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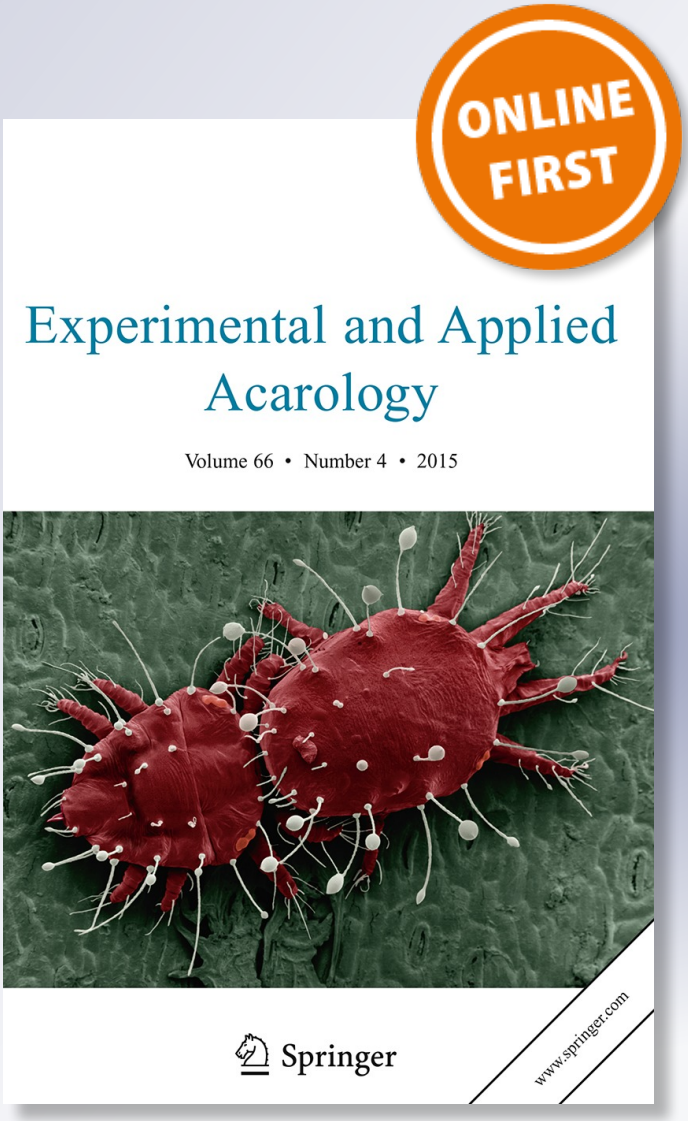

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Effects of temperature on feeding duration, success, and efficiency of larval western black-legged ticks (Acari: Ixodidae) on western fence lizards

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Abstract The western black-legged tick (*Ixodes pacificus*) is a common tick species throughout the western USA and is the major vector for *Borrelia burgdorferi*, the Lyme disease causing bacterium. Western fence lizards (*Sceloporus occidentalis*) are a major host for juvenile *I. pacificus*, but are incompetent hosts for *B. burgdorferi*, which makes this host–parasite relationship of particular interest. In order to shed further light on this complex host–parasite relationship, we investigated the effects of temperature on feeding duration (number of days to repletion), success (number feeding to repletion), and efficiency (replete tick mass) of larval *I. pacificus*. Western fence lizards were experimentally infested with larval ticks and exposed to three constant temperatures (21, 27, 33 °C). Larvae feeding at 21 °C took approximately twice as long as larvae at 27 and 33 °C. Effects of temperature on feeding duration are likely mediated through effects on host blood circulation and functionality of tick salivary proteins. Our results here suggest temperature is another important factor influencing the feeding dynamics of *I. pacificus*, and likely other tick species. Future research is needed to clarify the exact mechanisms behind temperature effects on tick feeding.

