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OVERWINTERING ECOLOGY AND PHYSIOLOGY
OF *AMBLYOMMA AMERICANUM* AND *DERMACENTOR VARIABILIS*
(ACARI: IXODIDAE) IN CENTRAL OHIO

DISSertation

Presented in Partial Fulfillment of the Requirements for the
Degree of Doctor of Philosophy in the Graduate School of
The Ohio State University

By
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* * * * *

The Ohio State University
1998

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ABSTRACT

Understanding overwintering behavior, physiology and ecology in ticks is critical for managing vector borne diseases and controlling tick populations. Winter exposure results in cumulative mortality of approximately 12% per month on *D. variabilis*. Mortality in the more southerly distributed tick, *A. americanum*, is likewise cumulative; however it is much more severe than for *D. variabilis* during Ohio winters. This cumulative effect of winter exposure implicates factors other than freezing for mortality. Bioenergetic studies of overwintering ticks revealed an increase in lipid content throughout winter as compared to laboratory maintained ticks with the same age, lineage and feeding history. This increase in lipid content does confer protection against extreme cold temperatures (-11°C) during *in vitro* studies and provides a readily accessible energy store upon initiation of activity in the spring. Diapause does not appear to occur in laboratory or local strains of *D. variabilis* or laboratory strains of *A. americanum*, and permits ticks to relocate to different hibernacula throughout winter. Using a flow through respirometer, CO2 levels, burst and interburst duration were measured with no significant differences occurring between laboratory maintained or overwintering ticks. Likewise, questing and feeding abilities between overwintering and laboratory maintained ticks throughout the winter revealed no significant differences. Overwintering site selection was determined using confining arenas and revealed that all
ticks remain on the soil surface protected by the naturally occurring layer of litter. While overwintering ticks decrease hemolymph osmolarities during winter and gain weight, their ability to absorb water from saturated atmospheres is limited by temperature. Low temperatures (below 15°C) do not permit active water vapor sorption in either A. americanum or D. variabilis indicating that the water vapor sorption mechanism in ticks is conserved between genera.

Ticks are not randomly distributed in the field. Significantly more ticks were discovered within one meter of the road edge than between 1 and 2 m. while tick numbers remained constant in areas not associated with roads. Marked ticks were recovered near the road edge and had traveled between 9 and 65.5 m from the nearest release point indicating that D. variabilis adults assume a more active host searching strategy when a host is detected from immediate or remote locations than previously recognized. Roads did not act as barriers to tick movement as a similar number of marked ticks was recovered on both sides of the road after release on one side. Evidence to support auto emissions and infrared radiation as attraction factors is presented as more ticks were recruited towards more heavily traveled roads. Behavioral and physiological experiments with detection and attraction of infrared are ongoing. Accumulation of ticks within 2 meters of a road edge may provide a “trap crop” for their control. Mowing road edges during peak abundance significantly reduces the number of ticks questing along roadsides.
I dedicate this dissertation to my family, especially my wife and parents, who have supported me throughout the years. My dedication also includes John Farr and Jan Humphreys who inspired my interest in biology and encouraged me to pursue a Ph.D.
ACKNOWLEDGMENTS

I would like to thank my committee members first, since they have worked with me on these projects for the past five years. Additionally, I would like to thank Woody Foster for his guidance and inspiration. Each of you has influenced me in positive ways and helped me find the path when it was difficult to see. So many of my peers have helped me with this and other projects that it is difficult to name them all. Special thanks go out to my wife, Steve Tammarriello, Joe Rinehart, Steve Chordas, Marcela Hernandez, Andrea Borton, Emmett Glass and Mohamed Selim (in no particular order). I must especially thank Glen and Laura for their close critique of my work. I hope these alliances last for a very long time. Finally, Richard Berry and Robert Restifo of the Health Department were always there to provide guidance and Susan Fisher played a very important role in broadening my knowledge base.
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CHAPTER 1

INTRODUCTION

Ticks are obligate blood feeding ectoparasites capable of serving as vectors and reservoirs of disease-causing micro-organisms. There are two main tick lineages, the Argasidae (soft ticks) and the Ixodidae (hard ticks). Of the approximately 825 species, three fourths are ixodids.

The ixodid lifestyle can be characterized various ways but the classical approach is to describe it by host utilization strategy (three, two and one host). The majority of ixodid ticks (approximately 625 out of 650 species) use a three-host life style that results in approximately 97% of their life being spent off the host (Needham and Teel 1991). While attached, ticks risk being dislodged by grooming and host immunological rejection. However, many species feed upon rodents whose individuals are short lived, seldom resulting in problems with immunological resistance.

Ticks are generally nidicolous, living in close association with the host and spend their off-host time in nests or burrows or non-nidicolous (exophilic), living in the environment during off-host periods (Sonenshine 1993). The off-host habitat for nidicolous ticks is usually more uniform (especially temperature and relative humidity) than for non-nidicolous ticks. Non-nidicolous ticks utilize more exposed habitats that
may experience severe abiotic extremes. Despite greater selection pressures, the majority of ixodid species are non-nidicolous (Sonenshine 1993). Thus understanding the strategies (physiological and ecological) employed by non-nidicolous ticks is critical for protecting humans and domestic animals from the diseases they vector.

As with other arthropods, factors such as host and water availability, temperature extremes, day length and suitable habitat limit the geographic distribution of ticks. Differential tolerance to stressful conditions between species (Needham and Teel 1991) and different aged ticks within species (Jaworski et al. 1984) contribute to availability of mechanisms and behaviors that permit survival.

Although off-host physiology and ecology have been studied since the early work by Lees (1946, 1969), specific behavioral and physiological responses to abiotic stressors deserve much more attention. Only a few papers have been published on how low temperatures and extended periods of inactivity during winter affect survival (Lee and Baust 1987, Burks et al. 1996a, 1996b, Dautel and Knülle 1996a, 1996b, Needham et al. 1996, Strey et al. 1996).

**TICK-BORNE DISEASES**

Both *Amblyomma americanum* and *Dermacentor variabilis* frequently parasitize humans in the United States and regionally compose the majority of ticks attaching to humans (Slaff and Newton 1993, Felz et al. 1996). Although they are not efficient vectors of *B. burgdorferi*, the causative agent of Lyme disease, (Sanders and Oliver 1995, Piesman and Happ 1997), *D. variabilis* is an efficient vector of *Rickettsia*
*Rickettsia*, the causative agent of Rocky Mountain spotted fever (RMSf) and both species appear to be vectors of *Ehrlichia chaffeensis*, the causative agent of ehrlichiosis (Sonenshine 1993). RMSf is the most severe illness of any rickettsiosis in man. The disease characteristics initially begin with fever, headache, arthralgia and myalgia and in most cases, a generalized rash. An incubation period of approximately seven days follows and is characterized by a sudden increase in fever, severe frontal headache, nausea, and severe arthralgia and myalgia. Other symptoms often include abdominal pain, vomiting, diarrhea and cough. If left untreated this illness usually results in death, which is most often attributed to rickettsial encephalitis (Sonenshine 1993). The clinical symptoms of ehrlichiosis resemble those exhibited by RMSf. Since this is a relatively new disease little is known concerning its pathogenicity in humans; however, it appears less severe than RMSf and if patients are left untreated mortality is observed less frequently.

**TICK ECOLOGY**

The distributions of the American dog tick, *D. variabilis*, and lone star tick, *A. americanum* overlap (Figs. 1.1, 1.2), but each utilizes distinctly different habitat types. The American dog tick prefers lush meadows to young secondary growth forests. In Ohio it is usually found in areas that have lain fallow for many years. They are frequently in ecotones, along trail edges, power lines and clearings, near outbuildings and homes (Sonenshine 1993). Much of the summertime vegetation within these areas is herbaceous consisting primarily of goldenrod, *Solidago* sp. These habitats vary
throughout summer, being moist in the spring and dry during the late summer. Absence of secondary growth contributes to dramatic fluctuations in micro-climatic conditions daily and seasonally (Harlan and Foster 1990). The life cycle of *D. variabilis* in Ohio requires 2 years with flat (unfed) larvae and adults overwintering; however, overwintering locations have not been reported (Sonenshine 1993). Small rodents are frequently used as hosts by nymphs and adults often use medium-sized rodents (Demaree 1986). In its southern distribution, the lone star tick, *A. americanum*, is mostly a woodland species Hair and Bowman (1986). It prefers ecotonal and second growth woods, characterized by Hair and Bowman (1986) as bottom oak-hickory. Few specimens are found in succeeding fields or meadows. This constant canopy of trees provides a more stable environment for this tick species where fluctuations between temperature and humidity are much less severe.

### OVERWINTERING MICROHABITAT

Specific tick overwintering habitats are not known for these species. Despite this lack of knowledge, all habitats can be divided into different zones. Sonenshine (1993) separates all habitats into three distinct strata. Macroclimate is the zone above the vegetation layer (this includes mature trees but not saplings), mesoclimatic includes the enclosed vegetation (including small saplings), and microclimate encompasses the soil as well as the dead and decaying vegetation interface between the soil and atmosphere. Microhabitats dramatically influence the tick hydration state and are extremely important for rehydration (Lees 1969, Hair et al. 1975) and overwintering success.
(McEnroe and McEnroe 1973, McEnroe 1978). Using macroclimatic conditions to predict survival has enjoyed limited success since correlations between macro- and microhabitats have not been made. Analyzing vegetation type, geography and lyme disease cases shows more promise to predict where *I. scapularis*, the blacklegged tick, could exist (Kitron and Kazmierczak 1997). Glass et al. (1994) also used global information systems (GIS) in Maryland to predict *I. scapularis* abundance based primarily on vegetation type. Glass et al. (1994) also observed a positive correlation with well-drained sandy soils in Maryland while Duffy et al. (1994) reported that few *I. scapularis* were found in similar conditions on Shelter Island, NY. Conflicting reports suggest how little we understand about the micro-habitat requirements of individual tick species and how challenging it will be to utilize GIS to predict where ticks should be found. Identifying microhabitat requirements for individual tick species is critical if we are going to accurately predict geographical distributions and future hotspots for each disease.

