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Eric L. Rulison  
*University of Rhode Island*

Roger A. LeBrun  
*University of Rhode Island, lebrun@uri.edu*

Howard S. Ginsberg  
*University of Rhode Island, hginsberg@uri.edu*

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# Effect of Temperature on Feeding Period of Larval Blacklegged Ticks (Acari: Ixodidae) on Eastern Fence Lizards

ERIC L. RULISON,<sup>1,2</sup> ROGER A. LEBRUN,<sup>1</sup> AND HOWARD S. GINSBERG<sup>1,3,4</sup>

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**ABSTRACT** Ambient temperature can influence tick development time, and can potentially affect tick interactions with pathogens and with vertebrate hosts. We studied the effect of ambient temperature on duration of attachment of larval blacklegged ticks, *Ixodes scapularis* Say, to eastern fence lizards, *Sceloporus undulatus* (Bosc & Daudin). Feeding periods of larvae that attached to lizards under preferred temperature conditions for the lizards (WARM treatment: temperatures averaged 36.6°C at the top of the cage and 25.8°C at the bottom, allowing behavioral thermoregulation) were shorter than for larvae on lizards held under cool conditions (COOL treatment temperatures averaged 28.4°C at top of cage and 24.9°C at the bottom). The lizards were infested with larvae four times at roughly monthly intervals. Larval numbers successfully engorging and dropping declined and feeding period was longer after the first infestation.

**KEY WORDS** *Ixodes scapularis*, *Sceloporus undulatus*, attachment, temperature, feeding period

The feeding period of hard ticks varies with host species, tick species, tick stage, and to some extent with environmental temperature (Apanaskevich and Oliver 2014). Sweatman (1970) studied feeding periods of *Hyalomma aegyptium* L., which attaches primarily to tortoises (Paştiu et al. 2012), and found shorter larval feeding periods on reptiles as ambient temperatures increased from 20 to 30°C, but not above that temperature, and not on homeothermic hosts. Hagra and Khalil (1988) found no association of ambient temperature with feeding period of camel ticks, *Hyalomma dromedarii* Koch, on rabbits. In contrast, Lysyk (2008) found that feeding periods of *Dermacentor andersoni* (Stiles) on cattle differed at different ambient temperatures. Apparently, the relationship of tick attachment time to temperature differs among tick species and on different hosts. The natural situation is further complicated, especially for ectothermic hosts, by the fact that neither ambient nor body temperatures are constant.

Nevertheless, ectothermic animals thermoregulate to some extent. For example, eastern fence lizards, *Sceloporus undulatus* (Bosc & Daudin), maintain constant body temperatures behaviorally by modifying basking time or microhabitat use (Bowker et al. 1986, Adolph 1990, Angilletta et al. 2002). *S. undulatus* serves as a host for blacklegged ticks, *Ixodes scapularis* Say, over much of eastern and central North America

(McAllister et al. 2013). Ogden et al. (2004) showed that many aspects of development time of *I. scapularis* are determined by temperature, as is broadly true of physiological processes, so one might speculate that feeding rate might also be temperature related, and that feeding period on *S. undulatus* would vary at different ambient temperatures.

We studied the feeding period of larval *I. scapularis* on *S. undulatus* in the laboratory by allowing ticks to attach to lizards held at different ambient temperatures, as part of a study on tick–lizard relationships and *Borrelia burgdorferi* transmission. The reservoir competence results were reported by Rulison et al. (2014). We provided temperature gradients along with climbing opportunities and shelters to allow behavioral thermoregulation by the lizards. Finally, we allowed larvae to attach to the lizards four times over a period of 4 mo to assess the effects of repeated infestations.

