

Larval thermal characteristics of multiple ixodid ticks

Alicia M. Fieler^a, Andrew J. Rosendale^{a,b}, David W. Farrow^a, Megan D. Dunlevy^a, Benjamin Davies^a, Kennan Oyen^a, Yanyu Xiao^c, Joshua B. Benoit^{a,*}

^a Department of Biological Sciences, University of Cincinnati, Cincinnati, OH 45221, USA

^b Department of Biology, Mount St. Joseph University, Cincinnati, OH, USA

^c Department of Mathematical Sciences, University of Cincinnati, Cincinnati, OH 45221, USA

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ABSTRACT

Temperature limits the geographic ranges of several tick species. Little is known about the thermal characteristics of these pests outside of a few studies on survival related to thermal tolerance. In this study, thermal tolerance limits, thermal preference, and the impact of temperature on activity levels and metabolic rate were examined in larvae for six species of ixodid ticks. Tolerance of low temperatures ranged from -15 to -24 °C with *Dermacentor andersoni* surviving the lowest temperatures. High temperature survival ranged from 41 to 47 °C, with *Rhipicephalus sanguineus sensu lato* having the highest upper lethal limit. *Ixodes scapularis* showed the lowest survival at both low and high temperatures. Thermal preference temperatures were tested from 0 to 41 °C. The majority of species preferred temperatures between 17 and 22 °C, while *Dermacentor variabilis* preferred significantly lower temperatures, near 12 °C. Overall activity was measured across a range of temperatures from 10 to 60 °C, and most tick species had the greatest activity near 30 °C. Metabolic rate was the greatest between 30 and 40 °C for all tick species and was relatively stable from 5 to 20 °C. The optimal temperature for tick larvae is likely near the thermal preference for each species, where oxygen consumption is low and activity occurs that will balance questing and conservation of nutrient reserves. In summary, tick species vary greatly in their thermal characteristics, and our results will be critical to predict distribution of these ectoparasites with changing climates.

1. Introduction

Ticks are blood-feeding, ectoparasitic arthropods that are known for spreading a wide range of diseases through very specific vector and host interactions (Wikel, 2013). Ticks require a blood meal at each mobile life stage and often acquire pathogens from vertebrate hosts as larvae or nymphs, transmitting these pathogens in subsequent life stages (Riek, 1964). Lyme disease is one of the most commonly reported vector-borne illnesses in the United States (Stafford et al., 1998). Agents of spotted fever rickettsioses including *Rickettsia rickettsii* and *R. parkeri* are transmitted by *Dermacentor variabilis*, *D. andersoni*, *Amblyomma maculatum* and *Rhipicephalus sanguineus sensu lato* throughout North America (Parola et al., 2005). Tick-borne diseases have been consistently on the rise; recent studies have shown that the proportion of *Borrelia*, Lyme disease causing pathogen, carrying ticks in Iowa has increased from 8% to 23.5% between 1998 and 2013 (Oliver et al., 2017) and the number of Lyme disease cases has been steadily increasing over time (Stafford et al., 1998). In addition, the occurrence rate of *Anaplasma*

phagocytophilum and *Ehrlichia chaffeensis*, transmitted by *I. scapularis* and *A. americanum*, respectively, has increased by more than 2-fold from 2000 to 2007 (Dahlgren et al., 2011). There are a multitude of putative reasons for this increased disease incidence, including improved recognition of tick-borne diseases, encroachment of humans into tick habitats, and climate change increasing the duration of the active season and geographic distribution of ticks (Lindgren et al., 2000).

The historic geographic distributions of many tick species are well defined in the USA (Fig. 1); however, current tick distributions are shifting and this is predicted to continue, given climate change projections (CDC, 2018a; CDC, 2018b). Previous studies have shown strong links between species geographic distributions and the range of temperatures they can tolerate (Sunday et al., 2011). Therefore, predicting future changes in tick distributions may largely depend on the species-specific thermal tolerance characteristics. Previous studies of tick thermal and stress physiology have demonstrated that ticks vary in their abilities to tolerate extreme temperature and humidity (Rosendale et al., 2019; Rosendale et al., 2016a, 2016b; Springer et al., 2015; Yoder and

* Corresponding author.

E-mail address: joshua.benoit@uc.edu (J.B. Benoit).

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Benoit, 2003; Needham and Teel, 1991; Yano et al., 1987). Tick development has been directly tied to temperature, where continual temperatures over 4 °C and under 32 °C are critical for molting and reproductive output of *I. scapularis* (Ogden et al., 2004). These temperature ranges for development are likely similar in other tick species, but lower temperature threshold can be as high as 12 °C for some species (Lysyk, 2014; Yano et al., 1987).

Extreme temperatures limit survival of various species of ticks, (Rosendale et al., 2016a; Holmes et al., 2018). When exposed to such temperature extremes in field conditions, ticks are susceptible to overheating in the summer, freezing in the winter, and dehydration throughout the year (Eisen et al., 2016; Rosendale et al., 2016a, 2016b; Yoder and Benoit, 2003). Importantly, each bout of stress seems to yield a quantifiable expenditure of energy reserves, which cannot be

replenished until the tick locates a new host and obtains a bloodmeal (Rosendale et al., 2017; Randolph, 2004; Vandyk et al., 1996). Extreme high temperatures lower questing activity in ticks (Loye and Lane, 1988). This is likely because ticks will return to the duff layer of the soil to lower their temperature and rehydrate, which will decrease the probability of locating a host (Tomkins et al., 2014). This behavioral thermal regulation has been well-documented in other organisms but is not without cost; animals that seek thermal refugia often exhibit lower overall activity patterns, which reduces feeding and mating success (Nakayama and Miyatake, 2010). For ticks, host-seeking behavior also declines at low temperatures (Dautel et al., 2008); therefore, seasonal variations in temperature, and also photoperiod, determines activity patterns of ticks. These specific behavioral changes can ultimately impact the dynamics of tick-borne diseases by regulating host-tick

