

## Microhabitat choice and host-seeking behaviour of the tuatara tick, *Amblyomma sphenodonti* (Acari: Ixodidae)

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**Abstract:** Understanding the factors that influence patterns of ectoparasite infestation within wildlife populations involves knowledge of the mechanisms that influence host infestation. For ectoparasitic ticks, knowing where ticks might occur in the off-host environment and how they locate their hosts is essential to understanding patterns of ectoparasite infestation. The tuatara tick (*Amblyomma sphenodonti*) parasitises the tuatara (*Sphenodon punctatus*) on Stephens Island, New Zealand. We completed a series of laboratory microcosm experiments to examine daily activity patterns, microhabitat preferences and host-seeking behaviour by *Amblyomma sphenodonti*. Firstly, to determine the diel activity pattern of ticks, we observed the behaviour of ticks every 2 h over a 48-h period. We then tested the preferences of ticks for soil moisture, soil texture and shade by offering different pairs of substrate conditions. Last, to determine what cues ticks used to locate their hosts, we tested the response of ticks to filter paper infused with host scent or excrement. Ticks were most active at night. They also showed a significant preference for moister, coarser and shaded substrates 12 h after the start of the experiment. Ticks did not show an immediate response to either of the two host stimuli, but after 12 h showed a significant preference for host scent and avoided host excreta. We suggest that the microhabitat preferences of ticks reflect conditions within host refuges (burrows), and that the delayed response to host odour suggests ticks could use host scent to identify substrates frequented by hosts.

**Keywords:** host-parasite ecology; reptile; *Sphenodon*; ticks; tuatara

### Introduction

Understanding the factors that influence patterns of ectoparasite infestation in wildlife populations requires knowledge of how parasites are passed from one host individual to another. Ticks spend alternate periods on the host while feeding and off the host while moulting to the next life-cycle stage or laying eggs (Oliver 1989). Thus, infestation of hosts with ticks requires host contact with environments containing ticks. The distribution of ticks depends on where they detach from their host, their ability to locate and survive in suitable microhabitats, and their ability to relocate a new host (Randolph 2004). In particular, ticks are extremely susceptible to desiccation while off their hosts (Needham & Teel 1991), their off-host survival increasing with humidity (Bertrand & Wilson 1996; Lindsay et al. 1998; Civitello et al. 2008). However, ticks have limited energetic reserves, so they must find a host before their metabolic stores become depleted (Oliver 1989). Thus, to ensure their survival, ticks should detach in microhabitats that minimise desiccation risk, while maximising contact with new hosts.

To mediate the competing needs of shelter and host-seeking, ticks may adopt either of two main strategies. One that is widespread among mammal and bird ticks is to inhabit high humidity microclimates in litter or low understorey vegetation, and to move periodically into more desiccating microclimates higher in the vegetation to increase the chance of contacting a host (Camin & Drenner 1978; Vail & Smith 2002). Ticks may also use kairomones such as host scents or excrement to identify and aggregate for periods of time in exposed microhabitats that are used frequently by hosts (Carroll et al. 1995, 1998). Ticks

then use a variety of cues, such as heat (Oorebeek et al. 2009), carbon dioxide (Anderson et al. 1998) or vibration (Vassallo & Pérez-eid 2002), to locate a host. The second host-seeking strategy of ticks is to occupy host refuges. Host refuges may provide a sheltered environment for ticks, reducing desiccation, and maximising potential contact with new hosts. Ticks can enhance their likelihood of detaching into a host refuge, by synchronising drop-off rhythms with times when hosts are in resting sites (Mather & Spielman 1986; Matuschka et al. 1990, 1991). Again, host cues such as scents or excrement may be used to identify areas frequented by hosts.

Methods usually employed by researchers for identifying the location of bird and mammal ticks in the off-host environment (e.g. dragging fabric through vegetation, and carbon dioxide traps; Miles 1968; Ginsberg & Ewing 1989) are ineffective for locating reptile ticks since they use different cues for host seeking than mammal and bird ticks. Instead, researchers have examined the behaviour and habitat preferences of reptile ticks when offered simulated alternative microhabitats, to deduce their likely microhabitat choice and host-seeking behaviour in the field (Klomp & Bull 1987; Chilton & Bull 1993; Duffield & Bull 1996).

We investigated the microhabitat preferences and host-seeking behaviour of the tuatara tick, *Amblyomma sphenodonti* (Dumbleton, 1943; Acari: Ixodidae), which is host specific to *Sphenodon punctatus* (Reptilia: Rhynchocephalia). It is a three-host tick that develops from an egg into a larva, nymph and then adult, and feeds on tuatara blood during each stage (Heath 2006). Tuatara ticks can survive for long periods off their hosts (nymphs up to 177 days; adult females up to 405

days; adult males up to 582 days) under constant conditions in the laboratory ( $18^{\circ}\text{C}$ , 90–95% humidity) (Heath 2006). However, where they persist in the environment and how they find their hosts are unknown. On Stephens Island, tuatara take refuge in burrows, which are also used for nesting by seabirds (fairy prions, *Pachyptila turtur*) (Markwell 1997). Tuatara are infested by ticks in a variety of habitats on the island, including closed-canopy forest, coastal shrub, and open pasture (Godfrey et al. 2008). The prevalence of tick-infested hosts is similar among these habitats, although individual tick loads on tuatara are higher in pastures, despite lower host density (Godfrey et al. 2008). This suggests that ticks survive well in a variety of habitats that differ substantially in vegetation structure, macroclimatic conditions and host density.

