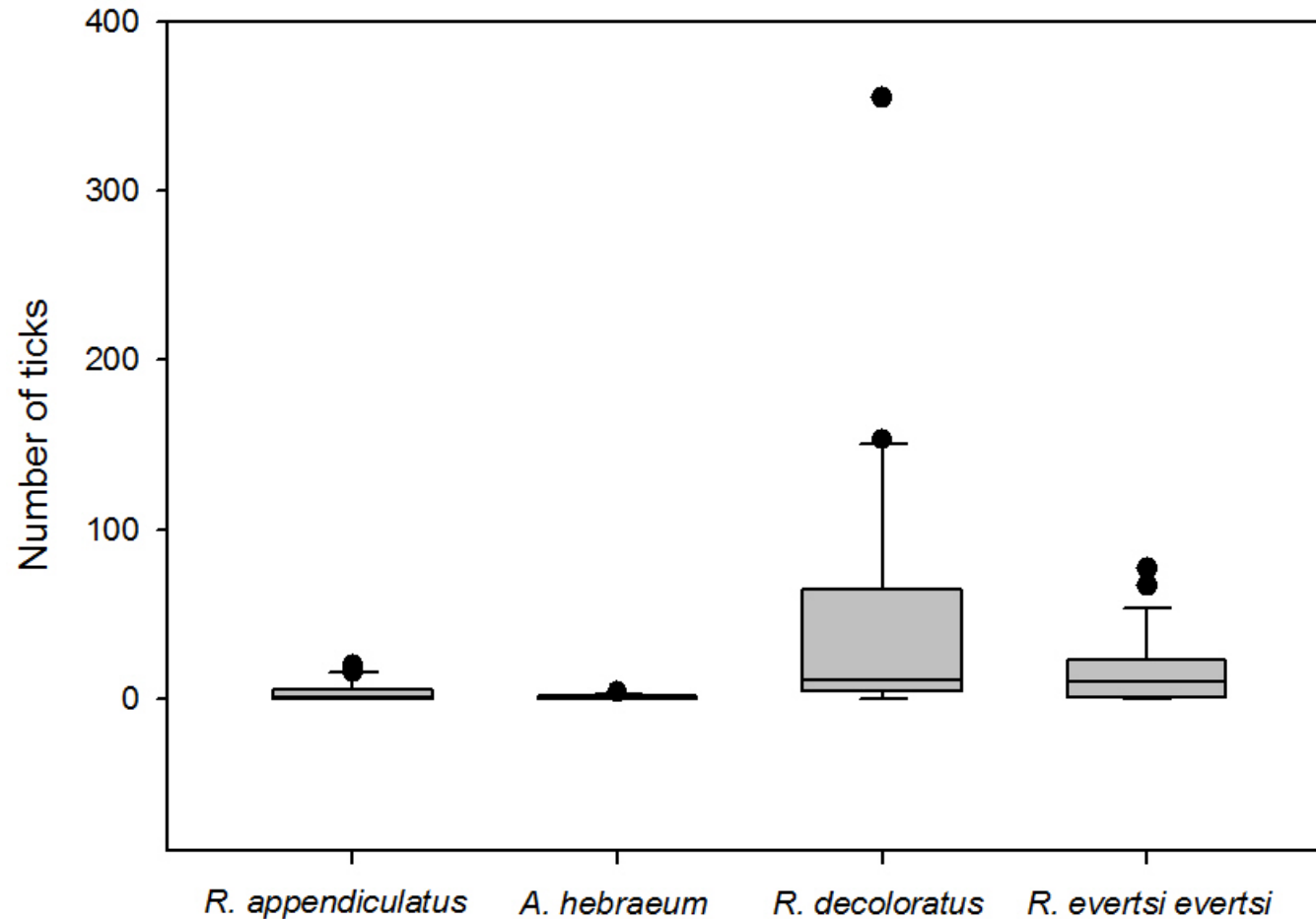


Figure 4: Boxplot depicting the significant difference in abundance between individual tick species in the grassland of the sable antelope breeding enclosures on the Farm Hoopdal KQ96. The middle bar, the box and the whiskers refer to the median, the interquartile range, and the interoctile (80%) range, the dots are extremes or outliers.

1.4.2 *Amblyomma hebraeum*

A. hebraeum larvae were collected throughout the year in the multi-species herbivore enclosure, whilst none were collected in December 2011, April and August 2012 and between October 2012 and May 2013 and in July 2013 in the sable antelope breeding enclosures (Figs. 5 and 6). Of all the *A. hebraeum* larvae collected, significantly more were found in the multi-species enclosure versus the mono-species enclosure in both the woodland ($V=198.0$, $p<0.05$, Fig. 7) and the grassland habitats ($V=174.5$, $p<0.01$, Fig. 8). There was no difference in the abundance of ticks found in woodland versus grassland habitats in the multi-species enclosure ($V=158.0$, $p=0.83$), or the mono-species enclosure ($V=59.0$, $p=0.12$). Although not significant ($p\leq 0.119$), there was a reduction in the number of *A. hebraeum* collected in the sable antelope breeding camps over the duration of the study period.

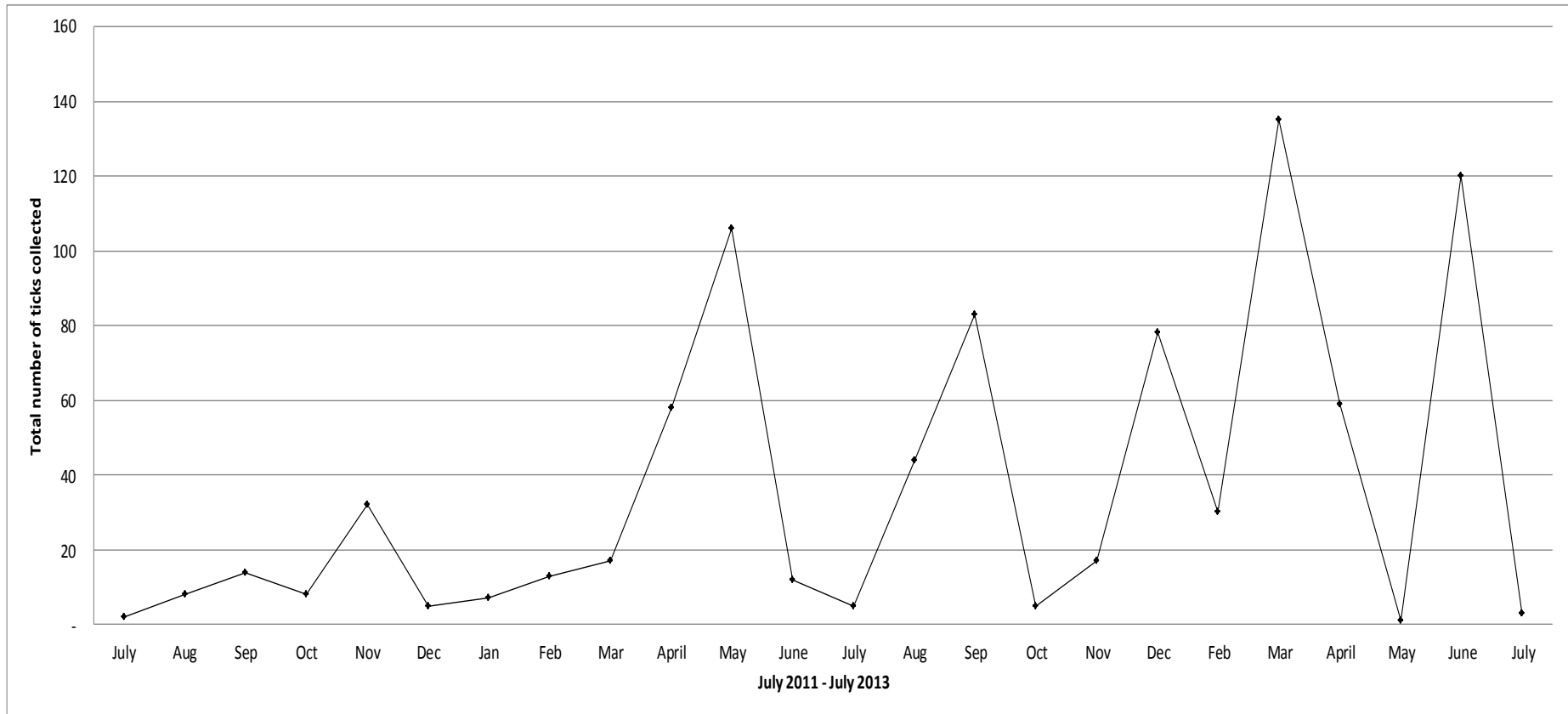


Figure 5: Seasonal abundance of *Amblyomma hebraeum* larvae collected monthly by drag-sampling the vegetation in the multi-species herbivore enclosure on the farm Hoopdal KQ96 between July 2011 and July 2013

