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

Natasha Bansal (tashu.vet@gmail.com)
Massey University Institute of Agriculture and Environment 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

Numerous laboratory and fewer field-based studies have found that ixodid ticks develop more quickly and survive better at temperatures between 18 and 26°C and relative humidity between 75% and 94%. *Ixodes anatis* Chilton, 1904, is an endophilic, nidicolous species endemic to North Island brown kiwi (*Apteryx mantelli,* NIBK*)* and the tokoeka (*Apteryx australis*) and little is known about the environmental conditions required for its development. 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 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 life cycle stage of ticks takes to complete is determined by interactions between temperature and moisture (relative humidity, RH) in the off-host habitat [1-3]. During protracted off-host (questing) and engorged periods of their life, ticks are more prone to desiccation [4] and their ability to perform bodily functions largely depends on water vapour absorption [5]. 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 [6-11]. 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 these higher temperatures. 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 [12-16].

I. 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 [17, 18]. It is an endophilic, nidicolous species which has only been recovered either from the body of the hosts or within their burrows. *I. anatis* of all stages are prevalent in kiwi burrows at some sites throughout the year [19, 20].

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. arboricola*, and *I. trianguliceps* [21] or species in other genera such as *Amblyomma* and *Archaeocroton* [21, 22] 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; Figure 1). In all experiments, the pre-moult period was defined as the time from when a fully 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 ecdyse. For females, preoviposition was the time from the moment the female was placed in the incubator to the time it started laying eggs, and oviposition was the time taken for the female from the start to stop of egg laying.

Tick collection

Ticks were collected from NIBK inhabiting a high-density population of one bird per hectare on Ponui Island (Inner Hauraki Gulf, New Zealand; 36.8622° S, 175.1842° E; Figure 1) [23]. These birds had been observed to have high densities of ticks, with up to 250 individuals recorded on one host [18, 24]. Between April and June 2016 (for the laboratory experiments) and March 2018 (for the field experiment), detached, free -walking engorged ticks were collected. No ticks were forced off the hosts but were found walking on their feathers, on the surface of their bodies, on bird handlers and inside the bags used to cover the birds during handling. We assumed these ticks would have been naturally leaving the hosts after being

satiated. All ticks used also looked fully engorged to the eye. Ticks were separated into the three stadial groups (larva, nymph, adult female), placed in plastic containers with fresh vegetation to provide moisture and stored at 4^{o}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; Figure 1).

Tick identification

Only two species of ticks have been found from kiwi at the study site *Haemaphysalis longicornis* and *I. anatis* and as NZ has only one species of *Haemaphysalis* present,that genus is readily separated from the genus *Ixodes* based on palpal morphology. This and other features separating the species of *Ixodes* in NZ (shape of scutum, presence or absence of coxal spurs, etc.) were understood by the authors and taken into consideration when ticks were collected and identified, using keys in Dumbleton [17]. Only those ticks identified as *I. anatis* were used in this study.

Pilot experiment

To test the combined effect of temperature and humidity on the stages of the tick we needed to provide ticks with different RHs and place these at different temperatures. Winston and Bates [25] developed protocols to create various RHs for exactly this purpose by dissolving enough solid salt to super saturate distilled water at boiling point. The basic principal of this mechanism is that any saturated salt solution, when placed at a constant temperature produces a fixed vapour pressure (vp) which is in equilibrium with the vp of water and thus expresses a fixed relative humidity [25, 26]. We conducted a pilot test using salt solutions from their protocols [25, 26] with the idea to be able to produce a range of RHs for further experiments with our ticks. The salt solutions we used to achieve the required RH are given in Table 1. These solutions were placed at the bottom of sealed plastic containers with mesh lids and an iButton Hygrochron^{TM} Temperature/Humidity Loggers (Model DS1923; Maxim Integrated, San Jose, California) was hung from the lid, so it was at the same level as the ticks. The entire setup was placed in fixed temperature incubators, and the temperatures selected were 5˚C, 10˚C, 15˚C, 20˚C, 25˚C and 30˚C. The hygrometers were set to record temperature and humidity every 10 minutes for a week. Despite numerous attempts, not all the salt solutions produced the desired RHs reported in Winston and Bates [25] and therefore we used the actual RHs achieved (Table 1) as our final RHs for the main laboratory experiment.