In this study, we examined three components of tick behaviour to provide some insights into their microhabitat preferences and host-seeking behaviour. Firstly, we examined the diel activity patterns of ticks to identify when they were most active, and thus the optimal time to conduct behavioural experiments. This investigation also allowed us to examine how well tick activity was synchronised with host behaviour. We predicted that ticks would show higher levels of activity at night when tuatara were also active (Walls 1983). Secondly, we examined the preferences of ticks for different microhabitats. We tested the influence of variation in soil moisture, soil texture and shade to deduce the characteristics of habitats that ticks would prefer in their off-host environment in the field. We predicted that ticks would reduce their desiccation risk by choosing moist, coarse substrates, and would demonstrate a preference for shade, as found within burrows. Lastly, we tested the response of ticks to two different host stimuli (host scent and host excrement) to determine whether they used these cues for host-seeking or selecting microhabitats frequented by hosts. We expected that if ticks used these cues to seek hosts actively, we would observe an immediate attraction to host stimuli. Alternatively, a delayed response may be expected if ticks use host scent to find areas frequented by hosts, and where an immediate response to the stimulus is not as crucial for locating an optimal microclimate as for attaching to a passing host.

## Methods

We conducted the study on Stephens Island (Cook Strait, New Zealand,  $40^{\circ}40'\text{S}$ ,  $174^{\circ}0'\text{E}$ ) in November 2005, November 2006 and March 2007. At the start of each study period, we manually removed nymphs (engorged and unfed) and adult male and female ticks from tuatara in wild populations. The numbers of each tick life-cycle stage included in the experiments depended on the local abundance of ticks at the time the experiments were conducted. Female ticks were rare in both March and November, so were rarely included in our experiments. Although manual removal of ticks is not ideal, since manually removed ticks may behave differently from naturally detached and newly moulted ticks, we were unable to retain tuatara for long periods to wait for ticks to detach naturally, because of ethical and logistical restrictions. Therefore, we carefully removed ticks from hosts using fine-tipped forceps, gripping the ticks at the site of attachment to ensure they were removed with their mouthparts fully intact. Damaged ticks were not used in experiments. For our microhabitat choice experiments, we assumed that all life-cycle

stages of ticks and individuals in all stages of engorgement should show preferences for a suitable microclimate. Because ticks were randomly assigned to treatments, any potential effect of level of engorgement on tick microhabitat selection was minimised. In the host-seeking-behaviour experiments, we tested for differences in the response to stimuli of different life-cycle stages and stages of engorgement, before testing for an overall effect.

Ticks were kept in individual 1.5-ml Eppendorf vials containing a small strip of filter paper moistened with distilled water. We conducted all experiments within 20 days of tick capture, under ambient light conditions in an unheated room when temperatures were  $10\text{--}26^{\circ}\text{C}$  (mean  $17.8^{\circ}\text{C}$ ).

### Diel activity patterns

In November 2005, we examined diel patterns of tick activity. We collected 23 male ticks from tuatara on 18–19 November 2005, the last tick being collected at 1230 hours on the 19th. At 1930 hours on 19 November, each tick was placed in a separate Petri dish (90-mm diameter), which was sealed with Parafilm to maintain constant ambient humidity. At 2200 hours and every 2 h over the next 48 h we recorded whether each tick was inactive (legs curled) or active (legs spread out). If the tick was active, we recorded whether it was moving (i.e. mobile) or sedentary. The six observations between dawn and dusk (0800–1800 hours) were considered to be daytime, whereas those between dusk and dawn (2000–0600 hours) were considered to be night-time observations. At night, ticks were checked under dim torchlight. We analysed patterns of tick activity in a generalised linear mixed-effects model (lmer) in R (R Core Development Team 2007). We constructed separate models, with a binomial response variable for tick activity (active or inactive), and among active ticks, tick mobility (moving or sedentary). Observation time was included in all models as a covariate, trial day as a fixed effect (0–24 h after the start of the trial = day 1; 24–48 h = day 2), and an interaction between these effects. Sequence of observations and tick identity were random effects in the models.

### Microhabitat choice

In November 2006 and March 2007 we conducted three experiments to test the microhabitat preferences of detached ticks. We collected loose topsoil from the forest floor on Stephens Island (about 2 kg in each experimental period), dried it for 4 days in a solar oven, and used it as the substrate in all experiments. For each trial we divided a 90-mm diameter Petri dish into two halves with a small strip of cardboard (88 × 5 mm). Each half was filled with a different substrate type (depending on the experiment; see below) and compacted so it was level with the top of the divider. Thus, each experiment compared different pairs of conditions. The mass of soil used to fill the dishes was consistent among trials within experimental combinations. Substrates were prepared within 4 h of the beginning of a trial, and one tick was used in each trial. Ticks could move around the entire test arena.

