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Population dynamics of the cattle tick *Rhipicephalus* (*Boophilus*) *microplus* in a subtropical subhumid region of Argentina for use in the design of control strategies

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Abstract. The population dynamics of *Rhipicephalus microplus* (Ixodida: Ixodidae) in northwest Argentina was analysed to support the design of strategic methods for its control. Both parasitic and non-parasitic phases were studied. The seasonal activity of *R. microplus* in its parasitic phase was characterized by three peaks in abundance: the first in mid–late spring; the second in summer, and the third in autumn. The non-parasitic phase of *R. microplus* was characterized by a long total non-parasitic period observed after exposures of females from mid-summer to early autumn, a short total non-parasitic period observed after exposures of females from late winter to late spring, a short period of larval longevity in early and mid-summer, and no hatch of the eggs produced by females exposed in mid- and late autumn and winter. Treatments of cattle administered during the period from late winter to late spring will act on small cohorts of *R. microplus*, preventing the emergence of larger generations in summer and autumn. A 17-week spelling period starting in late spring and early summer will be necessary to achieve optimal control of *R. microplus* free-living larvae. If spelling begins in mid- or late summer or in autumn, the required period will be 26–27 weeks.

Key words. *Rhipicephalus microplus*, cattle tick, control, ecology, Argentina.

Introduction

The cattle tick *Rhipicephalus* (*Boophilus*) *microplus* (Canestrini) is by far the most important tick species to affect cattle in the world. The direct effects caused by the parasitism of *R.* (*B.*) *microplus* ticks and the haemoparasites they transmit constitute a major constraint to cattle production in tropical and subtropical areas (Jongejan & Uilenberg, 2004). The economic losses in cattle production caused by the parasitism of *R.* (*B.*) *microplus* are associated with decreases in weight gain and milk production, and increases in hide damage, mortality, morbidity and control costs (acaricides, manpower, maintenance of plunge dips), as well as with the direct relationship between tick load and the occurrence of screwworm myiasis in cattle (Spath *et al.*, 1994; Reck *et al.*, 2014a). Moreover, the widespread use of chemical ectoparasiticides for tick control has resulted in increasing problems related to multi-drug resistance to practically all of the commercially available acaricides, accumulation of chemical residues in meat or milk, and contamination by release of chemical compounds to the environment (Frisch, 1999; George *et al.*, 2008; Nari Henrioud, 2011; Guerrero *et al.*, 2012; Reck *et al.*, 2014b).

Relevant changes in what is known about the cattle tick and its environment in the American continent have occurred in the last decades. *Rhipicephalus* (*B.*) *microplus* was traditionally considered as a tick species distributed in tropical and

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subtropical areas of Asia, the Americas, Africa, northeastern Australia and New Caledonia (Estrada-Peña *et al.*, 2006; Madder *et al.*, 2012), but since the reinstatement of *Rhipicephalus* (*Boophilus*) *australis* Fuller by Estrada-Peña *et al.* (2012), the current distribution of R (B.) *microplus* encompasses tropical and subtropical areas of America, Africa and south-eastern Asia, whereas *R.* (*B.*) *australis* is present in Australia, New Caledonia and also in Southeast Asia (Estrada-Peña *et al.*, 2012; Burger *et al.*, 2014). This taxonomic reassessment has had profound implications for knowledge of the ecological and epidemiological traits of boophilid species. For example, a significant proportion of current knowledge on the ecology of *R.* (*B.*) *microplus* was generated in Australia (Hitchcock, 1955; Snowball, 1957; Wilkinson & Wilson, 1959; Wilkinson, 1961, 1970; Harley, 1966; McCulloch & Lewis, 1968; Sutherst *et al.*, 1978, 1988; Sutherst & Bourne, 2006, among others) and then extrapolated to American and African countries where it was used for ecological inferences and the formulation of tick control strategies, but currently all the Australian information applies only to *R.* (*B.*) *australis*. Furthermore, in the particular case of livestock areas of northern Argentina, much of the previously forested landscape has been converted into grassland for livestock forage after the introduction of non-native megathermic pastures (Boletta *et al.*, 2006; Zak *et al.*, 2008). This habitat modification has a potential impact on tick ecology, not only as a result of modifications of microclimatic conditions that act on free-living tick stages, but also in an increased tick–host encounter rate because the introduced pastures allow a higher rate of stocking (cows/ha) than forested areas (Nava *et al.*, 2013). All these facts evidence the need for new ecological studies of *R.* (*B.*) *microplus* in South America in order to design sustainable strategies for its control, and to preclude the extrapolation of ecological data previously obtained for a different species [*R.* (*B.*) australis)] or for *R.* (*B.*) *microplus* in an environmental scenario which has been subjected to meaningful transformations (e.g. Ivancovich, 1975; Guglielmone *et al.*, 1981, 1990; Ivancovich *et al.*, 1982, 1984; Guglielmone, 1992; Mangold *et al.*, 1994).

The cattle tick *R.* (*B.*) *microplus* has a one-host life cycle that comprises a parasitic phase and a non-parasitic phase (Nuñez *et al.*, 1982). During the parasitic phase, larvae, nymphs and adults feed, moult (larvae and nymphs) and mate (adults) on the same host, and engorged females drop off the host to oviposit in the environment. Preovipositional development and oviposition of engorged females, incubation of eggs, and host-seeking by larvae are the components of the non-parasitic phase. The duration of the parasitic phase is relatively constant with a mode of approximately 23 days (Nuñez *et al.*, 1982), whereas the extent of the non-parasitic phase varies within and among localities because it is strongly influenced by environmental factors such as climate and vegetation.

Knowledge of the population dynamics of a tick is a key factor in the design of strategic methods of control that minimize the number of acaricide applications. Previous studies performed in northwest Argentina have shown that treatments of cattle in late winter and spring have a significant effect on the small first spring generation of *R.* (*B.*) *microplus* that preclude the development of the larger autumn generation (Nava *et al.*, 2014, 2015). The control schemes designed in these works were based on information on seasonal variations in population abundances of *R.* (*B.*) *microplus*. Further studies on control strategies may benefit from updated and accurate knowledge on the population dynamics of *R.* (*B.*) *microplus* under certain conditions. In order to provide a framework for the strategic control of *R.* (*B.*) *microplus*, this work is intended to describe the population dynamics of this tick species through the analysis of both parasitic and non-parasitic tick stages in a livestock area of northwest Argentina characterized by a subtropical, subhumid climate and the introduction of megathermic pastures.