Keywords Ectoparasite · *Ixodes pacificus* · Parasitism · *Sceloporus occidentalis*

Introduction

Ixodid ticks generally exhibit a three-host life cycle, feeding on distinct host individuals, and sometimes distinct host species, during the larval, nymphal, and adult stages. The western black-legged tick (*Ixodes pacificus*), distributed throughout the western USA,

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feeds on lizards, birds, rodents, and other small mammals as larvae and nymphs, and primarily on large mammals as adults (Castro and Wright 2007). The western fence lizard (*Sceloporus occidentalis*) is a major host for larval and nymphal *I. pacificus*, estimated to host up to 90 % of all juveniles in some habitats (Casher et al. 2002). The host–parasite relationship between these species has been studied in detail because *S. occidentalis* are refractory to infection with the Lyme disease causing spirochete, *Borrelia burgdorferi* (Lane and Loye 1989; Lane and Quistad 1998), for which *I. pacificus* is the major vector (Burgdorfer et al. 1985; Lane and Burgdorfer 1986). Juvenile ticks carrying *Borrelia* that feed on these lizards are cleansed of infection through complement-mediated innate immunity (Kuo et al. 2000). Recent attention has therefore focused on the role of *S. occidentalis* in Lyme disease ecology, and studies have shown that removal of *S. occidentalis* from oak woodland communities can have dramatic and complex effects on the prevalence of the bacteria (Swei et al. 2011). Studying the fine details of the *Ixodes* feeding mechanisms and the responses of the host may help elucidate the complexities inherent in this host–parasite relationship.

Aside from the immune response to *Borrelia*, one major way in which lizard hosts differ from small mammals and birds involves thermoregulation. Lizards are poikilothermic ectotherms, meaning that their body temperature varies throughout the day and they derive the majority of their heat from external sources (Pough et al. 2004). Ticks feeding on lizards will also experience these daily fluctuations in temperature and may be exposed to cool environmental temperatures in certain habitats. In contrast, ticks feeding on homeothermic endotherms, such as mammals and birds, will experience far lower variation in temperature and may even be buffered from cold environmental temperatures by the warm blood, fur, or feathers of the host. Because ticks, like lizards, are ectothermic, physiological processes such as feeding, digestion, and excretion should be affected by temperature. Sweatman and Gregson (1970) showed that low body temperatures in tortoises led to decreased feeding dynamics (inactivity, decreased pharyngeal pumping, and decreased salivary ejection) of attached nymphal ticks (*Hyalomma aegyptium*). More recently, in a study on eastern fence lizards (*Sceloporus undulatus*) and black-legged ticks (*Ixodes scapularis*), Rulison et al. (2014) investigated the effects of host behavioral thermoregulation and repeated infestations on larval tick feeding duration at "warm" (36.6–25.8 °C) and "cool" (28.4–24.9 °C) temperatures. They found that ticks fed to repletion faster at "warm" temperatures and repeated infestations resulted in increased feeding duration and decreased feeding success of ticks. Even in endothermic hosts, for example sheep, lower ear temperatures increased tick feeding duration, while acclimatization to these lower temperatures increased engorgement weight (Norval 1978). These effects might be even more pronounced on ectothermic hosts like *S. occidentalis*. Could low temperatures negatively affect feeding such that the ticks obtain less blood or even become unable to feed to repletion? Does feeding on a host with a low body temperature prolong feeding?

We sought to answer these questions by examining the effects of temperature on the success, efficiency, and duration of larval *I. pacificus* feeding on *S. occidentalis*. Feeding success is defined as the proportion of ticks that successfully feed to repletion after attaching to a host; efficiency is the average body mass of the replete ticks, with heavy ticks having high efficiency because they obtain more blood from the host; duration is the average time (in days) to repletion after infesting a host. We tested the hypothesis that low temperatures inhibit rate-sensitive processes involved in feeding of ticks by infesting captive *S. occidentalis* with larval *I. pacificus*, incubating them at low, intermediate, and high temperatures, and calculating feeding duration, success, and efficiency. If the

hypothesis is supported, then low temperatures should increase feeding duration while reducing feeding success and efficiency.