We started all trials between 2200 and 0300 hours, during the nocturnal activity period of the ticks (see Results). Under dim red light we placed each tick in the middle of a dish (on the divider), ensured it was active and moving, and then sealed the dish with Parafilm. After 12 h, we recorded the side of the dish selected by the tick and assumed this reflected a preference for its daytime resting microhabitat. Individual ticks were not used to test the same combination of conditions (e.g.

20% vs 40% soil moisture) more than once, but the same ticks were sometimes used in different combinations of conditions within the same experiment (e.g. 20% vs 40% and 0% vs 40% soil moisture). Overlapping subsets of all available ticks were used in three different microhabitat experiments. The number (given below) of ticks (and different tick life-cycle stages) used in each experiment depended on the availability of ticks and the opportunity for conducting trials. Times with appropriate temperatures to conduct trials were weather dependent. Dishes were cleaned with 70% ethanol and soil was replaced between trials.

### **Experiment 1 – Soil moisture**

We examined the response of ticks to differences in soil moisture on five days in November 2006 using 62 ticks (28 unfed nymphs, 10 engorged nymphs, and 24 males) and on three days in March 2007 using 40 ticks (4 unfed nymphs, 10 engorged nymphs, 20 males and 6 females). Oven-dried soil was sifted through a 1-mm-mesh screen and distilled water was added to make 0, 20, 40 or 60% soil moisture by weight. We recorded the side chosen by each tick when offered alternative soil moistures of 0 or 20%, 0 or 40%, 0 or 60%, 20 or 40%, and 40 or 60%, with between 46 and 64 trials for each of the five experimental combinations.

### **Experiment 2 – Soil texture**

We examined the response of ticks to three different soil textures; fine (<1 mm), medium (1–5 mm) and coarse (>5 mm). We had three different experimental combinations (fine or medium, fine or coarse, and medium or coarse) in trials conducted on two days in March 2007 using 29 ticks (9 engorged nymphs and 20 males). Unfed nymphs were too small to relocate in the coarser substrates and were not used in this experiment. Since ticks showed no obvious preferences for a specific level of soil moisture (see Results), we made the soil up to 40% moisture with distilled water (as described above) for all trials.

### **Experiment 3 – Shade**

We examined the response of ticks to different light levels on four days in March 2007 using 32 ticks (10 engorged nymphs, 20 males and 2 females). We used finely sieved soil (<1 mm) with 40% soil moisture (as described above) on both sides of the divider but excluded light from one half with a black felt pouch. We orientated the shaded half of dishes randomly within trials, and dishes were placed away from direct sunlight so that heat would not confound the experiment.

### **Analysis**

We analysed the preference of ticks within each combination of conditions with a chi-squared goodness-of-fit test assuming an equal probability that ticks would choose either side of the Petri dish. When comparing preferences between different combinations in the same experiment, we used a generalised linear mixed-effects model (lmer in R; R Core Development Team 2007), since the same ticks were sometimes used in different experimental combinations within the same experiment. The preference (e.g. for moist soil) was a binomial dependent variable, and tick identity was a random effect. Treatment and tick life-cycle stage, and an interaction between these terms, were the fixed effects.

### **Host-seeking behaviour**

#### *Experiment 1 – Host scent*

We tested whether ticks responded to host scent in 90-mm-diameter Petri dishes over five nights in November 2006. We used 94 ticks in this experiment (27 unfed nymphs, 23 engorged nymphs, and 44 males). We collected host odour by holding a piece of filter paper (70-mm diameter) against the skin of a tuatara for 1 min (30 s on the dorsal surface, and 30 s on the ventral surface). Scent was collected from one tuatara for each piece of filter paper. For a negative control, we held a piece of filter paper on a recently dead fairy prion (*Pachyptila tutur*) for 1 min. Fairy prions were considered to be appropriate negative controls with which to compare tick responses to odours they may commonly encounter, but should have little biological relevance for the host-seeking behaviour of tuatara ticks. They are commonly found dead on the island in November and although they share burrows with tuatara, tuatara ticks are not known to parasitise fairy prions (Dumbleton 1943). Latex gloves were used to hold all filter papers. Subsequently, each piece of filter paper was cut in half and stored in a separate sealed plastic bag for use within 4 h.

Trials were started between 2300 and 0530 hours under dim red light. Two halves of filter paper, with alternative stimuli and moistened with three drops of distilled water, were placed on opposite sides of the Petri dish in three experimental combinations (tuatara or control, prion or control, and tuatara or prion). We placed one active, mobile tick in the centre of each Petri dish, and observed it for 2 min, recording which stimulus it contacted first, and how long it spent in contact with each stimulus. A 2-min period should represent the immediate response of the tick to a new stimulus. Then we sealed the dish with Parafilm and left it out of direct sunlight for 12 h, before recording which side of the dish the tick was on and whether it was in contact with the filter paper. This measurement was considered to reflect the delayed response of the tick to the stimulus. Petri dishes were cleaned with 70% ethanol between trials.