Materials and methods

The study was carried out at Leales (27°11′ S, 65°14′ W), Tucumán Province, Argentina. This site belongs to the Chaco Phytogeograpic Province *sensu* Cabrera (1976), and has a subtropical and subhumid climate with an average annual rainfall of 900 mm concentrated from October to March. Cattle were kept in a pasture composed of *Chloris gayana* Kunth (Gramma Rhodes) and fed on corn silage and expeller soybean meals at an 80 : 20 proportion and given access to water *ad libitum*. Micro-climate data were recorded using HOBO® data loggers (U23-002 Pro v2; Onset Computer Corp., Bourne, MA, U.S.A.). The sensors of the data loggers were placed at ground level, where *R.* (*B.*) *microplus* develops the non-parasitic phase of its lifecycle. Temperature and relative humidity (RH) were measured daily once every hour and data were used to calculate saturation deficit according to the formula presented by Randolph & Storey (1999). Saturation deficit is a measure of the atmosphere's drying power which integrates temperature and RH, and is a key factor in the modulation of the lifespan of free-living stages of ticks (Teel, 1984; Randolph & Storey, 1999; Perret *et al.*, 2000; Sutherst & Bourne, 2006).

The seasonal dynamics of all parasitic stages of *R.* (*B.*) *microplus* were determined by monthly examination of Braford cattle over 2 years. Counts of *R.* (*B.*) *microplus* females (4.5–8.0 mm long) were performed each month on one side of 10 animals not subjected to treatments with acaricides from August 2013 to August 2015. Tick infestation was evaluated in two temporal blocks: (a) August 2013 to July 2014, and (b) August 2014 to July 2015. A cohort of 10 Braford calves aged 9 months at the beginning of the counts was examined for tick infestation in each temporal block. The number of ticks collected on cattle was multiplied by two for statistical analyses. Prevalence (number of hosts infested/number of hosts examined), mean number of ticks (number of ticks/number of hosts examined, including both infested and non-infested hosts) and median values and first and third quartiles (Q1–Q3) were calculated. Data were subjected to the Shapiro–Wilk test for normality prior to statistical analysis. Monthly differences in tick distributions on hosts were tested using the Kruskal–Wallis test with *a posteriori* Dunn's multiple comparison (Zar, 1999). Tick aggregation level among cattle was estimated by the variance to mean ratio and the index of discrepancy (D) (Poulin, 1993, 2007). Briefly, a D-value of 0 constitutes null aggregation (all hosts have equal levels of infestation) and a D-value of 1 indicates complete aggregation (all members of a parasite population are concentrated on one individual host). This index quantifies aggregation as the distance between the observed

Table 1. Prevalence (P), mean number, median and interquartile range [IQR (first–third quartiles)], index of discrepancy (*D*) and variance : mean ratio (V : M) for *Rhipicephalus* (*Boophilus*) *microplus* (females, 4.5–8.0 mm long) infestation on cattle.

	Block I∗						Block II∗				
	P, %	Mean†	Median (IQR)	*D*	V : M		P, %	Mean†	Median (IQR)	*D*	V : M
August 2013	90	9.1^{d}	7 (2–14)	0.45	7.5	August 2014	10	0.2^{d}	0 (0–0)	0.80	2
September 2013	70	4.7^{d}	4 (0–8)	0.46	4	September 2014	10	4.8^{d}	4 (0–6)	0.51	5.6
October 2013	60	3.4^{d}	3 (0–4)	0.54	5.4	October 2014	80	3.6^{d}	4 (2–6)	0.35	1.9
November 2013	90	57.9^{bc}	54 (30–82)	0.33	26.2	November 2014	100	80.4^{b}	74 (42–110)	0.36	29.9
December 2013	100	50.1^{bc}	53 (18–82)	0.36	25.2	December 2014	80	8.2^{d}	9 (6–10)	0.36	3.3
January 2014	90	45.7^{bc}	50 (20–68)	0.36	23.1	January 2015	100	42.4^{c}	38 (12–54)	0.33	21
February 2014	90	77.5^{ab}	82 (46–103)	0.31	28.5	February 2015	90	9.2^{d}	9 (4–14)	0.35	4.7
March 2014	90	35.8^{bc}	42 (19–51)	0.28	11.8	March 2015	100	54.4^{bc}	66 (26–80)	0.29	20.5
April 2014	90	120^{a}	114 (91–153)	0.24	29.2	April 2015	100	267.4^{a}	191 (102–430)	0.29	151.5
May 2014	90	119.8^{a}	104 (64–149)	0.29	50.5	May 2015	90	164.6^{a}	146 (36–266)	0.27	107.6
June 2014	90	28.5^{cd}	30 (22–32)	0.33	7.8	June 2015	10	0.2^{d}	0 (0–0)	0.82	2
July 2014	0	0	0	—	—	July 2015	10	0.4^{d}	0 (0–0)	0.79	3.1

∗A cohort of 10 Braford calves aged 9 months at the beginning of the counts was examined for tick infestation in each temporal block.
†Kruskal–Wallis test with *a posteriori* Dunn's multiple comparison. Numbers with different superscripts are significantly different ($P < 0.01$).

parasite distribution and a perfectly uniform distribution, and can be employed to compare distributions that vary in prevalence or mean number of parasites per host (Poulin, 1993, 2007). Calculations were made with Quantitative Parasitology Version 3.0 (Rózsa *et al.*, 2000).

Engorged females of *R.* (*B.*) *microplus* were exposed each month in pastures of *C. gayana* to evaluate the developmental dynamics of non-parasitic lifecycle stages. The exposures were made in 21 series of 20 *R.* (*B.*) *microplus* females each from January 2013 to May 2015. Ticks were obtained from naturally parasitized cattle in the study area. Engorged females were enclosed in stainless steel wire mesh envelopes placed under the grass and protected from direct solar irradiation as described in Nava *et al.* (2013). The biological parameters of *R.* (*B.*) *microplus* measured in each exposure included the proportion of females ovipositing (PFO), the preoviposition period (POP) (time from female exposure until beginning of oviposition), the proportion of egg clusters hatching (PECH), the incubation period of eggs (time from laying of the first egg until hatch of the first egg), larval longevity (time from the first egg hatch to death of last larva), and total non-parasitic period (TNPP) (time from exposure of the female to death of last larva). Statistical comparisons among series were performed using one-way analysis of variance (ANOVA) ($P < 0.01$) with Tukey's post hoc test (Zar, 1999). Finally, stepwise multiple regressions were generated to determine the relationships between free-living developmental phases of *R.* (*B.*) *microplus* ticks and microclimate variables. Incubation period of eggs and larval longevity were the criterion variables, and temperature, RH, saturation deficit and incubation period of eggs (in the case of larval longevity) were regarded as predictor variables. Data were log-transformed to match regression assumptions, and the relationship with the highest coefficient of determination (r^2) was chosen as the best descriptor.