Survival is dependent on microhabitat conditions (Sonenshine 1993). While some information has been accumulated for summer (Short et al. 1989, Steele et al. 1990, Chilton and Bull 1993, Duffield and Bull 1996, Bertrand and Wilson 1997, Randolph and Rogers 1997), little information is available detailing the requirements of tick overwintering microhabitats (Daniels et al. 1996, Burks et al. 1996). Seasonal differences within an attic habitat have shown that microhabitat requirements are limiting tick distributions and are ultimately the limiting factor regulating survival (Dautel and Knülle 1998). No other specific microhabitats are reported for overwintering non-nidicolous ticks, despite its importance in regulating population Figure 1.1.
Figure 1.1. Distribution of the American dog tick, *Dermacentor variabilis*, in the United States (modified from Drummond 1998).
Figure 1.2. Distribution of the lone star tick, *Amblyomma americanum* in the United States (modified from Drummond 1998).
levels. Ticks are often collected during spring in central Ohio covered in dried mud, indicating that they utilize microhabitats throughout winter within or on the soil surface.

SPECIES DISTRIBUTION

The American dog tick's geographic range is throughout the southeast and extends northward into Canada in the Great Lakes region and also includes a human introduced population along the western seaboard (Figure 1.1). In Ohio, established populations are not common north of the 0°C winter isotherm, except in the northwest corner where lake effect moderates temperature extremes. American dog ticks are found in a variety of environments but are most often encountered in field-forest ecotones (Sonenshine 1993). The lone star ticks range covers much of the southeastern United States, from the Atlantic coast west to central Texas and the Iowa border, and continues north into areas of coastal New York (Figure 1.2). Few *A. americanum* are submitted to the Vector-Borne Disease Unit of the Ohio Department of Health for identification, and only recently has *A. americanum* been documented as established in southwestern Ohio (OVBDP 1997).

The differences in range between these two species have traditionally been attributed to ability to tolerate low temperatures. Differences in inherent cold-hardiness has been observed between these and other species (Burks et al. 1996, Needham et al. 1996, Strey et al. 1996) with *D. variabilis* more cold hardy than *A. americanum* (Burks et al. 1996). This small difference in cold-hardiness may have large biological implications in range limitation. This is one question I address in this dissertation.
OVERWINTERING PHYSIOLOGY

Arthropods respond to low temperature in three ways: they enter dormancy (covered in the next section), remain active or die. Both dormant and active (mobile) arthropods exhibit two distinct overwintering physiological responses during winter: freeze tolerance and freeze intolerance. Those that can survive body water freezing are tolerant and those that cannot are freeze intolerant (Lee 1991). Most arthropods are freeze intolerant including ticks. Both freeze tolerance and intolerance has been studied extensively, but studies of low temperature effects on arthropods were not detailed and organized until vital groundwork was conducted by R. W. Salt (Ring and Riegert 1991).

During the early years of cryo-entomological work, hemolymph supercooling was perceived as a measure of cold tolerance and seemed to answer many of the questions concerning arthropod overwintering. Surprisingly, hemolymph does not freeze at 0°C, but instead solutes permit the aqueous solution to cool below the freezing point. Different arthropod species possess hemolymph with different supercooling capacities and greater supercooling can occur via low temperature preadaption (Storey and Storey 1991). Cryoprotectants such as complex carbohydrates, free amino acids and polyols are commonly used to influence supercooling. Glycerol is the most common cryoprotectant molecule (Lee 1991) and has been identified from nympha and adult mites collected in the Antarctic. *Alaskozetes antarcticus* nymphs exhibit relatively high (0.5M) hemolymph concentration of glycerol as well as novel antifreeze proteins (Young and Block 1980. Block and Duman 1989). Despite its abundant use as a cryoprotectant in insects
(Denlinger 1998), only trace amounts of glycerol have been identified from ticks (Lee and Baust 1987).

Injury from cold exposure occurs in most arthropods without ice formation and well above the supercooling point, so supercooling has little influence on cold hardiness (Denlinger 1991). Mortality during exposure to cold can directly result from ice nucleation or the inability of physiological mechanisms to function. Mortality occurs through these two mechanisms in freeze intolerant species. Water does not spontaneously freeze with ease. Instead a nucleus must be present around which ice crystals form. In fact, small volumes of pure water do not freeze until -39°C (Angell 1982). Freeze tolerant species can utilize strategically placed ice nucleators to avoid freezing, so ice forms in less vital compartments (Bale et al. 1989). Ice nucleation also causes cells to desiccate in response to ice crystals forming nearby, which further decreases the supercooling point of the intracellular fluid. Freeze intolerant species cannot, or do not, regulate where ice forms within their bodies, and if their systems continue to function at low temperatures, ice formation within cells will result in death. Arthropods that remain active during winter must produce different physiological compounds to survive cold temperature extremes. Some arthropods initiate alternative pathways that permit specialized overwintering responses. This is recognized in arthropods as a specific form of dormancy called diapause. Although diapause is frequently observed in insects, sometime conferring increased cold tolerance (Denlinger 1998), little information detailing the diapause status of overwintering ticks is available.
TICK DORMANCY

Ticks, as well as other sedentary arthropods in temperate zones, exhibit a distinct period of activity when conditions are favorable and are inactive when conditions become less so. This period of inactivity is usually mediated by behavioral and physiological responses to the environment and is often referred to as dormancy (Denlinger 1991). Dormancy is characterized by a decrease in metabolic activity and is often confirmed by measuring respiratory CO$_2$ production or O$_2$ consumption. Dormancy initiation can be immediate, or preprogrammed responses to environmental cues or adverse conditions. Immediate responses sometimes called quiescence, are usually brief and involve abrupt inactivity. This type of dormancy is vital for survival of many species because it permits arthropods to become inactive during inclement weather, i.e. frost in early fall. Diapause is a much more strict form of dormancy, although it can be facultative. Environmental conditions, usually day length and temperature, preprogram facultative diapause. Preprogramming is usually acquired by exposure to some environmental stimulus and this stimulus differs among insect species. Behavioral diapause permits arthropods to prepare for upcoming adverse climatic conditions such as increases or decreases in temperature or drought. Obligatory diapause occurs during the same period of the arthropod's life cycle regardless of environmental conditions. If weather conditions remain predictable, this form of diapause is highly advantageous to arthropod survival; however, obligatory diapause is not plastic and does not permit arthropods to monitor and modify their biology to brief changes in weather conditions. Despite the existence of two separate types of diapause in arthropods.
characteristics between the two are shared. The characteristics described thus far are decreased metabolic rate, increase in biological storage compounds and arrest in development or reproduction (Denlinger 1985).

Although diapause is understudied in ticks and traditional definitions may not describe what we observe, two types of diapause are thought to occur. Both types appear to be facultative, although developmental may occur in morphogenetic diapause. Behavioral diapause is the suppression of host-seeking activity and refusal to feed, while morphogenetic diapause is characterized by delayed development (Belozerov 1982, Sonenshine 1993). Although behavioral diapause occurs much more often it has been studied less. Behavioral diapause in ticks is potentially the most important aspect in the ability of ticks to colonize such diverse habitats (Hoogstraal 1978) and deserves much more attention.

Understanding tick dormancy is critical from basic scientific and economic viewpoints. Basic questions include: are ticks utilizing diapause to overwinter or are they simply quiescent? Are energetics (utilizing and storing glycogen and lipids) as vital for tick survival during dormancy as they are during active periods? Economic questions revolve around predicting tick abundance based on dormancy survival, and inducing/breaking diapause (or lack of diapause) as a potential control measure. To explore these and other questions, my research has been focused on the ability of two important tick species to survive harsh environments, particularly exposure to cold temperatures during winter. The specific goals of my research are:
1) To compare survivability of tick species in Ohio that have different cold-hardiness traits, and evaluate winter survival for predicting spring abundance, as well as to examine specific microhabitat selections during overwintering.

2) To characterize bioenergetics of these two ticks during winter and determine if total lipid content affects cold hardiness.

3) To characterize water relations during winter and determine the critical temperature for water vapor absorption.

4) To determine if the American dog tick utilizes a traditional diapause during winter.

5) To evaluate roadside attraction in Ohio *D. variabilis* and to determine if "trap crop" tactics are viable for their control.

REFERENCES


CHAPTER 2

OVERWINTERING OF NYMPHAL AND ADULT AMBLYOMMA AMERICANUM
AND DERMACENTOR VARIABILIS (ACARI: IXODIDAE) IN OHIO.

ABSTRACT

Numerous overwintering reports concentrate on a single tick species within two or more distinctly different ecological environments. This project sets forth to characterize overwintering in two tick species, *A. americanum* and *D. variabilis*, at one geographic location (Central Ohio). Laboratory strain and local *D. variabilis* strains were compared. Survival was monitored throughout each winter between 1995 and 1998 with midwinter (January) and Spring (March) receiving the most attention. Mortality throughout the study differed statistically between species and years. Significantly more *D. variabilis* mortality was observed during the winter of 1995 than 1996 but not during the winter of 1997. Mild winters (like those of 1996) may decrease mortality and result in higher tick numbers during the following activity period. *Dermacentor variabilis* appears to be innately more cold tolerant than *A. americanum*, as survival between species during warmer winters was not significantly different. Adults of both species
overwintered on the soil surface immediately below the litter layer, which provides some protection from low temperature. This data suggests that temperature tolerance is a limiting factor in southern species' distributions northward.

