Aspects of the development of *Ixodes anatis* under different environmental conditions in the laboratory and in the field

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Natasha Bansal
Massey University Institute of Agriculture and Environment

tashu.vet@gmail.com
Corresponding Author

ORCiD: https://orcid.org/0000-0003-0903-0401

William Pomroy
Massey University Institute of Veterinary Animal and Biomedical Sciences

Allen C Heath
AgResearch Ltd

Isabel Castro
Massey University Institute of Agriculture and Environment

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Abstract

Background

As parasites spend a large amount of their life cycles on their hosts, to gain a better understanding of how host-parasite systems work, information about the life cycle of the parasite is important. Numerous laboratory and few field-based studies have explored the influence of microclimates on developmental times of different stages of various species of ixodid ticks and found that most of these species develop quicker and survive better at temperatures between 18 and 26°C and relative humidity between 75% and 94%. *Ixodes anatis* Chilton, 1904, or kiwi tick, is an endophilic, nidicolous species endemic to North Island brown kiwi (*Apteryx mantelli*, NIBK) and the Tokoeka (*Apteryx australis*). Little is known about the environmental conditions that are ideal for the development of the kiwi tick. Our aims in this study were to determine and compare the conditions of temperature and RH that ensured the best survival, and the shortest interstadial periods for the kiwi tick, in the laboratory and outdoors inside artificial kiwi burrows.

Methods

We collected free walking engorged ticks off wild kiwi hosts and placed them in the laboratory at various fixed temperature and humidity regimes. We also placed sets of different stages of these ticks in artificial kiwi burrows and in both cases, recorded the times taken for the ticks to moult to the next stage.

Results

We found that temperature had a larger impact on the mouls between stages than relative humidity, and larvae and nymphs both showed optimum development between 10-20°C, which is lower than many other species of Ixodid ticks. However, larvae moulted quicker and survived better when saturation deficits were <1-2 mmHg (RH>94%) while for nymphs the optimum saturation deficits were 1-10 mmHg.

Conclusions

We believe that the kiwi tick has adapted to stable, but relatively cool and humid conditions in the burrows reflecting the evolutionary consequences of its association with the kiwi.
Introduction
The amount of time that each stage of ticks takes to completion is determined by the interactions between temperature and moisture (relative humidity, RH) in the off-host habitat (King, Gettinby, and Newson, 1988; Randolph, 2004; Tukahirwa, 1976). During protracted off-host (questing) and engorged periods of their life, ticks are more prone to desiccation than when feeding (e.g. Apanaskevich and Oliver 2014) and their ability to perform bodily functions largely depends on water vapour absorption (Gaede and Knülle, 1997). Thus, optimum developmental conditions ensure faster progress to the next stage of the life cycle and better chances of survival. Numerous laboratory-based studies have explored the response of different species of Ixodid ticks to microclimates and their influence on developmental times (Arthur and Snow, 1968; Heath, 1979, 1981; Kahl and Knülle, 1988; Lees, 1946; Needham and Teel, 1991; Padgett and Lane, 2001; Troughton and Levin, 2007; Yoder, Hedges, and Benoit, 2012). Most of these studies agree that, for optimum development, ixodid ticks prefer temperatures between 18 and 26 °C and relative humidity between 75% and 94%. Some of these studies showed that an increase in temperature within the preferred range reduced moulting times, and that while some species were able to tolerate temperatures up to 38 °C, mortality rates increased. At lower temperatures such as 4-8 °C some species continue development, but at a greatly reduced rate and with higher mortality. Similar results have been demonstrated in the small number of field studies that have been conducted with various species (Campbell and Glines, 1979; Koch and Tuck, 1986; Norval, 1977; Ogden et al., 2004; Troughton and Levin, 2007).

Ixodes anatis Chilton, 1904, is a host-specific ixodid tick found on apterygid birds; the North Island brown kiwi (Apteryx mantelli, NIBK) and the Tokoeka (Apteryx australis), and therefore is endemic to New Zealand (Dumbleton, 1953; Heath, 2010). It is an endophilic, nidicolous species which has only been recovered off the hosts or within the burrows of these birds. Ixodes anatis of all stages are prevalent in kiwi burrows throughout the year (Swift et al. 2015; Bansal et al., 2019; pers. obs.). Our aims in this study were two-fold: to determine in the laboratory the conditions of temperature and RH that ensured the best survival, and the shortest interstadial periods for the kiwi tick, and to contrast these with those of ticks of different stages placed in artificial kiwi burrows outdoors. To
date, little is known about the environmental conditions that are ideal for the development of *I. anatis* and therefore our null hypothesis was that this species would behave comparably to other species with similar ecological requirements such as *I. uriae*, *I. aboricola*, and *I. trianguliceps* (Gray et al., 2014) or species in other genera such as *Amblyomma* and *Archaeocroton* (Barker and Burger, 2018; Gray et al., 2014) which are all examples of nidicoles.