Results

Quantitative data for monthly rates of infestation of *R.* (*B.*) *microplus* females on cattle in each temporal block are presented in Table 1. There was an increase in abundance between mid–late spring and autumn and then a decrease towards winter and early spring. The highest levels of infestations were found in April and May (autumn) in both temporal blocks. Values recorded in April and May were statistically different from values recorded in the other months (Table 1). The observed pattern of seasonal dynamics of *R.* (*B.*) *microplus* in its parasitic phase is depicted in Fig. 1. In results for levels of aggregation, the variance to mean ratio was always >1 (Table 1), indicating that the distribution of ticks among cattle was aggregated. Values of *D* ranged from 0.23 to 0.54 in block I, and from 0.27 to 0.82 in block II (Table 1). The lowest values of *D* (lower level of aggregation) were mostly recorded in the autumn months, when the largest abundances of ticks on cattle were observed.

Weekly mean ground-level temperatures and saturation deficits (mmHg) recorded from January 2013 to June 2015 at the study site are shown in Fig. 2. Data on the seasonal dynamics of the biological parameters of *R.* (*B.*) *microplus* (PFO, POP, PECH, incubation period, larval longevity and TNPP) in its non-parasitic phase obtained during this study are detailed in Table 2. In most exposures, PFO values were ≥0.90. The exceptions were the exposures of June 2013, June 2014 and March 2015, in which PFO values were 0.80, 0.70 and 0.75, respectively. The shortest POPs were recorded in the late spring and summer exposures, whereas the longer POPs were recorded in the winter exposures. Preovipositon periods ranged from 5 to 7 days in summer and late spring, and from 16.2 to 25.5 days in winter (Table 2). Analyses of PECH data showed no hatches in series 4–7, 13–16, 20 and 21. These series corresponded to exposures in mid-autumn and winter, when mean monthly temperatures were below 20 °C (Fig. 2). In the remaining series, PECH values ranged from 0.60 to 1 (Table 2).

The results of the multiple statistical comparisons among series for incubation period, larval longevity and TNPP are presented in Table 2. The general trend showed that incubation periods in late spring and early summer exposures were significantly shorter than in late summer, mid-spring and early autumn exposures (Table 2). The longest periods of larval longevity were

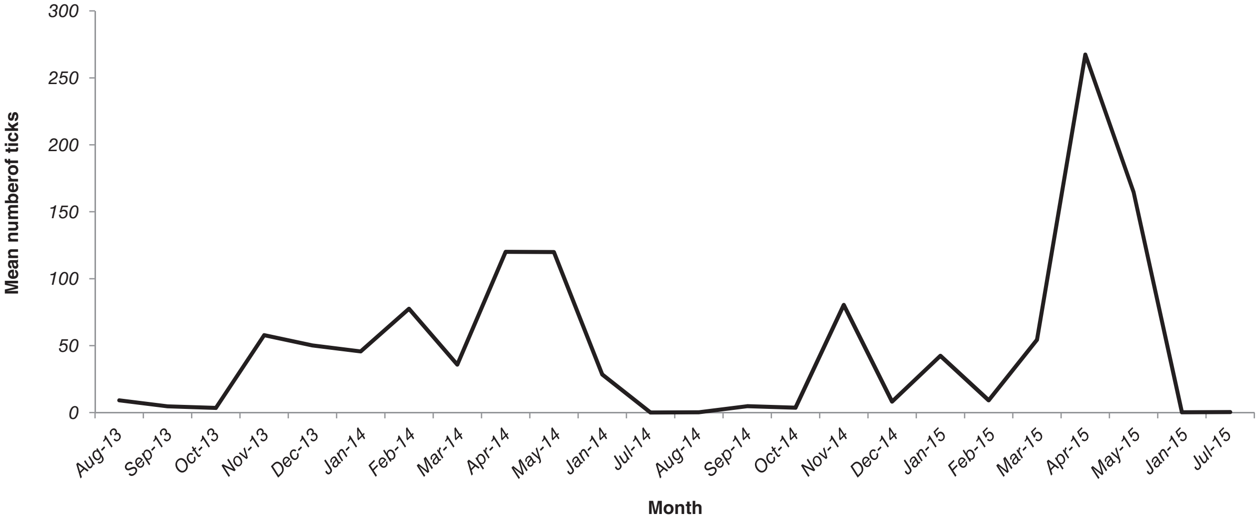

Fig. 1. Seasonal dynamics of *Rhipicephalus* (*Boophilus*) *microplus* infestation on cattle in a subtropical, subhumid region of Argentina.

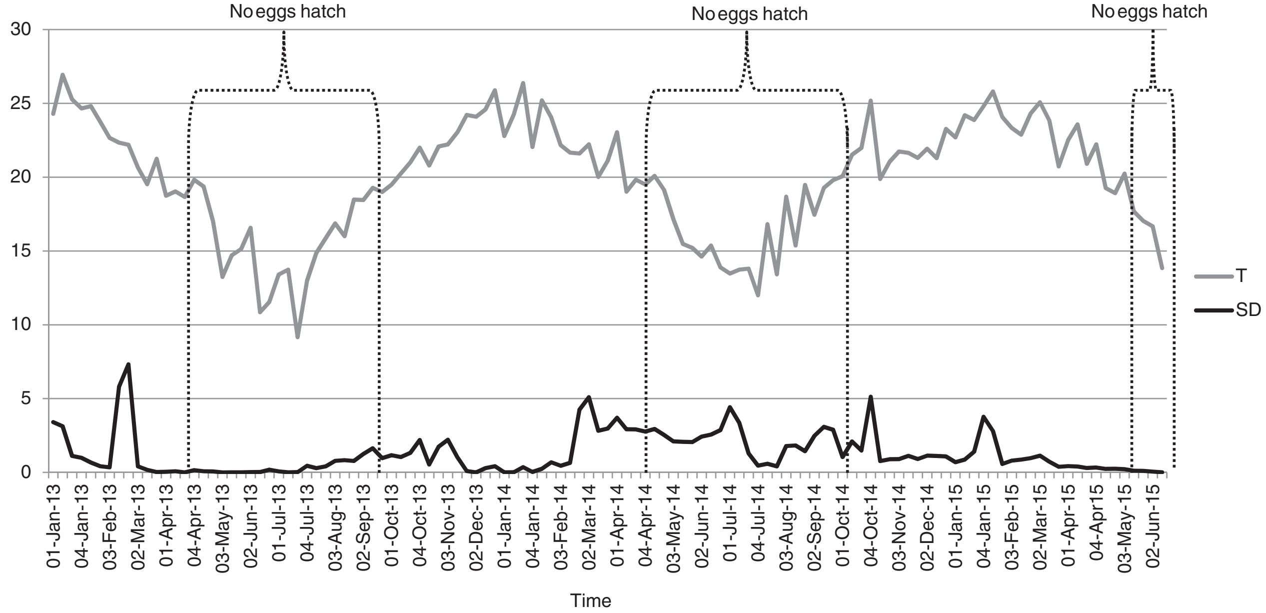

Fig. 2. Weekly mean ground-level temperatures and saturation deficits registered during the study period at Leales, Tucumán Province, Argentina. T, temperature (°C); SD, saturation deficit (mmHg).