INTRODUCTION

Most non-nidicolous ticks utilize discrete activity periods to avoid harsh conditions or periods when host availability is limited. Many temperate species are not active during winter presumably because temperatures are often below activity thresholds (Clark 1995) or they enter diapause (Belozerov 1982). Understanding these periods of inactivity when ticks are sessile and highly vulnerable is essential for developing novel control strategies.

Dermacentor variabilis larvae and adults overwinter, but cohort activity begins and ends during different seasons throughout its variable distribution. Nymphs are seldom observed before larvae indicating that there is little nymphal overwintering (Campbell. 1979). The 2-year life cycle starts with replete females in the summer. Eggs hatch into larvae that overwinter until warmer temperatures permit activity. In southern localities, larval activity begins during January, however, in northern localities larval activity can be delayed for several months usually commencing in May (Sonenshine, 1993). Larvae rapidly feed and molt into nymphs and their numbers remain high throughout the summer, declining during the fall and ending in November. In the north, nymphal activity peaks in June and July and ends in early September. The entire nymphal cohort in the north is thought to be derived from overwintered larvae.
Replete nymphs molt into adults during the late summer and these unfed adults overwinter. Throughout the southern range of *Amblyomma americanum* all stages can overwinter and many of the stages remain active for long periods with no distinct peaks. This implies a one or two year cycle. Sempter and Hair (1973) showed that free-living nymphs remain active from March to October as reflected in numbers of nymphal ticks infesting deer throughout this period. Larvae and adults show distinct peaks in activity. Larval activity peaks in late July or early August and continues until mid-November while adult activity peaks in June and declines until the later weeks of August (Hair and Bowman, 1986).

The American dog tick, *Dermacentor variabilis*, is well-established in Ohio and other northern regions. It is located throughout the eastern United States from the southern border of New York west to the Mississippi River and south to central Texas. Its range extends north into the Great Lakes region to southern Canada. Recent introductions have permitted additional populations to flourish along the western coast in California and Oregon (Sonenshine 1993). Its range has increased north and small changes in distribution have been documented in Pennsylvania (Snetsinger et al. 1993). This broad distribution permits us to classify *D. variabilis* as a highly plastic species, able to survive in southern and northern climates. The lone star tick, *A. americanum*, on the other hand, is a southern tick species established throughout the southeast, west to central Texas and north to Iowa (Sonenshine 1993). In fact, the entire *Amblyomma* genus is classified as tropical (Cooley and Kohls 1944). Historically, the range of *A. americanum* was not considered further north than Kentucky, southern Pennsylvania and New Jersey. Today, its range extends along the eastern seaboard as far north as Rhode
Island and it has recently become established in southern Ohio, Clermont Co. (Ohio Vector-borne Disease Unit, 1997) and New York (Means and White 1997).

Tick distributions are thought to be regulated by differential ability to endure cold temperatures (Gilot et al. 1992). Cold-hardiness, the ability to survive low temperatures, and supercooling have been examined in vitro to compare survival between species (Dautel and Knüelle 1994, Burks et al. 1996a, Burks et al. 1996b, Dautel and Knüelle 1996a, 1996b. 1997, Needham et al. 1996, Strey et al. 1996, VanDyk et al. 1996). While important for understanding the effects of low temperatures, these assays provide cursory information or the effect of relatively warmer microhabitat temperatures (0-5°C) experienced over long periods during winter. Dermacentor variabilis is slightly more cold hardy than A. americanum although both species survive well below the temperatures experienced during a normal winter. Spontaneous freezing and direct chilling injury are not significant mortality factors for either of these species during winter in Ohio (Burks et al. 1996). Mortality increased for nymphs chilled while in direct contact with ice, although the temperature where similar mortality occurred was lower for D. variabilis than for A. americanum. This suggests that inoculative freezing is a more important cause of overwintering mortality than direct freeze injury and D. variabilis is slightly more cold tolerant than A. americanum potentially permitting their range expansion to the north.

The objective of this study is to compare survival of southern (A. americanum) and northern (D. variabilis) tick species in Ohio and speculate about the impact winter conditions have historically had on populations of A. americanum. Is winter mortality affected by naturally occurring low temperatures? Where are overwintering hibernacula
and what temperatures occur there? Can winter temperatures be used to predict adult tick abundance the following season? In addition, nymphal survival during consecutive winters was reevaluated and predictions for spring abundance of both tick species are made. Answering these question will provide a clearer picture of how ticks survive winter in temperate regions.

MATERIALS AND METHODS

Overwintering experiments for *D. variabilis* were conducted in central Ohio during the winters of 95-97, and for *A. americanum* during the winters of 95-96. All ticks were placed into field situations during October except during the release of field collected *D. variabilis* during 95-96 (winter of 95) and 97-98 (winter of 97). Photoperiods were kept at 14:10 (L:D) while ticks were maintained indoors or during immature feedings except when field collected ticks were use during 1997. photoperiods were natural. All ticks were placed in areas where native ticks normally occur at Alum Creek State Park (Delaware Co.) or a private residence in Reynoldsburg (Franklin Co. Ohio).

Ticks. Adult ticks used during this experiment originated from laboratory cultures maintained for many generations at The Ohio State and Oklahoma State Universities or from field collections. Laboratory ticks were reared on New Zealand White (NZW) rabbits several months prior to release and were approximately one month post-molt when placed in the field. Ticks collected from the field were all *D. variabilis*, and were collected locally July of the year of release. They were either placed in arenas on the
date of collection or housed in rearing chambers until the last week in October. All
nymphal ticks were fed as larvae on NZW rabbits in August of the year under study and
were 1-2 months post-molt. Large tick numbers were difficult to obtain since less than
100% of attached larvae or nymphs molt. Although large numbers of immatures were
placed on hosts prior to the experiment, numbers placed in the field varied depending on
successful feeding. Eight hundred and sixty *A. americanum* and 2262 *D. variabilis* adults
were used during this three-year study. Of these, 438 *A. americanum* and 701 *A.
*americanum* were sampled in January while 422 *A. americanum* and 1561 were sampled
in March. One hundred and ninety nine *D. variabilis* adults and 98 *A. americanum* adults
were monitored throughout winter within a controlled environment. Two thousand and
fifteen *A. americanum* and 601 *D. variabilis* nymphs were used for overwintering
studies while 439 *A. americanum* and 524 *D. variabilis* nymphs were monitored under
controlled conditions. Numbers for adult ticks sampled during the most frequent months
do not add up because samples were taken during additional months during winter.

*Tick Arenas and Confining Units.* To determine tick location (within soil strata)
throughout the winter, ticks were housed in open bottom arenas. Arenas were
constructed from three-pound coffee cans (15 cm deep) by removing the top and bottom
seals to form a cylinder. These cylinders were driven into the ground until only a few
centimeters were visible above the soil surface. This allowed similar compactness and
organic matter on both sides of the arena. The total available area, encompassed by the
arena edges, was 177 cm². Ticks were sealed into arenas using fiberglass window
screening and *Liquid Nail™*. All arenas were checked monthly for leaks and leaky
arenas were withdrawn from the study. Since ticks remained on the soil surface
throughout the winter, adult specimens were subsequently sewn into squares of sheer curtain material and placed under the litter layer (winter 1997). Nymphs were placed in squares under leaf litter during the winters of 1995 and 96.

**Overwintering Location.** Entire arenas were removed by digging around the perimeter of the coffee can to a depth below the bottom opening. This maintained original orientation of arena constituents until they could later be observed. Soil samples, still confined by arena walls, were dissected under cold conditions (3-5°C) in a walk-in refrigerated room. To access the contents of the arena, tin snips were used to cut through the arena wall longitudinally and the arena sides were bent open to remove the cylinder of soil. This cylinder was then teased apart. As the soil on the surface of the cylinder was removed, the soil beneath the surface would also be removed before any additional surface was examined. Therefore the cylinder was essentially sliced (although forceps were used to pick away each slice) into longitudinal pieces so overwintering depth could be determined.

**Overwintering Survival.** Ticks collected in the cold room from soil dissections were relocated to ambient indoor temperatures (approximately 23°C) and placed under hydrating conditions. Survivorship, within 2 h, was evaluated by placing each tick in an open area to see if it displayed coordinated movement. Walking ticks were considered alive while those unable to move in a coordinated manner (moving a single or multiple legs but unable to walk) were considered dead or moribund. No moribund ticks recovered their ability to walk.

**Fungus assay.** During the winter of 1996, ticks of both species (alive and dead) were covered with white hyphae along with organic matter from within the arena.
Isolations of this material were made from each of the affected ticks and many particles of organic matter that appeared to maintain fungus. For isolation the surface of each tick was sterilized in 70% ethanol for 3 minutes and then washed in distilled water for 2 minutes. Surface sterilization was done to help eliminate spores and growing fungi to facilitate isolation from inside the cuticle. Using sterile techniques, ticks were sliced in half and place on Potato Dextrose Agar (PDA). PDA is a generic medium that will provide nutrition for most species of fungi. Cultures were incubated at room temperature (approximately 24°C) and checked every 48 h for growth. Subcultures were made when fungi began to sporulate so vegetative growth could be used in entomopathogenic assays. Three *D. variabilis* females were used in each assay and each assay was completed 5 times. Ticks were placed on the surface of PDA at the time of inoculation, after vegetative cultures were established and on mature cultures. Additionally, ticks were placed on established vegetative and mature cultures for 10 min. to attempt inoculation. These ticks were returned to isolated rearing chambers maintained with natural lighting conditions, room temperature (approx. 24°C) and 93% R.H. (Winston and Bates, 1960).