**Materials And Methods**

**Experimental Design**

Two series of experiments were conducted to determine the optimum developmental conditions for *I. anatis*. In the first, engorged larvae, nymph and adult were incubated under laboratory conditions (Laboratory Experiments) and in the second, engorged ticks were maintained in artificial kiwi burrows (Field Experiments) in a forested area close to the Laboratory (40.3709° S, 175.6303° E; Fig. 1). In all experiments, the pre-moult period was defined as the time from when an engorged tick was placed in the incubator or burrow to the time it started moulting. Moulting duration was the time from when the tick started moulting until the time the new stage first appeared. Moulting success was the proportion of ticks that were able to successfully ecydose.

**Tick collection**

Ticks were collected from NIBK inhabiting a high-density population of 1 bird per hectare on Ponui Island (Inner Hauraki Gulf, New Zealand; 36.8622° S, 175.1842° E; Fig. 1) (Cunningham, Castro, and Alley, 2007). These birds had been observed to have high densities of ticks, with up to 250 individuals recorded on one host (Castro, 2006; Heath, 2010). Between April and June 2016 (for the laboratory experiments) and March 2018 (for the field experiment), detached, engorged ticks were collected off the birds, bird handlers, and the bags used to cover the birds during handling for transmitter change or as part of other experiments, Ticks were separated into the three stadia groups (larva, nymph, adult female), placed in plastic containers with fresh vegetation to provide moisture and stored at 4°C, for a mean duration of five days (± 5 days), until they arrived in the laboratory at Massey University, Palmerston North (546 km distant from the study site; Fig. 2,1).

**Tick identification**

Two species of ticks are known from kiwi at the study site and were differentiated on the basis of the
position of the ventral anal groove as described in Dumbleton (1953). In I. anatis the groove is anterior to the anus (Prostriata) and in Haemaphysalis longicornis is posterior to the anus (Metastriata) (Fig. 2). Only Ixodes anatis were used in this study.

Laboratory Experiment 1 – Effects of a range of temperatures at a single high humidity

Individual engorged larvae and nymphs were placed into transparent, screw-lid, 15 ml plastic bottles. A ten mm hole was drilled in the bottle lids and then covered with mesh cloth to allow passage of air. A total of 40 bottles (20 replicates for each stage) were then placed in 1000 ml closed plastic storage containers with water at the bottom to produce a high relative humidity (RH). Each plastic container was placed into the respective incubators pre-set to 9.2°C, 16.5°C, 21°C and 23.5°C. Ticks were examined every second day under a stereomicroscope at 4-10X to check for movement and moulting progress. Only 5 engorged adult females were available for study, and were kept, one per bottle, at 16.5°C which was closer to the average environmental temperature for Ponui Island (Dixon, 2015). These were also examined every second day noting the start and completion of egg laying. In this experiment the null hypothesis was that there would be no difference between the oviposition times at the different temperatures than at a constant high RH.

Laboratory Experiment 2 – Effects of a range of temperatures and humidity

Engorged larvae and nymphs were individually placed into small fabric mesh pockets which were suspended above the saturated salt solutions (Table 1, Fig. 3). These were then incubated at a range of temperatures (5°C, 10°C, 15°C, 20°C, 25°C and 30°C). There were 20 replicates of engorged larvae and 10 replicates of engorged nymphs for each humidity and temperature combination. In addition, 12 engorged adult females were available and divided into four groups of three. Two batches of three were incubated at 15°C and 93% and 96% RH respectively; one at 10°C and 94% RH and one at 20°C and 85.5% RH. Eggs obtained from these female ticks were subsequently divided into batches and placed in mesh bags (50 eggs/bag) at all temperature and RH combinations (Table 1). Temperatures and RH were measured every hour using iButton Hygrochron™ Temperature/Humidity Loggers (Model DS1923; Maxim Integrated, San Jose, California). The ticks were observed every two days for evidence of development, for a total of six months. In this experiment the hypothesis was that both larvae and
nymphs of I. anatis, would have more successful and faster developmental times at temperatures between 15ºC – 20ºC and RH above 90% than in conditions outside this range.