observed in larvae produced by females exposed in mid–late summer and early autumn, whereas the shortest periods were detected for larvae produced by females exposed in spring (Table 2). Longer TNPPs were observed for series in which females were exposed in summer and early autumn (series 1–3, 11, 12 and 19), for which maximum TNPP values ranged from 161 to 188 days. The shortest TNPP corresponded to the series in which females were exposed in spring (series 9, 10 and 17), in which maximum TNPP values ranged from 87 to 120 days. Mean, maximum and minimum TNPP values can be seen in Table 2. Variation in TNPP in *R.* (*B.*) *microplus* (disaggregated in POP, incubation period and larval longevity) in relation to the months in which engorged females were exposed is depicted in Fig. 3.

For the regression analysis, mean values for microclimatic variables to which egg masses were exposed were calculated from the date of the laying of the first egg to the date at which the first egg hatched. Values for microclimatic variables to which the hatched larvae derived from each female were exposed were calculated from the date at which the first egg hatched to the date of death of the last larva. These periods correlate with the study definitions for the incubation period of eggs and larval longevity. The regression analysis showed that incubation period has a negative linear relationship with temperature, with a high

Table 2. Dynamics of the non-parasitic phase of *Rhipicephalus* (*Boophilus*) *microplus* in a subhumid subtropical region of Argentina.

Series	Date of exposure	PFO	POP, days, mean (range)	PECH	IP,∗ days, mean (range)	LL,∗ days, mean (range)	TNPP,∗ days, mean (range)
1	23 January 2013 (SU)	0.90	5	0.90	35	96.2 (59–136)^{a}	136.2 (99–176)^{c}
2	27 February 2013 (SU)	0.95	5.2 (5–7)	0.95	59.8 (59–63)^{b}	102.5 (83–110)^{a}	167.5 (147–174)^{a}
3	26 March 2013 (AU)	1	7	1	86.7 (82–106)^{a}	60.4 (12–71)^{cd}	154.1 (124–161)^{ab}
4	22 April 2013 (AU)	1	10.3 (10–16)	0	—	—	—
5	23 May 2013 (AU)	1	16.6 (8–25)	0	—	—	—
6	28 June 2013 (WI)	0.80	29.7 (20–40)	0	—	—	—
7	21 August 2013 (WI)	0.90	16.2 (7–21)	0	—	—	—
8	23 October 2013 (SP)	1	9 (7–14)	0.60	60 (57–69)^{b}	37.8 (23–52)^{e}	106.5 (105–117)^{d}
9	27 November 2013 (SP)	1	8.2 (8–11)	0.80	34.4 (22–36)^{de}	38.2 (28–52)^{e}	80.4 (70–87)^{e}
10	18 December 2013 (SP)	1	5.2 (5–9)	0.65	28.2 (16–30)^{e}	40.8 (14–57)^{e}	75.2 (49–92)^{e}
11	25 February 2014 (SU)	1	6	0.80	51^{c}	90.5 (61–130)^{a}	147.5 (119–188)^{bc}
12	26 March 2014 (AU)	1	5.35 (5–12)	0.90	87.5 (84–108)^{a}	69.9 (56–85)^{bc}	162.8 (159–175)^{a}
13	29 April 2014 (AU)	1	8	0	—	—	—
14	27 May 2014 (AU)	1	7.35 (7–14)	0	—	—	—
15	24 June 2014 (WI)	0.70	25.5 (24–31)	0	—	—	—
16	23 September 2014 (SP)	0.90	8.5 (7–14)	0	—	—	—
17	3 December 2014 (SP)	1	7	0.90	40^{d}	52.7 (45–73)^{de}	99.7 (96–120)^{d}
18	27 January 2015 (SU)	1	7	1	31^{e}	74.5 (21–94)^{bc}	115.1 (62–132)^{d}
19	26 March 2015 (AU)	0.75	11.4 (11–15)	0.73	47.8 (46–56)^{cd}	85.3 (10–129)^{ab}	144.5 (65–184)^{bc}
20	29 April 2015 (AU)	0.90	15	0	—	—	—
21	20 May 2015 (AU)	0.90	12	0	—	—	—

∗ANOVA with Tukey's post hoc test. Numbers with different superscripts are significantly different ($P < 0.01$).

PFO, proportion of females ovipositing; POP, preoviposition period; PECH, proportion of egg clusters hatching; IP, incubation period of eggs; LL, larval longevity; TNPP, total non-parasitic period; SP, spring; SU, summer; AU, autumn; WI, winter.

coefficient of determination ($R^2 = 0.82$). This means that as the temperature (T) increases, the incubation period decreases. The following regression equation described the influence of temperature (T) on incubation period (IP):

$$\mathrm{IP} = 6.143 \; - \; 3.361 \; \times \; \mathrm{T}.$$

With regard to the relationship between larval longevity (LL) and these predictor variables, the best regression model ($R^2 = 0.79$) includes both T and IP. There is a negative relationship between LL and T and IP. The regression equation for this is:

$$\mathrm{LL} = 5.177 \; - \; 1.988 \; \times \; \mathrm{T} \; - \; 0.487 \; \times \; \mathrm{IP}$$

Discussion

The seasonal activity of *R.* (*B.*) *microplus* in its parasitic phase in a subtropical, subhumid region of Argentina was characterized by three peaks in abundance. The first of these occurred in mid–late spring, the second in summer, and the third and largest peak in abundance was observed in autumn (Fig. 1, Table 1). In the study area, *R.* (*B.*) *microplus* appears to undergo at least three generations per year. These results coincide with previously recorded data on the seasonality of *R.* (*B.*) *microplus* on cattle in more arid areas of northwest Argentina and in localities in Brazil and Uruguay between the latitudes of 24° S and 32° S (Guglielmone *et al.*, 1981, 1990; Brum *et al.*, 1987; Evans, 1992; Guglielmone, 1992; Mangold *et al.*, 1994; Nari, 1995; Martins *et al.*, 2002). Thus, the seasonal pattern observed during this work is representative of the population dynamics of *R.* (*B.*) *microplus* on cattle in this species' southernmost range of distribution in South America.