#### *Experiment 2 – Host excrement*

In a second experiment carried out in November 2006, we examined the response of 119 ticks (35 unfed nymphs, 30 engorged nymphs and 54 males) to host excrement. We collected fresh excrement (a combination of faecal material and uric acid) from tuatara (host stimulus) and fairy prions (negative control) from the forest floor, and made a 20% solution of each, using distilled water. We placed two halves of filter paper (70-mm diameter) on opposite sides of each Petri dish, and infused each with three drops of either distilled water (control) or the excremental solution from tuatara or fairy prion in three experimental combinations (tuatara and control, prion and control, and tuatara and prion). We then monitored tick behaviour as in Experiment 1 above.

We compared the preferences of different tick life-cycle stages with chi-squared tests when sample sizes were sufficient, or with Fisher's exact tests. When all tick life-cycle stages and stages of engorgement showed a similar response, we grouped them together to analyse for an overall preference within each experimental combination using a chi-squared goodness-of-fit test assuming an equal probability that ticks would choose either side. When comparing preferences after 12 h, only ticks contacting the stimulus were considered in the analysis.

## Results

### Diel activity patterns

The proportion of ticks that were active varied significantly between daytime and night-time (mixed-effects model:  $\chi^2 = 76.76$ , df = 1,  $P < 0.001$ ), with almost all ticks active during many night-time observations, and generally 40% or fewer ticks active during daytime observations (Fig. 1a). Overall, there were more observations of active ticks on the first day of observations than the second ( $\chi^2 = 7.12$ , df = 1,  $P = 0.007$ ) (Fig. 1a), but there was no interaction between time of day and trial day on the proportion of ticks that were active ( $\chi^2 = 0.47$ , df = 1,  $P = 0.488$ ). A significant interaction was found between time of day and trial day for the proportion of active ticks that were mobile ( $\chi^2 = 5.98$ , df = 1,  $P = 0.014$ ). On the first day more active ticks were mobile during daytime observations, whereas on the second day, more active ticks were mobile during night-time observations (Fig. 1b).

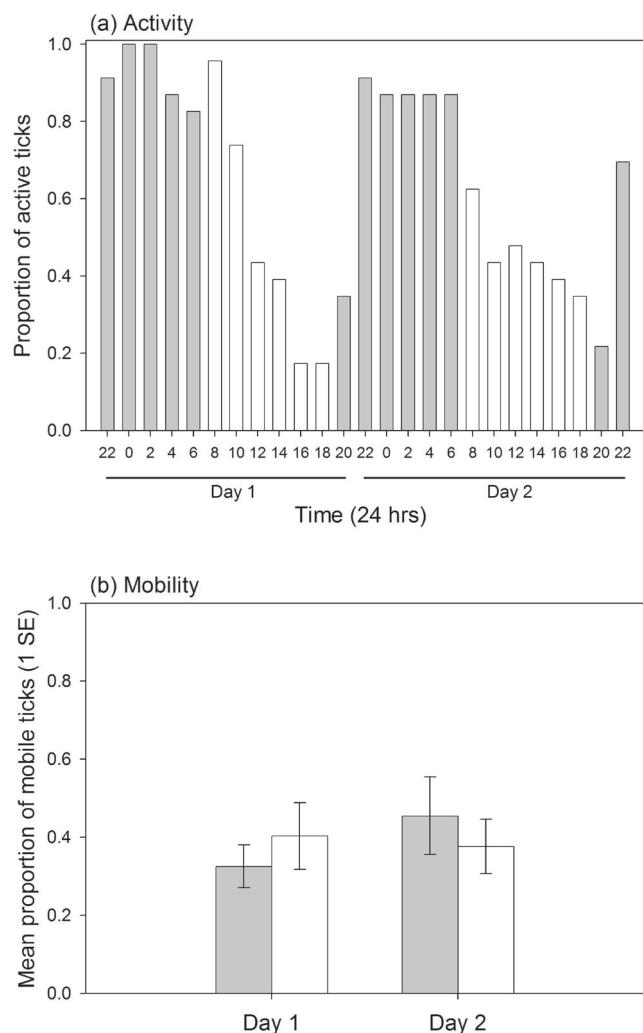
### Microhabitat choice

#### Experiment 1 – Soil moisture

No differences were found in the soil moisture preferences of ticks in trials conducted in November 2006 and March 2007 (Table 1), so we combined data from both periods in analyses. In all comparisons, ticks significantly avoided dry soil (0%) in preference for moister soil (20, 40 or 60% soil moisture) (Table 1). However, preferences for the moister side of the Petri dish were independent of the level of soil moisture (20%, 40% or 60% soil moisture) (mixed-effects model:  $\chi^2 = 2.17$ , df = 2,  $P = 0.337$ ), and all tick life-cycle stages showed a similar preference ( $\chi^2 = 0.50$ , df = 3,  $P = 0.918$ ). When ticks were offered different levels of soil moisture (20 or 40%, and 40 or 60%), they showed no significant preference for either side of the Petri dish (Table 1).