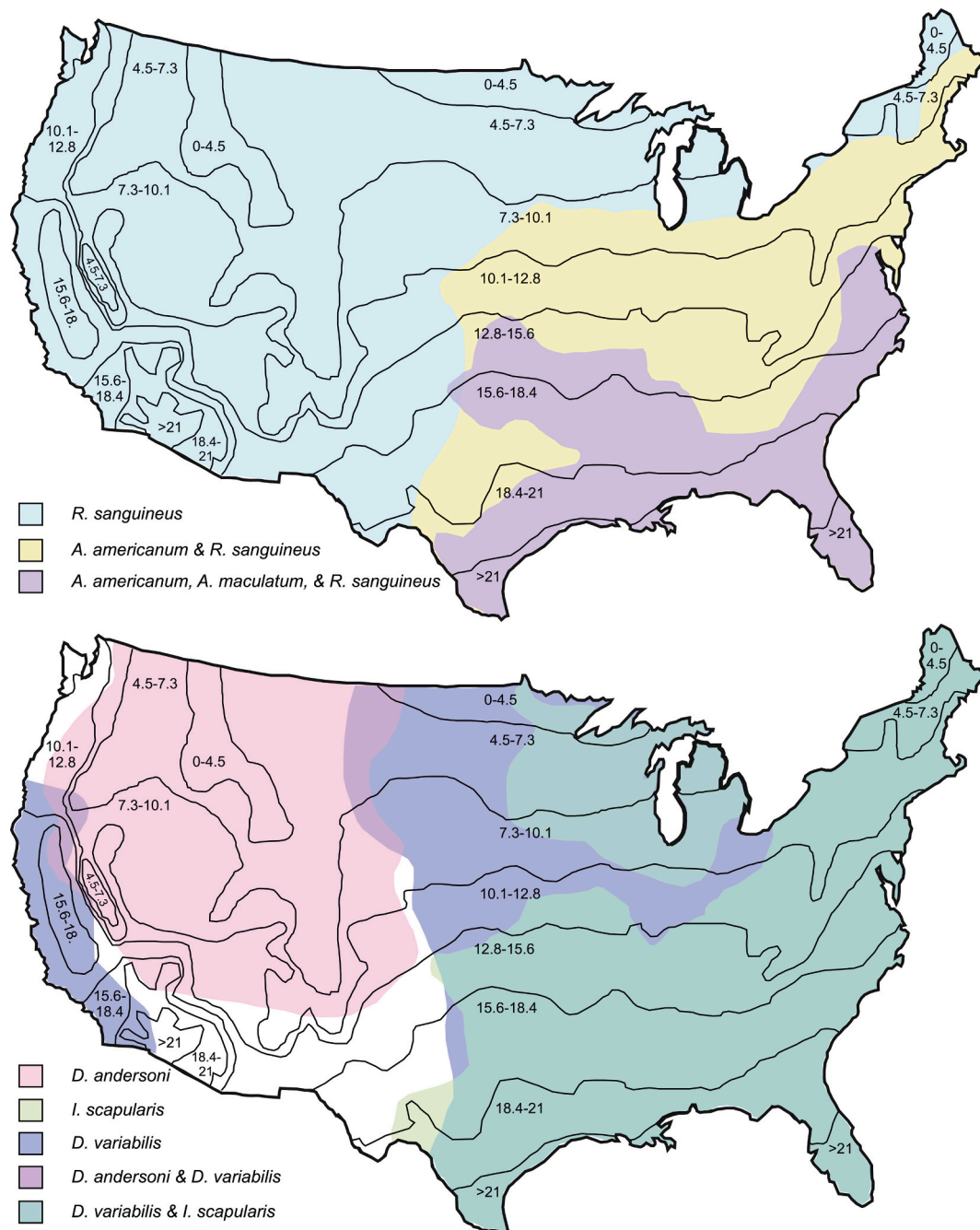


Fig. 1. Geographic distribution of Ixodidae in the continental United States. Distribution data based on data available from the Centers for Disease Control and Prevention (CDC, 2018b). Isotherm lines represents annual average temperature (°C).

interactions (Gilbert, 2010). Climate change also influences human behavior; increasing the number of humans outside, wearing fewer layers of clothing and subsequently leading to higher exposure to ticks and potential diseases (Gilbert, 2021; Wikel, 2018; Ostfeld and Brunner, 2015).

A consistent distribution shift of ticks and tick-borne diseases has been identified due climate change, which will impact future tick-host-pathogen transmission dynamics (Gray et al., 2009). Studies on thermal characteristics of ticks have almost solely focused on survival during exposure to lower and upper lethal temperatures and supercooling points without study of additional physiological and behavioral processes that could be impacted by extreme temperatures (Neelakanta et al., 2010; Knülle and Dautel, 1997; Lee and Baust, 1987). The purpose of this study is to examine thermal characteristics of ticks, including limits of survival, effects on metabolic rates and activity patterns, and preferred temperature ranges of various tick species, to predict the impact on tick-borne disease dynamics and distributions. We examined the larvae of six tick species commonly found within the United States. These tick larvae are usually present during the spring to late summer (Ogden et al., 2005; Kollars et al., 2000; Vandyk et al., 1996; Davidson et al., 1994; Garvie et al., 1978). Larval presence has also been noted, based on collection, as early as the end of winter and as late as the end of fall depending on the specific region of the United States. Importantly, overwintering as larvae can occur in some species (Davidson et al., 1994; Kollars et al., 2000), but likely not in others (Ogden et al., 2005; Ostfeld et al., 1996). This indicates that tick larvae are likely to experience thermal stresses, potentially both heat during the summer and cold during the winter. There are limited studies on tick thermal characteristics (Rosendale et al., 2019; Holmes et al., 2018; Vandyk et al., 1996); however, existing data indicates that global shifts in tick distributions correspond to climate change (Gray et al., 2009) and establishing baseline comparative studies on tick thermal tolerance is therefore necessary. Overall, our study expands the understanding of the effects that temperature has on the physiology and behavior of larval ticks. The most dramatic difference among species was seen at low temperatures, suggesting that winter conditions may have a strong influence on tick behavior and distributions. Based on these studies, tick thermal preference, where ticks usually chose to remain for extended periods, will be near the optimal temperature that balances tick activity, such as questing, and reduces the utilization of the limited nutrient reserves to survive long periods from larval emergence until feeding on the first host.