The dynamics of the non-parasitic phase of *R.* (*B.*) *microplus* in the subtropical, subhumid region of Argentina are principally characterized by the following traits: (a) a long TNPP observed after exposures of female ticks in February and March (mid-summer to early autumn); (b) a short TNPP observed after exposures of female ticks in October–December (late winter to late spring); (c) short longevity of larvae active in early and mid-summer (December–February); (d) a long incubation period of eggs produced by engorged females exposed from mid-summer to early autumn; (e) a short incubation period of eggs produced by engorged females exposed from mid-spring to early summer, and (f) no hatch of eggs produced by females exposed in mid- and late autumn and winter. This ecological pattern of the non-parasitic phase of *R.* (*B.*) *microplus* in a subtropical, subhumid locality of Argentina is similar to those described in certain localities of Brazil and Uruguay (Nari *et al.*, 1979; Cardozo *et al.*, 1984; Evans, 1992), which are also located in the meridional margins of *R.* (*B.*) *microplus* distribution in South America. When the findings of the current work were compared with results from previous studies on the non-parasitic phase of *R.* (*B.*) *microplus* performed in Argentina, the present data were found to be in agreement with the results reported by Nava *et al.* (2013) for other localities also belonging to the Chaco Phytogeograpic Province but characterized by more arid conditions. The principal difference between the current findings and earlier data refers to the description of the non-parasitic phase of the *R.* (*B.*) *microplus* life cycle in localities in northeast Argentina

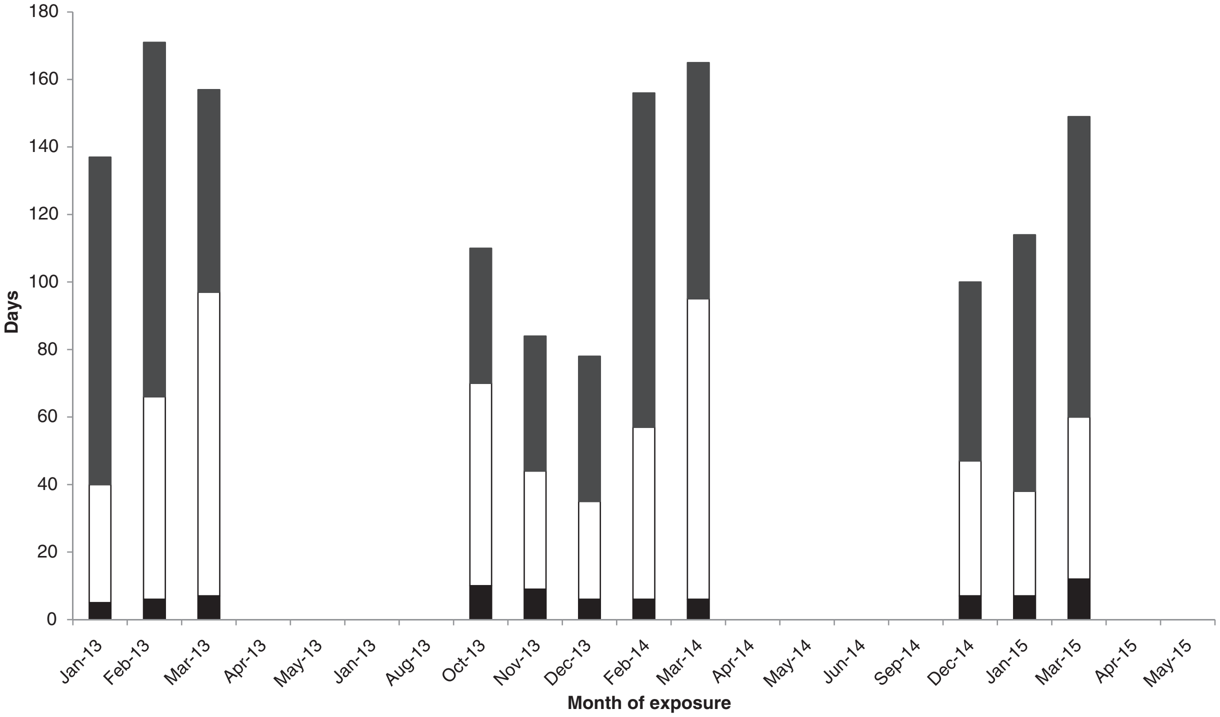

Fig. 3. Variation in the total non-parasitic period (TNPP) of *Rhipicephalus* (*Boophilus*) *microplus* in relation to months in which engorged females were exposed in a subtropical, subhumid region of Argentina. The TNPP is disaggregated in the preoviposition period, incubation period of eggs and larval longevity. Black, preoviposition period; white, egg incubation period; grey, larval longevity.

(Corrientes and Formosa Provinces) given by Ivancovich *et al.* (1982, 1984). In this region of Argentina, engorged female ticks lay eggs that successfully develop to larvae regardless of the time of year at which female ticks are exposed (Ivancovich *et al.*, 1982, 1984). This is not the case in northwest Argentina, where eggs fail to hatch in winter, which implies the cessation of the lifecycle of *R.* (*B.*) *microplus* in the coldest part of the year and a lower number of annual generations (three) than in northeast Argentina (four or five) (Ivancovich *et al.*, 1984; Guglielmone & Nava, 2013; Nava *et al.*, 2013; this study).

The sum of the values of POP and incubation period recorded for the exposures of each series (Table 2) explains the intervals between peaks in abundance of *R.* (*B.*) *microplus* on cattle (Fig. 1) The mean values of these periods (although it should be noted that the time required for larvae to mature from hatching to beginning host-seeking activity is absent from this calculation) range from 35 to 70 days in spring exposures, from 40 to 65 days in summer exposures, and from 60 to 95 days in autumn exposures. The high longevity of larvae originating from females exposed in mid-summer explains the high TNPP values of these cohorts (Table 2). Other cohorts with similarly high TNPP values are those originating from females exposed in early autumn, but in this case the high TNPP value is explained by a long incubation period (Table 2). The lowest TNPP values correspond to the cohorts originating from females exposed in spring, the larvae of which are active in early and mid-summer. These low TNPP values are determined by a short incubation period and larval longevity (Table 2).