## Materials and Methods

Ten *S. undulatus* (one male and nine females) were collected from scattered sites in southern New Jersey and brought to the laboratory at the University of Rhode Island. Each lizard was placed in a 10-gallon aquarium within an insert (just slightly smaller than the aquarium) made of hardware cloth that was held above ≈2 cm of water at the bottom of the aquarium (to catch dropping ticks). The lizards readily climbed on the hardware cloth, and each was provided with a wooden shelter, a feeding dish, and a water bowl. Water, live mealworms, and house crickets were supplied ad libitum, with occasional supplementation with a nutrient blend. The room was maintained at a photoperiod of 15:9 (L:D) h using full-spectrum lamps

<sup>1</sup> Department of Plant Sciences and Entomology, Woodward Hall, University of Rhode Island, Kingston, RI 02881.

<sup>2</sup> Current address: Caesar Kleberg Wildlife Research Institute, Texas A&M University-Kingsville, Kingsville TX 78363.

<sup>3</sup> USGS Patuxent Wildlife Research Center, Woodward Hall – PSE, University of Rhode Island, Kingston, RI 02881.

<sup>4</sup> Corresponding author, e-mail: hginsberg@usgs.gov.

Table 1. Environmental conditions in laboratory trials (mean  $\pm$  SE)

Trial	Temperature ( $^{\circ}$ C)				Relative humidity (%)
	Top	Middle	Bottom	Lizard*	
WARM treatment					
A	37.4 $\pm$ 0.21	30.4 $\pm$ 0.18	27.1 $\pm$ 0.19	–	51.2 $\pm$ 1.25
B	36.3 $\pm$ 0.45	29.4 $\pm$ 0.11	26.1 $\pm$ 0.11	28.9 $\pm$ 0.31	52.8 $\pm$ 2.18
C	35.6 $\pm$ 0.30	28.5 $\pm$ 0.24	24.8 $\pm$ 0.27	28.4 $\pm$ 0.45	44.8 $\pm$ 1.39
D	36.9 $\pm$ 0.35	29.0 $\pm$ 0.16	25.3 $\pm$ 0.19	29.3 $\pm$ 0.71	31.9 $\pm$ 1.36
COOL treatment					
A	29.2 $\pm$ 0.11	27.3 $\pm$ 0.12	25.4 $\pm$ 0.17	–	81.1 $\pm$ 0.21
B	28.7 $\pm$ 0.09	26.5 $\pm$ 0.10	25.4 $\pm$ 0.08	25.4 $\pm$ 0.09	69.0 $\pm$ 1.79
C	27.4 $\pm$ 0.30	25.1 $\pm$ 0.24	24.0 $\pm$ 0.24	24.2 $\pm$ 0.21	68.6 $\pm$ 2.26
D	28.4 $\pm$ 0.16	26.0 $\pm$ 0.22	24.6 $\pm$ 0.17	24.7 $\pm$ 0.14	40.9 $\pm$ 2.53

\* Lizard skin surface temperature.

(ReptiSun, Zoo Med Laboratories Inc., San Luis Obispo, CA) with basking spot lamps used to increase temperature at the tops of the WARM treatment aquaria. The WARM treatment provided a broad range of temperatures that spanned preferred ranges for *S. undulatus* (Bowker et al. 1986, Angilletta et al. 2002). Optimal body temperatures vary geographically and with type of activity (e.g., maximum sprint speed, 28–38 $^{\circ}$ C, endurance 25–36 $^{\circ}$ C), although throughout its range *S. undulatus* generally maintains body temperatures within a few degrees of 32.8 $^{\circ}$ C, which is associated with energy assimilation (Angilletta 2001a,b). Each lizard was infested with larvae by placing  $\approx$ 100 *I. scapularis* larvae (from a laboratory culture) on the lizard and holding the lizard and ticks within a PVC tube (with the ends sealed with mesh, or within a stocking) for several hours before placing the lizard in its cage. The water in each aquarium was checked daily for engorged larvae that had dropped, which were counted and then collected for other studies.

The four infestations (trials) were on A) 30 July, B) 7 September, C) 3 October, and D) 2 November 2012. Five lizards were assigned to the WARM treatment (with basking lamps) and five to the COOL treatment (without basking lamps). After the first infestation (trial A), lizards were reassigned randomly to treatments for subsequent infestations. Lizards were also exposed to nymphal *I. scapularis* from the same laboratory culture (10–15 per lizard) on 5 and 9 July and on 17 August 2012 using similar methods, as part of a reservoir competence experiment. The lizards were treated in accordance with IACUC protocols from the University of Rhode Island (AN08-04-017, AN09-04-016, and AN11-04-012) and USGS Patuxent Wildlife Research Center.