Laboratory Experiment – 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). These were then incubated at a range of temperatures (5ºC, 10ºC, 15ºC, 20ºC, 25ºC and 30ºC). There were 20 engorged larvae and 10 engorged nymphs for each humidity and temperature combination. In addition, 12 engorged adult females were available and divided into four groups of three. To measure preoviposition and oviposition time, two of the groups were incubated at 15ºC and 93% and 96% RH respectively; one at 10ºC and 94% RH and one

at 20ºC 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, 2). Temperatures and RH were measured every hour using iButton Hygrochron^{TM} Temperature/Humidity Loggers. The ticks were observed every two days for evidence of development, for a total of six months. In this experiment the hypothesis was that larvae, nymphs and females 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 120-150mm diameter and 600mm deep (Figure 2). A larger chamber was constructed at the end to mimic a typical kiwi-constructed burrow [D. Vieco-Galvez, unpublished data]. Ten nymphs and 20 larvae were placed in each burrow in mesh pockets (one for each stage). These ticks were checked every two to three days to record moulting. Temperature and RH were recorded every hour using iButton Hygrochron^{TM} Temperature/Humidity Loggers. For this experiment, we expected both the stages to follow the same pattern as found in the laboratory experiment.

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, where applicable. We also carried out linear regressions to test the significance of temperature and RH on preoviposition and oviposition times in females.

The saturation deficit (SD), which is the amount of water vapour required to saturate air, (in mm of Hg) was calculated using the formula: $SD = (1- RH/100) * 4.9463e^{0.0621T}$ (where RH is relative humidity in %, e is the mathematical constant 'Euler's number' and T is temperature in ºC) [27]. For both the laboratory experiment and the field experiment results were reported using both RH and the corresponding SD at the given temperatures.

Results

Laboratory Experiment

Percentage survival of larvae and nymphs and the duration range of pre-moult are summarised in Table 2. 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 mmHg SD.

The larvae only moulted at RH > 94% at 10°C with a mean pre-moult period of 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 2). 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 2-6mmHg 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 Anova ($F_{(1,44)}$ =4.061, p<0.01). At 25°C, and 64% RH (ca. 8-10mm Hg SD) 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. Overall, a variable proportion of nymphs started premoult at each RH but only a small number completed the process (Table 2). At SD of > 3 mmHg, the nymphs showed evidence of fungal growth. 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 2). 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 ($F_{(1,28)}$ =4.19, p <0.01) less time to moult (Table 2). At 20°C and an SD between 6-8mmHg all nymphs started premoult but only 20% successfully completed the process, which they did over 9 days (Table 2). 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 2-3mm 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 Hg SD) 90% completed a moult.

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 and only two of three placed at 15°C and 96.37% RH laid eggs. As the temperature increased, the pre-oviposition period significantly decreased (F stat= 196, df = 2, p = 0.005) while the RH had no significant effect (Table 3). The remaining females that did not lay eggs, died within 60 days of being placed in the incubator. 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 overall mean temperature over the three months was 11°C (range: 10-13) and mean RH was 67% (range: 65-69). The mean (± Standard Deviation) temperature over all burrows was 13ºC (± 0.27) for June, 11ºC (± 0.15) for July and 10ºC (± 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 (Figure 3). 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 (Figure 4).

Discussion

Our experiment did not support our initial hypothesis that *I. anatis* would act comparably to most other nidicolous tick species in terms of conditions of preferred temperature and humidity.

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 at temperatures 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. Unfortunately, the lack of a set of constant RH at each temperature, prevented us from comparing between the different conditions, to help us to discern the ideal range of conditions required for *I. anatis* life cycles. Females laid eggs at all temperatures and over 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. However due to the very small number of females tested, this result serves only as a loose guideline and needs to be further explored. The failure of eggs to hatch at the different temperature and RH combinations could be due to a number of reasons. The prolongation in duration of oviposition 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 the surface area of the egg clumps and subjected them to increased dehydration. However, this requires further investigation.

Under field conditions, the temperatures in the burrows varied only slightly across the three months of the study, which were at the lower end of the favourable range for both larvae and nymphs found in the laboratory experiment. However, 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. Vieco-Galvez, personal communication, 2018] we know that while the external temperature fluctuates, the diurnal temperature within the burrow remains relatively constant, although the burrows do show a seasonality over the year. Moreover, 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 as those in the field

(10°C and 62.1%-83% RH; SD 1-4 mmHg) did not survive. 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. [15] reported that even small fluctuations or changes in temperature and humidity can affect the developmental times in ticks. Therefore, it is also possible that these differences between lab and field results may have been caused by our routine checks as larvae are less tolerant to minor changes in temperature and RH [28]. In addition, another study by Padgett and Lane [29] found that when larvae were left undisturbed, they had a higher success of moulting that the ones that were disturbed more often. For example, some ticks observed in the laboratory would have been exposed to more severe changes of temperature and humidity when extracted to assess survival than those in the field as some were kept at considerably higher or lower temperatures than the general laboratory environment.