The regression analysis allows understanding of the relationships between the dynamics of the non-parasitic phase of the *R.* (*B.*) *microplus* lifecycle described above and some abiotic and biotic factors such as microclimate variables and incubation period of eggs. There was a negative linear relationship between incubation period and temperature, as expected: the higher the temperature, the shorter the incubation period. This relationship has been described previously for *R.* (*B.*) *microplus* in other ecological areas of the Southern Cone of America, such as in northern Argentina, southeast Brazil, southeast Paraguay and Uruguay (Ivancovich, 1975; Nari *et al.*, 1979; Ivancovich *et al.*, 1982, 1984; Cardozo *et al.*, 1984; Evans, 1992; Brizuela *et al.*, 1996; Nava *et al.*, 2013), and also for the closely related species *R.* (*B.*) *australis* [formerly: *R.* (*B.*) *microplus*] in Australia (Harley, 1966; Sutherst *et al.*, 1988). However, the linear relationship between incubation period and temperature occurred only above a threshold temperature value because eggs did not hatch at monthly mean temperatures of <20 °C (Fig. 2). The failure of eggs to hatch occurred with both low (April–September 2013) and high (April–September 2014) saturation deficit values (Fig. 2), which suggests that, at least under the microclimatic conditions of a subtropical subhumid area of Argentina, temperature is the principal factor explaining the cessation of the lifecycle of *R.* (*B.*) *microplus* during the coldest months of the year.

The extension of larval longevity was explained by temperature and incubation period of eggs. Within a range of temperatures >20 °C, increasing temperatures resulted in correspondingly shorter periods of longevity. This relationship between temperature and larval longevity has already been described in *R.* (*B.*) *microplus* by other authors (Ivancovich, 1975; Cardozo *et al.*, 1984; Davey *et al.*, 1994; Brizuela *et al.*, 1996). This study also found a negative relationship between larval longevity and incubation period. At similar conditions of temperature (or microclimatic factors), a longer period of incubation may negatively affect larval longevity. The survival of larvae hatching in late winter or early spring is negatively affected by the long period of stress to which overwintering eggs are subjected. Sutherst & Bourne (2006) demonstrated that, in relation to the effects of microclimatic factors, eggs and unfed larvae can be considered as a single entity as stress is transferred from the eggs to the larvae. Davey & Cooksey (1989) and Sutherst & Bourne (2006) experimentally demonstrated a strong negative relationship between the exposure time of eggs and the longevity of larvae originating from those eggs in *R.* (*B.*) *microplus* and *R.* (*B.*) *australis* [named as *R.* (*B.*) *microplus*], respectively. Also in agreement with the results described in this work, field studies on the non-parasitic phase of *R.* (*B.*) *microplus* performed in southern Brazil by Gonzales *et al.* (1985) and De Souza *et al.* (1988) found the longevity of larvae derived from eggs with long incubation periods to be lower than that of larvae derived from eggs with short incubation periods.

Unexpectedly, no significant statistical relationship was found between saturation deficit and the biological parameters of *R.* (*B.*) *microplus* considered as criterion variables. This result is likely to have emerged because the saturation deficit at the study area does not reach values high enough to constitute a limiting factor. Using methods identical to those employed in this work, Nava *et al.* (2013) recorded monthly mean saturation deficit values of >5 mmHg several times during a study period of 20 months in more arid areas of the Chaco Biogeographic Province in northern Argentina. However, in the subtropical, subhumid area of northern Argentina in which the current study was conducted, saturation deficit reached monthly mean values of >5 mmHg in only 3 months out of a total period of 40 months of data recording. This comparison supports the hypothesis that saturation deficit has a lesser influence on the biology of *R.* (*B.*) *microplus* in the subtropical subhumid region of northwest Argentina than in other subtropical areas in which the cattle tick is present.

The current study has generated empirical data which may be used to design strategic methods for the control of *R.* (*B.*) *microplus*. Such strategies aim to achieve high levels of efficacy while minimizing the number of treatments with chemical compounds required. The population dynamics of *R.* (*B.*) *microplus* in a subtropical, subhumid area of Argentina are characterized by the cessation of the lifecycle during the coldest period of the year, which leads to low levels of infestation in pastures in winter and early spring. Treatments of cattle that are concentrated from late winter to late spring will act on these small cohorts of *R.* (*B.*) *microplus*, thereby preventing the emergence of larger generations in summer and autumn. This strategy to control *R.* (*B.*) *microplus* was adopted by Nava *et al.* (2014, 2015) in the same area in which the current study was performed, and demonstrated acceptable levels of control with just three applications of acaricides in 1 year. Complementarily or alternatively, the use of chemical acaricides could be minimized or prevented through the use of strategies for the control of cattle tick populations that deny access to hosts to free-living larvae by means of pasture spelling (Sutherst *et al.*, 1979; Norton *et al.*, 1983). With this method, questing larvae die by starvation and desiccation. Data on the TNPP constitute basic information for the planning of tick control protocols by pasture spelling. The spelling period required to achieve total control of *R.* (*B.*) *microplus* ticks depends on the time of year at which pasture spelling begins. According to the present results, a 17-week spelling period starting in late spring or early summer will be necessary to achieve optimal control of *R.* (*B.*) *microplus* free-living larvae, but if spelling begins in mid- to late summer or in autumn, the period required will be 26–27 weeks. These results are aligned, in general terms, with those obtained by Nava *et al.* (2013) in more arid areas of the Chaco Phytogeograpic Province. Future studies should evaluate the efficacy of pasture spelling to control *R.* (*B.*) *microplus* in standard cattle production systems in northwest Argentina by considering as parameters the data obtained in this study.

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References

Boletta, P.E., Ravelo, A.C., Planchuelo, A.M. & Grilli, M. (2006) Assessing deforestation in the Argentine Chaco. *Forest Ecology and Management*, **228**, 108–114.

Brizuela, C.M., Ortellano, C.A., Sanchez, T.I., Osorio, O. & Walker, A.R. (1996) Formulation of integrated control of *Boophilus microplus* in Paraguay: analysis of natural infestations. *Veterinary Parasitology*, **63**, 95–108.

Brum, J.G.W., Ribeiro, P.B., Costa, P.R.P. & Gonzales, J.C. (1987) Flutuçao sazonal de *Boophilus microplus* (Canestrini, 1887) no município de Pelotas, RS. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, **39**, 891–896.

Burger, T.D., Shao, R. & Barker, S.C. (2014) Phylogenetic analysis of mitochondrial genome sequences indicates that the cattle tick, *Rhipicephalus* (*Boophilus*) *microplus*, contains a cryptic species. *Molecular Phylogenetics and Evolution*, **76**, 241–253.