*Weather and statistics.* Soil temperature at each site was measured using *Hobotemps*™ (Onset Corp.) placed under leaf litter, during the 95-96 winter to compare microhabitat temperatures between sites. Degree days were calculated as described by Pedigo (1989) where maximum and minimum daily temperatures were summed. This quantity was divided by two to obtain the average per day and zero was subtracted from the average, as zero degrees was considered the minimum temperature threshold for metabolism. If an average temperature was below zero, no degree-days are considered
accumulated. These daily averages were summed by month. Additionally, soil and atmospheric temperatures were recorded by Ohio Agricultural Research and Development Center (OARDC) Weather Station in Columbus, OH (39° 59' N, 83° 01' W) at an elevation of approximately 270 m. It can be accessed at http://sun1.oardc.ohio-state.edu/weather. These soil and atmospheric temperatures are graphed in Figures 2.1, 2.2 and 2.3. For survival estimates, Microsoft Excel™ was used to obtain all descriptive statistics. Subsequently, Z tests were performed on all proportions to compare differential survival (Freund and Wilson 1993).

RESULTS

Mortality varied between sites, years, species and sex. Despite these discrepancies, behavior between both species was consistent throughout each of the winters. All adult ticks within arenas overwintered between the leaf layer (and other organic debris) and the soil surface. An average of seventy-eight percent (66% for A. americanum and 90% for D. variabilis) of live ticks was recovered in this manner. No ticks were found “buried” in soil without some extended contact with the soil surface. The remaining 22% were found in natural cracks within the soil and along a very small opening located within the first several centimeters of the can/soil interface. This space never extended the entire length of the can and was not responsible for any tick escapes. Although location of dead ticks was not recorded, movement to subterranean habitats did not appear to increase survival as many of the ticks recovered from either micro-habitat were dead. During the 1996 winter, temperatures rose above freezing directly after snow
Figure 2.1. Soil and air temperatures recorded in Columbus, OH during October through March of 1995/96.
Figure 2.2. Soil and air temperatures recorded in Columbus, OH during October through March of 1996/97.
Figure 2.3. Soil and air temperatures recorded in Columbus, OH during October through March of 1997/98.
had fallen during one sampling period. When the ticks were recovered, the inside surface
of the arena was saturated and small pools of water had accumulated on parts of the soil
surface. No ticks were found submerged in water, although several were located close to
pools and had elevated the posterior portions of their bodies causing the anterior to be in
contact with the substrate. This posturing was only observed in a single arena during one
sampling period and it could not be determined if the ticks were drinking, avoiding
contact with water or some other behavior.

**General Mortality Comparisons.** Overwintering mortality was significantly
different at each site. Significantly more ticks died at Alum Creek than at Reynoldsburg
during midwinter and spring (Figure 2.4) as well as for ticks maintained under controlled
conditions. Significantly more tick mortality was observed at Reynoldsburg than under
controlled conditions during midwinter; however, significantly more mortality was
observed when *D. variabilis* were held under controlled conditions than when placed
outside at Reynoldsburg by the end of winter in 1996. No significant mortality
differences were seen during October, December or March between sexes; however,
significantly less female mortality was observed during November and February and less
male mortality in January (Figure 2.5). Mortality was significantly greater for *A.
americanum* during midwinter and spring (Figure 2.6) and field collected ticks
consistently exhibited more mortality than laboratory originating ticks (Figure 2.7).
Significantly more mortality was observed during the winter in 1996 when compared to
1995 in both January and March and less mortality was observed at the end of winter in
1995 and 1996 when compared to 1997 (Figure 2.8). Summary statistics and Z values
are presented in Table 2.1.
Specific Mortality Comparisons (Alum Creek). Significantly greater mortality was observed for *A. americanum* during January of 1995 and 1996. All *A. americanum* died by March in 1995 while several *D. variabilis* survived. In 1996, no significant differences were detected between species at the end of winter (March). Mortality was significantly higher for both species in 1995 than in 1996 except for *D. variabilis* during January. Significantly more mortality was observed for both species of overwintering ticks during the winters of 1995 and 96 when compared to controls (Table 2.2a).

Specific Mortality Comparisons (Reynoldsburg). Significantly greater *A. americanum* mortality was observed in Reynoldsburg during January and March, 1995 and 1996. Mortality was significantly greater in January and March for *D. variabilis* in 1995 when compared to 1996 and in March for *A. americanum* when both years were compared. Significantly less mortality was observed in 1996 than 1997 for *D. variabilis*. No significant differences in mortality were detected between 1995 and 1997 for *D. variabilis* or 1995 and 1996 for *A. americanum*. Mortality was significantly less for all ticks maintained in a controlled environment when compared to Reynoldsburg (Table 2.2b).

Specific Mortality Comparisons (Overwintering Location and Field vs. Laboratory). Mortality was significantly less when ticks were maintained in a controlled environment. Location within the field did not significantly affect overwintering mortality as 92 and 91% of the ticks placed in bags or arenas were dead when sampled in February. Significantly more field collected ticks died by the end of winter than the laboratory strain(2.2c).
Nymphal Mortality Comparisons. Nymphal mortality was significantly greater for both species during 1995 than 1996. When nymphs were held in controlled environments significantly less mortality was observed. Few nymphs (3) of either species survived the winter of 1995 and no significant differences were observed between species during 1995. Significantly fewer *A. americanum* than *D. variabilis* died during the winter of 1996.

Additional overwintering observations. A single species of fungus was isolated from 8 dead *D. variabilis*, 5 dead *A. americanum*, 3 live *D. variabilis* and 5 live *A. americanum* as well as most of the vegetative matter. No pathogenicity was observed on any of the 75 live ticks tested. The fungus was identified as belonging to the genus *Trichoderma*, which specialize in chitin degradation.

Temperature. Degree days (DD) between Alum Creek and Reynoldsburg varied throughout winter (Figure 2.9). By the end of winter, 173 more DD were observed at Reynoldsburg than Alum Creek. During the month of October slightly more DD were observed at Alum Creek (253.3) than at Reynoldsburg (248.8), however by January more DD accumulated at Reynoldsburg than Alum Creek.
Figure 2.4. Total tick mortality comparisons by site throughout winter for *Amblyomma americanum* and *Dermacentor variabilis* adults.
Figure 2.5. Total tick mortality comparisons between sex throughout winter for *Amblyomma americanum* and *Dermacentor variabilis* for all sites.
Figure 2.6. Total tick mortality comparisons between species throughout winter within both sites.
Figure 2.7. Total Dermacentor variabilis mortality comparisons between source throughout winter including both sites and sexes.
Figure 2.8. Total tick mortality comparisons between years throughout winter including both sites and species.
Table 2.1. Z test (one-tailed) comparisons of the inequality of two mortality proportions (θ) by site, sex, year, species and source at the α=0.05 level. Alum = Alum Creek State Park. Rey. = Reynoldsburg. Cont. = control. * = significantly different. NS = no significant difference. A.a. = *Amblyomma americanum.* D.v. = *Dermacentor variabilis.* Field = field collected adult ticks in July returned outdoors in July. Lab. = laboratory strain adults fed in July and Aug. and placed outside in Oct.

<table>
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<th>Comparison</th>
<th>Month</th>
<th>θ₁</th>
<th>θ₂</th>
<th>Z</th>
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<tr>
<td><strong>Site</strong></td>
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<tr>
<td>Alum vs. Rey.</td>
<td>Jan.</td>
<td>0.72</td>
<td>0.56</td>
<td>8.89*</td>
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<td>0.34</td>
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<td>Alum vs. Rey.</td>
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<td>0.92</td>
<td>0.44</td>
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<td>0.66</td>
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<td>Mar.</td>
<td>0.76</td>
<td>0.76</td>
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<tr>
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<td>Jul.</td>
<td>0.95</td>
<td>0.92</td>
<td>3.61*</td>
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<td>95 vs. 96</td>
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<td></td>
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Table 2.2. Z test (one-tailed) comparisons of the inequality of two mortality proportions (θ) by species within one site, by year within one species and by site within one species overwintering in different habitats originating from different sources at α=0.05 level. Alum = Alum Creek State Park. Rey. = Reynoldsburg. Cont. = control. * = significantly different, NS = no significant difference. A.a. = *Amblyomma americanum*. D.v. = Dermacentor variabilis. Field = field collected adult ticks in July returned outdoors in July. Lab. = laboratory strain adults fed in July and Aug. and placed outside in Oct.

<table>
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<th>θ₂</th>
<th>Z</th>
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<td>0.38</td>
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* indicates statistical significance.
Table 2.3. Z test (one-tailed) comparisons of the inequality of two mortality proportions (θ) for nymphs at α=0.05 level. Rey. = Reynoldsburg, Cont. = control. * = significantly different, NS = no significant difference. A.a. = *Amblyomma americanum. D.v. = *Dermacentor variabilis.

<table>
<thead>
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<th>Month</th>
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<th>θ₂</th>
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<td>0.21</td>
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<td></td>
</tr>
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<td>95 vs. 96</td>
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<td>0.36</td>
<td>-11.54*</td>
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Figure 2.9. Degree-day accumulation within the overwintering microhabitat at Alum Creek and Reynoldsburg during October through February 1995/96.
DISCUSSION

Ticks experienced dramatically different atmospheric temperatures during each winter. Despite these differences, soil appeared to buffer extreme shifts in temperature and remained above freezing throughout most winters (Figure 2.1-2.3). Overwintering microhabitats are consistently more mild than mesoclimatic habitats and although microhabitat importance is relevant to location (Bertrand and Wilson 1996, 1997), temperatures within microhabitats appear to be the primary determining factor for mortality. When winter was severe (compare soil surface temperatures), as it was in 1995, *A. americanum* mortality was significantly greater than *D. variabilis* (*Z* = 2.25 Alum, 10.43 Reynoldsburg); however, when winter was more mild, as it was in 1996, no significant differences between species were observed within 1 of the 2 sites (*Z* = -0.59 Alum, 2.26 Reynoldsburg). This indicates that extremely low temperatures are potentially more detrimental to *A. americanum* than *D. variabilis* and adds support the hypothesis that the lone star tick’s range is limited by temperature.