In studies with kiwi-occupied burrows [19, 20] larvae were most prevalent 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 [30] and deposited waste material.

In general, in many species of Ixodidae, immature stages survive better at moderate to high RH (>90%) and between 18°C to 25 °C but die off rapidly if the RH declines to 75% RH at these same temperatures [16, 29, 31, 32]. 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 [8, 28, 32, 33].

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 [34, 35]. Despite the original example of this species of tick being found on a duck, the kiwi tick is almost exclusively host-specific [aberrant hosts are very rare; 18] 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.

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.

Conclusion

We found that the optimum temperature preferred by *I. anatis* to complete development is between 10°C to 15°C which is lower than that of many other species of ixodid ticks. We suggest that 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. 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.

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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Author contribution

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

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Tables

Table 1: The list of saturated solutions used at different temperatures to achieve required RH. Numbers in the table represent mean RH with standard deviation.

	5°C		10°C		15°C		20°C		25°C		30°C	
	Expected	Actual	Expected	Actual	Expected	Actual	Expected	Actual	Expected	Actual	Expected	Actual
Chloride KCl							70	64.9 (± 0.61)			70	66.8 (± 0.87)
Chloride	75	77.1 (± 0.62)	75	62.1 (± 0.47)	75	66.8 (± 1.18)	75	63.3 (± 0.58)	75	73.9 (± 0.56)	75	72.9 (± 0.6)
sium nide			85	83.4 (± 0.48)			85	66.3 (± 0.71)	85	63.9 (± 0.71)		
sium ride									85	83.6 (± 0.98)	85	84.6 (± 0.17)
sium rate	95	92.4 (± 1.86)	95	95.5 (± 0.86)	95	93.9 (± 0.1.53)			90	87.8 (± 1.2)	90	81.6 (± 2.01)
sium hate	99	64 .7 (± 0.50)	99	96.4 (±0.45)	99	96.4 (± 2.56)	99	85.6 (± 1.58)	95	67.5 (± 1.82)	95	72.9 (± 1.60)
sium omate									99	97.6 (± 0.23)	99	98.8 (± 1.80)
nitrite							65		65		65	

Temp (ºC)	RH (%)	Saturation Deficit (mm of Hg)	Larvae				Nymphs			
			Premoult		Moulting		Premoult		Moulting	
			Time (days)	Survival %	Time (days)	Survival %	Time (days)	Survival %	Time (days)	Survival %
5	64 (± 0.50)	1-2		0		0		0		0
	77 (± 0.62)	1-2		0		0		0		0
	92 (± 1.86)	<1		0		0		0		0
10	62 (± 0.47)	3-4		0		0	71 ± 1.70	100	6.3 ± 1.7	100
	83 (± 0.48)	1-2		0		0	71 ± 0.84	100	7.4 ± 0.8	100
	94 (±0.45)	<1	64 ± 4.39	100	9.8 ± 3.7	100	78 ± 0.84	100	6.6 ± 0.8	100
	95(± 0.86)	<1	78 ± 1.23	100	18.3 ± 5.02	100	78 ± 1.33	100	7 ± 1.3	100
15	67 (± 1.18)	4-5		0		0	71	100	7	30
	94 (± 0.1.53)	<1	57 ±1.41	100	14.9 ± 1.4	100	50 ± 0.95	100	6.7 ± 0.9	100
	96 (± 2.56)	<1	57 ± 1.33	100	14.75 ± 1.3	100	36 ± 0.84	100	7.4 ± 0.8	100
20	63 (± 0.58)	6-8		0		0	62 ± 1.51	100		0
	65 (± 0.61)	6-8		0		0	66 ± 1.03	100	9	20
	66(± 0.71)	5-6	52 ± 0.67	100	14.15 ± 0.7	100	38 ± 0	100	12.6 ± 2.95	100
	86 (± 1.58)	2-3	73 ± 0.82	100	6.6 ± 0.8	100	66 ± 1.93	100	14 ± 4.8	100
25	64 (± 0.71)	8-10	35	60	7	60	35 ±15.65	50	8.8 ± 2.5	10
	67(± 1.82)	8		0		0	56	10		0
	74 (± 0.56)	6		0		0	70	100		0
	84 (± 0.98)	3-4		0		0	35 ± 1.64	50	7	30
	88 (± 1.2)	3		0		0	70 ± 2.54	90	9 ± 1.7	90
	98 (± 0.23)	<1		0		0	28	70	7	40
30	67 (± 0.87)	10-12		0		0		0		0
	73(± 1.60)	8-10		0		0		0		0
	73 (± 0.6)	8-10		0		0		0		0
	82 (±	5-6		0		0		0		0