**Field Experiment**

Engorged larvae and nymphs were placed in artificial burrows (n = 12) from June to August (Southern Hemisphere winter) 2018. At Massey University, horizontal burrows were dug in a forest environment consisting of clay/silt loam-type soil, and imitated natural burrows. These simulated burrows were approximately 120–150 mm diameter and 600 mm deep (Fig. 4). A larger chamber was constructed at the end to mimic a typical kiwi-constructed burrow (de Vieco, 2019). Ten nymphs and 20 larvae were placed in each burrow in mesh pockets (one for each stage: Fig. 3D). These ticks were checked every two to three days to record moulting. Temperature and RH were recorded every hour using iButton Hygrochron™ Temperature/Humidity Loggers. For this experiment, we expected both the stages to follow the same pattern as found in laboratory experiment 2.

**Statistical analysis**

One-way ANOVA were carried out in R Core Team (2013) to test for significance between the number of days taken to start and complete moult for the different stages as well as egg laying for the adult ticks, where applicable.

The saturation deficit (SD), which is the amount of water vapour required to saturate air, (in mm of Hg) was calculated using the formula: 

\[
SD = (1 - \frac{RH}{100}) \times 4.9463e^{0.0621T}
\]  

(where RH is relative humidity in %, e is the mathematical constant ‘Euler’s number’ and T is temperature in ºC) (Randolph and Storey, 1999). For laboratory experiment 2 and the field experiment results were reported using both RH and the corresponding SD at the given temperatures.

**Results**

**Laboratory Experiment 1**

The time taken for completion of moulting of both stages decreased with increasing temperature (Fig. 5) but there was no significant difference in these times between the larval and nymphal stages (t-test, t = -0.63, df = 38.6, p > 0.05). Females kept at 16.5 ºC showed no oviposition.

**Laboratory Experiment 2**

Percentage survival of larvae and nymphs and the duration range of pre-moult are summarised in
Table 1. None of the larvae or nymphs showed signs of development at 5 °C, even after 120 days of observation, and all ticks placed at 30 °C died within 20 days regardless of environmental humidity. Both larvae and nymphs survived between 10 °C to 20 °C, with nymphs tolerating a wider range of temperatures than larvae. The greatest overall survival and shortest moulting times for both larvae and nymphs happened at 10 °C and 94-95% RH, representing a SD < 1 mmHg. Larvae at > 5 mmHg SD did not survive, nor did nymphs at > 10 mmHgSD.

The mean premoult period for larvae at 10 °C and > 94% RH was 60 days (range: 64–80) and the cumulative time for all larvae to complete a moult was a mean of 14 days (range: 5–21) (Table 1). At an RH > 93% at 15 °C, the mean premoult period for larvae was 56 days (range: 54–57) and mean moulting duration was 15 days (range: 14-17). At 20 °C and between 2–6 mmHg SD larvae had a mean premoult period of 73 days (range: 73–75), compared to a mean of 52 days at 5-6 mmHg, which was a statistically significant difference (p < 0.01). At 25 °C, and 64% RH (ca. 8–10 mm HgSD) only 60% of larvae showed signs of development with a 35-day premoult period and seven days moulting duration. Larvae at all other experimental temperatures and RHs did not develop.

The nymphs were more tolerant to a greater range of temperature and RH than were larvae. All nymphs at 10 °C started premoult with a mean of 75 days (range: 69–80) and completed moulting with a mean of seven days (range: 4-9) irrespective of RH (Table 1). At 15 °C, the premoult period for nymphs at 67% RH (SD ~ 4-5 mmHg) was 71 days but only 30% of these completed moulting over a seven-day period. However, at 15 °C and a SD of < 1 mmHg, nymphs took significantly (ANOVA, p < 0.01) less time to moult (Table 1). At 20 °C and an SD between 6-8 mmHg all nymphs started premoult but only 20% successfully completed the process, which they did over 9 days (Table 1). At 66% RH (SD between 5–6 mmHg), all the nymphs moulted but with a large variation in time (mean 14 days; range: 7-42). At 86% RH (SD between 2-3 mm of Hg), all nymphs had a premoult period of 38 days, taking 13 days (range: 7-14) to complete the moult. At 25 °C and 88% RH (3 mm HgSD) 90% completed a moult. Overall, a variable proportion of nymphs started premoult at each RH but only a small number actually completed the process (Table 1). At SD of > 3 mmHg, the nymphs showed evidence of fungal growth.
All six female ticks at 15 °C and 93.86% RH and the three at 20 °C and 85.55% RH laid around 600 to 750 eggs each. Only one of the three placed at 10 °C laid eggs and only two of those placed at 15 °C and 96.37% RH. As the temperature increased, the pre-oviposition period significantly decreased ($R^2 = 0.89$) (Table 2). No eggs hatched under any of the experimental conditions.