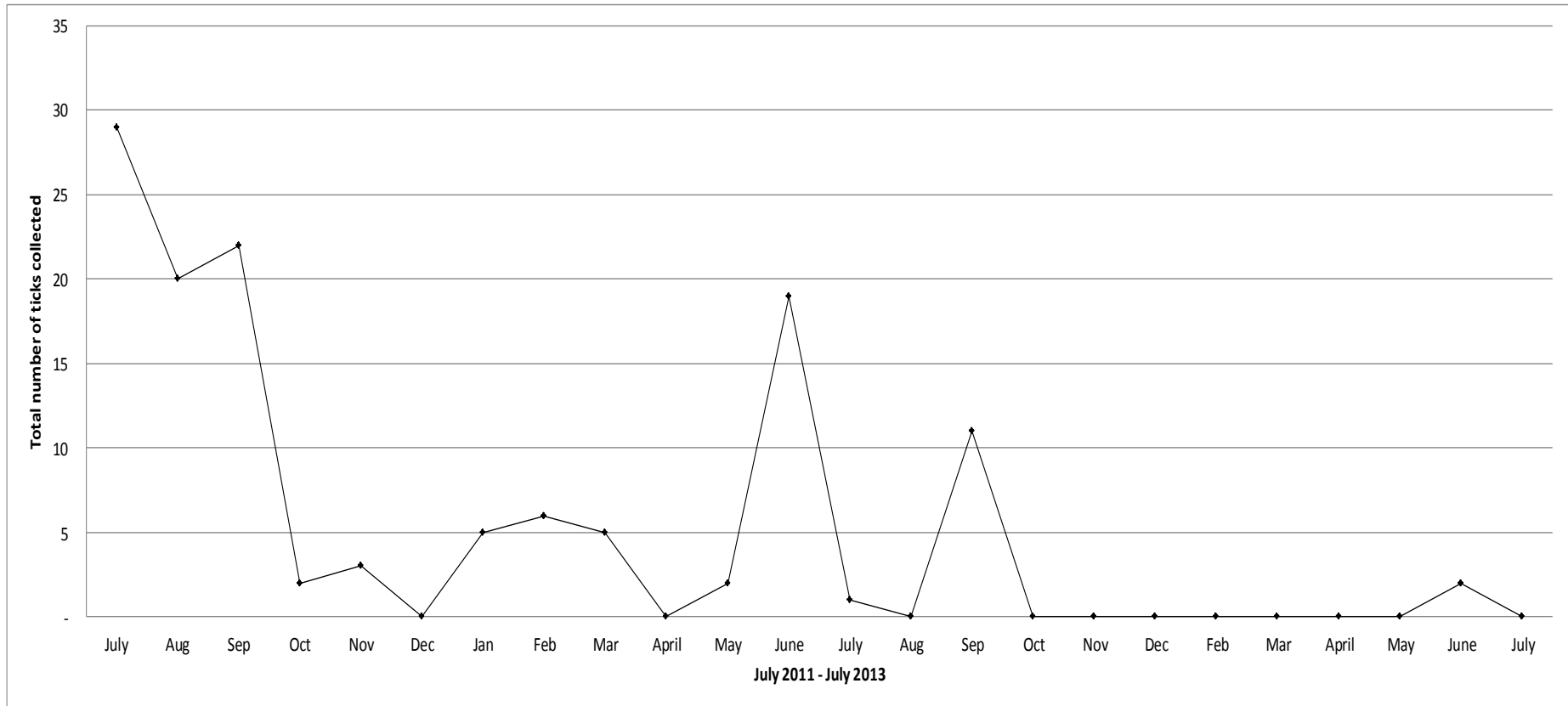


Figure 6: Seasonal abundance of *Amblyomma hebraeum* larvae collected monthly by drag-sampling the vegetation in the sable antelope breeding enclosures on the farm Hoopdal KQ96 between July 2011 and July 2013

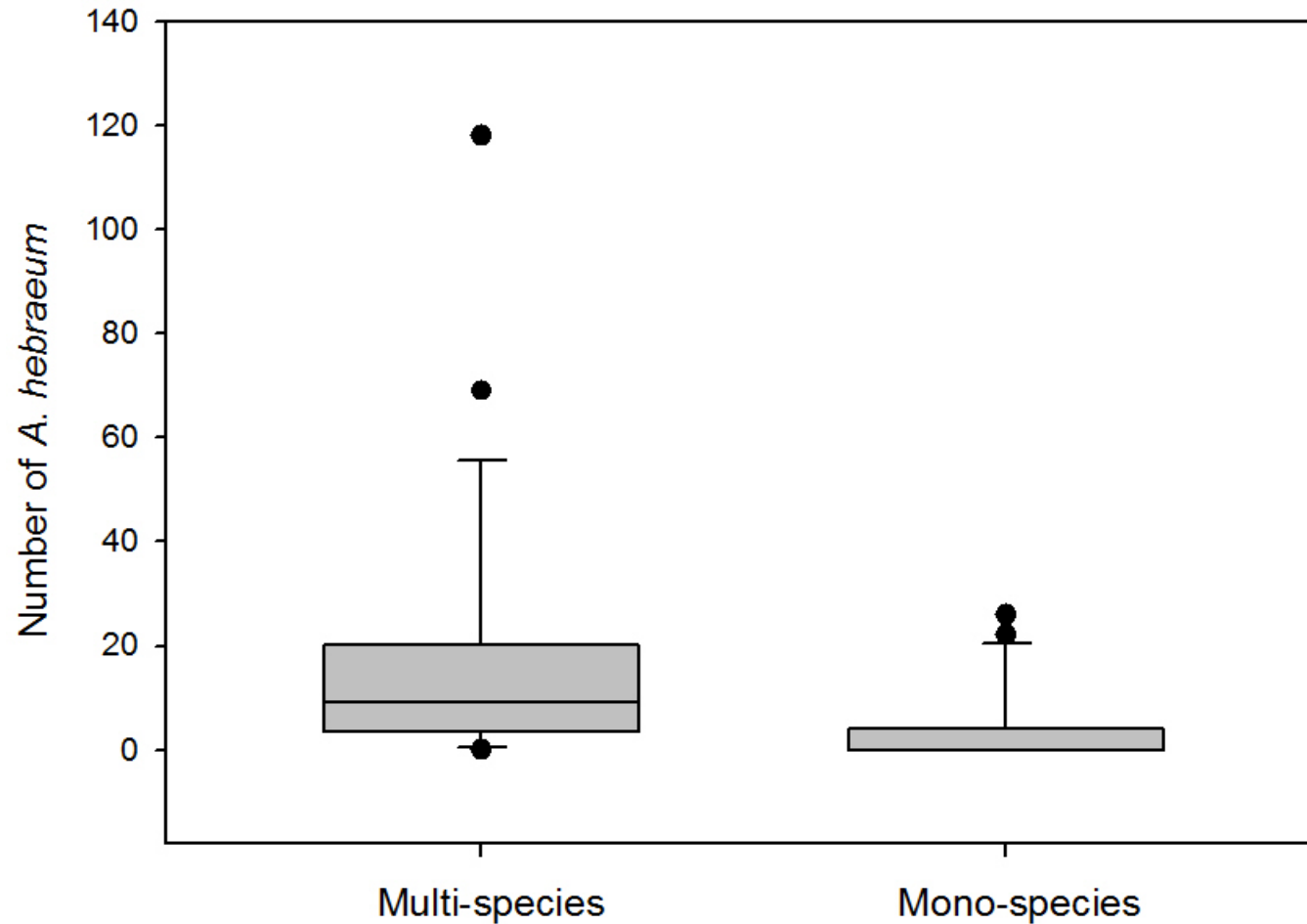


Figure 7: Boxplot depicting the number of *Amblyomma hebraeum* larvae collected from the vegetation in the woodland in the multi-species enclosure versus the sable antelope enclosures on the farm Hoopdal KQ96. The middle bar, the box and the whiskers refer to the median, the interquartile range, and the interoctile (80%) range, the dots are extremes or outliers.