2.01)					
85 (± 0.17)	4-5	0	0	0	0
99 (± 1.80)	<1	0	0	0	0

Table 2: Survival and development time of *I. anatis* engorged larvae and nymphs under tested laboratory conditions. There were 20 larvae and 10 nymphs at each temperature and RH combination. Pre-moult in this table refers to the number of ticks that survived and started moulting and moulting refers to the actual time of moulting from attachment to complete emergence. Saturation deficit of air in each chamber was calculated using the formula from Randolph and Storey (27).

Table 3: The development times for the 12 female engorged ticks (three at each chamber) at given temperature and RH regimes.

ºC	% RH	SD	preoviposition period (in days)	oviposition period (in days)	success rate %
		Mm of Hg			
10	94	<1	33	7	33.3
15	94	<1	27	9	100.0
15	96	<1	27	9	66.7
20	86	2-3	19	9	100.0

Figures

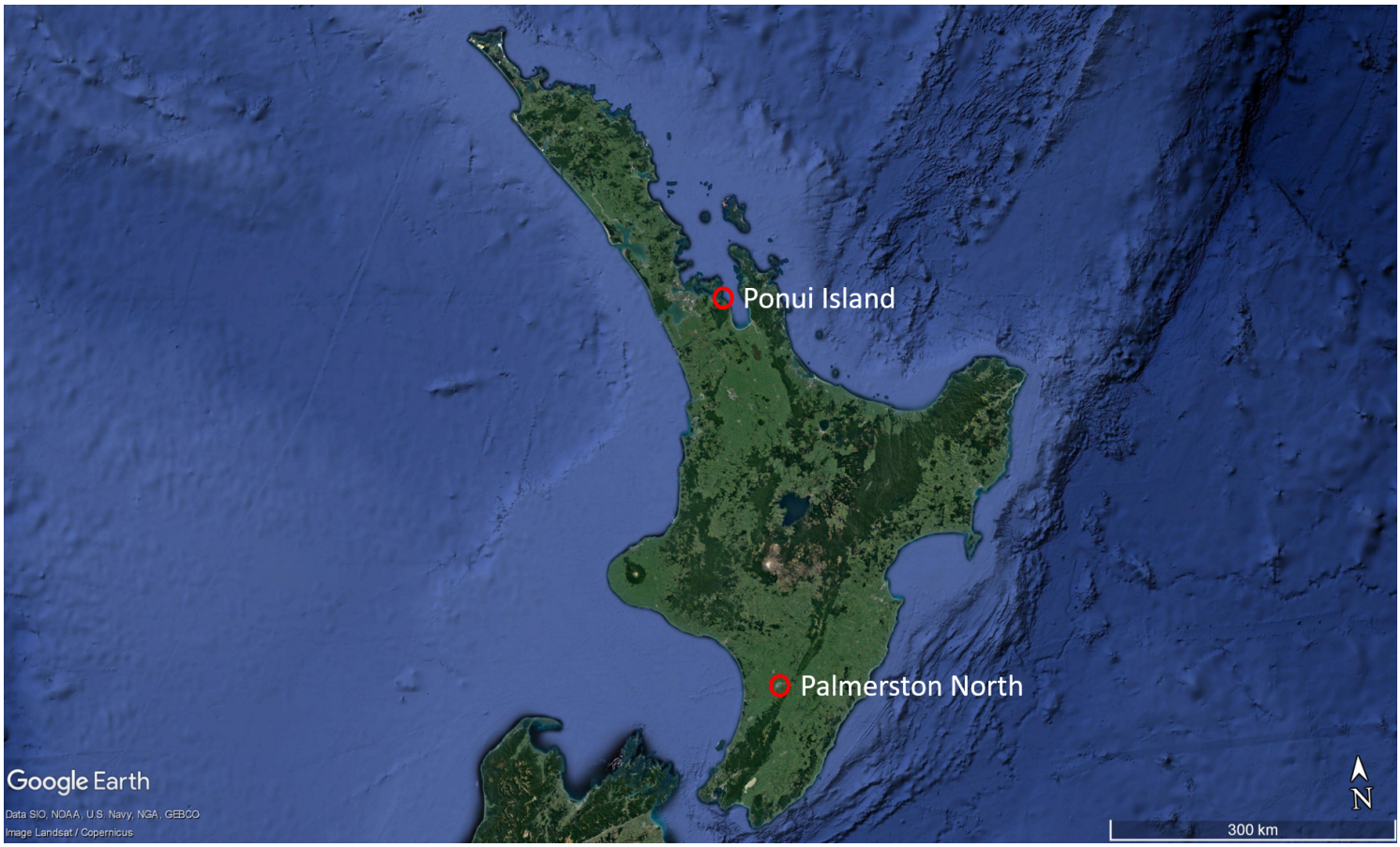

Figure 1

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

An example of a kiwi burrow (with a kiwi in it), which was a model for the burrows dug for the field studies at Massey University.