Materials and methods

Animal collection and maintenance

Adult *I. pacificus* ticks were collected by dragging fleece flags through trailside grasses in a riparian coastal habitat (Vredevoe et al. 1999) near Los Osos, CA, USA. Field-collected female and male ticks were then bred on bulls (*Bos primigenius taurus*) at the California Polytechnic State University beef unit, and larvae were harvested as in Pollock et al. (2012). Replete female ticks were housed individually in 20 ml plastic vials (Wheaton Science Products, Millville, NJ, USA) with mesh lids in incubators until oviposition. Larval emergence occurred at approximately 6–8 weeks post-oviposition. Vials contained a mixture of plaster of Paris with activated charcoal to prevent desiccation and retard mold growth. Incubator conditions were 22.7 °C and 100 % humidity under 8:16 light/dark photoperiod. Larvae were held in the laboratory for approximately 4 weeks post-emergence before placement on lizards to ensure readiness for host feeding.

In July 2009, 21 adult male *S. occidentalis* were collected by hand-held noose from the Chimineas Ranch unit of the Carrizo Plain Ecological Reserve in San Luis Obispo County, CA and transported back to the California Polytechnic State University, San Luis Obispo campus in cloth bags, where snout–vent length (SVL, ±0.5 cm) and body mass (±0.1 g) were measured.

Experimental infestations

Lizards were randomly assigned to one of three treatment groups, 33 °C (N = 7), 27 °C (N = 7), or 21 °C (N = 7). Following assignment, the SVL and body masses of the lizards were checked to ensure that each treatment group were size matched. Each lizard was then placed into a 2.5-l beaker containing an open micro centrifuge tube with 50 tick larvae. Fine mesh was secured around the top of the beaker with rubber bands to prevent tick escape from the beakers. Infestation trials spanned 48 h, during which beakers were periodically misted with distilled water to prevent desiccation of the larvae. To permit host basking during the infestation trials, beakers were placed close to a 60-W incandescent lamp for approximately 12 h each day. At the termination of the infestation trials, lizards were removed and the number of residual unattached ticks was recorded. Lizards were placed into individual 13 × 8 × 8 (cm) metal mesh cages elevated above tubs filled with approximately 4 cm of water such that any ticks dropping off the host lizards would fall into the tubs and float until they were retrieved daily by the investigators. The sides of the tubs were coated with Fluon (Bioquip, Rancho Dominguez, CA, USA) to prevent tick escape. Tubs were placed in environmental chambers (8:16 light/dark) at one of the three temperature treatment groups, 33, 27, and 21 °C. The preferred basking body temperature of *S. occidentalis* is 35 °C (McGinnis 1966), similar to the highest treatment group. Body temperatures of lizards found resting under objects on the ground have been found below 23 °C during the active season, similar to the lowest treatment group (Brattstrom 1965). Water was offered ad libitum and 2–3 crickets were offered per day to ensure animals were fed to satiety. Replete (fully fed) and unfed

tick numbers were quantified daily as ticks dropped off into the water for approximately 36 days, until all ticks were collected. Replete tick larvae were stored in separate 20 ml vials for each lizard. At the termination of the experiment the total number of unfed and replete ticks was quantified and the replete tick larvae were weighed on a microbalance (to the nearest 0.0001 g).

Data analysis

Feeding duration was defined as how many days it took for tick larvae to feed to repletion. To determine feeding duration, we used the following equation: [day 1 $\times$ (# replete ticks) + day 2 $\times$ (# replete ticks) + $\cdots$ day i $\times$ (# replete ticks)]/total # of replete ticks. Feeding success was defined as the percent of tick larvae that were able to feed to repletion after attaching to the host lizard and was calculated as: (# of replete larvae/total # of larvae attached) $\times$ 100. Feeding efficiency was determined by dividing the total mass of all replete ticks by the total number of replete tick larvae to obtain an average replete tick mass. All comparisons of feeding duration, success, and efficiency across temperatures were analyzed using ANOVA with Tukey post hoc comparisons. Statistics were performed using SAS version 9.3 (Cary, NC, USA). All P values were considered significant at the $\alpha = 0.05$ level.