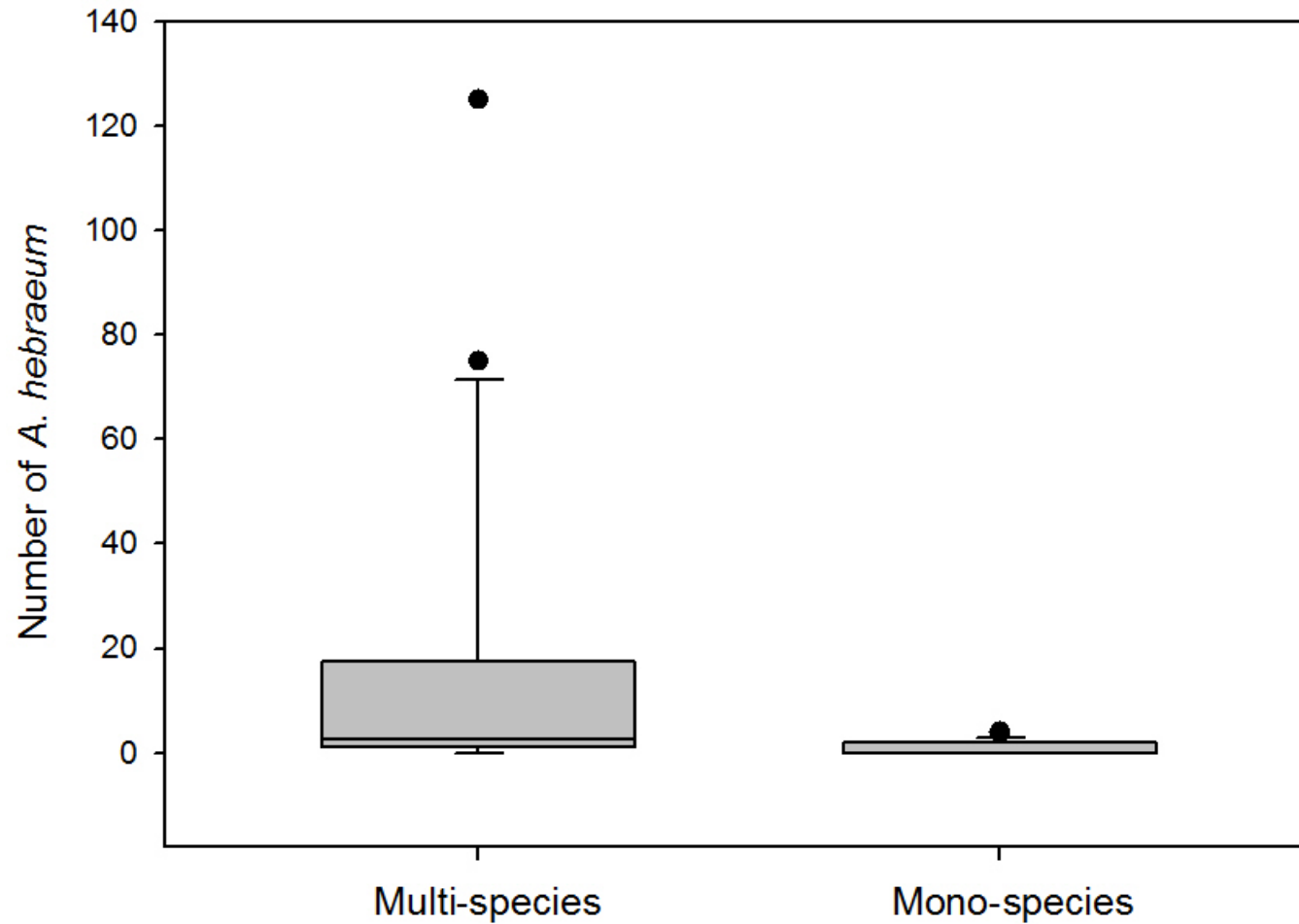


Figure 8: Boxplot depicting the number of *Amblyomma hebraeum* larvae collected from the vegetation in the grassland in the multi-species enclosure versus the sable antelope enclosures on the farm Hoopdal KQ96. The middle bar, the box and the whiskers refer to the median, the interquartile range, and the interoctile (80%) range, the dots are extremes or outliers.

1.4.3 *Rhipicephalus appendiculatus*

Significantly more *R. appendiculatus* larvae were collected in the multi-species herbivore enclosure versus the mono-species enclosures in both woodland habitat ($V=231.0$, $p<0.001$, Fig. 9) and grassland habitat ($V=195.5$, $p<0.01$, Fig. 10), and 91.09% of all *R. appendiculatus* collections came from the multi-species enclosure.

There was no significant difference in the total number of *R. appendiculatus* larvae collected in woodland versus grassland habitats in the multi-species enclosure (Wilcoxon signed rank test (paired by month) $V=157.5$, $p=0.15$), or the mono-species enclosures ($V=138.0$, $p=0.44$). *R. appendiculatus* accounted for 24.19% of all ticks collected and was the only tick species of which nymphs (4.1% of all *R. appendiculatus* collected) and adults were frequently recovered. Nymphs were collected in peak numbers from April to September and none were collected between October and March 2011/2012 or 2012/2013. The numbers of *R. appendiculatus* larvae collected in the multi-species herbivore enclosure had a distinct peak between March and May 2012 with a similar trend in 2013, with absolute peaks in April of both years (Fig. 11). The total numbers of *R. appendiculatus* larvae collected in the breeding enclosures is graphically depicted in Figure 12 but the numbers collected were too low to significantly determine the seasonality. Peak numbers of *R. appendiculatus* larvae were collected in the sable antelope enclosures in September 2011, April 2012 and again in March 2013.

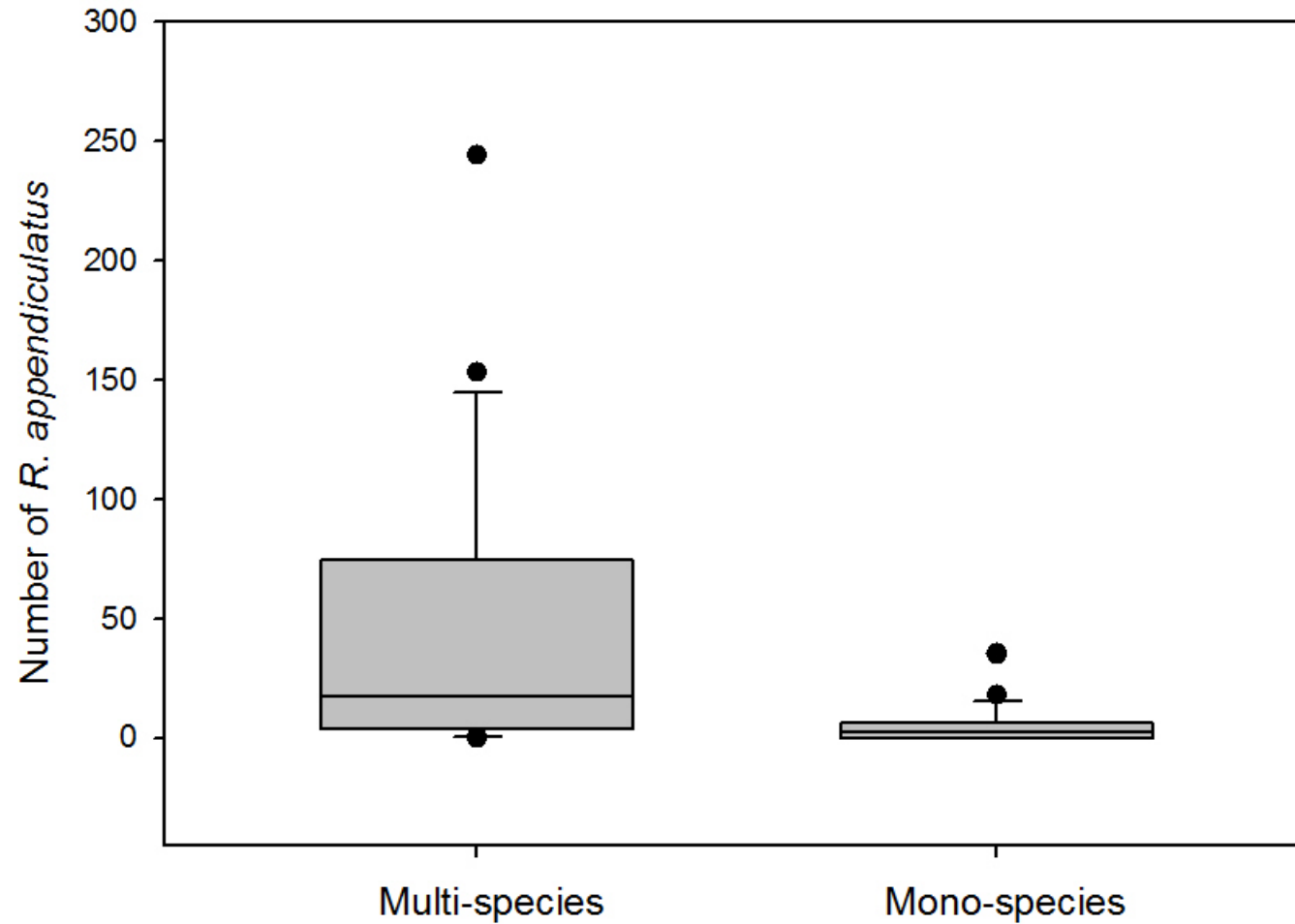


Figure 9: Boxplot depicting the number of *Rhipicephalus appendiculatus* larvae collected from the vegetation in the woodland in the multi-species enclosure versus the sable antelope enclosures on the farm Hoopdal KQ96, The middle bar, the box and the whiskers refer to the median, the interquartile range, and the interoctile (80%) range, the dots are extremes or outliers.



Figure 10: Boxplot depicting the number of *Rhipicephalus appendiculatus* larvae collected from the vegetation in the grassland in the multi-species enclosure versus the sable antelope enclosures on the farm Hoopdal KQ96. The middle bar, the box and the whiskers refer to the median, the interquartile range, and the interoctile (80%) range, the dots are extremes or outliers.