2. Methods

2.1. Ticks

Larvae of several species, including *Amblyomma americanum*, *Amblyomma maculatum*, *Rhipicephalus sanguineus sensu lato*, *Ixodes scapularis*, *Dermacentor variabilis*, and *Dermacentor andersoni*, were reared from eggs. Engorged females were acquired from laboratory colonies at the Oklahoma State University (OSU) Tick Rearing Facility (Stillwater, OK, USA). Adult ticks are maintained in these colonies at 14:10 h, light:dark (L:D), 93–97% relative humidity (RH), and $25 \pm 1^\circ\text{C}$. Mated females were fed on sheep (*Ovis aries*) until repletion. For our study, fed females were sent to our laboratory within one-two days of drop off from the host and, upon arrival, the engorged females were placed in closed chambers containing a supersaturated solution of potassium nitrate, 93% RH (Winston and Bates, 1960). Females were allowed to lay eggs, and the eggs developed at $26 \pm 1^\circ\text{C}$, 15:9 h L:D, and 93% RH for several weeks. Multiple females ($N = 3$) were used for each tick species to prevent results based upon a single clutch of eggs. After emerging, larvae were kept at these conditions until being used in experiments 2–4 weeks post-hatching.

2.2. Thermal tolerance

Limits of thermal tolerance were determined based on studies of cold tolerance in *D. variabilis* (Rosendale et al., 2016a). Briefly, larvae were subjected to high or low temperatures via 2 h exposures to cold (0 to -25°C) or heat (35 to 47°C). Groups of 10 ticks ($N = 10$ groups) were placed in 1.5 cm^3 tubes which were then arranged into foam-plugged 50 mL tubes, which were suspended in an ethylene glycol:water (60:40) solution. Although these changes are likely more abrupt than those that ticks would experience on a daily scale (Rosendale et al., 2016a; Yu et al., 2014), these baseline survival rates tend to be representative of heat and cold tolerance and allow our results to be compared to previous studies that have used similar methods (Yu et al., 2014). Temperature was regulated ($\pm 0.1^\circ\text{C}$) with a programmable bath (Arctic A25; Thermo Scientific, Pittsburgh, PA, USA) and relative humidity was maintained with a small vial of a supersaturated solution of potassium nitrate (93% RH). After temperature treatments, larvae were returned to rearing conditions and allowed to recover for 24 h prior to survival assessment. Ticks were marked as surviving if the larvae could move five body lengths following probing and breathing from the individual assessing survival.

2.3. Thermal preference

To determine the temperature preferred by the larval ticks, a thermal preference arena was constructed. The arena consisted of a 30 cm long piece of clear plastic tubing (2.5 cm diameter, PETG) that was heated at one end with water (45°C) circulated from a programmable bath and cooled on the other end with an ice bath. The whole length of tubing was placed within insulated boxes with the metal plate below to allow for temperature transfer. This setup resulted in one end of the arena being at 43°C while the other end was at 0°C and the middle was $\sim 24^\circ\text{C}$. The arena was visibly marked along its length into multiple sections with known temperatures. Groups of 10 ($N = 10$ groups) ticks were released into the center of the arena and allowed to freely move for 2 h. Relative humidity was maintained with container of supersaturated solution of potassium nitrate (93% RH) placed within the insulated boxes. A total of 5 trials were performed and at the end of each trial, the number of ticks in each section was recorded. At the end of each assay, ticks were probed to ensure that individuals were capable of movement. Temperature was validated at the site where the tick larvae stopped movement with a temperature probe (Omega) at the completion of each assay.

2.4. Oxygen consumption

The effect of temperature on O_2 consumption in larval ticks was examined using microrespirometers constructed and utilized as previously described (Rosendale et al., 2017). Groups of 10–20 larvae ($N = 10$ groups) were positioned within the microrespirometers such that $\sim 0.02\text{ cm}^3$ of space was available for the ticks. Microrespirometers were positioned in heated or cooled baths (0 – 60°C) and the complete apparatus was allowed 15 min to equilibrate prior to measurements being taken. Measurements were conducted across a range of temperatures based on standard methods that establish thermal performance curve (Sinclair et al., 2016). Of importance, temperatures near the maximum could represent the high oxygen consumption, but are far above the optimal temperature for each species and mortality could occur due to prolonged exposure. CO_2 production, measured through the movement of KOH, was measured every hour for 6 h. Assuming that one mole of O_2 is consumed for every mole of CO_2 that is released, oxygen consumption was calculated by using the distance travelled by the KOH and was expressed as $\text{nl O}_2\text{ mg}^{-1}\text{ wet mass tick}^{-1}\text{ h}^{-1}$. At the end of each assay, ticks were probed to ensure that individuals were capable of movement.

2.5. Activity

Activity was monitored using a Locomotor Activity Monitor from Trikinetics Inc. (Waltham, MA, USA) and the DAMSystem3 Data Collection Software (TriKinetics). Larvae ($N = 20$) were individually placed in 10 cm glass test tubes which consisted of tightly packed cotton at the top and bottom so that the ticks were contained in a 12.5 cm³ space in the middle. The tubes were loaded horizontally into the locomotor activity monitor and the entire apparatus was placed inside an insulated plastic container that contained water to maintain a high relative humidity (>97% outside of vials and over 93% in all vials) throughout the trial. Water that was heated or cooled with a water bath was circulated through plastic tubing within the insulated container to generate temperatures ranging from 10 to 60 °C $\pm$ 2 °C. Activity was measured by a sensor that counted the number of times the tick crossed the center of the tube; this number is directly related to the activity and movement of the ticks. All ticks were monitored for a 6-h period. Similar to the experiments on oxygen consumption, activity levels near the thermal maximum are likely to be the greatest, but could be far above the optimal to balance questing, nutrient reserve levels, and prolonged survival.

2.6. Statistical analyses

Summary statistics are reported as means $\pm$ standard error (SE). For survival data, the proportion of larvae that survived in each tube was recorded and all survival data were arcsin-square root transformed prior to analysis. Using Prism (GraphPad; San Diego, CA, USA), survival, activity, and oxygen consumption were analyzed using a two-way analysis of variance (ANOVA) with species and temperature as fixed factors, and pairs of means were compared among species using Tukey's *post-hoc* tests. LT₅₀ in relation to thermal tolerance was established with a Probit analysis in R. For thermal preference, means were compared using a one-way ANOVA with a Tukey's *post-hoc*.

3. Results

3.1. Thermal tolerance

The lower lethal temperature (LLT), where no larvae survived a 2 h exposure, ranged among species from -15 to -24 °C (Fig. 2, Table 1) with *A. americanum* and *I. scapularis* having the highest LLT and *D. andersoni* having the lowest. The temperature resulting in 50% mortality (LLT₅₀) varied ($F_{5,43} = 175$, $P < 0.0001$) among species with *R. sanguineus sensu lato* and *I. scapularis* having the highest LLT₅₀ and *D. andersoni* having the lowest (Table 1). There was a significant effect of temperature ($F_{10,603} = 379.4$, $P < 0.0001$), species ($F_{5,603} = 231.2$, $P < 0.0001$), and a significant temperature*species interaction ($F_{50,603} = 19.7$, $P < 0.0001$). Survival following thermal stress varied among all species ($P < 0.01$, all cases) with the exception of *R. sanguineus sensu lato* and *I. scapularis*, which did not differ from each other ($P = 1.0$).