#### Experiment 2 – Soil texture

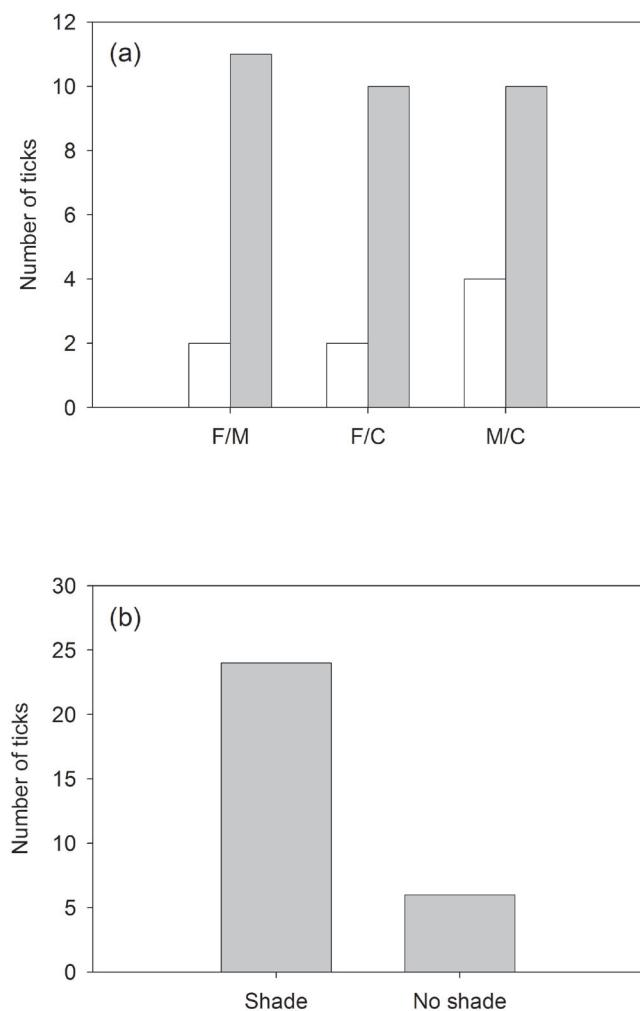
In all cases, ticks were found more frequently on the coarser substrate offered (Fig. 2a), although the results were not significant for the comparison of medium- and coarse-grain soils (Table 2). There were no differences in preference between



**Figure 1.** Diel activity patterns of tuatara ticks (*Amblyomma sphenodonti*). (a) The proportions of ticks that were active over a 48-h period, with observations starting at 2200 hours, during the day (0800–1800 hours, white bars) and night (2000–0600 hours, grey bars), and (b) the mean proportions of active ticks that were mobile on each day, during the day (white bars) and night (grey bars).

**Table 1.** Summary of results of soil moisture preference experiments for the tuatara tick (*Amblyomma sphenodonti*). The number of unfed nymphs ( $n$ ), engorged nymphs ( $n_e$ ), males ( $M$ ), females ( $F$ ) and the total number of ticks ( $N$ ) tested in each trial is given (both years combined); dry is the total number of ticks that selected the drier side of the Petri dish, and moist is the number of ticks that were on the moister side of the dish. ‘Years’ shows the results of a Pearson’s chi-squared test (corrected for continuity) testing for differences in tick preference between experiments conducted in November 2006 and March 2007. Where no chi-square value is given, a Fisher’s exact test was performed instead; ‘Preference’ shows the results of a chi-squared test for given probabilities (data combined from both years). In all tests, degrees of freedom are 1. P-values in bold are significant ( $P < 0.05$ ).

Soil moisture trials	$N$	$n_e$	$M$	$F$		dry	moist	Years		Preference	
								$\chi^2$	$P$	$\chi^2$	$P$
0 / 20%	10	14	22	2		48	9	39	-	1.000	18.75
0 / 40%	15	13	22	3		53	15	38	0.532	0.465	9.98
0 / 60%	14	9	20	3		46	10	36	0.125	0.722	14.69
20 / 40%	13	14	27	5		59	30	29	0.407	0.523	0.02
40 / 60%	16	13	31	4		64	27	37	0.106	0.743	1.56



**Figure 2.** Number of tuatara ticks (*Amblyomma sphenodonti*) found on (a) the finer (white bars) and coarser (grey bars) of paired substrates in the preference experiment. Substrates are: fine or medium (F/M), fine or coarse (F/C) and medium or coarse (M/C), and (b) shaded and unshaded substrates in the shade preference experiment.

**Table 2.** Summary of results of soil texture experiments for the tuatara tick (*Amblyomma sphenodonti*). The number of engorged nymphs ( $n_e$ ) and males ( $M$ ) tested in each trial is given; ‘Preference’ shows the results of a chi-squared test for given probabilities (data combined from both years). ‘Life-stages’ shows the results of a Fisher’s exact test testing for differences in tick preference between different tick life-cycle stages. In all tests, the degrees of freedom are 1.

Soil texture trials	$n_e$	$M$	Preference		Life stages $P$
			$\chi^2$	$P$	
Fine/Medium	5	10	6.23	<b>0.012</b>	0.070
Fine/Coarse	4	9	5.33	<b>0.020</b>	1.000
Medium/Coarse	4	10	2.57	0.108	0.520

males and engorged nymphs in any combination of soil textures, although the sample sizes were small (Table 2).