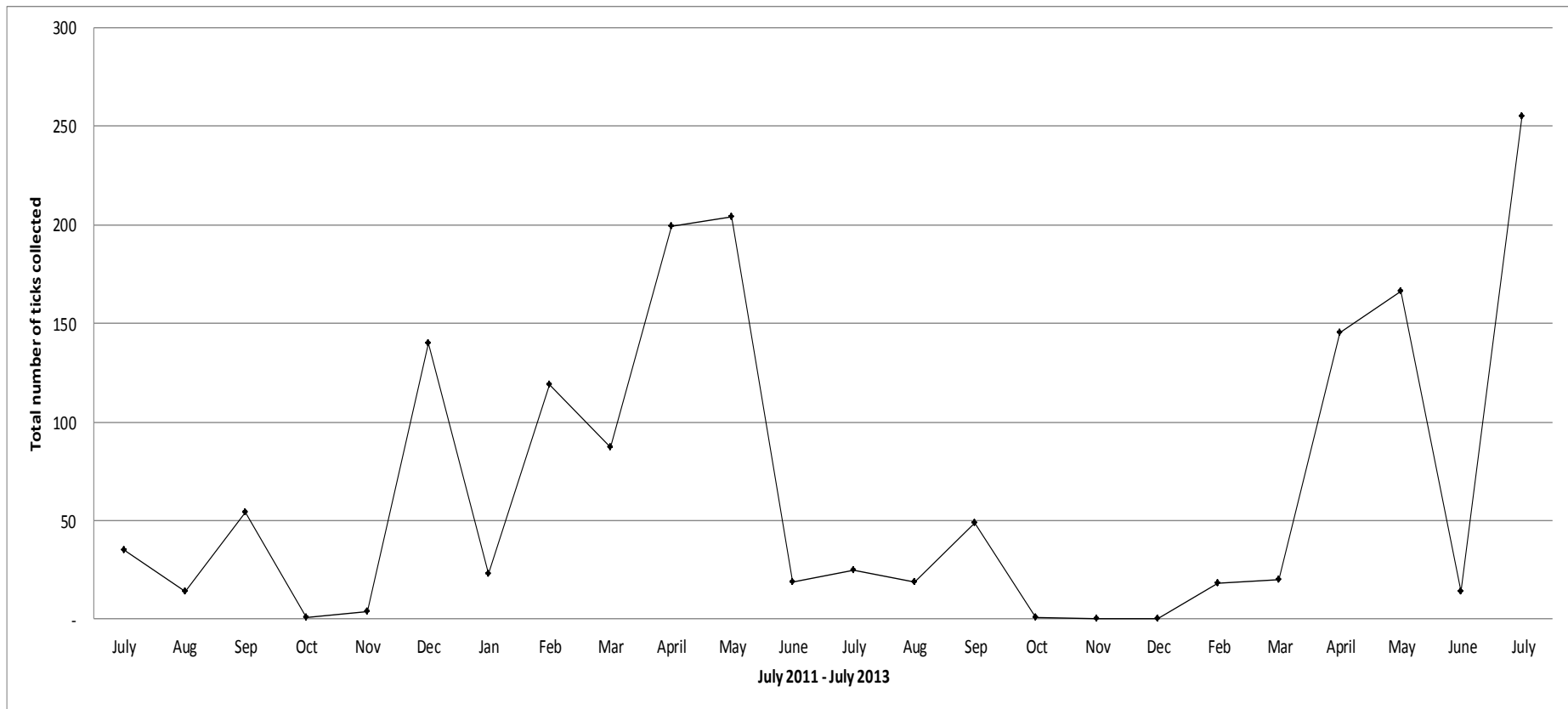


Figure 11: Seasonal abundance of *Rhipicephalus appendiculatus* larvae collected on the farm Hoopdal KQ96 in the multi-species herbivore enclosure between July 2011 and July 2013

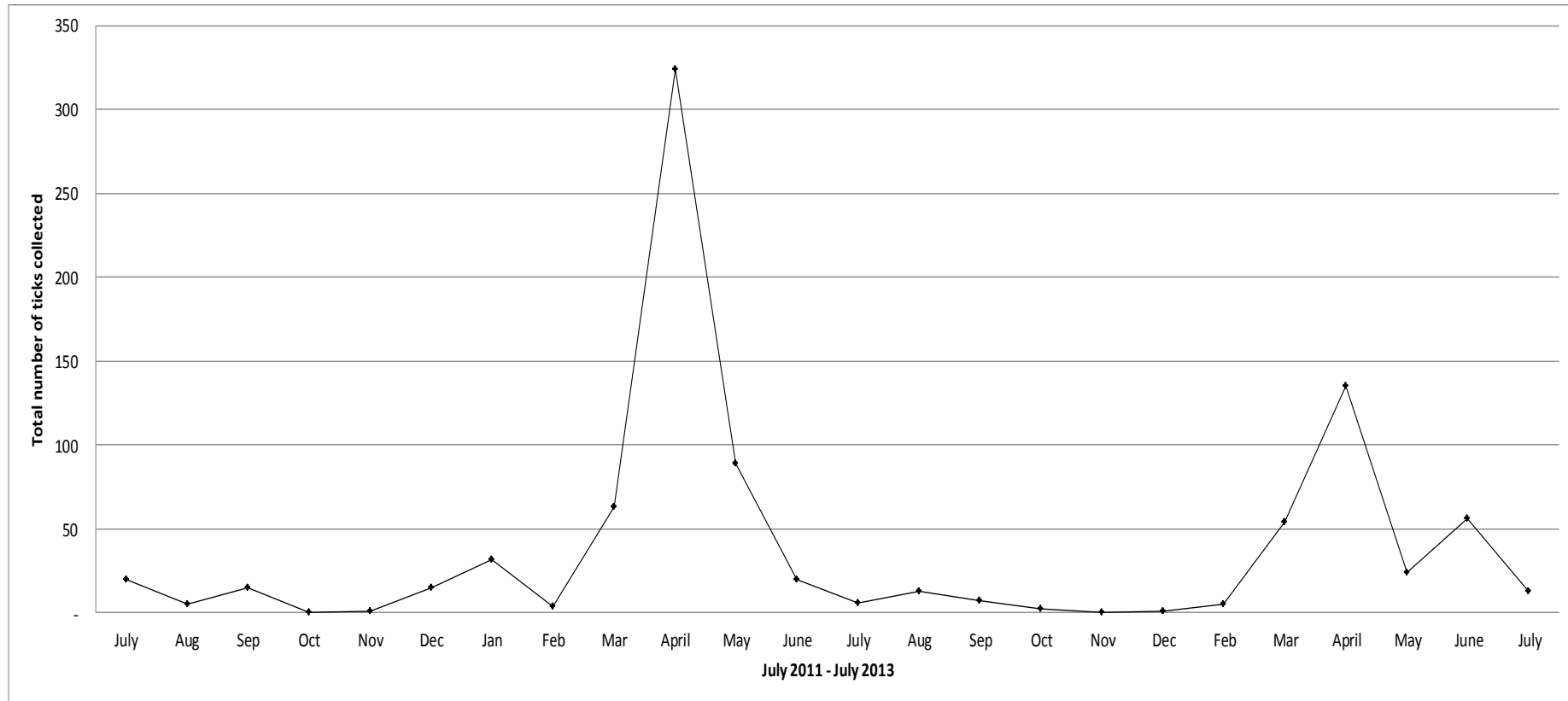


Figure 12: Seasonal abundance of *Rhipicephalus appendiculatus* larvae collected monthly by drag-sampling the vegetation in the sable antelope breeding enclosures on the farm Hoopdal KQ96 between July 2011 and July 2013

1.4.4 *Rhipicephalus decoloratus*

R. decoloratus was collected throughout the year in both the multi-species herbivore enclosure as well as the sable breeding camps. It was the most abundant tick in the sable antelope breeding enclosures, accounting for 65.38% of all ticks collected in these enclosures whilst it only accounted for 24.25% of the total ticks collected in the multi-species herbivore enclosure. Again, there was no significant difference between the abundance of *R. decoloratus* found in woodland versus grassland habitats for either the multi-species enclosure ($V=81.0$, $p=0.09$), or the mono-species enclosures ($V=87.5$, $p=0.21$). However, in contrast to *R. appendiculatus* and *A. hebraeum* (where more ticks were found in the multi-species enclosure than the mono-species enclosures), there was no significant difference in the abundance of *R. decoloratus* larvae found in multi-species enclosures versus mono-species enclosures for either woodland ($V=104.0$, $p=0.31$) or grassland ($V=1123.5$, $p=0.66$).

R. decoloratus larvae were collected throughout the year with peak collections in April and May 2012 and February and April 2013 in the multi-species herbivore enclosure, and in November 2012 and between October and December 2013 in the sable breeding enclosures. There was a significant annual increase in the mean number of *R. decoloratus* larvae collected in the breeding enclosures ($p \leq 0.020$). There was no significant difference between the total numbers of *R. decoloratus* larvae collected in the multi-species enclosure and the sable antelope breeding camps. The seasonality of *R. decoloratus* in the two enclosures is graphically depicted in Figures 13 and 14.