The upper lethal temperature (ULT), where no larvae survived a 2 h exposure, ranged among species from 41 to 47 °C (Fig. 3) with *I. scapularis* having the lowest ULT and *R. sanguineus sensu lato* having the highest. The temperature resulting in 50% mortality (ULT₅₀) varied ($F_{5,35} = 35.0$, $P < 0.0001$) among species with *I. scapularis* having the lowest ULT₅₀ and *R. sanguineus sensu lato* and *A. maculatum* having the highest (Table 1). There was a significant effect of temperature ($F_{8,425} = 229.2$, $P < 0.0001$), species ($F_{5,425} = 44.9$, $P < 0.0001$), and a significant temperature*species interaction ($F_{40,425} = 9.0$, $P < 0.0001$). Overall survival varied among the species with *I. scapularis* having the lowest survival and *R. sanguineus sensu lato* and *A. maculatum* having the greatest.

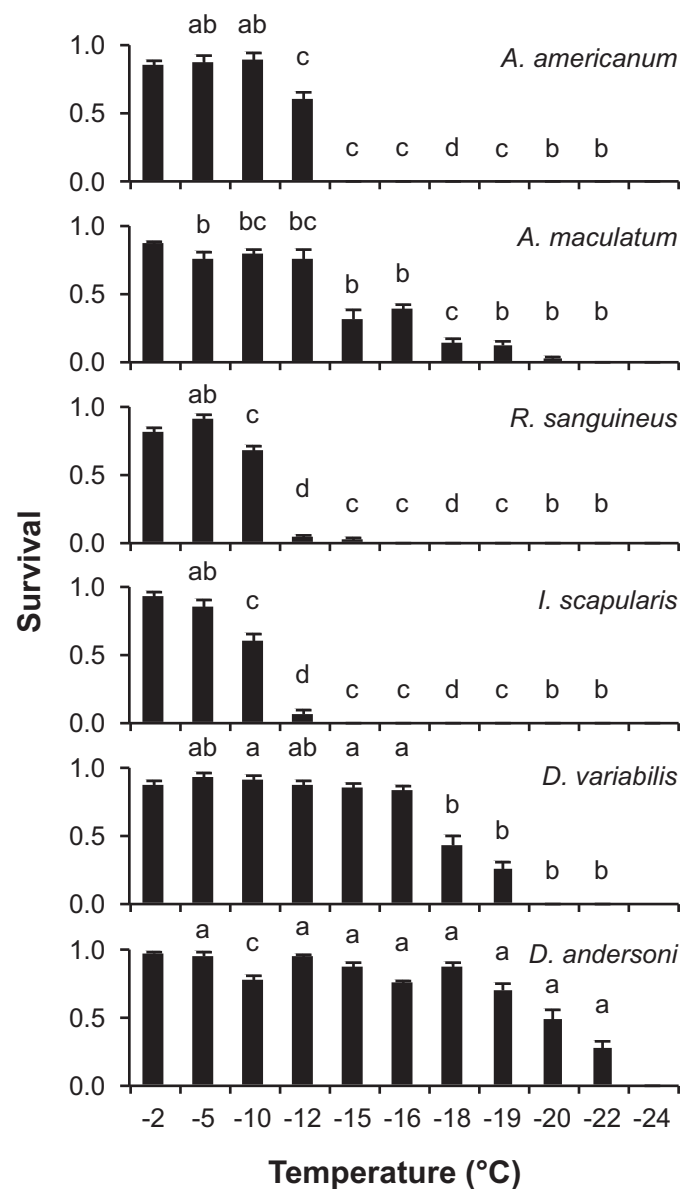


Fig. 2. Survival of ixodid larvae exposed to cold stress. Tick larvae were exposed to low temperatures for 2 h and survival was assessed after 24 h of recovery. For each temperature, values (mean $\pm$ SE of proportion surviving, ten groups of ten ticks that do not share a letter are significantly different among species, $P < 0.05$).

Table 1
Thermal tolerance of larval ixodid ticks (°C).

	LLT	LLT ₅₀	ULT	ULT ₅₀
<i>A. americanum</i>	-15	-11.4 $\pm$ 0.5 ^a	45	40.5 $\pm$ 0.2 ^a
<i>A. maculatum</i>	-22	-12.1 $\pm$ 0.3 ^a	46	42.3 $\pm$ 0.3 ^b
<i>D. andersoni</i>	-24	-19.7 $\pm$ 0.3 ^b	45	41.1 $\pm$ 0.2 ^a
<i>D. variabilis</i>	-20	-16.9 $\pm$ 0.4 ^c	43	40.2 $\pm$ 0.2 ^a
<i>I. scapularis</i>	-15	-8.5 $\pm$ 0.3 ^d	41	38.0 $\pm$ 0.2 ^c
<i>R. sanguineus sensu lato</i>	-16	-7.9 $\pm$ 0.3 ^d	47	42.3 $\pm$ 0.3 ^b

Values (mean $\pm$ SE) that do not share a letter are significantly different.

3.2. Activity

To examine the effects of temperature on tick activity, larvae were placed in horizontally positioned tubes at temperatures ranging from 10 to 60 °C, and their activity (number of times they moved across the