Field Experiment
Of the 12 burrows, only 11 were included in the analysis because Burrow 4 collapsed 16 days into the experiment. The mean (± Standard Deviation) temperature over all burrows was 13°C (± 0.27) for June, 11°C (± 0.15) for July and 10°C (± 0.09) for August. The mean RH over all the burrows was 66% ± 0.26 for June 68% ± 0.08 for July and 68% ± 0.17 for August (Fig. 6). The corresponding SD of all the burrows ranged between 3-4 mmHg. Of the 220 engorged larvae placed in the burrows 218 (99.1%) moulted to nymphs. Of 110 engorged nymphs, 101 (91.8%) survived to moult, the remaining nine died after 40 days without completing the development. Larvae in 10 out of 11 burrows had a premoult period of 66 days and took seven days to complete the moult. In Burrow 10, the larval premoult period was 70 days with a duration overall of five days. Nymphs in six burrows had a 70-day pre-moult period with 75 days for the remainder. All nymphs with exception of those in Burrows 3 and 5 finished moulting in eight days. Nymphs took 8 days to moult, with exception of those in Burrows 3 and 5 which took six days (Fig. 7).

Discussion
Our experiment proved our initial hypothesis that Ixodes anatis would act comparably to most other nidicolous tick species in terms of conditions of preferred temperature and humidity, false. Temperature had a larger impact on development than RH on engorged larval and nymphal stages of I. anatis.

Under laboratory conditions, the requirements for larvae were narrower than for nymphs. Engorged larvae showed optimum development (moulting times and survival) at 10-20 °C when SDs were < 1-2 mmHg (RH > 94%). Engorged nymphs survived and moulted up to 25 °C but, like larvae, appeared to favour a range of 10-20 °C, although with the ability to survive a somewhat drier atmosphere, tolerating a SD range of 1-10 mmHg. Females laid eggs at all temperatures and the range of humidity
tested, although the pre-oviposition period was from six to 14 days longer at SD of < 1 mmHg as compared to 2–3 mm of Hg. The prolongation of development at the lower temperature may have exposed the eggs to a greater decline in their water balance than would have occurred at higher temperatures. Also, breaking the eggs into smaller batches would have possibly increased surface area and subjected them to increased dehydration, however, this requires further investigation.

Under field conditions, the temperatures in the burrows varied slightly across the 3 months with a mean temperature of 11 °C (range: 10-13), mean RH of 67% (range: 65–69) and a calculated SD between 3-4 mm of Hg, which were at the lower end of the favourable range for both larvae and nymphs, but ambient humidity was a little drier than the larvae would seem capable of tolerating. Having said this, the RH was measured in the burrow air, not at the soil surface which may have been slightly more humid. From previous experiments conducted on burrows (D. Galvez, personal communication, 2018) we know that while external temperature fluctuates, the diurnal temperature within the burrow remains relatively constant. In addition, over the year, the microclimate in a burrow is not as extreme as in the external environment and remains within a range of ± 6 units for both temperature and humidity.

In the burrows, the developmental success rate for both larvae and nymphs was very high (99% and 91.8% respectively). In the laboratory however, larvae exposed to similar conditions (10 °C and 62.1%-83% RH; SD 1-4 mmHg), did not survived. It is possible that engorged larvae in burrows were in closer contact to available soil moisture, and able to absorb it in through the cuticle or experience reduced water loss. Larvae in laboratory chambers were surrounded by humid atmospheric air, but at a level perhaps less than that experienced by larvae in burrows. Ogden et al., (2004) reported that even small fluctuations or changes in temperature and humidity can affect the developmental times in ticks. It is also possible that these differences may have been caused by our routine checks as larvae are less tolerant to minor changes in temperature and RH (Chilton and Bull, 1993), and another study by Padgett and Lane (2001) found that when larvae were left undisturbed, they had a higher success of moulting that the ones that were disturbed more often.