Cabrera, A.L. (1976) Regiones Fitogeográficas Argentinas. *Enciclopedia Argentina de Agricultura, Jardinería*, Tomo 2. Editorial Acme, Buenos Aires.

Cardozo, H., Nari, A., Franchi, M., López, A. & Donatti, N. (1984) Estudio sobre la ecología del *Boophilus microplus* en tres áreas enzooticas del Uruguay. *Veterinaria (Montevideo)*, **20**, 4–10.

Davey, R.B. & Cooksey, L.M. (1989) Effects of prolonged exposure at low temperature on *Boophilus microplus* (Acari: Ixodidae). *Journal of Medical Entomology*, **26**, 407–410.

Davey, R.B., Pound, J.M. & Cooksey, L.M. (1994) Comparative reproduction and nonparasitic development of *Boophilus microplus* and hybridized *Boophilus* ticks (Acari: Ixodidae) under natural field conditions in subtropical South Texas. *Experimental and Applied Acarology*, **18**, 185–200.

De Souza, A.P., Gonzales, J.C., Ramos, C.I., Paloschi, C.G. & De Moraes, N.A. (1988) Fase de vida livre do *Boophilus microplus* no planalto Catarinense. *Pesquisa Agropecuária Brasileira*, **23**, 627–434.

Estrada-Peña, A., Bouattour, A., Camicas, J.L. *et al.* (2006) The known distribution and ecological preferences of the tick subgenus *Boophilus* (Acari: Ixodidae) in Africa and Latin America. *Experimental and Applied Acarology*, **38**, 219–235.

Estrada-Peña, A., Venzal, J.M., Nava, S. *et al.* (2012) Reinstatement of *Rhipicephalus* (*Boophilus*) *australis* (Acari: Ixodidae) with redescription of the adult and larval stages. *Journal of Medical Entomology*, **49**, 794–802.

Evans, D.E. (1992) Tick infestation of livestock and tick control methods in Brazil: a situation report. *Insect Science and its Application*, **13**, 629–643.

Frisch, J.E. (1999) Towards a permanent solution for controlling cattle ticks. *International Journal for Parasitology*, **29**, 57–71.

George, J.E., Pound, J.M. & Davey, R.B. (2008) Acaricides for controlling tick on cattle and the problem of acaricide resistance. *Ticks: Biology, Diseases and Control* (ed. by A.S. Bowman & P. Nuttall), pp. 408–423. Cambridge University Press, Cambridge, MA.

Gonzales, J.C., da Silva, N.R., Francon, N. & Pereira, J.H. (1985) A vida libre do *Boophilus microplus* (Can. 1887). *Arquivos da Faculdade de Veterinária UFRGS*, **3**, 21–28.

Guerrero, F.D., Lovis, L. & Martins, J.R. (2012) Acaricide resistance mechanisms in *Rhipicephalus* (*Boophilus*) *microplus*. *Revista Brasileira de Parasitologia Veterinária*, **21**, 1–6.

Guglielmone, A.A. (1992) The level of infestation with the vector of cattle babesiosis in Argentina. *Memorias do Instituto Oswaldo Cruz*, **87**(Suppl. 3), 133–137.

Guglielmone, A.A. & Nava, S. (2013) Epidemiología y control de las garrapatas de los bovinos en la Argentina. *Enfermedades Parasitarias con Importancia Clínica y Productiva en Rumiantes: Fundamentos Epidemiológicos para su Diagnóstico y Control* (ed. by A. Nari & C. Fiel), pp. 441–456. Editorial Hemisferio Sur, Buenos Aires.

Guglielmone, A.A., Hadani, A., Mangold, A.J., De Haan, L. & Bermudez, A. (1981) Garrapatas (Ixodoidea-Ixodidae) del Ganado bovino en la provincia de Salta: especies y carga en 5 zonas ecológicas. *Revista de Medicina Veterinaria*, **62**, 194–205.

Guglielmone, A.A., Mangold, A.J., Aguirre, D.H. & Gaido, A.B. (1990) Ecological aspects of four species of ticks found on cattle, in Salta, Northwest Argentina. *Veterinary Parasitology*, **35**, 93–101.

Harley, K.L.S. (1966) Studies on the survival of the non-parasitic stages of the cattle tick *Boophilus microplus* in three climatically dissimilar districts of north Queensland. *Australian Journal of Agricultural Research*, **17**, 387–410.

Hitchcock, L.F. (1955) Studies on the non-parasitic stages of the cattle tick, *Boophilus microplus* (Canestrini) (Acarina: Ixodidae). *Australian Journal of Zoology*, **3**, 295–311.

Ivancovich, J.C. (1975) Bioecología de la garrapata del ganado *Boophilus microplus* (Canestrini, 1888). *Revista de Investigaciones Agropecuarias*, **12**, 1–54.

Ivancovich, J.C., Braithwaite, G.B. & Barnett, S.F. (1982) *Comportamiento de los Estados no-parasitarios de la Garrapata del Ganado* Boophilus microplus *(Canestrini). I-El Colorado (Provincia de Formosa), Boletín de la Estaciòn Experimental Regional Agropecuaria Presidente Roque Sáenz Peña 82*. Instituto Nacional de Tecnología Agropecuaria, EERA Roque Sáenz Peña, Sáenz Peña, Argentina.

Ivancovich, J.C., Braithwaite, G.B. & Barnett, S.F. (1984) *Comportamiento de los Estados no-parasitarios de la Garrapata del Ganado –* Boophilus microplus *(Canestrini). II-El Sombrerito (Provincia de Corrientes). III-Bartolomé de las Casas (Provincia de Formosa). IV. Siete Diferentes Localidades*, *Boletín de la Estaciòn Experimental Regional Agropecuaria Presidente Roque Sáenz Peña 88*. Instituto Nacional de Tecnología Agropecuaria, EERA Roque Sáenz Peña, Sáenz Peña, Argentina.

Jongejan, F. & Uilenberg, G. (2004) The global importance of ticks. *Parasitology*, **129**, 1–12.

Madder, M., Adehan, S., de Deken, R., Adehan, R. & Lokossou, R. (2012) New foci of *Rhipicephalus microplus* in West Africa. *Experimental and Applied Acarology*, **56**, 385–390.

Mangold, A.J., Aguirre, D.H., Gaido, A.B. & Guglielmone, A.A. (1994) Seasonal variation of ticks (Ixodidade) in *Bos taurus x Bos indicus* cattle under rotational grazing in forested and deforested habitats in northwestern Argentina. *Veterinary Parasitology*, **54**, 289–395.

Martins, J.R., Evans, D.E., Ceresér, V.H. & Correa, C.L. (2002) Partial strategic tick control in a herd of European breed cattle in the state of Rio Grande do Sul, southern Brazil. *Experimental and Applied Acarology*, **27**, 241–251.