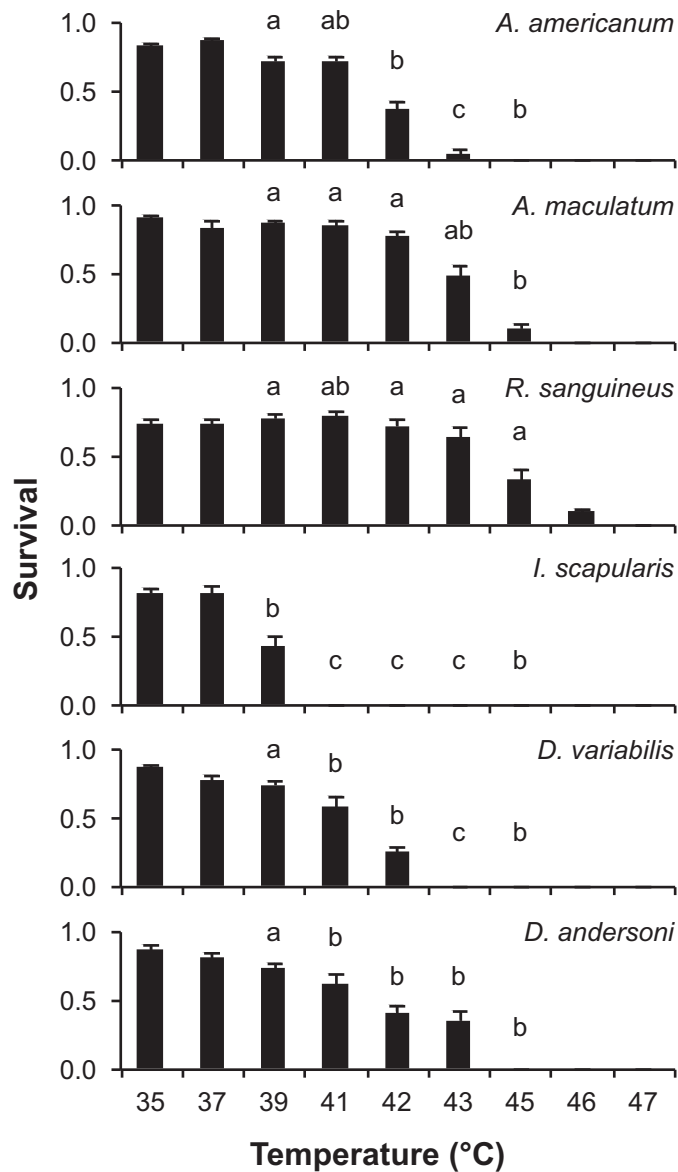


Fig. 3. Survival of ixodid larvae exposed to heat stress. Tick larvae were exposed to low temperatures for 2 h and survival was assessed after 24 h of recovery. For each temperature, values (mean $\pm$ SE of proportion surviving, ten groups of ten ticks that do not share a letter are significantly different among species, $P < 0.05$).

center of the tube) was monitored for 6 h. were used in the analysis. Activity was greatest at 30 °C for most species, with *D. variabilis* and *A. americanum* having greatest activity at 40 °C (Fig. 4). Activity was essentially reduced for all species at 10 and 60 °C. At 10 °C, larvae that were warmed after the experiment regained the ability to move. The reduced activity at 50 and near cessation at 60 °C is likely a result tick death, where activity was initially noted but no ticks survived experiments. Importantly, mortality begins to occur for most species after exposure to 40 °C for extended periods, thus the increased activity near the high thermal threshold is likely driven by ticks attempting to move to lower more tolerable temperatures, rather than optimal levels. There was a significant effect of temperature ($F_{6, 598} = 44.7$, $P < 0.0001$), species ($F_{5, 598} = 4.3$, $P = 0.0007$), and a significant temperature*species interaction ($F_{30, 598} = 6.2$, $P < 0.0001$). Variation in activity levels occurred among several species at 20, 30, and 40 °C.

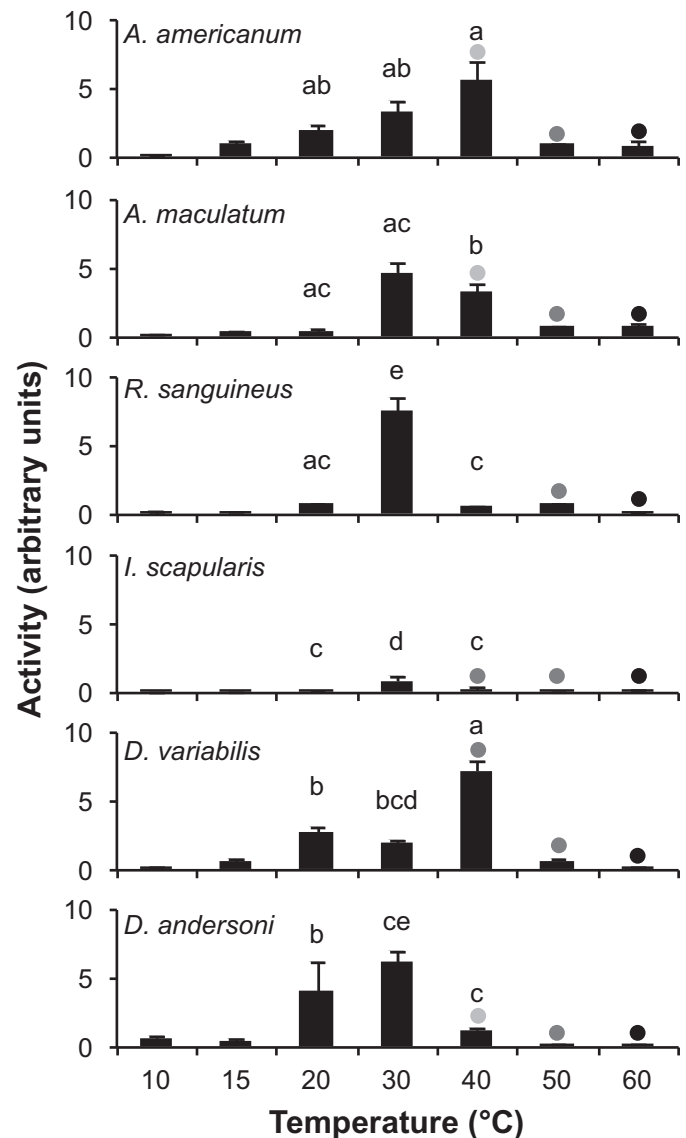


Fig. 4. Activity of ixodid larvae at various temperatures. Activity levels were monitored for 6 h periods. For each temperature, values (mean $\pm$ SE) that do not share a letter are significantly different among species. Values without letters did not differ among any of the species ($P < 0.05$). Dots represent where low (less than 30%, light gray), moderate (30–80%, gray), and high (over 80%) mortality occurs based on observations in Fig. 3.