Temperature and relative humidity were measured using Temp/RH pens (Traceable ISO 17025 Calibrated Humidity/Temperature Pens, Control Co., Friendswood, TX). In total, 14 Temp/RH pens were distributed to record temperatures at aquarium tops (on the screen top of the aquarium), middle areas (midpoint on the side of the insert within the aquarium for temperature and RH readings), and bottoms (temperature at the bottom outside the aquarium). Starting with trial B, lizard skin surface temperatures

were measured daily using an infrared laser thermometer (model IRT0421 Digital Noncontact Infrared Thermometer with Integrated Laser Sight, Kintrex, Vienna, VA).

We tested the differences in tick feeding periods between WARM and COOL treatments by comparing the cumulative 12-d drop-off curves (cumulative proportion of engorged larvae that dropped off from lizards in each treatment each day for 12 d) using Kolmogorov-Smirnov two-sample tests (Steel and Torrie 1980). We used one-tailed tests to determine whether ticks dropped off earlier in the WARM treatment than in the COOL treatment.

## Results and Discussion

Temperatures in the WARM treatment averaged 36.6 $^{\circ}$ C at the aquarium top down to 25.8 $^{\circ}$ C at the bottom, while the COOL treatment averaged 28.4 $^{\circ}$ C at the top and 24.9 $^{\circ}$ C at the bottom (Table 1). Lizard skin surface temperatures averaged 28.9 $^{\circ}$ C in the WARM treatment and 24.8 $^{\circ}$ C in the COOL treatment.

Feeding periods were shorter in the WARM treatment than in the COOL treatment (Fig. 1). Mean feeding periods in trial A were 4.7 d in the WARM treatment and 7.3 d in the COOL treatment; trial B, 6.4 in WARM and 7.9 in COOL; trial C, 7.5 in WARM and 8.0 in COOL; trial D, 6.0 in WARM and 9.9 in COOL. Feeding period was significantly shorter in the WARM treatment in trial A (Kolmogorov-Smirnov test, one-tailed,  $D = 0.864$ ;  $n_1 = 73$ ;  $n_2 = 75$ ;  $P < 0.005$ ), trial B ( $D = 0.615$ ;  $n_1 = 16$ ;  $n_2 = 26$ ;  $P < 0.005$ ), and trial D ( $D = 0.96$ ;  $n_1 = 11$ ;  $n_2 = 25$ ;  $P < 0.005$ ). The difference was not significant in trial C ( $D = 0.389$ ;  $n_1 = 4$ ;  $n_2 = 18$ ;  $P > 0.1$ ), presumably because of the small sample size.

The number of ticks that successfully engorged and dropped off declined markedly after the first trial (Fig. 1). We cannot confirm this result quantitatively because we applied  $\approx$ 100 larvae to each lizard, but we did not count the actual number of larvae applied. Nevertheless, the dramatic decline after the first trial in the number of larvae engorging and dropping suggests that some biological phenomenon was responsible. One possible explanation is that *S. undulatus* mounts an immune response to feeding by *I. scapularis*