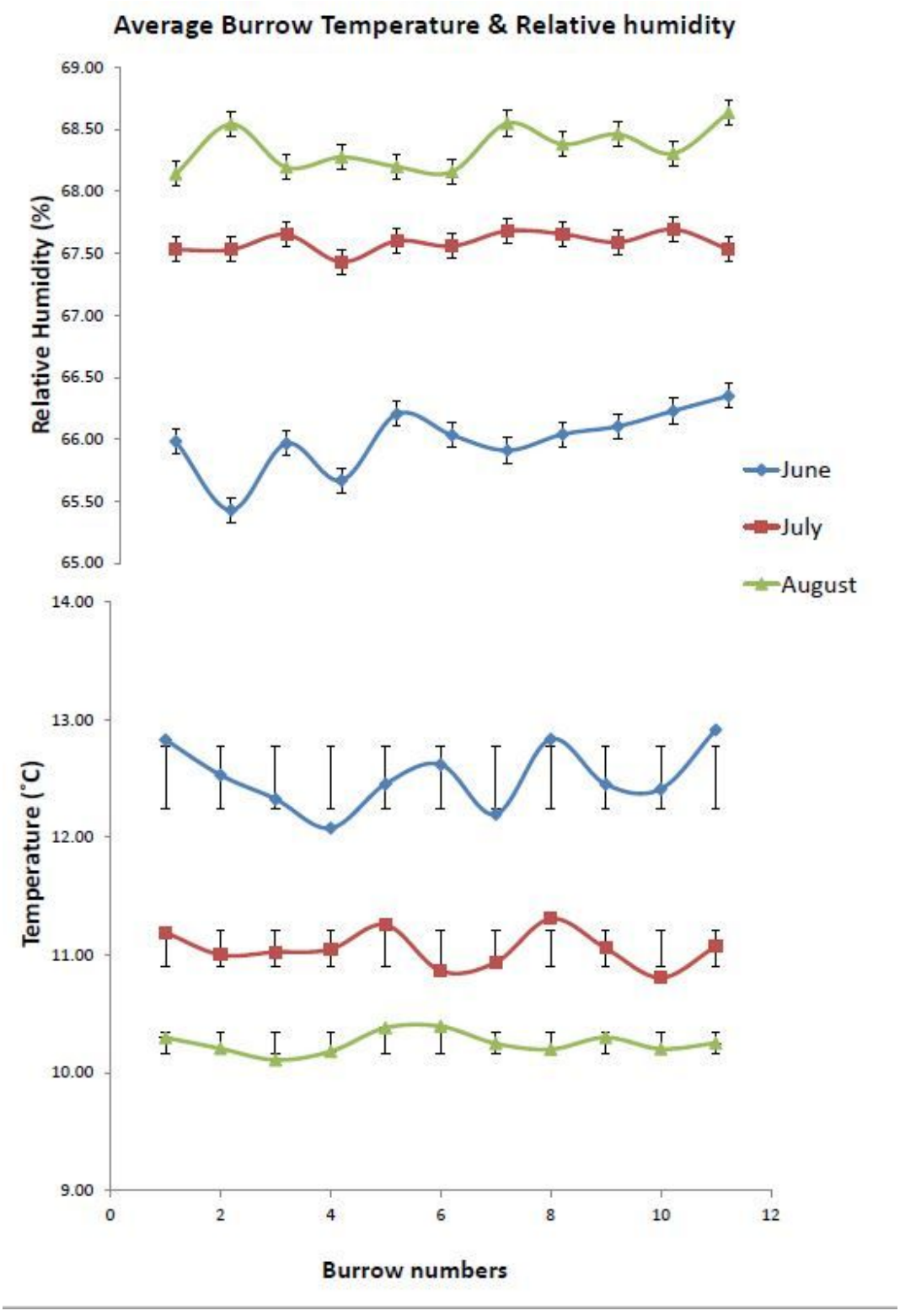

Figure 3

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

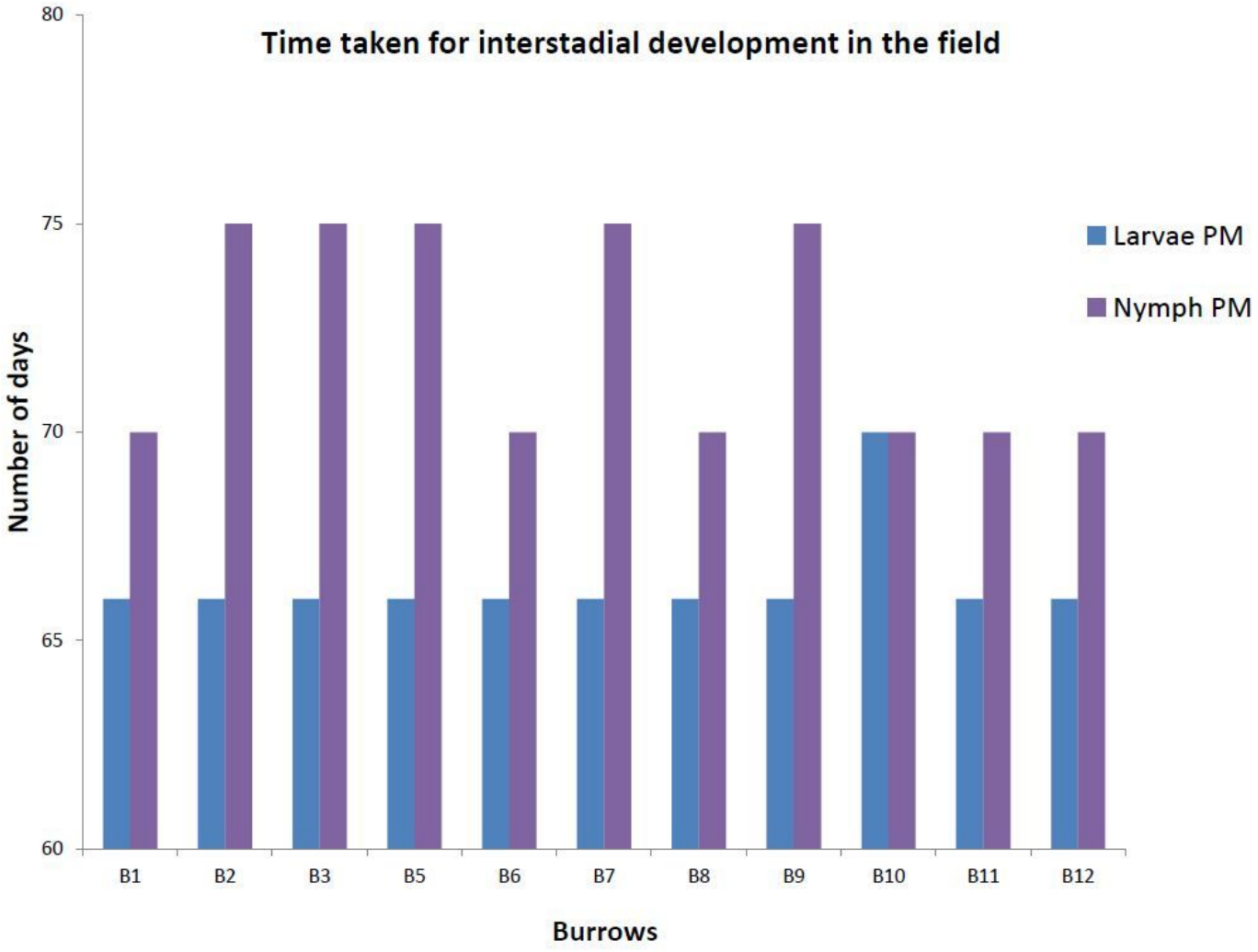

Figure 4

Time taken (in days) for development of immature stages of I. anatis in the field experiments. Where PM= premoult duration.

Supplementary Files

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- GraphicalAbstract.png