3.3. Oxygen consumption

The effect of temperature on oxygen consumption rates was examined by placing microrespirometers containing groups of larvae at various temperatures. Metabolic rate was greatest at 40 °C for most species, with *I. scapularis* and *A. maculatum* having highest rates at 30 °C (Fig. 5). Oxygen consumption stopped and/or dropped below our ability to measure for all species at 0 °C. At the upper thermal temperature, a drastic reduction in oxygen consumption occurred at 50° and no respiration was detected at 60 °C (Fig. 5). This rapid decline is likely due to mortality of ticks during prolonged exposure to high temperatures. Mortality, albeit at low levels, also occurs at 40 °C, further suggesting that the greatest oxygen consumption levels are unlikely to be optimal, and instead demonstrate the linear relationship between temperature and metabolic rate and the inability of ticks to regulate body temperature and oxygen consumption in these conditions. There was a significant effect of temperature ($F_{5, 175} = 149.2$, $P < 0.0001$), species ($F_{5, 175}$

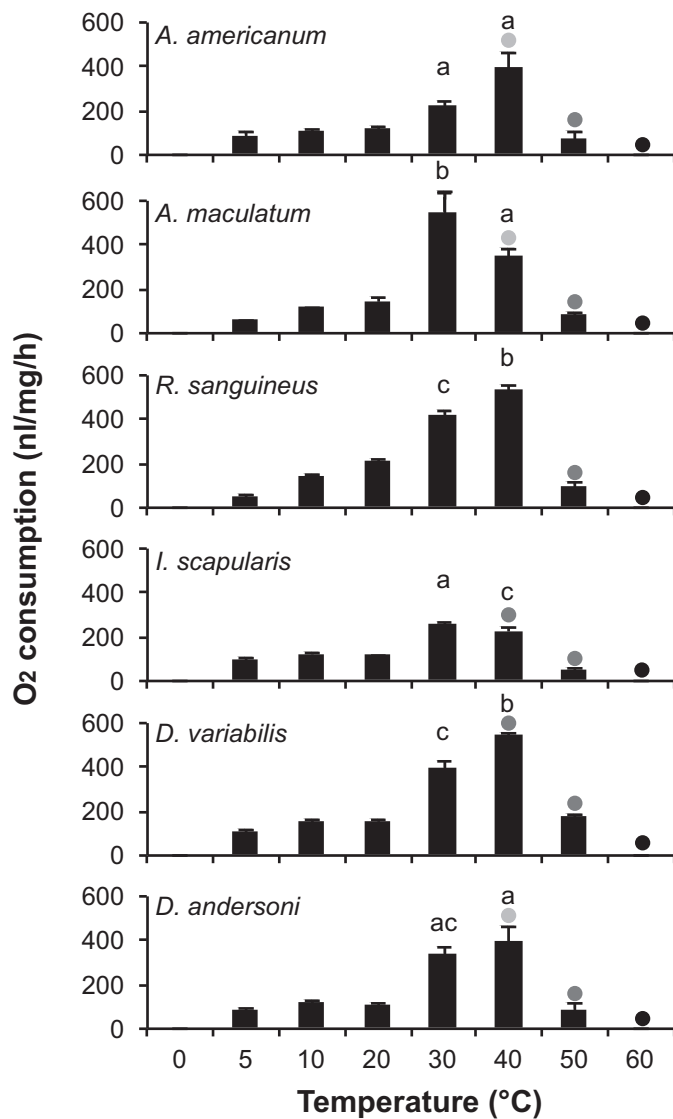


Fig. 5. Effect of temperature on oxygen consumption of ixodid ticks exposed to various temperatures. Metabolic rate was monitored for 6 h. For each temperature, values (mean $\pm$ SE) that do not share a letter are significantly different among species ($P < 0.05$). Dots represent where low (less than 30%, light gray), moderate (30–80%, gray), and high (over 80%) mortality occurs based on observations in Fig. 3.

$= 13.3$, $P < 0.0001$), and a significant temperature*species interaction ($F_{25, 175} = 5.1$, $P < 0.0001$). Variation in metabolic rate occurred among species at 30 and 40 °C. Importantly, oxygen consumption at temperatures from 5 to 20 °C remained relatively low, suggesting that shifts in oxygen consumption below 20 °C are minimal.

3.4. Thermal preference

To determine the preferred temperature of the different tick species, larvae were positioned in the center of a length of tubing that was cooled (0 °C) at one end and heated (43 °C) at the other and allowed 2 h to move to their preferred location. Although there was some variation among species as to which temperature had the greatest proportion of individuals, most larvae aggregated in the 15–25 °C temperature range (Fig. 6). The notable exception was *D. variabilis*, which had a higher proportion of individuals at the low temperature range. When the total distribution of the individuals was considered, there was significant ($F_{5, 273} = 10.9$, $P < 0.0001$) variation among the species, with *D. variabilis*

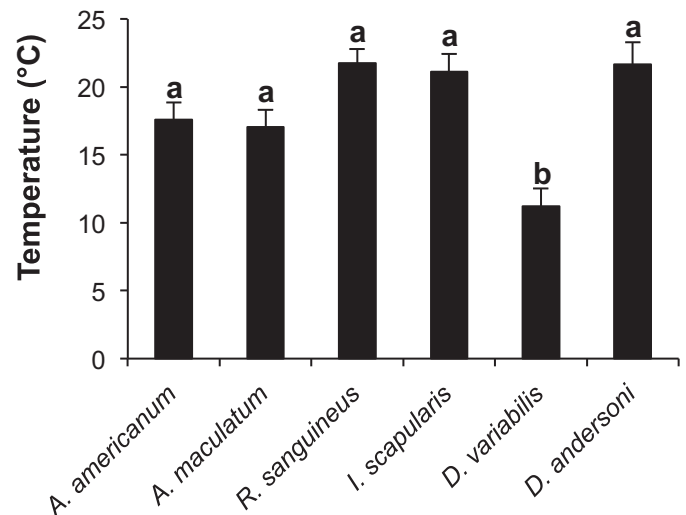


Fig. 6. Thermal preference of ixodid ticks. Ticks were subjected to temperatures ranging from 0 to 41 °C for 2 h. For each temperature, values (mean $\pm$ SE) that do not share a letter are significantly different among species ($P < 0.05$).

being lower ($P < 0.05$, all cases) than all other species (Fig. 6). Importantly, when ticks were examined for activity following each assay, all individuals were capable of movement into other areas indicating no ticks were permanently immobilized by temperature.