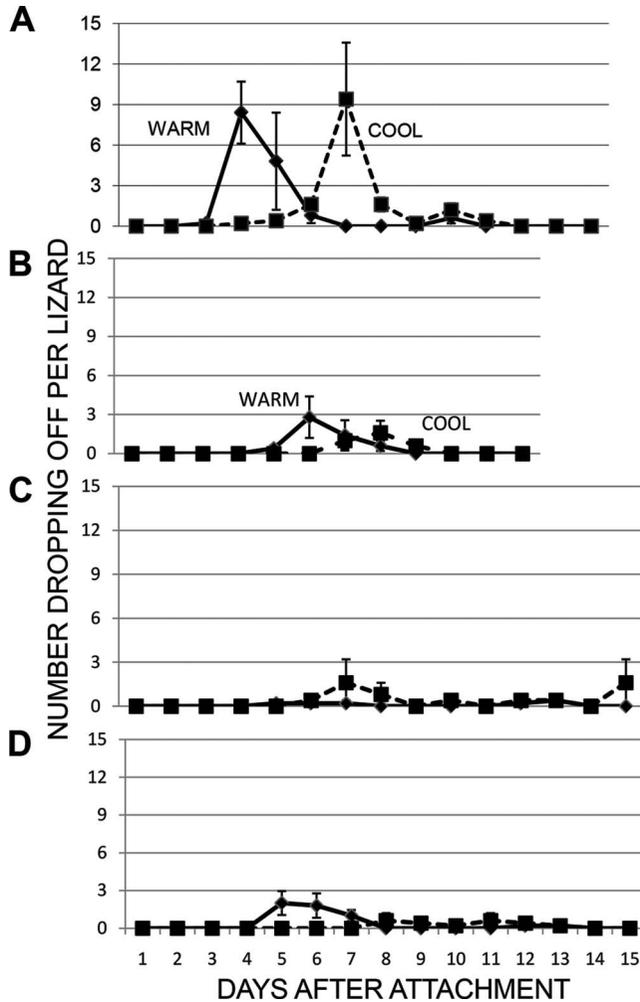


Fig. 1. Drop-off times of engorged larval *I. scapularis* after four successive infestations of eastern fence lizards. Data points are mean numbers of engorged larvae dropping off per lizard  $\pm$ SE.

*laris*. Immune responses of vertebrate hosts to tick feeding vary among tick and vertebrate species (Brosard and Wikel 1998, Wikel 2014), ranging from strong responses, such as that of guinea pigs to *Dermacentor variabilis* (Say) (Trager 1939), to modest responses, such as that of *Peromyscus leucopus* (Rafinesque) to *I. scapularis* (Mather and Ginsberg 1994). Galbe and Oliver (1992) reported different levels of immune response of different host species to feeding by larval *I. scapularis*, but they did not study *S. undulatus*. Another possible explanation is that the larvae used in later infestations might have simply been older than in the initial infestation, and had more difficulty attaching to the lizards. This might also explain the increase in the feeding period after trial A (mean feeding periods: A = 6.0 d, B = 7.0 d, C = 7.9 d, and D = 7.2 d). A third possibility is that the lizards might have developed a search image and simply eaten more of the applied larvae after the first trial, or removed them by grooming. Finally, these lizards might have been exposed to tick feeding before capture, which would

complicate the interpretation of our results. In any case, the dramatic change in feeding pattern after the first infestation suggests that this phenomenon is worthy of further study.

In addition to the effect on feeding period, ambient temperature can affect relationships between ticks and pathogens. The skin surface temperatures of lizards in our WARM treatment were considerably warmer than those in the COOL treatment (Table 1). These larvae had all fed on lizards that had been exposed to nymphs infected with *B. burgdorferi* (strain B31), but none of the 159 nymphs tested (that had developed from these larvae) were infected with *B. burgdorferi*, based on qPCR tests with a probe for the 16S rDNA of *B. burgdorferi* (Rulison et al. 2014), regardless of temperature. Therefore, maintenance at these different temperatures did not affect the reservoir competence of *S. undulatus* for *B. burgdorferi*.

Our results show that feeding periods of larval *I. scapularis* on *S. undulatus* are shorter at higher temperatures. Our temperature treatments were within

the 20 to 30°C range in which Sweatman (1970) found similar temperature effects for *H. aegyptium* on lizards. These results do not imply an effect of temperature on *B. burgdorferi* transmission because *S. undulatus* is not a competent reservoir. However, in ectotherms that serve as reservoirs of pathogens, such as wall lizards (*Podarcis* spp.) for *Borrelia lusitaniae* (Ragagli et al. 2011), ambient temperature could plausibly affect transmission dynamics.

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