Results

The number of tick larvae that attached to their host ranged from 17 to 43, but on average was 29 larvae. Two lizards had no ticks attach and, therefore, were not included in analyses. Qualitatively, tick larvae incubated at 33 °C fed to repletion and dropped off their hosts first, followed by larvae incubated at 27 °C. Larvae at 21 °C took the longest to engorge and drop off their host (Fig. 1).

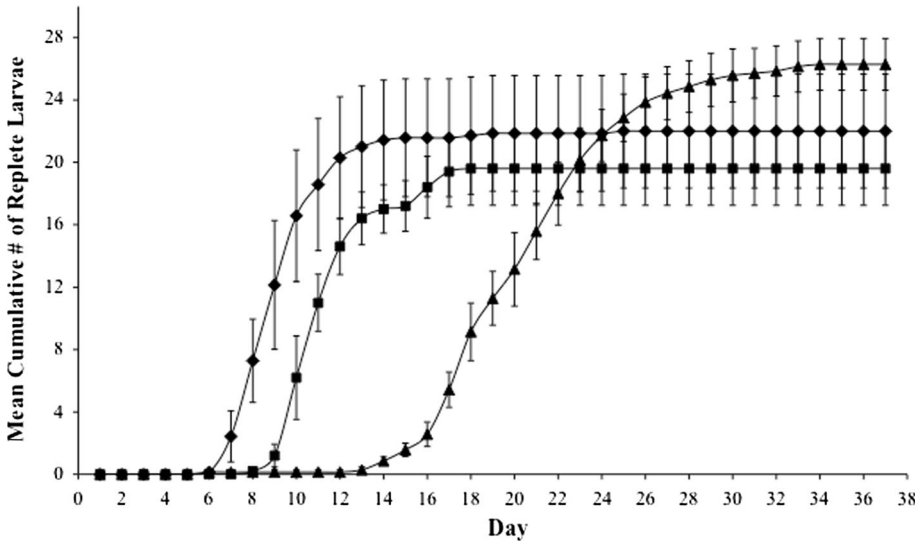

Fig. 1 Cumulative number of replete tick larvae at 33 °C (*diamond*), 27 °C (*square*), and 21 °C (*triangle*). Values are shown as means ± 1 SEM. Larvae fed significantly faster at 33 and 27 °C compared to 21 °C

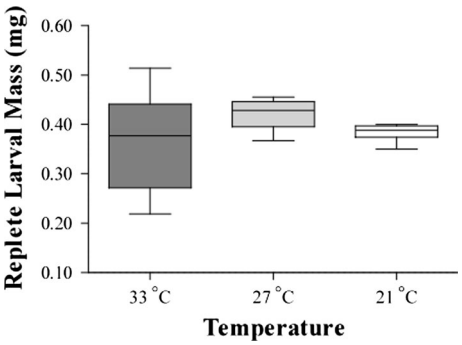

Fig. 2 *Box plots* of mean body masses (feeding efficiencies) for replete tick larvae fed at 33, 27, and 21 °C. *Whiskers* represent the range of body masses recorded for each temperature. Body masses were measured after all larvae in an incubator had fallen off their respective hosts. There were no significant differences in larval body masses (feeding efficiencies) among temperatures

Average feeding duration was 10.0, 11.7, and 20.8 days when larvae were incubated at 33, 27, and 21 °C, respectively. Feeding duration was significantly higher for larvae incubated at 21 °C compared to those incubated at 33 and 27 °C ($F_{2,16} = 132.19$, $P < 0.001$; Fig. 1). Feeding duration between tick larvae feeding at 33 and 27 °C did not differ significantly ($F_{2,16} = 2.94$, $P = 0.11$; Fig. 1). Feeding success was not affected by temperature (mean feeding success: 33 °C = 82.0 %, 27 °C = 82.1 %, 21 °C = 80.4 %; $F_{2,16} = 0.69$, $P = 0.52$). Feeding efficiency was also unaffected by temperature ($F_{2,16} = 0.93$, $P = 0.42$), with mean replete tick larval masses of 0.35, 0.42, and 0.38 mg for 33, 27, and 21 °C, respectively (Fig. 2).