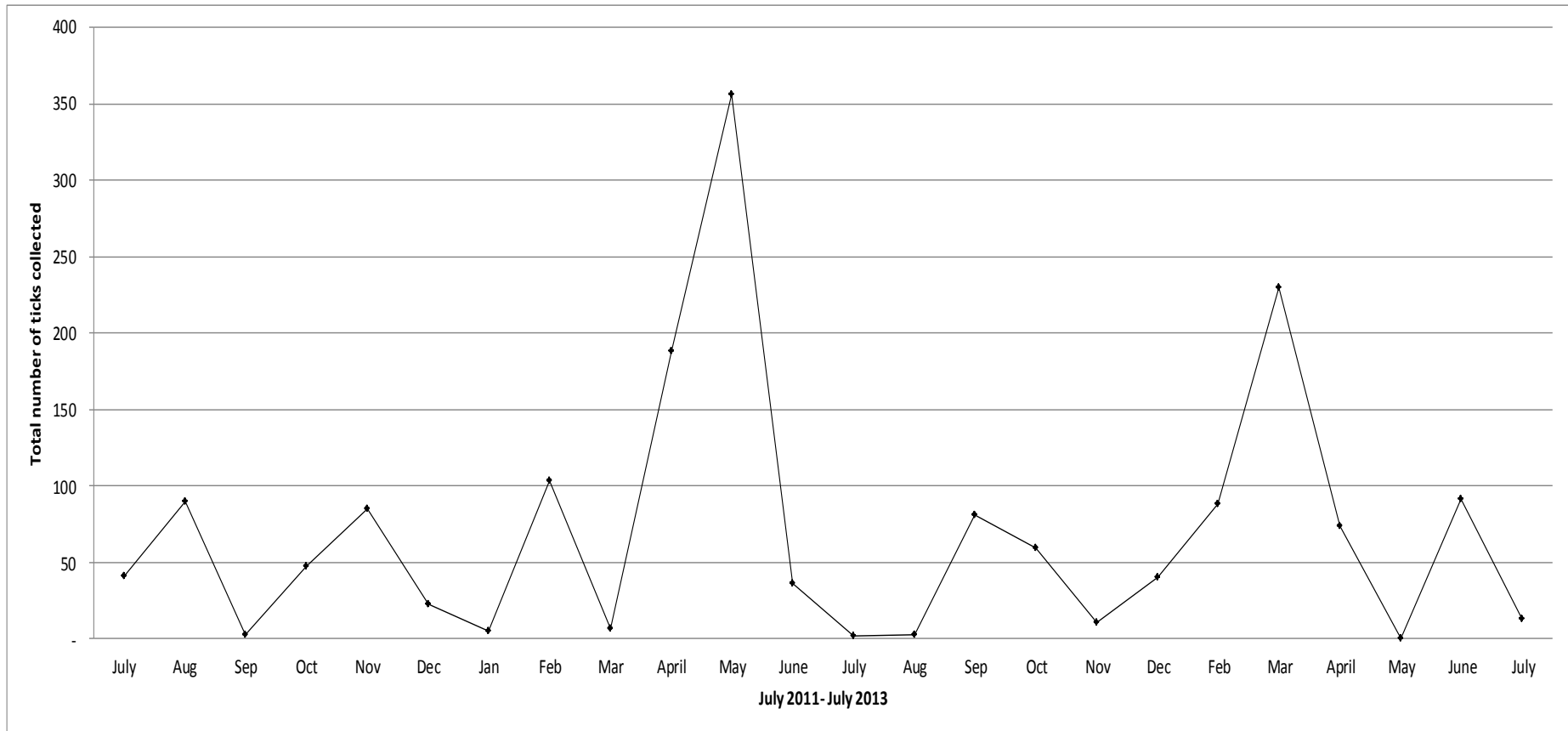


Figure 13: Seasonal abundance of *Rhipicephalus decoloratus* larvae collected in the sable antelope breeding enclosures on the farm Hoopdal KQ96 between July 2011 and July 2013

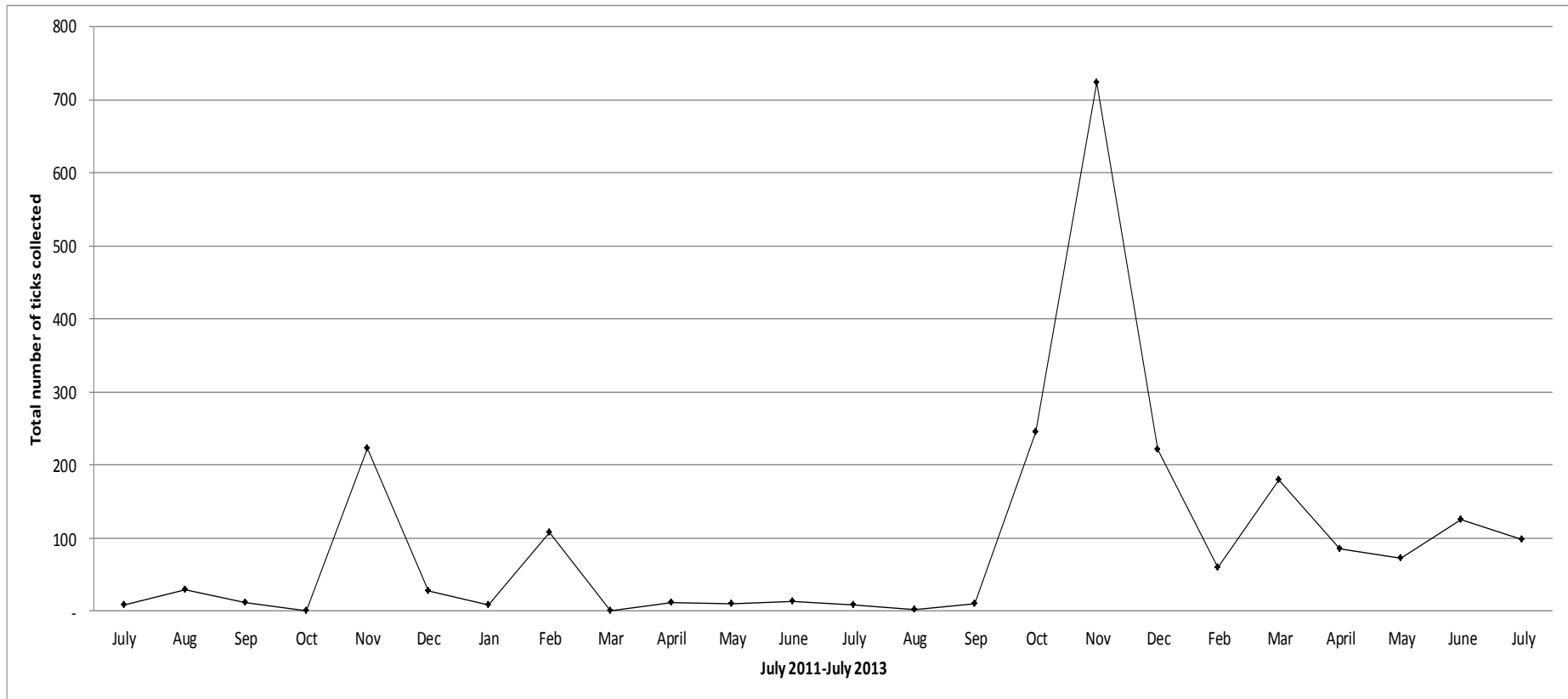


Figure14: Seasonal abundance of *Rhipicephalus decoloratus* larvae collected in the multi-species herbivore enclosure on the farm Hoopdal KQ96 between July 2011 and July 2013

1.4.5 *Rhipicephalus evertsi evertsi*

R. evertsi evertsi was the second most commonly collected tick species and accounted for 27.56% of the total ticks collected (Tables 1.1 and 1.2). Although it was collected throughout the year in both the multi-species enclosure and the sable antelope enclosures, there were distinct peaks in numbers between February and June 2012 and again between December 2012 and June 2013 (Figs. 15 & 16). More *R. evertsi evertsi* larvae were found in grassland than in woodland in the multi-species enclosure ($V=57.0$, $p<0.01$, Fig. 17), but there was no difference in the abundance of ticks between habitats in the mono-species enclosures ($V=110.0$, $p=0.86$). When looking at the difference between enclosure types, there was no significant difference in the abundance of ticks between enclosures in woodland habitat ($V=149.0$, $p=0.47$), however, there were significantly more ticks found in grassland in the multi-species enclosure than in the mono-species enclosures ($V=220.0$, $p<0.05$, Fig. 18). Of the total numbers of *R. evertsi evertsi* larvae collected 71.42% were recovered in the multi-species herbivore enclosure (Tables 1.1 and 1.2). This tick was the second most abundant species collected in the intensive breeding enclosures where it accounted for 23.47% of the ticks collected (Table 1.2).