4. Discussion

The ability to withstand unfavorable environmental conditions undoubtedly contributes to the widespread geographic distributions of ticks and their success as disease vectors. Temperature fluctuations and other bouts of stress affect tick questing behaviors, metabolic rates, and energy reserves (Rosendale et al., 2017; Rosendale et al., 2016a, 2016b; Gilbert, 2010; Randolph, 2004). We examined thermal tolerances of ixodid ticks by determining their lower lethal temperatures (LLT) and the upper lethal temperatures (ULT) after exposure to a wide range of temperatures, allowing comparisons among several species. The majority of ticks had a high survival at -12 °C, but decreased dramatically below -16 °C. *A. americanum* and *I. scapularis* exhibited the lowest tolerance to cold with a LLT_{50} near -12 °C whereas *D. andersoni* exhibited the greatest tolerance to low temperatures at -22 °C. Given that *D. andersoni* is found throughout the colder northern regions and higher elevations of the Rocky Mountains, our data confirms that *D. andersoni* has the physiological adaptations required to survive at the low temperatures that characterize their geographic range (James et al., 2006). In this study, we did not examine how periods of rapid cold hardening, cold acclimation, or dormancy could impact larvae cold tolerance. It is likely that these processes would have varying impacts on the cold tolerance of tick larvae as some species have shown increased cold tolerance following hardening, acclimation, or diapause and others do not (Rosendale et al., 2019; Holmes et al., 2018; Vandyk et al., 1996). Future studies that examine comparative cold tolerance among tick larvae following rapid cold hardening, cold acclimation or dormancy induction will provide additional insight into their overwintering survival and thermal tolerance. Studies featuring acclimation, either short hardening periods or longer acclimation, will likely be critical to establish the true thermal limits as shifts in tolerance of a few degrees can significantly impact the survival in specific geographic regions (Kellermann et al., 2017; Gunderson and Stillman, 2015; Sgro et al., 2010). In addition, differences between populations should be assessed as most ticks reside across broad latitudes, where it is very likely that northern and southern populations have differences in cold or heat

tolerance as noted in other invertebrates (Hallas et al., 2002; Hoffmann et al., 2002).

R. sanguineus sensu lato exhibited the highest upper lethal temperature of approximately 47 °C and *I. scapularis* exhibited the lowest at 41 °C. These results are similar to previous studies by Yoder et al. (2009) with *R. sanguineus sensu lato* having an especially high heat tolerance. Of interest is that *R. sanguineus sensu lato* is a peridomestic association, where ticks can be associated with kennels and houses (Gray et al., 2013). In these environments, temperatures could be extremely low or high, sometimes to a greater extent than the exterior environment, if climate control is not used. *Amblyomma* which are primarily located on the southeastern areas of United States are also described as being relatively heat tolerant which is supported by this study and previous survival estimates (Yoder et al., 2009). The physiological mechanisms setting the upper lethal temperatures of ticks are not well-studied, but some prior research suggests heat-shock proteins and other stress proteins contribute to their survival at high temperatures (Villar et al., 2010). Our results show relatively little variation in upper lethal temperatures of ixodid ticks which vary from a low of 41 °C in *I. scapularis* to a high of 47 °C in *R. sanguineus sensu lato*. We see much greater variability in lower lethal temperatures, between −24 °C in *D. andersoni* and −15 °C in *A. americanum* and *I. scapularis*. The lower temperature thresholds were comparable to those observed in previous studies on *Ixodes* thermal tolerance (Vandyk et al., 1996). The reduced cold tolerance of *I. scapularis* larvae may be the fact that peak levels of larval abundance are mainly found mid-summer and either the larval stage for species or this specific strain is not adapted to cold exposure (Ogden et al., 2004; Ostfeld et al., 1996).

Our results on heat tolerance match global geographic patterns in thermal tolerance limits of diverse terrestrial ectotherms, where heat tolerances are fairly invariant and cold tolerance limits vary strongly across latitudinal gradients (Sunday et al., 2012). Several reasons may underlie this pattern. First, it may be that biophysical limitations for heat tolerance of all organisms, given that proteins tend to denature around 45 °C, setting an immovable upper threshold for heat tolerance of most animals (Hochachka and Somero, 2002). Another potential explanation is that maximum high temperatures are relatively invariant across the earth, leading to uniform heat tolerance across broad geographic gradients and increasing cold tolerance at high latitudes (Williams et al., 2015). A third reason is that species from warm origins have maintained greater heat tolerance than required but that geographic range expansion exhibits strong selection on cold tolerance, leading to increased cold tolerance and relaxed selection on heat tolerance (Lancaster, 2016). Lastly, host temperature during blood feeding could directly set the upper limit of ticks as heat stress from a bloodmeal is extensive (up to 40–44 °C) and can vary between host species (Benoit et al., 2011; Benoit et al., 2019). Ticks are likely to retreat to cooler areas if temperatures are too high and only quest when thermal conditions are more favorable (Sonenshine and Roe, 2013), which likely reduces the selective pressure for high thermal tolerance. Additionally, dermal gland secretions can provide metastriate hard ticks with a mechanism to prevent overheating for short periods of time (Yoder et al., 2009; Maldonado-Ruiz et al., 2021). For ticks, it makes sense that their thermal tolerances would be matched to those of the diverse organisms they parasitize, likely driving the observed patterns in lower and upper lethal limits. Relative humidity also has a major impact on questing and other aspects of tick biology (Rosendale et al., 2016b; Randolph, 2004), where dry conditions suppress questing during warmer periods.

Ticks are hardy and can survive over a year between blood feedings (Rosendale et al., 2017; Lighton and Fielden, 1995). This extreme resistance to starvation likely depends on minimizing unnecessary activity and lowering overall metabolic rates. Metabolic rate is highly temperature-dependent, particularly in ectotherms, which cannot regulate body temperature independently of environmental temperatures (Addo-Bediako et al., 2002). This increase in metabolism is also directly correlated to oxygen consumption and is an important factor in

regulating chemical reactions needed to maintain homeostasis (Hawkins, 1995). Warmer environmental temperatures will therefore increase kinetic energy, driving faster rates of biochemical reactions and subsequently higher metabolic rates (Hochachka and Somero, 2002). Measurements of metabolic activity across a range of temperatures, therefore, generates a temperature-dependent curve, which is bounded by the hottest and coldest temperatures an organism can withstand (Somero, 2011; Huey and Kingsolver, 1989; Kellermann et al., 2019). Thermal metabolic performance curves can be used as much better indicators, rather than single measurements, for the predictions of organismal survival and behavioral thermal regulation with changing climate (Sinclair et al., 2016; Sinclair et al., 2015). Importantly, hard ticks are different than other arthropods as individuals cannot feed to replenish nutrient reserve levels until a host is located (Rosendale et al., 2019), which also triggers development to the next instar. Thus, a balance between activity, metabolism, and off-host survival is critical for ixodid ticks. This indicates that the thermal optimum, typically defined as the greatest metabolic rate or activity in relation to a specific temperature, is unlikely to be the most ecologically-relevant optimal temperature for tick survival which is likely closer to their preferred temperatures. Therefore, thermal preference may be a better metric for estimating tick survival rather than thermal optimums.