While extremely cold winters appear to be a detriment to survival, extremely mild winters may be even more so. Significantly greater mortality was observed during 1997 than 1995. When *D. variabilis* was compared at the end of winter at Reynoldsburg between 1995 and 1997, no significant differences were observed, while significantly greater *D. variabilis* mortality was observed during 1995 than 1996, and in 1997 than 1996. Winters that are not too cold or too warm offer the best survival conditions. Significantly less mortality was even observed during the winter of 1996 in overwintering ticks than in controls. Most likely, lower temperatures decrease the
metabolic rate and utilize less energy while not being so cold as to induce freeze
damage. Similar decreases in aging can be accomplished through placing ticks inside a
refrigerator.

Although some differences were observed between sex, mortality was unaffected
during October, December and March. Female mortality was significantly less in
November and February, while male mortality was significantly less in January.
Needham et al. (1996) and Burks et al. (1996) both demonstrated that males were more
cold hardy than females and because this study demonstrates that only during one month
(January) male mortality was significantly less than female, cold-hardiness may not be
extremely relevant during most winter months. To support this contention, Lindsay et al.
(1998) demonstrated that female *Ixodes scapularis* survived in greater numbers in each
of four habitat types during consecutive years in Ontario.

Although meso and macro habitats between Alum Creek and Reynoldsburg
appeared similar, tick mortality throughout winter was significantly different. In all cases
significantly more tick mortality was observed at Alum Creek than at Reynoldsburg
(Table 2.1). The site at Reynoldsburg was situated at approximately a 10° angle sloping
upward toward the north. This provided a south facing exposure for extended periods
during the day and microhabitat temperatures reflected this increase. Degree-days were
greater at Reynoldsburg which reflected the milder microhabitat conditions experiences
by this southward facing slope (Figure 2.9). Microhabitat differences do not always
reflect macroclimatic conditions (and vice versa) and predicting tick presence or
population numbers based solely on macroclimatic factor may be extremely difficult.
Field collected *D. variabilis* exhibited significantly greater mortality than laboratory reared ticks during every month of winter (Table 2.1). This is most likely a function of different age. ticks going through winter when field collected ticks were utilized. Laboratory *D. variabilis* were all from the same genotype and of consistent age (1 month post molt). As ticks age they are less able to adapt to adverse conditions (see Jaworski et al. 1984 and Chapter 3). Overwintering mortality for laboratory strains throughout this study is probably lower than what would normally be expected in a wild population. Natural populations of ticks have different aged members as described by Sonenshine et al. (1966) and older ticks would be more prone to death if winters were extremely cold or mild.

Nymphal mortality between years varied greatly. During 1995, only three *D. variabilis* and no *A. americanum* survived throughout the winter. However, during 1996 significantly more *A. americanum* nymphs survived than *D. variabilis* nymphs. Therefore, the statement by Campbell (1979) that few nymphs overwinter, should be qualified. Nymphal survival is dependent on overwintering conditions. Although *A. americanum* is not established throughout Ohio, survival by nymphs does not appear to be influencing colonization. During the summer of 1997, the Ohio Department of Health detected the first established population of *A. americanum* in southern Ohio (Vector-borne Disease Unit, of the Ohio Department of Health 1997). Potentially, the winter of 1996 was sufficiently mild to permit overwintering of nymphal and adult *A. americanum* but when extremely warm or cold winters return this population may become locally extinct (selected against).
Few sources of tick location during overwintering exist within the literature. In Japan, the majority of overwintering ticks *Haemaphysalis longicornis* was obtained from 5-15 cm deep under shaded trees (Shiraishi et al. 1989). While several of the ticks in our experiment may have reached depths of 5 cm, none was found at depths greater than 10 cm. This slight increase in insulation, as offered by the soil between the surface and the tick, probably does not affect winter survival. The depth of soil freezing, as observed during the winter of 1995-1996, exceeded the depth of the arena edges and the cylinders had to be chipped out of the surrounding frozen soil to obtain samples during these periods. Potentially the ticks in the experiment performed by Shiraishi et al. (1989) moved to these depths by following naturally occurring cracks in the soil and if they moved to a depth below the soil freezing point mortality would decrease. Tick morphology is not suited for a fossorial life as their first pair of legs function more like antennae (for host detection) than for digging. Being buried may actually have deleterious effects as there are numerous periods of freezing and thawing associated with winter in Ohio. A tick may become trapped within the soil when freezing occurs. The inoculating effects of being in contact with ice during freezing temperatures have been shown to decrease lower lethal temperatures (Burks et al. 1996). Being on the surface permits ticks to move from microenvironments where freezing occurs to areas with fewer ice crystals. The results of this experiment demonstrate that at least these two ixodid tick species do not bury themselves.

Because microhabitat selection was in direct contact with the soil just beneath the litter layer, mortality between bags (placed within this habitat) and arenas was compared during 1997. No significant differences were observed between the two indicating that
indeed the overwintering site for *D. variabilis* is in this stratum. Overwintering in this specific area allows ticks to modify their specific location. When conditions become saturated during winter, ticks are able to move above the water level and avoid becoming supersaturated. Potentially the posturing viewed in 1996 was to avoid such conditions.

The identification of a specialized chitin degrading fungus on dead overwintering ticks demonstrates that even during low temperatures associated with winter, dead arthropods do not last long when in contact with moist soil. This may be the reason that less than 100% of the ticks placed in the field were recovered (dead or alive) in the middle or end of winter. Isolations from live ticks indicate that spores were present in the exoskeleton and were resistant to surface sterilization. While this fungus species was not acrachnopathic, researchers should remain vigilant in looking for inquisitive fungi and other parasitic organisms to test as novel control organisms.

One of the least understood aspects of tick biology is overwintering, potentially because ticks are difficult to rear and are not easily tracked when activity ceases. The data here suggest that microhabitat selection of overwintering *A. americanum* and *D. variabilis* increase their chances of survival. Species range is directly influenced by the ability to survive low temperatures experienced in overwintering hibernacula. Surprisingly, extremely warm temperatures throughout winter increase mortality and temperatures that remain slightly above freezing promote survival. Because survival was drastically different between two ecologically similar sites, predicting species distribution using macroclimatic conditions may prove to be extremely difficult. Future studies should be directed at understanding overwintering within microhabitats. Although extremely large tick numbers were not used in this study, conclusions
concerning many of the basic survival criteria were described. This study fell short in understanding the physiological mechanisms that ticks utilize to avoid freeze injury and survive during winter conditions. Winter physiology has been virtually untouched by tick researchers even though it is potentially the most important aspect of arthropod overwintering. Chapters two through five focus on bioenergetics, water balance and metabolic rates of these two species during winter.

REFERENCES


CHAPTER 3

THE EFFECT OF AGE AND OVERWINTERING EXPOSURE ON LEVELS OF FAT, GLYCOGEN AND COLD-TOLERANCE IN AMBLYOMMA AMERICANUM AND DERMACENTOR VARIABILIS (ACARI: IXODIDAE).

ABSTRACT

Efficient management of metabolic reserves is essential for the ixodid tick gorging/fasting lifestyle. However, little information detailing the biochemical and physiological processes used is available. Amblyomma americanum and Dermacentor variabilis metabolic reserves were assayed throughout the winter of 1996 in Reynoldsburg, Ohio (an eastern suburb of Columbus). Lipid and glycogen levels were monitored in overwintering adults during January and March 1997 and compared to same age ticks maintained in the lab at 23°C. Overwintering ticks possessed much higher lipid and glycogen levels during midwinter (January) than laboratory maintained ticks. Overwintering A. americanum exhibited higher lipid and glycogen levels than those maintained in the controlled environment and glycogen levels also increased in D. variabilis; however, lipid levels in overwintering and laboratory maintained D. variabilis
were similar by the end of winter. Increased cold tolerance was observed in physiologically younger but same (chronological) aged ticks. Lipid levels increased for physiologically younger ticks indicating that lipids may play a role in cold hardiness. Lipid storage may increase tick survival by providing abundant energy reserves and potential cryoprotection during cold exposure.

INTRODUCTION

During winter, *Amblyomma americanum* and *Dermacentor variabilis* maintain temperature dependent activity and do not enter diapause (Stewart et al. 1998 and Chapter 5). This activity utilizes reserves accumulated during nymphal feeding. Hard ticks feed only once within each stage, molting to the next stage or laying eggs when ample blood is imbibed. This punctuated feeding does not permit hard ticks to engage in preparation for winter. In most arthropods, reserves accumulate within the fat body throughout extended feeding periods.

Unlike insects, ticks do not possess discrete fat bodies (Amosova 1983). The insect fat body, as defined by Chapman (1974), consists of a compact mass or loose aggregation of cells suspended in the hemocoel. It is usually found just beneath the body wall and occasionally surrounding the gut. Tick fat bodies are much less organ-like, consisting of extremely dispersed strands of cells attached most commonly to the tracheal branches but also to internal organs, especially the reproductive system (Sonenshine 1991). The fat body of insects functions in production and storage of lipid.
complex carbohydrate, and amino acid synthesis (Friedman 1985) and is analogous to the mammalian kidney (Wigglesworth 1967). Tick fat body functions similarly with one exception. Although the fat body appears to synthesize complex carbohydrates in minute quantities, the primary function of tick fat body appears to be associated with lipid synthesis and storage as well as protein synthesis. Gut wall cells are potential storage sites for glycogen (Sonenshine 1991).