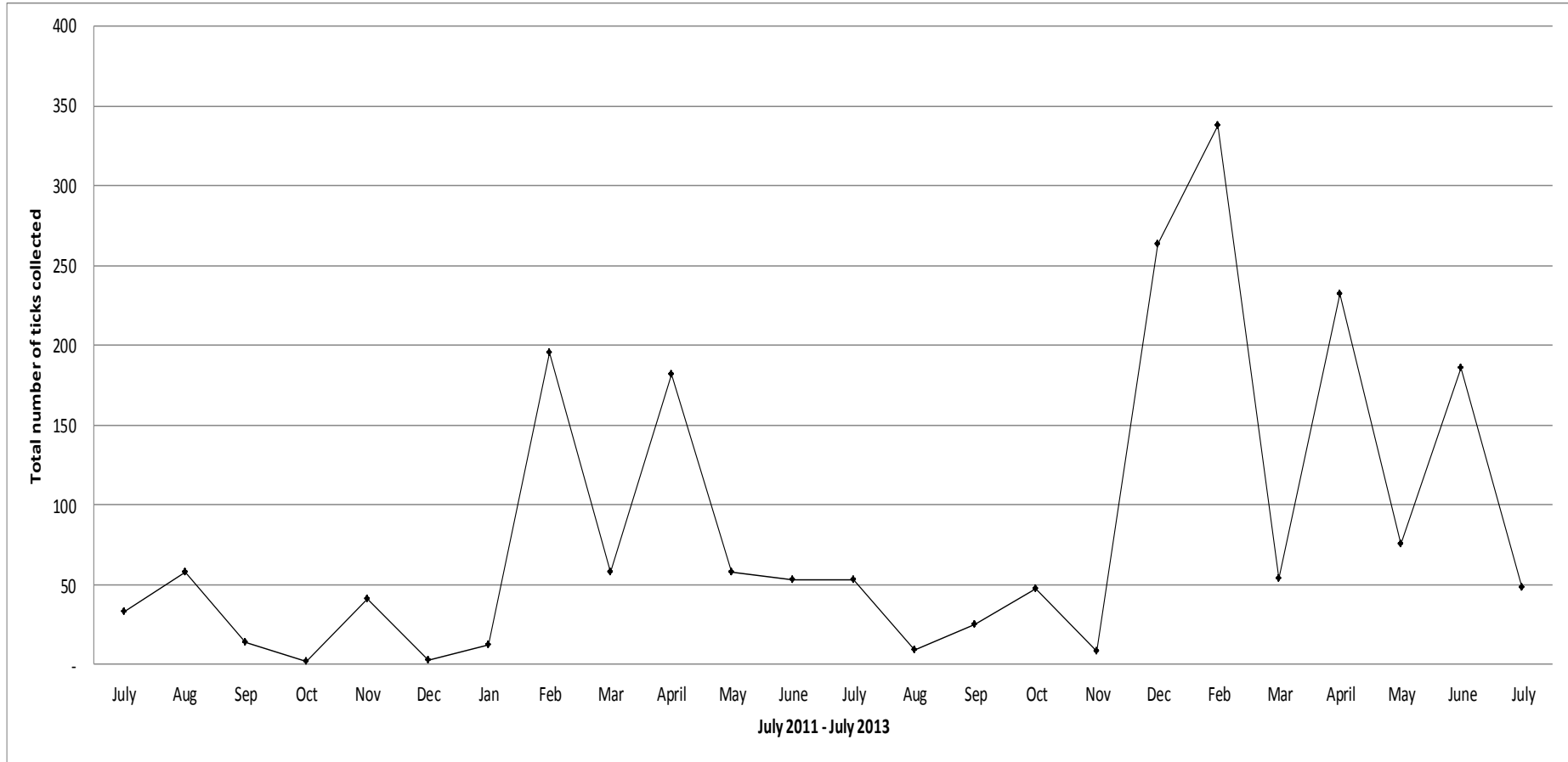


Figure 15: Seasonal abundance of *Rhipicephalus evertsi evertsi* larvae collected monthly by drag-sampling the vegetation in the multi-species herbivore enclosure on the farm Hoopdal KQ96 between July 2011 and July 2013

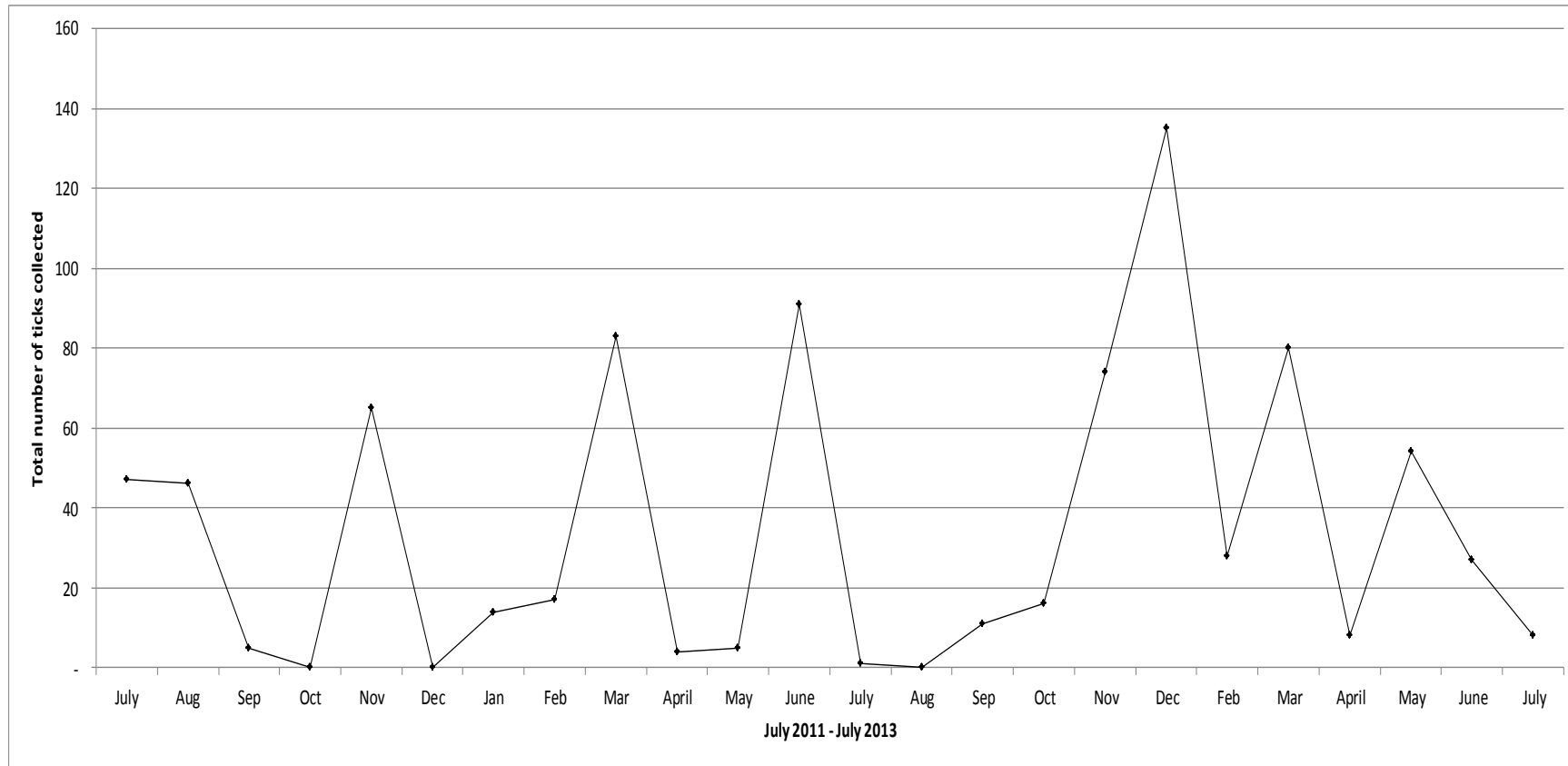


Figure 16: Seasonal abundance of *Rhipicephalus evertsi evertsi* larvae collected monthly by drag-sampling the vegetation in the sable antelope enclosures on the farm Hoopdal KQ96 between July 2011 and July 2013