#### Experiment 3 – Shade

Ticks were found significantly more frequently on the shaded than unshaded side of the Petri-dish ( $\chi^2 = 10.8$ ,  $df = 1$ ,  $P = 0.001$ ) (Fig. 2b). There was no difference in the preference for shade between male ticks and engorged nymphs ( $\chi^2 = 0.05$ ,  $df = 1$ ,  $P = 0.816$ ).

#### Host-seeking behaviour

##### Experiment 1 – Host scent

Ticks showed no significant preference for which stimulus they first contacted, and no difference in the mean time spent in contact with either stimulus in the first two minutes of the trials (Table 3a, paired *t*-test; ‘tuatara or control’:  $t = -1.23$ ,  $df = 48$ ,  $P = 0.223$ ; ‘prion or control’:  $t = -0.482$ ,  $df = 13$ ,  $P = 0.637$ ; ‘tuatara or prion’:  $t = -0.914$ ,  $df = 13$ ,  $P = 0.337$ ). After 12 h, 25–26% of the ticks tested were in contact with a stimulus (Table 4a). In the ‘tuatara or control’ trial, significantly more ticks were in contact with the tuatara stimulus ( $n = 13$ ) than the control stimulus ( $n = 4$ ) (Table 4a). Each of the tick life-cycle stages showed a consistent response in the experiment (Table 4a). Too few ticks were in contact with the paper in the other trials to test for a significant preference (Table 4a).

##### Experiment 2 – Host excrement

Ticks showed no preference for which stimulus they first contacted, or which stimulus they spent most time in contact with during the first two minutes of any set of trials (Table 3b); (paired *t*-tests for time in contact; ‘tuatara & control’:  $t = -0.246$ ,  $df = 30$ ,  $P = 0.807$ ; ‘prion & control’:  $t = -0.054$ ,  $df = 30$ ,  $P = 0.95$ ; ‘tuatara & prion’:  $t = 0.74$ ,  $df = 30$ ,  $P = 0.465$ ). After 12 h, 18–40% of the ticks tested were in contact with a stimulus (Table 4b). In the ‘tuatara & control’ trial, ticks were significantly more frequently in contact with the control stimulus ( $n = 15$ ) than the tuatara stimulus ( $n = 1$ ) (Table 4b). Ticks did not show a significant preference for either stimulus in the other trials; each tick life-cycle stage showed a consistent response in the experiment (Table 4b).

#### Discussion

Tuatara ticks were most active at night, coinciding with the predominantly nocturnal activity of their hosts (Walls 1983). All tested life-cycle stages of the ticks showed strong preferences for moist soil, coarse substrates, and shaded habitats. Their responses to host-related stimuli were unclear, although there was evidence of attraction to host scent and avoidance of host excreta.

#### Diel activity patterns

Detached male tuatara ticks showed a consistent cycle in activity over the 48 h of the study, with a higher proportion of ticks active during night-time observations than during the day. Nocturnal activity coincides with the time when tuatara are most active and mobile (Walls 1983), and could be an adaptation to maximise the likelihood of ticks finding a host. The nocturnal activity of ticks could also be an adaptation to minimise their desiccation risk, by being more active when temperatures are cooler overnight. There were no consistent

**Table 3.** Summary of results of host-seeking behaviour experiments, testing tuatara tick (*Amblyomma sphenodonti*) responses to (a) host scent, and (b) host excreta within the first two minutes of the experiment. ‘First choice’ is the stimulus the tick first contacted, ‘Most time’ is the stimulus the tick was in contact with for the most time.  $N_{exp}$  is the number of ticks tested in each trial;  $N$  is the number of ticks that contacted a stimulus within the first two minutes of the experiment. ‘Preference’ shows results of a chi-squared test for given probabilities. ‘Life stages’ shows results from a Fisher’s exact test comparing differences in response to stimuli between different tick life-cycle stages. The numbers of nymphs ( $n$ ), engorged nymphs ( $n_e$ ) and males ( $M$ ) that contacted a stimulus within the first two minutes of the experiment are shown. In all tests, the degrees of freedom are 1.

	$N_{exp}$	Life stages						Preferences			
					First choice	Most time	First choice			Most time	
		$n$	$n_e$	$M$	$P$	$P$	$N$	$\chi^2$	$P$	$\chi^2$	$P$
<b>(a) Host scent</b>											
Tuatara/Control	63	14	8	27	0.856	0.575	49	0.18	0.668	1.00	0.317
Prion/Control	15	3	3	8	0.230	0.143	14	0.29	0.593	0.00	1.000
Tuatara/Prion	15	3	5	6	0.413	0.625	14	1.14	0.285	1.14	0.285
<b>(b) Host excreta</b>											
Tuatara/Control	40	9	7	16	0.531	1.000	32	0.13	0.723	0.03	0.857
Prion/Control	40	8	7	16	0.717	0.799	31	0.03	0.857	0.29	0.590
Tuatara/Prion	39	9	5	17	0.368	0.873	31	0.81	0.369	2.13	0.144

**Table 4.** Summary of results of host-seeking-behaviour experiments, testing the responses of tuatara ticks (*Amblyomma sphenodonti*) to (a) host scent, and (b) host excreta 12 h after the start of an experiment.  $N_{exp}$  is the number of ticks tested in each trial;  $N$  is the number of ticks that were in contact with a stimulus at the end of the experiment. ‘Preference’ shows results of a chi-squared test for given probabilities. ‘Life stages’ shows results of a Fisher’s exact test comparing differences in response to stimuli between different life-cycle stages of ticks. The numbers of nymphs ( $n$ ), engorged nymphs ( $n_e$ ) and males ( $M$ ) on a particular side of a dish at the end of the experiment are shown. In all tests, degrees of freedom are 1. Results are not shown where there was an insufficient sample size to test for preferences ( $n < 10$ ).  $P$ -values in bold are significant ( $P < 0.05$ ).