Unfed *A. americanum* can live for at least three years outdoors in Oklahoma (Hoch et al. 1971). Two studies have dealt with lipid utilization over time while maintained at constant (25°C) or field temperatures. Jaworski et al. (1984) and Williams et al. (1986) both found that male and female *A. americanum* lost as much as 50 to 75% of lipid stores (although not significantly different) during a 12 month period and those ticks kept outside utilized less lipid over time. All told the rate of lipid metabolism is directly related to temperature in adult *A. americanum*.

Compared to insects, little is known about the bioenergetics of overwintering in ticks. One of the most understood insects is the golden rod gall fly, *Eurosta solidaginis* (Baust and Nishino 1991). This minute dipteran builds up lipid and glycogen reserves prior to the onset of winter then lives off them from October to March in Ohio, and potentially longer further north. Metabolism in poikilothermic animals is environmental temperature regulated, and likewise lipid metabolism for *Eurosta* was highly correlated to winter temperatures. These fly larvae emerged from winter hibernacula having used approximately 25% of their lipid reserve. When temperatures were elevated during winter, lipid reserves decreased accordingly (Lee et al. 1995).
The ability of ticks to withstand cold temperatures has recently gained interest with acarine researchers (see chapter 2). Burks et al. (1996) showed a slight, but significant, increase in cold hardiness when *A. americanum* adults were held four weeks at 4°C, while nymphal *A. americanum*, *D. variabilis* and *I. scapularis* showed little response when treated with the same pre-exposure. They suggest that low temperature acclimation results in cryoprotectant production, which improved survival. Metabolic rates should be slower at lower temperatures to reduce energy utilization and conserve lipid and glycogen.

The purpose of this study was to measure lipid and glycogen reserves during an Ohio winter to determine if there are differences with their production or utilization that contribute to survival. Additional experiments were conducted to determine if increased lipid levels may play a role in cryoprotection.

**MATERIALS AND METHODS**

*Ticks.* Ticks originated from a colony maintained at The Ohio State and Oklahoma State Universities, or were collected nearby areas. Colony immatures were fed on New Zealand White (NZW) rabbits and held during all laboratory off-host time periods in rearing chambers at 23°C (+/-2°C), 14:10 (L:D) and approximately 93% R.H. (Winston and Bates, 1960). Overwintering adults were fed as nymphs on NZW rabbits during September and placed in arenas during October, at Reynoldsburg, an eastern suburb of Columbus, as described by Stewart et al. (1998). Additionally, 73 male and 87 female field-originating *D. variabilis* were also collected near overwintering locations on
August 8, 1996 and placed in arenas on the same day. Ticks remained undisturbed for extended periods during winter under laboratory or outside conditions until being retrieved, weighed and stored frozen (-20°C). Eight to 15 ticks were utilized for each sample as survival was not 100% throughout winter (Chapter 2). Laboratory ticks were maintained for 3.5, 6 and 9 months for both species and overwintering colony and field samples were taken in January and March 1997 for *D. variabilis* and March for *A. americanum*.

*Age-Related cold-tolerance.* Cold-tolerance was evaluated for different aged laboratory maintained ticks by assaying survival at a temperature slightly above the lower lethal temperature for newly molted ticks (Burks et al. 1996). Using these temperatures should assure some survival of each species. Therefore, cold tolerance assays were conducted at -9 and -11°C for *A. americanum* and *D. variabilis*, respectively. Different age ticks were placed in glass test tubes (5 ml) and confined to the bottom 2 cm with a cotton plug. These tubes were then suspended in refrigerated baths with the tube opening protruding from the cooled ethanol. An equilibration time of 10 min was added to the incubation time of 2 h for each tube to bring the total submergence time to 2 h 10 min. After exposure to cold, ticks were placed at room temperature (approximately 25°C) for 24 h to reacclimatize. Moribund ticks were classified as dead and those that responded to stimulants as alive. Chi square goodness of fit tests were used to determine significance (Sokal and Rohlf. 1981).

*Determination of glycogen content.* After removal from overwintering arenas, ticks were weighed and placed in a -20°C freezer until glycogen or lipid analysis. Ticks were weighed before freezing to minimize condensation when they were returned to
warmer environments. To prepare samples, ticks were thawed at room temperature for 1 h and refrigerated until analysis. Because of the difficulty in crushing hydrated ticks, they were wetted with 0.1 ml of 2% aqueous sodium sulfate solution and cut into >25 pieces using iris scissors in 1.5 ml tubes. This increased surface area and exposed body contents. This sample preparation is the only deviation from the method described by Van Handel (1985a) for mosquitoes. After the ticks were macerated, another 0.1 ml of 2% sodium sulfate solution was added to totally immerse the remaining tick parts. To precipitate the glycogen from this solution, 1 ml of methanol was added and these samples were then transferred to glass test tubes. Each sample was then vortexed to ensure mixing. Glycogen is absorbed by the sodium sulfate so each sample was centrifuged for approximately 2 min to precipitate the sodium sulfate. The supernatant was discarded leaving behind the sodium sulfate, glycogen and other tick tissue. Anthrone reagent, prepared as described by Van Handel (1985a), was then added to each tube to facilitate the reaction, which manifested itself as a color change within the solution. After heating, optical densities were read at 625 nm with a spectrophotometer. To determine how optical densities correlated to glycogen levels, known glucose dilutions were prepared. Resulting optical densities produced a regression equation of \( Y = 107.14(X) - 1.62 \) (Figure 3.1B). This equation was used to determine glycogen quantities in unknown samples. To determine percent glycogen within each tick, glycogen quantities were divided by tick wet weights.

*Determining of lipid content.* Tick samples were thawed and macerated as above and analyzed using a procedure described by Van Handel (1985b). Lipids were extracted using chloroform methanol (1:1) and brought into solution with hot sulfuric
acid, which converts unsaturated lipids into water-soluble sulfonic derivatives. Vanillin-phosphoric acid reagent was then used to react with these sulfonic derivatives and this reaction was manifested as a color change. Optical densities were determined within 5 min using a spectrophotometer at 525 nm. To determine how optical densities correlated to lipid levels, known lipid dilutions were prepared. Resulting optical densities produced a regression equation of \( Y = 123.18(X) + 0.83 \) when the overwintered ticks were assayed and \( Y = 138.08(X) + 0.92 \) when the pre-exposed ticks were assayed (Figure 3.1A). Appropriate equations were used to determine lipid quantities in unknown samples and lipid contents were averaged from 12 ticks for each pre-exposure. To determine percent lipid, within each tick, lipid quantities were divided by tick wet weights.

**Pre-exposure effects on lipid content and cold tolerance.** Newly molted lab colony female *D. variabilis* (within one week) were placed at 15, 23 or 31°C for 63 days. Photoperiod and relative humidity among all three temperature treatments were maintained at 12:12 (L:D) and 93% R.H. At the end of the incubation period, 15 ticks from each group were assayed for lipid content. The remaining ticks were assayed for cold tolerance. Because survival was extremely high in 2-month old *D. variabilis* challenged at -11°C (Table 3.1), tick groups were split in half and assayed at -11 and -12.5°C to provide a more challenging environment for these young ticks.

**RESULTS**

*Age-related cold tolerance.* Cold tolerance decreased with age. When ticks were challenged at -9°C for 2 hours, significantly more *D. variabilis* females survived, than
than died ($x^2=13.76, d.f.=1, \alpha=0.05$). Female *A. americanum* that had aged 9 months at the same temperature demonstrated significantly less survival ($x^2=10.29, d.f.=1, \alpha=0.05$). The only other significant differences in survival were seen in 2 month old *A. americanum*, where significantly more survived exposure ($x^2=16.44, d.f.=1, \alpha=0.05$) and 9 month old *D. variabilis*, where significantly fewer survived ($x^2=25.0, d.f.=1, \alpha=0.05$) (Table 3.1).

**Glycogen reserves during winter.** Glycogen content increased in all ticks after 3.5 months regardless of environmental conditions (Figs. 3.2 and 3.3). Overwintering male *D. variabilis* glycogen levels increased from 0.4% to 1.4% while lab-maintained male levels increased to only 0.8%. Similarly, overwintering female glycogen levels increased from 0.6 to 1.1%. while laboratory-maintained female levels only increased to 0.9%. By the end of winter, or 6.5 months in laboratory maintained ticks, glycogen reserves remained higher in overwintering ticks. Overwintering *D. variabilis* females had 0.7% while laboratory females had only 0.5%. Overwintering *A. americanum* females had 0.9%, while laboratory females exhibited an average level of 0.5% (identical to levels immediately after molting). Overwintering *D. variabilis* males also maintained higher levels, 1.4%, than laboratory maintained males, 0.6%. and *A. americanum* males exhibited similar results with overwintering specimens showing 1.5% and those laboratory-maintained with 0.8% whole body glycogen.

**Lipid reserves during winter.** Similar to glycogen levels, lipid content increased in all but laboratory maintained *D. variabilis* males, for the first 3.5 months after ecdysis, regardless of environmental conditions (Figs. 3.4 and 3.5). More lipid was observed in overwintering than lab-maintained *D. variabilis*. Overwintering *D. variabilis*
males exhibited 10.0% whole body lipid content while laboratory-maintained males had only 5.5%. By midwinter, lipid levels in field-collected males were comparable (9.4%) with laboratory-maintained ticks. Similarly, although not as dramatically, overwintering *D. variabilis* females exhibited 6.6% whole body lipids while laboratory *D. variabilis* female lipid levels were slightly less (6.0%). Field-collected overwintering *D. variabilis* females possessed fewer lipids than either colony tick average (5.2%). Although no lipid assays were conducted on *A. americanum* during midwinter, estimations from overwintering lipid content (Fig. 3.5B) are similar to those observed in the laboratory (10.1% for males and 6.4% for females). Cooler temperature pre-exposures increased survival when ticks were challenged at cold temperatures (Table 3.2).