Discussion

Feeding duration, efficiency, and success are likely important factors for tick populations. If juveniles are able to successfully feed and engorge then they will be able to drop off and molt, thus potentially allowing for more adults to emerge. The feeding duration and the ability of ticks to successfully feed depends upon various factors, such as host species, host immune function, tick species, tick life stage, and temperature (Apanaskevich and Oliver 2014). For example, in rabbits there was no relationship between environmental temperature and feeding duration of camel ticks (*Hyalomma dromedarii*; Hagras and Khalil 1988). However, in cattle the feeding duration of wood ticks (*Dermacentor andersoni*) differed among temperatures and cattle strains (Lysyk 2008). Clearly there are several factors influencing the ability of ticks to acquire a sufficient blood meal. However, the majority of studies focus on endothermic species, such as mammals. In contrast, studies involving ectothermic species have been limited. Studies on *S. occidentalis* and *I. pacificus* have found effects of host sex, reproductive state, and hematocrit on feeding duration (Pollock et al. 2012; Pittman et al. 2013), but the effects of temperature on tick feeding had not been investigated until this study.

In the present study we demonstrate the importance of temperature on feeding duration, but not feeding efficiency and success, in a poikilothermic ectotherm. Although there was no significant effect of temperature on feeding efficiency, the variation in replete larval mass was greatest at 33 °C. This variation may be attributed to the correlation between

number of replete larvae and replete larval mass. Average replete larval mass decreased as the number of replete larvae dropping off the lizard host increased. Previous studies investigating effects of tick density on tick feeding efficiency have yielded mixed results, however, with some finding support for the idea that increases in tick density lead to decreases in feeding efficiency (Sutherst et al. 1973), some finding no relationship (Hazler and Ostfeld 1995), and others finding that increases in tick density lead to increases in feeding efficiency (Davidar et al. 1989; Ogden et al. 2002).

We found that the length of time required by larval *I. pacificus* ticks to feed to repletion on the lizard, *S. occidentalis*, increased at a low temperature. Feeding duration was longer at 21 °C (approximate body temperature of lizards resting under objects; Brattstrom 1965) than at 27 and 33 °C (approximate preferred body temperature of lizards; McGinnis 1966). Similar results have been shown in a closely related host–parasite relationship (*S. undulatus* and *I. scapularis*) found in the eastern USA, where ticks feed to repletion faster at "warm" temperatures (Rulison et al. 2014). Together, Rulison et al. (2014) and our study clearly demonstrate the importance of temperature on the feeding duration of ixodid ticks. However, the importance of feeding duration to the overall tick life cycle has yet to be directly demonstrated. It is very plausible that decreased feeding duration of larval ticks could increase survival to subsequent life stages. *Ixodes pacificus* larvae hatch in late summer, overwinter, and begin questing for hosts in early spring, such that peak larval densities occur in late-April to early-May (Padgett and Lane 2001; Eisen et al. 2002), coinciding with peak breeding activity of *S. occidentalis*, the primary larval host (Bromwich and Schall 1986; Casher et al. 2002). Replete larvae molt into nymphs during mid-summer and any larvae that do not manage to encounter a host, feed, and molt fail to survive (Padgett and Lane 2001). Therefore, because larval ticks feed to repletion faster at higher temperatures (i.e. −27 and 33 °C) and the development time of engorged larvae to nymphs is shorter at higher temperatures (Ogden et al. 2004), one could expect increased molting to the nymphal stage, and therefore, increased survival with warmer temperatures. The exact mechanisms behind how temperature can drive decreased feeding duration of ixodid ticks remains unclear, however.

One potential way in which low temperatures could increase tick feeding duration is through effects on parasite behavior and the host circulatory system. Low environmental temperatures have been shown to decrease tick feeding activity and coordination (Clark 1995), which in turn would require a longer period of time for ticks to obtain a sufficient blood meal. Temperature can also impact tick feeding via effects on the host circulatory system. Host blood flow is critical for feeding ticks, and in ectothermic species decreases in temperature result in decreased cardiac output, decreased heart rate, and increased circulation time (Baker and White 1970; Barron et al. 1987; Stinner 1987). Each of these would cause slower flow of blood to feeding ticks and, therefore, significantly increase the amount of time needed to feed to repletion.