In studies with kiwi-occupied burrows (Bansal et al., 2019; Swift et al., 2015) larvae were most
prevail from January to June (summer and autumn), and lowest in October (spring; usually a damper season). Nymphs, on the other hand, were less prevalent in January, with highest numbers from June to December. In the present study the artificial ‘burrows’ did not have any kiwi, which is very likely to have influenced temperature and humidity levels, both from physiological exhalations, body warmth (Calder, Parr and Karl, 1978) and deposited waste material.

In general, in many species of Ixodidae, immature stages of these ticks survive better at moderate to high RH (> 90%) and between 18 °C to 25 °C but die off rapidly at 75% RH at similar temperature conditions (Ginsberg et al., 2017; Needham and Teel, 1991; Padgett and Lane, 2001; Troughton and Levin, 2007). We found that the optimum temperature preferred by *Ixodes anatis* to complete development is between 10 °C to 15 °C which is lower than many other species of Ixodid ticks. Extended developmental times as a function of low temperature preference may be an adaptation for survival in burrows which are unoccupied for long periods as well as to the cold temperatures in New Zealand. However as in other species, the bioclimatic requirements of larvae are at the lower end of the range tolerated by the species overall. To a large extent this determines both seasonal patterns and habitat suitability for the species because, if larvae are disadvantaged, the life cycle can be disrupted. Nymphs, however, are generally more desiccation resistant and have a better tolerance of higher temperatures than do larvae, with engorged females capable of withstanding even greater bioclimatic extremes (Chilton and Bull, 1993; Heath, 1975, 1981; Needham and Teel, 1991).

The kiwi is a nocturnal animal and can range widely in search of food as well as use a multitude of burrows within its range (Dixon, 2015, Jamieson et al., 2016). The tick too is exclusively host-specific (aberrant hosts are very rare; see Heath 2010) and this suggests it would be an advantage for the tick to be sedentary and to be capable of sustained quiescence in the event of the spasmodic presence of hosts. There has been no success in finding questing *I. anatis* outside of kiwi burrows, reinforcing the inference of the tick’s sedentary nature and thus its adaptation to stable, but relatively cool and damp conditions in the burrows and reflecting the findings in this study as well as the evolutionary consequences of its association with the kiwi.

The best survival strategy for the kiwi tick is to have a mix of stages in each burrow, ready to take
advantage of the return of a host. A quicker development cycle for engorged larvae over the warmer time of year provides unfed nymphs that are able not only to withstand cooler times of the year but also the attendant added risks of dehydration. Unfed stages were not tested in these experiments and such a study would throw additional light on the biology of I. anatis in relation to its host.

Declarations

**Ethics approval and consent to participate**

Not applicable

**Consent for publication**

Not applicable

**Availability of data and materials**

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

**Competing interests**

The authors declare that they have no competing interests

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The project was self-funded with no outside funding.

**Author contribution**

Pilot experiments: NB. Experimental design: NB with input from WP and ACG. Lab work: NB. Fieldwork: NB and IC. Writing: NB with comments from IC, WP and ACG. Analysis: NB.

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Tables
Due to technical limitations, Tables 1 & 2 are only available for download from the Supplementary Files section.

Figures
Map showing the two sites used in experiments designed to find the best temperature and humidity conditions for the development of *Ixodes anatis*, the kiwi tick. Ponui Island is where the ticks were collected, and Massey University in Palmerston north is where the Laboratory Experiments as well as the Field experiment was conducted.
Figure 2

A figure showing the ventral view of the abdomen of ticks (basis capituli and legs are omitted) difference between the kiwi (left) and cattle tick (right) with respect to the placement of the anal groove used to identify the species.
Figure 3

The laboratory setup for the experiment. A- the ten chamber mesh pockets for larvae and nymphs, B- the mesh pockets suspended over the salt solution, C- the entire setup from B placed in an incubator and D- the mesh bags used for housing individual females. *D- same newly made mesh bags were also used for the field method.
Figure 4

An example of a burrow dug for the field studies at Massey University (photo by David de Vieco).
A box plot of the time taken in days for the moulting of engorged nymphs at three different temperatures. The boxes represent the inter-quartile range and the whiskers extend to the highest and lowest observations except for the empty circle on the top of 23.5°C which represents an outlier. The median is represented by the dark bar which is at the top of each box.
Figure 6

Average temperature and RH (± SE) in artificial kiwi burrows during June (blue), July (red) and August (green), 2018.
Figure 7

Time taken (in days) for development of immature stages of I. anatis in the field experiments

Supplementary Files
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Tables 1 - 2.docx
graphical abstract.png