By the end of winter (6.5 months in Fig. 3.4 and March in Fig. 3.5) lipid levels in overwintering *D. variabilis*, both in colony- and field-collected ticks, had fallen to levels near laboratory maintained ticks. Laboratory maintained males had 6.0%, overwintering field collected males 5.4%, and overwintering colony males 4.4% whole body lipids. Females maintained slightly lower lipid levels as laboratory ticks possessed 4.6%, overwintering colony ticks possessed 5.4%, and field-collected females exhibited 6.1%. In overwintering *A. americanum*, lipid levels far exceeded those in ticks maintained under laboratory conditions. Levels for males rose from 6.5% immediately after ecdysis to 12.2% by the end of winter, while lipid levels for laboratory-maintained ticks dropped to 6.1%. Lipid levels for overwintering females similarly increased from 4.9 to 7.6%, while levels in laboratory maintained ticks remain the same, 5.0%.
Effects of pre-exposure on lipid levels and cold tolerance. *Dermacentor variabilis* pre-exposed to 15.23 or 31°C produced average lipid levels of 4.1, 2.0 and 2.7%, respectively. Percent mortality at both -11 and -12.5°C challenges increased as pre-exposure temperatures increased. No significant differences were observed in survival in any of the pre-exposure/tolerance combinations except when ticks were pre-exposed to 15°C and then challenged at -11°C. Significantly ($\chi^2=9.8, d.f.=1, \alpha=0.05$) more *D. variabilis* females survived. Despite lack of statistically significant survival differences, the trend for pre-exposures was to possess more lipid after exposure to cooler temperatures and to better survive tolerance challenges when pre-exposed to colder temperatures (Table 3.2).
Figure 3.1. Standard curves for known lipid and glycogen concentrations. The thick line in A. represents the regression used for overwintering ticks while the thin line represents the regression used for lower lethal temperatures associated with lipid levels.
Figure 3.2. Percent whole body glycogen within laboratory maintained ticks throughout 9-months. Adult *Dermacentor variabilis* (A) and *Amblyomma americanum* (B) were assayed directly after molting (0.5), 3.5, 6.5 and 9 months.
Figure 3.3. Percent whole body glycogen within overwintering ticks at the beginning, middle and end of winter. Adult *Dermacentor variabilis* (A) and *Amblyomma americanum* (B) were assayed directly after molting (October), during midwinter (January) (A) and at the end of winter (March).
Figure 3.4. Percent whole body lipid within laboratory maintained ticks throughout a 9 month period. Adult *Dermacentor variabilis* (A) and *Amblyomma americanum* (B) were assayed directly after molting (0.5), 3.5, 6.5 and 9 months.
Figure 3.5. Percent whole body lipid within overwintering ticks at the beginning, middle and end of winter. Adult *Dermacentor variabilis* (A) and *Amblyomma americanum* (B) were assayed directly after molting (October), during the midwinter (January) (A) and at the end of winter (March).
Table 3.1. Age related mortality at -9 or -11°C. *Amblyomma americanum.* assayed at -9°C, and *Dermacentor variabilis,* assayed at -11°C. adults were challenged at 2, 6 and 9 months post molt.

<table>
<thead>
<tr>
<th>Sex/Age</th>
<th>Number Sampled</th>
<th>% Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. americanum</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male/2 months</td>
<td>25</td>
<td>68*</td>
</tr>
<tr>
<td>Female/2 months</td>
<td>17</td>
<td>88</td>
</tr>
<tr>
<td>Male/6 months</td>
<td>25</td>
<td>48</td>
</tr>
<tr>
<td>Female/6 months</td>
<td>32</td>
<td>44</td>
</tr>
<tr>
<td>Male/9 months</td>
<td>24</td>
<td>25</td>
</tr>
<tr>
<td>Female/9 months</td>
<td>29</td>
<td>24*</td>
</tr>
<tr>
<td><em>D. variabilis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male/2 months</td>
<td>23</td>
<td>78</td>
</tr>
<tr>
<td>Female/2 months</td>
<td>21</td>
<td>90*</td>
</tr>
<tr>
<td>Male/6 months</td>
<td>21</td>
<td>33</td>
</tr>
<tr>
<td>Female/6 months</td>
<td>25</td>
<td>72</td>
</tr>
<tr>
<td>Male/9 months</td>
<td>25</td>
<td>0*</td>
</tr>
<tr>
<td>Female/9 months</td>
<td>14</td>
<td>7*</td>
</tr>
</tbody>
</table>

* Significantly more survived than died  
* Significantly more were observed dead than alive
Table 3.2. Age related *Dermacentor variabilis* mortality after pre-exposure to 15, 23 or 31°C for 63 days. Lipid levels were determined for each pre-exposure and 2 hour cold tolerance assays were conducted at -11 or -12.5°C.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Number Sampled</th>
<th>% Lipid</th>
<th>-11°C Survival</th>
<th>-12.5°C Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>20</td>
<td>4.1</td>
<td>85</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>4.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>11</td>
<td>2.0</td>
<td>55</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>16</td>
<td>2.7</td>
<td>50</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>2.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
DISCUSSION

Age-related physiological changes have been known since initial studies on tick behavior and physiology (Lees 1946). Age does influence tick water balance (Needham and Teel 1991), and activity ceases while palp splaying (indicative of water absorption) increases as ticks age (Curran 1993). While significant contributions have been made on the effects of aging on water balance, the effects of age on cold tolerance have remained unstudied. Significantly fewer old ticks survived when challenged with cold temperatures while mortality decreased significantly decreased for young ticks challenged at low temperatures (Table 3.1). Because older ticks are more prone to desiccation than younger individuals (Williams et al. 1986), water kinetics between the cuticle are more efficient (i.e. cuticular and respiratory water loss). This decreased resistance to desiccating environments is analogous to decreases in low temperature tolerance because it demonstrates a decrease in overall hardiness when ticks are exposed to inclement conditions. Although Williams et al. (1986) additionally observed heme content decreases with the hemolymph, the largest difference observed with this and other tick studies on aging was in lipid and glycogen content (Figure 3.2). Greater lipid and/or glycogen levels within younger ticks may protect them from freeze injury. Potential hypothetical mechanisms for lipid protection will be discussed later; however, this difference in storage compound quantities remains the largest observed physiological differences between different aged ticks. Factors other than increased cuticular permeability may be contributing to decreased cold tolerance in older ticks.
Potentially increases in membrane permeability, decreases in quantity of stress proteins as well as other factors including decreases in hemolymph osmotic pressures (from reduced heme content) may be affecting water balance as well as cold tolerance.

Glycogen is a storage carbohydrate that can easily be mobilized for energy. In insects, glycogen is primarily stored in the peripheral fat body (Keeley 1985). Conversely, in ticks only small glycogen stores are in the fat bodies and most glycogen reserves are thought to be in gut cells (Sonenshine 1991). With this uncertainty whole body glycogen content was assayed in the current study to assure that all stores would be detected. Levels of glycogen increased within 3.5 months post ecdysis regardless of environmental condition (Figs. 3.2 and 3.3). Potentially, this is an artifact of the slow intracellular digestion utilized by ticks (Akov 1982). In ixodids, blood is concentrated only within the midgut and excess water is eliminated through the salivary glands. A few digestive cells are released into the midgut, where they are thought to lyse blood cells. After lysis, bloodmeal constituents are taken into the luminal cells through phagocytosis. These constituents, within the resulting swollen vacuoles, are slowly digested. This intracellular digestion is asynchronous; therefore, overall digestion proceeds at a very slow rate. Akov (1982) suggests this explains, at least in part, why ticks can endure extremely long periods of starvation.

Unlike 3.5 months, glycogen levels varied greatly depending on environmental exposure after 6.5 months. Overwintering ticks (Fig. 3.3) exhibited increased levels of glycogen. *Dermacentor variabilis* male glycogen levels remained consistent with levels observed at 3.5 months. This suggests that tick metabolism is very slow at the end of February, or that other sources of energy are being utilized. It is unlikely that glycogen
maintenance can be attributed to glycerol conversion since glycogen levels in the later portion of winter did not increase as they did during flesh fly diapause for example (Adedokun and Denlinger 1985) and glycerol does not appear to be used as a cryoprotectant in ticks (Lee and Baust 1987).

Glycogen is easily converted to glucose, and insects (Adedokun and Denlinger 1985) as well as other invertebrate species (Silva and Zancan 1994) appear to use glycogen reserves as their primary energy source during winter. Therefore, elevating glycogen levels during winter in ticks may be indicative of extremely low metabolic rates, utilization of other metabolic stores and/or conversion of other stored products into glycogen. Increasing lipid and glycogen levels have been suggested as a mechanism to postpone aging in specific strains of *Drosophila melanogaster* (Graves et al. 1992) by increasing desiccation resistance. Potentially ticks are similarly elevating glycogen levels to avoid physiological aging during winter. They then may be able to resume activity in spring with energy stores adequate to secure a host.