McCulloch, R.N. & Lewis, I.J. (1968) Ecological studies of the cattle tick, *Boophilus microplus*, in the north coast district of new South Wales. *Australian Journal of Agricultural Research*, **19**, 689–710.

Nari, A. (1995) Strategies for the control of one-host ticks and relationships with tick-borne diseases in South America. *Veterinary Parasitology*, **57**, 153–165.

Nari, A., Cardozo, H., Berdié, J., Canabez, F. & Bawden, R. (1979) Estudio preliminar sobre la ecología de *Boophilus microplus* (Can) en Uruguay. Ciclo no parasitario en un área considerada poco apta para el desarrollo. *Veterinaria (Montevideo)*, **15**, 25–31.

Nari Henrioud, A. (2011) Towards sustainable parasite control practices in livestock production with emphasis in Latin America. *Veterinary Parasitology*, **180**, 2–11.

Nava, S., Mastropaolo, M., Guglielmone, A.A. & Mangold, A.J. (2013) Effect of deforestation and introduction of exotic grasses as livestock forage on the population dynamics of the cattle tick *Rhipicephalus* (*Boophilus*) *microplus* (Acari: Ixodidae) in northern Argentina. *Research in Veterinary Science*, **95**, 1046–1054.

Nava, S., Mangold, A.J., Canevari, J., Morel, N. & Guglielmone, A.A. (2014) Strategic treatments with systemic biocides to control *Rhipicephalus* (*Boophilus*) *microplus* in northwestern Argentina. *InVet*, **16**, 23–30.

Nava, S., Mangold, A.J., Canevari, J.T. & Guglielmone, A.A. (2015) Strategic applications of long-acting acaricides against *Rhipicephalus* (*Boophilus*) *microplus* in northwestern Argentina, with an analysis of tick distribution among cattle. *Veterinary Parasitology*, **208**, 225–230.

Norton, G.A., Sutherst, R.W. & Maywald, G.F. (1983) A framework for integrating control methods against the cattle tick, *Boophilus microplus* in Australia. *Journal of Applied Ecology*, **20**, 489–505.

Nuñez, J.L., Muñoz Cobeñas, M. & Moltedo, H. (1982) Boophilus microplus*: La Garrapata Común del Ganado Vacuno*. Hemisferio Sur, Buenos Aires.

Perret, J.L., Guigoz, E., Rais, O. & Gern, L. (2000) Influence of saturation deficit and temperature on *Ixodes ricinus* tick questing activity in a Lyme borreliosis-endemic area (Switzerland). *Parasitology Research*, **86**, 554–557.

Poulin, R. (1993) The disparity between observed and uniform distributions: a new look at parasite aggregation. *International Journal for Parasitology*, **23**, 937–944.

Poulin, R. (2007) *Evolutionary Ecology of Parasites*, 2nd edn. Princeton University Press, Princeton, NJ.

Randolph, S.E. & Storey, K. (1999) Impact of microclimate on immature tick–rodent host interactions (Acari: Ixodidae): implications for parasite transmission. *Journal of Medical Entomology*, **36**, 741–748.

Reck, J., Marks, F.S., Rodrigues, R.O. *et al.* (2014a) Does *Rhipicephalus microplus* tick infestation increase the risk for myiasis caused by *Conchliomyia hominivorax* in cattle? *Preventive Veterinary Medicine*, **113**, 59–62.

Reck, J., Klafke, G.M., Webster, A. *et al.* (2014b) First report of fluazuron resistance in *Rhipicephalus microplus*: a field tick population resistant to six classes of acaricides. *Veterinary Parasitology*, **201**, 128–136.

Rózsa, L., Reiczigel, J. & Majoros, G. (2000) Quantifying parasites in samples of hosts. *Journal of Parasitology*, **86**, 228–232.

Snowball, G.J. (1957) Ecological observations on the cattle tick, *Boophilus microplus* (Canestrini). *Australian Journal of Agricultural Research*, **8**, 394–413.

Spath, E.J.A., Guglielmone, A.A., Signorini, A.R. & Mangold, A.J. (1994) Estimación de las pérdidas económicas directas producidas por la garrapata *Boophilus microplus* y las enfermedades asociadas en la Argentina. 1 parte. *Therios*, **23**, 341–360.

Sutherst, R.W. & Bourne, A.S. (2006) The effect of desiccation and low temperature on the viability of eggs and emerging larvae of the tick, *Rhipicephalus* (*Boophilus*) *microplus* (Canestrini) (Ixodidae). *International Journal of Parasitology*, **36**, 193–200.

Sutherst, R.W., Wharton, R.H. & Utech, K.B.W. (1978) *Guide to Studies on Tick Ecology*Commonwealth Scientific and Industrial Research Organization, Division of Entomology, Technical Paper No. 14. CSIRO, Canberra.

Sutherst, R.W., Norton, G.A., Barlow, N.D., Conway, G.R., Birley, M. & Comins, N.H. (1979) An analysis of management strategies for cattle tick (*Boophilus microplus*) control in Australia. *Journal of Applied Ecology*, **16**, 359–382.

Sutherst, R.W., Sutherland, I.D., Bourne, A.S., Maywald, G.F. & Stegeman, D.A. (1988) Ecology of the cattle tick (*Boophilus microplus*) in subtropical Australia. I. Introduction and free-living stages. *Australian Journal of Agricultural Research*, **39**, 285–297.

Teel, P.D. (1984) Effect of saturation deficit on eggs of *Boophilus annulatus* and *B. microplus* (Acari: Ixodidae). *Annals of the Entomological Society of America*, **77**, 65–68.

Wilkinson, P.R. (1961) The use of sampling methods in studies of the distribution of larvae of *Boophilus microplus* on pastures. *Australian Journal of Zoology*, **9**, 752–783.

Wilkinson, P.R. (1970) Factors affecting the distribution and abundance of the cattle tick in Australia: observations and hypotheses. *Acarologia*, **12**, 492–508.

Wilkinson, P.R. & Wilson, J.T. (1959) Survival of cattle ticks in central Queensland pastures. *Australian Journal of Agricultural Research*, **10**, 129–143.

Zak, M.R., Cabido, M., Cáceres, D. & Díaz, S. (2008) What drives accelerated land cover change in central Argentina? Synergistic consequences of climatic, socioeconomic, and technological factors. *Environmental Management*, **42**, 181–189.

Zar, J.H. (1999) *Biostatistical Analysis*, 4th edn. Prentice-Hall, Englewood Cliffs, NJ.

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