For all ticks, temperature was correlated with metabolic rate in a manner consistent with traditional ectothermic thermal performance curves (Angilletta, 2009; Kellermann et al., 2019). Ticks, however, have a standard metabolic rate that is lower than other arthropods due to a low ratio of actively respiring tissue in relation to their total body mass (Rosendale et al., 2019; Lighton and Fielden, 1995). Nevertheless, we found that maximum oxygen consumption across a range of environmental temperatures varied between species. The lowest peak metabolic activity was in *A. maculatum* which peaked at 30 °C, while *A. americanum* had the greatest oxygen consumption at 40 °C. This result was mirrored in activity rates, where *A. americanum* had higher activity levels at 40 °C than *A. maculatum*. *D. variabilis* also exhibited relatively high oxygen consumption at warmer temperatures, peaking at 40 °C, which also matched high activity levels at warm temperatures. Importantly, the greatest metabolic rate will not be the most optimal for survival off-host since ticks can experience extended bouts between meals (Rosendale et al., 2019). A lower metabolic rate, likely at temperatures closer to 20 °C, is critical for ticks to reduce consumption of nutrient reserves and allow sustained questing during prolonged periods of starvation. In our study, this balance was best illustrated by measurements of thermal preferences, which were near 20 °C for most species. Thus, dynamics between temperature and metabolic rates of ticks likely influence tick host-seeking patterns, which may be an important metric for predicting disease dynamics (Brites-Neto et al., 2015).

For all ticks measured, the greatest activity was within a temperature range of 30–40 °C, which corresponded to the peak in oxygen consumption. *D. variabilis* and *D. andersoni* which are more northern tick species, maintained significantly higher activity levels at 20 °C than their counterparts, which all had lower activity levels below 30 °C. Interestingly, for all ticks, activity was low overall but tended to be skewed towards warmer (30–40 °C) temperatures. This peak in activity is likely represents an attempt to find more tolerable temperatures to reduce energy expenditure and maximize survival between infrequent bloodmeals (Hawkins, 1995; Rosendale et al., 2019). Thermal preference of each species was much lower than the peak in activity, thus even though ticks show the greatest activity between 30 and 40 °C, lower activity between 20 and 30 °C likely represent optimal temperature where activities such as questing can still readily occur and prevent the rapid utilization of energy reserve.

A wide variety of invertebrates display strong thermal preferences due to specific thermoreceptors (Hamada et al., 2008). Preferential thermoregulatory behaviors have been linked to optimal performance levels in other ectoparasites, where a delicate balance between preventing the rapid decline of metabolic reserves and locating a new host

must be maintained (Abram et al., 2017). Preferential temperature ranges of ticks were between 15 °C and 25 °C with the exception of *D. variabilis*, which displayed a higher proportion of ticks at 12 °C. The low thermal preference in *D. variabilis* could be due to a higher tolerance to cold (Rosendale et al., 2016a; Yu et al., 2014) or an impaired ability to detect temperature. It is unlikely that *D. variabilis* was immobilized by the lower temperature since activity was noted as low as 10 °C. Interestingly, ticks tended to prefer slightly lower temperatures than many other arthropods, perhaps suggesting that ticks use behavioral thermoregulation to maintain lower metabolic rates during long periods between blood meals for preserve nutrient reserves. These thermal preferences match our predictions based on activity, oxygen consumption, and survival that ticks prefer temperatures near 20 °C to balance nutrient retention and allow questing.

Of all measured species, *I. scapularis* had the narrowest thermal tolerance breadth, with LLT₅₀ at −8.5 °C and ULT₅₀ at 38 °C and correspondingly the lowest oxygen consumptions across all temperatures. Because species typically inhabit areas with environmental temperatures within the range of tolerable physiological limits, narrow thermal tolerance breadths are often correlated with narrow geographic ranges (Sunday et al., 2014). Notably, the thermal tolerance breadths (the difference between the ULT, and LLT) for all measured ticks are extremely broad, compared with other terrestrial ectotherms (Sunday et al., 2011). Thermal tolerance breadths for ticks ranged from 56 °C (*I. scapularis*) to 69 °C (*D. andersoni*), which is among the greatest thermal tolerance breadths measured in terrestrial ectotherms. Given their broad geographic distributions, spanning from ~25° N to 50° N, one might expect ticks to possess broad thermal tolerance limits. Furthermore, as generalist ectoparasites, ticks likely maintain broad thermal tolerance limits which allow them to exploit diverse host species.

Overall, this study demonstrates a clear relationship between several thermal characteristics of ticks, including their upper and lower lethal limits, limits of activity, preference temperatures, and metabolic activity across a broad thermal gradient. This combined study of activity, oxygen consumption, and thermal tolerance indicates that the highest temperatures tolerated for most ticks is between 30 and 40 °C, where short periods over 40 °C can be tolerated by some species. Ticks that are more vulnerable to high temperatures, such as *D. variabilis* and *D. andersoni*, will likely have drastic reductions in their southern and elevational distributions as temperatures warm. More warm adapted species, *A. americanum*, *A. maculatum*, and *R. sanguineus sensu lato* are likely to move north or into higher elevations. These trends are supported by our survival data, which suggest overall lower survival in response to warm temperatures for *D. andersoni* and *D. variabilis*. As “sit and wait” parasites, ticks must spend periods of months to years between meals (Rosendale et al., 2019; Lighton and Fielden, 1995). Lower temperatures, likely those at the thermal preferences (15–20 °C) will balance metabolic output to conserve nutrient reserves, allowing ticks to survive between meals, with the ability to be active for periods of questing to locate a host. This indicates that the most ecologically optimal temperatures for ticks are far below those that represent their maximum activity and metabolic output and closer to thermal preferences. Tick thermal physiology has been scarcely studied and this work provides a better understanding of these temperature-related traits, allowing better predictions of future distribution and disease patterns when combined with other factors such as relative humidity and host availability.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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