	$N_{exp}$	Life stages				Preference		
		$N$	$n_e$	$M$	$P$	$N$	$\chi^2$	$P$
<b>(a) Host scent</b>								
Tuatara/Control	63	5	3	9	0.445	17	4.76	<b>0.029</b>
Prion/Control	15	3	1	1	-	5	-	-
Tuatara/Prion	15	4	0	1	-	5	-	-
<b>(b) Host excreta</b>								
Tuatara/Control	40	7	2	7	1.000	16	12.25	<b>&lt;0.001</b>
Prion/Control	40	7	3	2	1.000	12	0.00	1.000
Tuatara/Prion	39	3	3	1	1.000	7	-	-

temporal patterns in the proportion of active ticks that had their legs raised in a questing position, or were walking. These behaviours are probably stimulated by host-related cues rather than time of day. A reduction in tick mobility over time on the second day of the study may have been a behavioural adjustment to a host-free environment.

Since light was not controlled in this experiment, an alternative explanation of our results is that ticks were responding to light cues, rather than displaying a diel cycle in activity. Thus, ticks showed increased activity in the dark. This could suggest that ticks are more active in dark environments

(e.g. within burrows) where potential hosts are likely to be, thus enhancing their likelihood of contacting hosts in these environments. Although diel activity cycles are common among mammal ticks (Perret et al. 2003; Madden & Madden 2005), they are uncommon among reptile ticks, which display only low levels of activity and no daily cycle in activity (Petney & Bull 1984; Bull 1986; Lane et al. 1995). Further observations under controlled light conditions are required to determine whether the behaviour of *Amblyomma sphenodonti* observed in this study reflects a response to light levels, or represents an inherent diel activity cycle. Adaptation to host activity cycles would be

more evident if, under constant dark conditions, ticks showed increased activity during the day, when tuatara are more likely to be in their burrows. Despite this, the ticks showed a clear and consistent nocturnal activity pattern in the experimental containers that allowed us to focus our observations on that time period in subsequent experiments.

### Microhabitat choice

Tuatara ticks showed a strong preference for moist over dry soil, but showed no preference towards a particular level of soil moisture. Our findings supported the hypothesis that ticks should minimise their desiccation risk by selecting moist microhabitats. The duration of our experiment was probably insufficient to allow ticks to become completely dehydrated, and thus we cannot infer how the level of hydration affected moisture preference of ticks. However, our findings indicate that even relatively hydrated ticks (which were maintained in moist containers prior to the experiment) with varying levels of engorgement showed a strong preference for moisture. The ambient humidity on Stephens Island is generally high (80–90%) (C. Allen pers. comm.), although, microhabitat humidity at ground level could vary over a wider range than this, depending on exposure to sun and soil moisture. Soil collected from forests on Stephens Island varied from 33 to 76% moisture (mean 61%; Mulder & Keall 2001) but in the burrows of tuatara, humidity is close to 100% most of the time (G. Birchard pers. comm.). Thus, a number of microhabitats could provide adequate humidity for ticks on Stephens Island.

Ticks showed a strong preference for coarse over fine substrate. However, they showed no preference when different grades of coarse substrates were compared. The coarser substrates used in the experiment were derived from the loose topsoil and leaf litter, abundant on the forest floor and in the burrows of hosts on Stephens Island. Ticks may occupy the lower levels of the substrate (between larger particles), where they would be better protected from direct sunlight and drying conditions.

Ticks also showed a strong preference for shade. Again, this preference probably reflects their need to reduce desiccation risk. However, not all *Amblyomma* species show a preference for shade, and in a similar experiment, the reptile tick, *A. limbatum* Neumann 1899, was attracted to light (Klomp & Bull 1987), perhaps to contact diurnally active hosts. Thus, our finding could also reflect an adaptation of tuatara ticks to the nocturnal behaviour of the host species. In our light and shade experiment, only a fine substrate was provided, so it is possible that ticks would have a different preference in the presence of coarser substrate. Shade may only be important if other forms of shelter provided by a coarser substrate are not available. Experiments on the preference of ticks for shade using different substrate textures would test this hypothesis.

Our results suggest that tuatara ticks prefer microhabitats that have high humidity, coarse substrate and darkness. On Stephens Island, tuatara inhabit two main habitat types: forests and pastures (East et al. 1995). Each habitat type is structurally distinct. Pastures are dominated by thick tussock grasses (Brown 2000), which might provide sufficient humidity and shelter for ticks at the soil interface. However, the microclimatic conditions in the forest are probably more favourable to ticks, since leaf litter is widespread on the forest floor, and the forest canopy provides shade and probably reduces evaporation. Yet, despite differences in microclimatic conditions, vegetation structure and host density, both habitats support a similar prevalence of tick infestation (Godfrey et al. 2008).