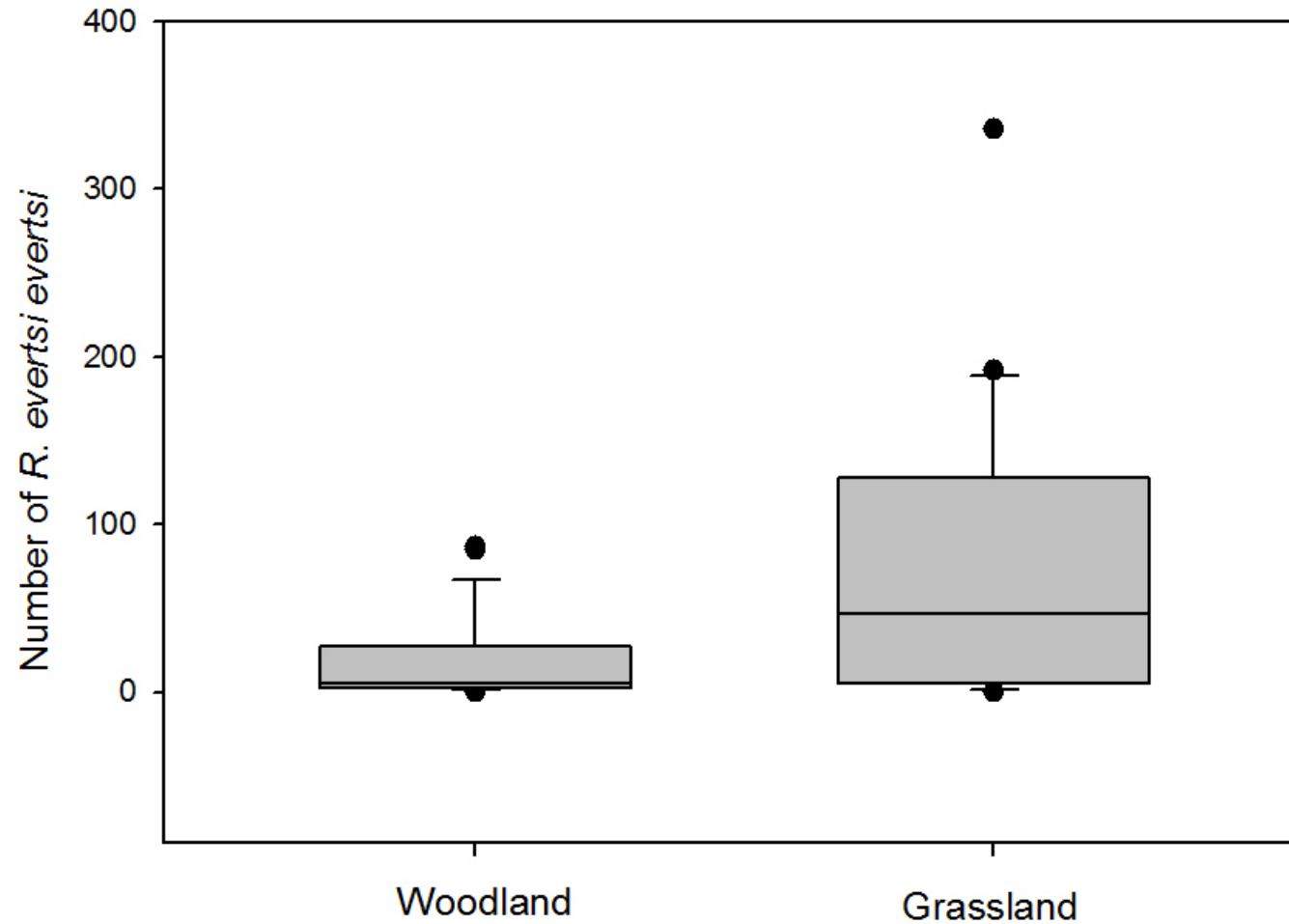


Figure 17: Boxplot depicting the number of *R. evertsi evertsi* larvae collected in grassland versus woodland in the multi-species enclosure on the farm Hoopdal KQ96. The middle bar, the box and the whiskers refer to the median, the interquartile range, and the interoctile (80%) range, the dots are extremes or outliers.

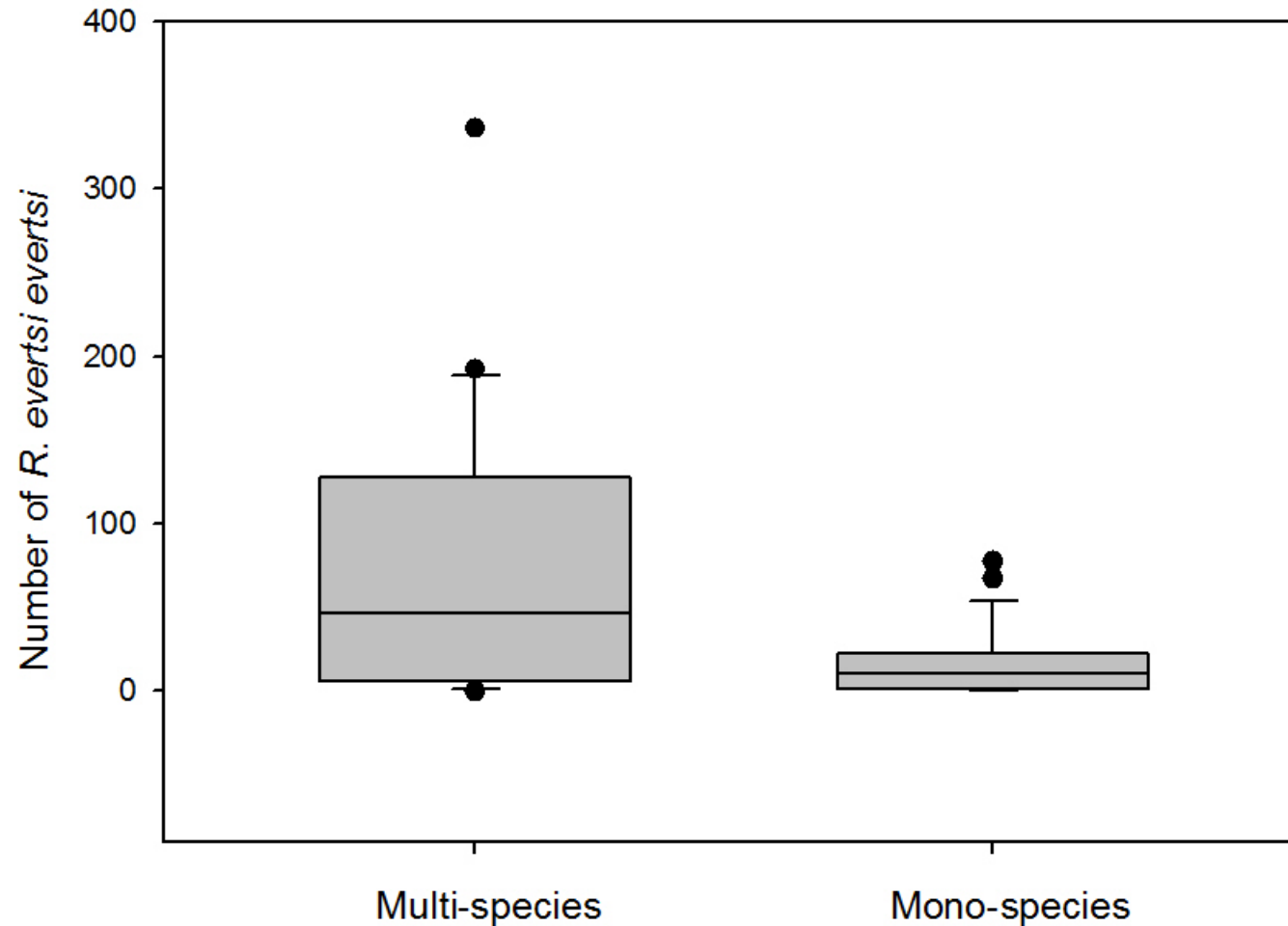


Figure 18: Boxplot depicting the number of *Rhipicephalus evertsii evertsii* larvae collected in grassland in the multi-species enclosure versus the sable antelope enclosures on the farm Hoopdal KQ96. The middle bar, the box and the whiskers refer to the median, the interquartile range, and the interoctile (80%) range, the dots are extremes or outliers.

1.4.6 Less commonly collected ticks

Four of the eight species of ixodid ticks recovered were collected too infrequently to determine their relative abundances or seasonal abundance. *R. zambeziensis* larvae were the most common of the less frequently collected ticks and most were collected in woodland habitats between March and May 2013. Larvae and a single nymph of *R. simus* were collected and all collections were made between March and May of both 2012 and 2013. Two *A. marmoratum* larvae were collected in the woodland of the sable breeding enclosures, one in July 2011 and the other in June 2012. Two adult female *H. elliptica* were collected in the multi-species enclosure in April 2012

1.5 DISCUSSION

The ticks most commonly collected on the Farm Hoopdal KQ 96 in this survey, namely *A. hebraeum*, *R. appendiculatus*, *R. decoloratus* and *R. evertsi evertsi*, were also the most commonly collected ticks by Schroder *et al.* (2006) on the same farm.

1.5.1 *Rhipicephalus decoloratus*

R. decoloratus was significantly more abundant than *A. hebraeum* and *R. appendiculatus* in both woodland and grassland habitats in the sable antelope breeding enclosures and accounted for 65.38% of the total number of ticks collected in the sable antelope breeding enclosures.

The larvae of *R. decoloratus* were present throughout the year but the largest numbers were collected between October and December 2012 during this study. This resembles the seasonality of *R. decoloratus* collected at Skukuza in the Kruger National Park, where peak numbers of larvae were collected in spring (September to November) and the lowest numbers in autumn and winter (Horak *et al.* 2011). Schroder *et al.* 2006 also collected *R. decoloratus* larvae throughout the year on Hoopdal.

Most, larger wild ruminants including impalas, greater kudu and sable antelopes, as well as Burchell's zebras have proved to be excellent hosts of the one-host tick *R. decoloratus* (Zieger, Horak, Cauldwell & Uys 1998b). The significantly greater

abundance of *R. decoloratus* in both woodland and grassland habitats in the sable antelope breeding enclosures when compared to the three-host ticks *A. hebraeum* and *R. appendiculatus* during this survey needs to be investigated further, but could be due to a combination of the development of resistance to the synthetic pyrethroids used to treat the sables and the suitability of the host for the tick as well as other factors such as habitat suitability.

Resistance to synthetic pyrethroids is one of the most serious problems in ticks worldwide (Rodrigues-Vivas, Trees, Rosado-Aguilar, Villegas-Perez & Hodgkinson 2011), and one which has been problematic for *R. decoloratus* in certain regions of South Africa for several years (Mekonnen, Bryson, Fourie, Peter, Spickett, Taylor, Strydom, Kemp & Horak 2003). Zieger *et al.* (1998c) also demonstrated that questing and parasitic populations of *R. decoloratus* were seemingly unaffected by the regular application of synthetic pyrethroids to sympatric cattle and ascribed this to the possible development of acaricide resistance. Treatment of ranched wildlife with acaricides remains problematic and most current practices, such as the method described in this study, involve opportunistic and inaccurate treatment regimes. Accurate treatment of wildlife with an acaricide is only possible when the animal is immobilised and weighed. The current high value of ranched sable antelopes in the commercial sector, coupled with the risks and costs associated with immobilisation have resulted in ranchers seeking less invasive acaricide administration methods. Acaricide treatments that are not accurately determined according to body weight of the target species result in the frequent administration of non-lethal doses of acaricide (synthetic pyrethroids in this case) to the target species, which in turn, may further accelerate the development of acaricide resistance in a tick population. The preferred attachment sites of *R. decoloratus* on cattle are the head, neck and shoulders. The method of acaricide application in the sable antelope breeding enclosures, as described above, may however, lead to the selection of highly resistant ticks as they frequently come into direct contact with the acaricide impregnated rolling pin, and those that survive this direct contact are thus likely to be highly resistant.