Another way low temperatures could affect feeding duration is through the rate of tick protein expression and activity. Upon attachment to their host, ticks produce a complex salivary cocktail of proteins, including anti-coagulants, anti-platelets, and vasodilators, which keep blood flowing to the feeding tick, and immunomodulators, which neutralize and counteract the host inflammatory and immune responses (Ribeiro and Francischetti 2003; Steen et al. 2006). Because these salivary components are proteins, they likely have optimal temperatures at which they function, and these salivary proteins could exhibit lower activity at low temperatures. Therefore, at 21 °C in this study, the decreased functionality of salivary proteins could have led to the increase in feeding duration due to the decreased ability to maintain sufficient blood flow to the feeding ticks. Some tick anti-

coagulants, for example, are known to be slow-binding competitive inhibitors that are heat labile and exhibit reduced activity at cooler temperatures (Waxman et al. 1990; Limo et al. 1991). Similar to the effect on hemostatic proteins of tick saliva, low temperatures could also decrease the functionality of immunomodulatory salivary proteins. As a result, ticks would have to extend their feeding period due to the difficulty in obtaining a sufficient blood meal while combating the host immune response.

Host lizards are also ectothermic, however, so their immune cells and proteins may be impacted by temperature as well. Indeed, immune function has been shown to decrease with decreasing temperature in several ectothermic species (Wright and Cooper 1981; Mondal and Rai 2001; Merchant et al. 2003; Merchant and Britton 2006). Overall, we believe it is unlikely that temperature-related effects on host immune function played a significant role in the observed decreases in feeding duration of ticks at 21 °C. Regardless of temperature, ticks fed successfully and to similar engorgement masses. One would expect differences in feeding success and efficiency among temperature groups if temperature-based effects on immune function were playing a role. Furthermore, no studies to our knowledge have directly demonstrated an immune response to ticks by lizards. In a study by Galbe and Oliver (1992), broad-headed skinks did not acquire resistance after multiple tick infestations, and ticks fed more efficiently on these lizard hosts compared to two mouse species and guinea pigs. Lastly, unlike the study by Rulison et al. (2014) on *S. undulatus* and *I. scapularis*, the lizards in our study are highly unlikely to have had any prior exposure to *I. pacificus* ticks because they were collected from a population in which tick parasitism almost never occurs. Therefore, any acquired resistance to ticks prior to the study is unlikely to have existed.

In conclusion, we have demonstrated a direct link between temperature and feeding duration of western black-legged ticks on western fence lizards, a poikilothermic ectotherm. The increased feeding duration at low temperature is likely driven by effects of temperature on host blood circulation and/or functionality of tick salivary proteins, although these hypotheses remain to be experimentally evaluated. Salivary proteins in ixodid ticks have recently been isolated and characterized (Francischetti et al. 2010) and future research should focus on the effects temperature may have on the kinetics of these proteins. While there is little evidence in the present study to suggest a role of host immune function on feeding of ticks, future studies should certainly explore potential temperature effects on the immune response of ectotherm hosts to tick feeding. Lastly, our results described here do not suggest an effect of temperature on *B. burgdorferi* transmission because *S. occidentalis* is an incompetent host. However, in ectotherms that do serve as hosts for *Borrelia* species, such as southeastern five-lined skinks and green anoles for *B. burgdorferi* (Levin et al. 1996), *Podarcis* species for *B. lusitaniae* (Richter and Matuschka 2006; Földvári et al. 2009), and *Lacerta* species for *B. burgdorferi* and *B. lusitaniae* (Majláthová et al. 2008; Földvári et al. 2009), temperature could plausibly affect transmission dynamics. Future research should investigate potential relationships between temperature, host competence, and tick transmission of *Borrelia*.

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Author contributions E.N.T., N.B.P., and E.G. conceived and designed the experiment. E.G. and N.B.P. performed the experiment. E.G. and N.B.P. analyzed the data and wrote the manuscript. N.B.P. and E.N.T. participated in manuscript revision.

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