A consistent microhabitat feature in both habitat types is the presence of burrows, which are occupied by both tuatara and nesting fairy prions (Newman 1987). Burrows provide a dark, moist environment with heterogeneous substrate, since leaf litter is commonly tracked into burrows by birds and tuatara (S. Godfrey pers. obs.). Burrows that are frequently used by tuatara also provide opportunities to relocate hosts, especially in areas with low host density. Other ectoparasitic species commonly use the burrows of host reptiles (Kerr & Bull 2006) and mammals (Butler & Roper 1996; Roper et al. 2002) in this way. Ticks of burrow-dwelling hosts normally synchronise their detachment with times when hosts are in refuges, or with physiological cues that indicate the host is at rest (Mather & Spielman 1986; Matuschka et al. 1990, 1991) thereby ensuring the tick detaches into the host refuge. Future studies of the drop-off rhythms of tuatara ticks could establish whether they too are synchronised with host refuge use.

### Host-seeking behaviour

Tuatara ticks did not show a significant immediate preference for either of the host stimuli that we provided within the first two minutes of the trials, but after 12 h, more had been attracted to the tuatara scent than to the control. Thus, ticks may ultimately be attracted towards the scent of their host, if not immediately. Our tests failed to show discrimination between prion and tuatara scents because of low response rates. In a similar experiment, the reptile tick, *Bothriocroton hydrosauri* Denny 1843, was attracted to host scent, and this attraction increased over time (Downes 1984). Host scent may therefore contribute to the selection of microhabitats by ticks. Some mammal and bird ticks use chemical stimuli from hosts to select positions from which they quest for hosts (Carroll et al. 1995, 1998; Yoder et al. 2003; Benoit et al. 2008). Other cues may be more important in eliciting an immediate response from tuatara ticks to the presence of a host.

In contrast to their response to host scent, tuatara ticks significantly avoided tuatara excrement. It is possible that tuatara excrement contains desiccating compounds, and therefore is avoided by ticks. For instance, uric acid, a highly concentrated nitrogenous, alkali waste product, is a component of tuatara excrement, and could act as a desiccant. Compounds in excrement of other host species can invoke an arrest response in ticks, so retaining ticks at sites such as bird nests where hosts are frequent (Yoder et al. 2003, 2008; Benoit et al. 2008). In contrast, tuatara defecate away from their burrows in open areas (S. Godfrey pers. obs.), making it unlikely that excreta would provide an appropriate cue for host-seeking by tuatara ticks.

If the behaviour of tuatara ticks is similar to that of other reptile ticks, it is possible that they use host scent to indicate where hosts live, but physical disturbance may be required to elicit a host-seeking response (Downes 1984; Bull 1986). Testing the effects of carbon dioxide, heat, vibrations and disturbance on host-seeking behaviour of tuatara ticks is required to determine what cues are used by ticks to find their hosts. It will also be important to test the distances that ticks can travel to find a host. If ticks inhabit host refuge burrows, they may be able to move towards hosts within the same burrow chamber, but movement between burrows may be inhibited by more desiccating conditions outside the burrows.

### Implications of tick behaviour for host-parasite ecology

Caution must be exercised when extrapolating the findings of our microcosm experiments to the natural behaviour of tuatara

ticks in the wild. In our experiments we tried to simulate the natural environment as closely as possible by using natural substrates. However, the natural environment is more complex than the controlled conditions in our microcosms, where ticks may respond differently to interactions between different factors. In particular, the persistence of host chemical cues on substrates may vary with soil moisture and air temperature. Furthermore, understanding how soil moisture and air temperature influence desiccation stress and survival of tuatara ticks would enhance our understanding of the ecology of this parasite.

Our experiments showed clear preferences by ticks for conditions that would reduce their desiccation risk in the off-host environment. These preferences most closely reflect conditions within host refuges. Ticks were also attracted to host scents, which would be strongest in their refuge burrows. Thus, our results suggest that tuatara ticks are probably nidicolous. Tuatara ticks might either detach within a burrow and remain there, or use host scents to locate a refuge near to where they detached outside a burrow. Like other reptile ticks, they probably adopt a ‘sit-and-wait’ host-seeking strategy within the host refuge (Bull 1986). A nidicolous lifestyle would allow ticks similar survival probabilities under different external environmental conditions, and at different host densities. Fewer burrows are available for tuatara (and their ticks) in the lower-host-density pasture populations on Stephens Island, but if burrows have similar occupancy rates across habitats, then transmission rates for ticks may be similar. If ticks rely mostly on host refuges for their transmission, then factors that influence host refuge use could also influence parasite transmission. Tuatara are territorial (Gillingham et al. 1995; Moore et al. 2009), and the defence and reuse of the same burrows may ensure the persistence of the parasites, independent of host density. Similarly, subordinate host individuals unable to defend burrows for their exclusive use may be a key component in the transmission of these ectoparasites between hosts (Godfrey et al. in press). Thus, microhabitat choice of ticks and territory structure of tuatara may have implications for parasite transmission.

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