Although host suitability may also play a role in the significantly increased abundance of *R. decoloratus* in the sable antelope breeding enclosures when compared to *A. hebraeum* and *R. appendiculatus*, it is unlikely that it is as significant a contributing factor to the observed abundance of this tick. Burchell's zebras, an excellent host of *R. decoloratus*, is one of the more abundant wildlife species in the multi-species enclosure, which they share with large numbers of impalas and kudus, also excellent hosts for *R. decoloratus*. With the abundance of such a variety of excellent hosts as opposed to the single host species in the sable antelope breeding enclosures one could have expected that more *R. decoloratus* larvae would have been collected from the multi-species enclosure.

Despite good breeding results for roan and sable antelopes in South Africa, confinement at high stocking rates may support the build-up of tick populations, as is the case with the population of *R. decoloratus* in this study, and increase the challenge on naïve calves from ticks possibly infected with tick-borne diseases (Nijhof *et al.* 2005). The increasing incidence of fatal babesiosis in farmed sables may be related to the gradual over-abundance of *R. decoloratus* in intensive breeding enclosures as recorded in this survey. Animals in these enclosures are also continuously exposed to a number of stress factors including both nutritional and social, as well as their own inability to select preferred habitat.

1.5.2 Other commonly collected ticks

Most of the preferred hosts of all stages of development of the three-host tick *A. hebraeum* are present on the farm Hoopdal in reasonable numbers, these include giraffes, elands, kudus, impalas, duikers and warhogs, while scrub hares (*Lepus saxatilis*), carnivores, ground-frequenting birds and leopard tortoises (*Stigmochelys pardalis*) are good hosts of the immature stages (Horak, MacIvor, Petney & De Vos 1987; Horak, Heyne & Donkin 2010; Horak *et al.* 2011). *A. hebraeum* larvae exhibited no significant seasonal abundance in this survey, however, the peak numbers collected in summer and lowest numbers collected in July coincide with similar surveys in other summer rainfall areas in South Africa (Horak *et al.* 2011). The largest numbers of *A. hebraeum* larvae were also collected during summer on Hoopdal by Schroder *et al.* (2006). Contrary to Horak *et al.* (2011), who collected significantly more *A. hebraeum* larvae in woodland than in grasslands in the Kruger National Park over a period of 164

months, there was no significant difference between the numbers of *A. hebraeum* larvae collected in woodlands and grasslands in this survey, in either of the enclosures. The survey duration of two years may be too short to draw any inferences from this finding. Significantly more larvae were collected in the multi-species enclosure than in the sable antelope breeding enclosures. Reasons for the difference in total numbers of *A. hebraeum* collected in the sable antelope breeding enclosures could be the absence of smaller mammals and ground-frequenting birds which are important hosts for the immature stages of the tick (Horak *et al.* 1987) and the regular synthetic pyrethroid application on the sable antelopes. Horak & Knight (1986) considered acaricidal treatment of sympatric cattle to be a more significant factor in reducing the numbers of *A. hebraeum* on kudu in the Eastern Cape Province South Africa than other factors such as the alteration of suitable habitat and availability of suitable hosts.

All stages of *R. appendiculatus*, a three-host tick, quest for their hosts from the vegetation (Spickett *et al.* 2011). These hosts include elands, kudu, waterbuck and smaller antelopes, which are all numerous on the farm Hoopdal KQ96. The peak in the numbers of *R. appendiculatus* larvae collected in April of both years during this survey was earlier than that recorded by Spickett *et al.* (2011) in the Kruger National Park, where larvae only peaked in June to August. The total numbers of *R. appendiculatus* larvae collected from woodland and grassland did not differ significantly in this survey. During a 164 month survey of ixodid ticks in the Kruger National Park the habitat distribution of *R. appendiculatus* was variable in response to spatial use of the habitat by their preferred and other hosts (Spickett *et al.* 2011).

Only 8.91% of the total of 2515 *R. appendiculatus* larvae were collected in the intensive breeding camps. The low numbers collected from these camps can possibly be attributed to the regular tick control regimen applied to the sables. Zieger *et al.* (1998c) found a similar reduction in numbers of *R. appendiculatus* on the vegetation and on impalas on a commercial game ranch in Zambia where intensive tick control was practiced on sympatric cattle as did Horak & Knight (1986) on kudu on a mixed cattle and game farm in the Eastern Cape Province, South Africa.

Rhipicephalus evertsi evertsi was the second most abundant tick collected overall in this survey, as well as being the second most abundant species collected in the sable antelope breeding enclosures. Burchell's zebras, greater kudu and impalas, as well as a few elands, all of which are preferred hosts of all stages of development of this two-host tick (Zieger et al. 1998b), are present on the property. The relative abundance of *R. evertsi evertsi* (23.47%) in the intensive breeding camps could also be attributed to the development of acaricidal resistance. The immature stages of this two-host tick remain on the host for longer than those of three-host ticks, and more than one life cycle can be completed annually, thus allowing more exposure over time to the acaricides with resultant perpetuation of the resistant genes in the population.

Only two *A. marmoreum* larvae were collected in this study, one in July 2011 and one in July 2012. Both these larvae were collected in the woodland habitat of one of the sable antelope breeding enclosures. Gallivan *et al.* (2011) collected most *A. marmoreum* larvae from woodlands between March and July during 164 consecutive months of drag-sampling in the Kruger National Park. The preferred host of the adults of this tick is the leopard tortoise (*S. pardalis*). The larvae of *A. marmoreum* infest a wide range of hosts including birds, hares and reptiles (Horak, McKay, Heyne & Spickett 2006). Its larvae in the sable antelope breeding enclosures is an incidental finding, and implies that tortoises, or a tortoise, was or had been present in the breeding enclosures.

Only two adult *Ha. elliptica* were collected in this study. The adults of this tick have frequently been recovered from a number of large and small carnivore species, including leopards (*Panthera pardus*) and black-backed jackals (*Canis mesomelas*), both of which occur erratically on the farm Hoopdal. The adults of *Ha. elliptica* were one of the most commonly collected species from the vegetation in the Kruger National Park by Gallivan *et al.* (2011).

Collections of *R. simus* were sporadic similar to the collections of Gallivan *et al.* (2011) in the Kruger National Park. Five of the seven *R. simus* collected in this study, including 1 nymph, were collected in woodland habitats, and two of the seven ticks recovered were collected in the multi-species herbivore enclosure. The preferred hosts of adult *R. simus* include Burchell's zebras and warthogs, and those of the immature stages rodents and scrub hares, which are all abundant on the farm Hoopdal (Walker *et al.* 2000; Horak, Fourie & Braak 2005).

Uys & Horak (2005) as well Schroder *et al.* (2006) collected small numbers *R. zambeziensis* from the vegetation in the Thabazimbi district as well as from that on the farm Hoopdal, both of which are considered as areas where the distribution of *R. appendiculatus* and *R. zambeziensis* overlap. In the present survey 14.7% of all *R. zambeziensis* larvae were collected in April and May 2012, and 67.2% between March and May 2013.

1.6 CONCLUSION

The commercial game farming practice of breeding rare and endangered antelopes such as sables in mono-species enclosures where strategic tick control is practiced may have a significant impact on the population dynamics of the one-host tick *R. decoloratus*, which possibly increases in abundance over time within these breeding camps. Observations made on other tick species such as *A. hebraeum* and *R. evertsi* suggest that this practice may also affect their population dynamics and this should be investigated further. The progressive development of over-abundance of any tick species, which is potentially a vector of fatal tick-borne diseases such as babesiosis and theileriosis, despite strategic control is of great concern. Recent studies on the evolution of acaricidal resistance in *Rhipicephalus microplus* suggest that alternative strategies to reduce acaricidal resistance, other than the rotation of synthetic pyrethroids, which is ineffective, should be sought (Rodriguez-Vivas *et al.* 2011). The breeding of wildlife species such as sable antelopes in isolated enclosures where tick control is practiced, is unlikely to prevent the loss of animals due to fatal tick-borne diseases in the long term. A reason for this is that the population dynamics of the tick vectors, which potentially transmit these diseases, are also affected, leading to their over-abundance, which in turn may lead to an increased challenge of tick-borne diseases such as babesiosis and theileriosis. When compared with the incidence of disease, the population dynamics of ticks in intensive breeding enclosures may give important insights into their potential vector status for babesiosis in sable antelopes. Alternative methods of tick control should also be sought, including rotational grazing systems and the correct wildlife species composition in enclosures.

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