Accumulation of lipids from digestion also occurs slowly in ticks (see previous discussion on digestion and glycogen), and for mosquitoes the end product of hemoglobin metabolism is lipid (Van Handel 1965). Digestion, similar to most functions in poikilothermic animals, is extremely temperature dependent. Conversion of digested products within ticks occurs at a temperature dependent rate, and this may explain why lipid accumulated in laboratory *A. americanum* (Fig. 3.4B) during the first 3.5 months appears equivalent to accumulation throughout the entire winter in overwintering *A. americanum* (compare levels in Fig. 3.4B with Fig. 3.5B). Because lipid levels for overwintering *A. americanum* were not assayed during January, this comparison may not
be accurate. It does appear, however, that laboratory maintained and overwintering *A. americanum* are following essentially the same digestion and conversions pathways, only at much different temperature-dependant rates. Tick accumulation of lipid during adverse conditions differs from other arthropods (Adedokun and Denlinger 1985, Lee et al. 1995). However, my results are consistent with previously reported tick lipid levels where they increased between 3-4 months and 6 months (Jaworski et al. 1984, Williams et al. 1986).

Lipid levels in *A. americanum* laboratory-maintained ticks were slightly higher in this study (6.4% female, 10.1% male) after 3.5 months than in Jaworski et al. (1984) (4.97% female, 6.29% male) after 3 months; however, the ticks within their study were maintained at a slightly higher temperature (25°C). This implicates increased metabolic rates in differential lipid content. By 6.5 months in this study, *A. americanum* levels (5.0% female, 6.1% male) were also similar to those reported by Jaworski et al. (1984) after 6 months (6.81% female, 6.47% male). Under warmer conditions, tick metabolism should be increased and reserves utilized at a much faster rate. The rate of metabolism in *A. americanum* seems to be particularly temperature sensitive and low temperature exposure may result in lipid accumulation. Alternatively, low temperatures may actually postpone lipid utilization resulting in higher content. Potentially, both scenarios are simultaneously affecting tick lipid content.

*Dermacentor variabilis* lipid content comparisons are more difficult because there are no other papers. Within this study, lipid levels of lab-maintained ticks were very similar to previous reports on *A. americanum* (Jaworski et al. 1984, Williams et al. 1986). *Dermacentor variabilis* showed slight increases in whole body lipids by 3.5
months in both overwintering and laboratory maintained females (Figs. 3.4A, 3.5A). By March (6.5 months in Fig. 3.4A) lipid levels substantially decreased. The sharp decline of lipid in overwintering *D. variabilis* is characteristic of a rapid shift in energy storage use. Ticks potentially utilize protein or carbohydrate reserves as suggested in flesh flies by Adedokun and Denlinger (1985).

Lipid accumulation within the first 3.5 months of adult life at 23°C and outdoors supports temperature-dependent processes. Females pre-exposed to 15°C for 2 months had higher lipid levels than females exposed to warmer temperatures (Table 3.2). Subsequent cold tolerance testing yielded significantly more survival with 15°C exposure. Lee and Baust (1985), Burks et al. (1996) and Needham et al. (1996) have also demonstrated increased cold tolerance or decreases in supercooling point with cold pre-exposure. Increased lipid levels may protect these arthropods from freeze injury. Lipids may confer protection by decreasing the freezing point, as is observed for thermal hysteresis proteins (Duman and Horwath, 1983). Potentially, lipids function in a similar manner by inhibiting ice crystals formation (Zachariassen and Husby, 1982). Alternatively, lipids sequestered by ticks within the highly amorphous fat body, other organs or within membranes could insulate cells from cold damage (lysis). Although neither of these explanations appears highly probable, pre-exposure to cold temperatures seems to confer increased lipid levels and cold tolerance. These higher levels of cold tolerance may alternatively be due to other factors such as the production of established cryoprotectant molecules (Storey and Storey, 1991).

Differences in survival (discussed in Chapter 2) do not appear to be influenced by differences in stored energy levels in either tick species during the 1996 winter.
Significantly more *A. americanum* mortality was observed during midwinter and at the end of winter (Table 2.2B); however, glycogen levels between species were not substantially different (Fig. 3.3) and lipid levels were substantially higher at the end of winter in *A. americanum* (Fig. 3.5). Levels of biological storage compounds appear similar between species throughout winter and it appears that starvation plays a very small role in the distribution of either species.

The data presented here raise numerous questions concerning energy storage compounds. Do overwintering ticks of other species similarly increase glycogen and lipid levels or is this not a temperature dependent function as this study suggests? Do increased lipid and/or glycogen levels confer cold protection as I suggested and if so, how? Are lipids actually protecting ticks from cold injury, or is it other cryoprotectants? Future studies should focus on lipid accumulation and its relevance to cold tolerance within ticks as well as in other arthropods as this appears to have the greatest relevance to understanding overwintering survival.

REFERENCES


CHAPTER 4

ACTIVE VAPOR SORPTION IN LONE STAR AND AMERICAN DOG TICK
ADULTS (ACARI: IXODIDAE) AT LOW TEMPERATURES AND HYDRATION
STATUS THROUGHOUT WINTER

ABSTRACT

Hard ticks absorb water from subsaturated air. This capability greatly extends their survival potential in ever changing stressful habitats. Temperature plays a vital role in the vapor uptake process. Neither *Amblyomma americanum* nor *Dermacentor variabilis* adults can gain water at near saturation when temperatures are below 16°C. Potentially these low temperatures decrease metabolism needed to produce salivary gland secretions, or water kinetics may be altered because mouthpart cuticle or structures associated with vapor uptake are nonfunctional. Despite this incapacity at low temperatures, ticks appear to gain net water throughout winter. Hemolymph osmolarities decreased until midwinter, showing that solute concentrations were diluted. This overall water gain may be facilitated passively in ticks by osmosis, as soil is frequently saturated unless frozen. If ticks gain water across the integument via osmosis, they may be unable to regulate water balance, which makes them more vulnerable to freeze injury.
INTRODUCTION

Hard ticks (Acari: Ixodidae) have a unique ability, shared with a few insects and isopods, to acquire water from subsaturated air (O'Donnell and Machin. 1988). This ability facilitates a gorging/fasting lifestyle and allows ticks to survive for years. Each tick feeds only once per life stage (larva, nymph, adult) up to 14 days, while the remainder of its time is spent searching for a host after molting or ovipositing (Sonenshine 1993). Needham and Teel (1991) estimate that three-host ticks spend >90% of their life off the host. As no food is imbibed during this period and most ticks are not known to drink (Kahl and Alikousti 1997), collecting moisture from subsaturated air is vital for survival. Despite the importance of this mechanism, most information concerning its mode of action has only recently been identified. The site of vapor uptake was discovered to be the mouth, rather than the general body surface in 1974 (Rudolph and Knülle). Since then several pivotal papers by McMullen et al. (1976), Rudolph and Knülle (1978), Needham et al. (1990), Sigal et al. (1991) and Gaede and Knülle (1997) have confirmed that active vapor sorption is oral and have shown it to be salivary gland driven, and mediated by salts secreted on the mouthparts during hydration.

Water relations in insects have received considerable attention even at low temperatures (Zachariassen 1991, Hadley et al. 1991, Quinlan and Hadley 1993, Hadley 1994, Roberts et al. 1994). Freeze intolerant insects have the most difficulty regulating their hydration status as they usually dehydrate throughout winter. Dehydration to some extent can be advantageous, as increased cryoprotectant concentrations decrease freezing points, or it can be disadvantageous when ice nucleators increase within the hemolymph.
Insects exposed to freezing temperatures (where water in the surrounding environment is in the form of ice) are especially vulnerable because hemolymph water content declines. The vapor pressure of ice is less than the vapor pressure of supercooled water at the same temperature. Put simply, liquid water moves across the semipermeable cuticular membrane down the gradient from the insect's hemolymph to ice crystals within the environment (Zachariassen, 1991). Arthropods may die not from direct freeze injury but from indirect cold injury due to desiccation. Freeze tolerant insects do not confront desiccation problems since their hemolymph is in vapor pressure equilibrium with ice. This phenomenon was demonstrated by Ring (1982) when he compared freeze intolerant and tolerant larvae of closely related insects. The freeze intolerant species lost up to 70% of its body water while the freeze tolerant species did not suffer a net water loss.

No tick studies have addressed hydration status during prolonged periods of low temperature exposure and only two studies (McEnroe 1971, Sauer and Hair 1971) have addressed the issue of temperature effects on active water absorption over a short duration. Using field-collected ticks McEnroe (1971) and Sauer and Hair (1971) detected no weight increase when specimens were predesiccated and placed at 5°C (95% R.H.); rather, predesiccated A. americanum gained significant net water at 15°C (95% R.H.). Similarly, D. variabilis gained water between 15-20°C, but not lower. These two species could not gain water below 5°C, but the threshold of 15°C was not determined.

These two species do not rehydrate under a temperature of approximately 15°C (McEnroe 1971, Sauer and Hair 1971); therefore exposures to low temperatures below this during winter should not permit net water gain. Ticks monitored during my survival
studies (Chapter 2) appeared turgid despite being exposed to overwintering temperatures that should not permit rehydration. Additionally, because ticks are freeze intolerant (Lee and Baust 1987), they should desiccate while exposed to frozen environments in winter. Several papers have dealt with cold-hardiness in hydrated ticks pre-exposed to room temperatures (approximately 25°C) or to lower temperatures for brief periods (Lee and Baust 1987, Burks et al. 1996, Dautel and Knüllle 1996, Needham et al. 1996, Strey et al. 1996), but none have assessed the effect of winter and prolonged low temperature exposure on hydration status.

The hypotheses for this chapter are: the critical temperature for net water gain in subsaturated environments in *D. variabilis* is lower than in *A. americanum*, and *D. variabilis* remains partially desiccated throughout winter to increase cold-hardiness. If these hypotheses are correct, low temperature exposure (freezing) may not directly determine the geographical range of ticks. Indirect effects, such as inability to gain water and differential hydration during winter may contribute to the ability of each species to survive in colder environments, which may ultimately dictate species range.

**MATERIALS AND METHODS**

Both sexes of *A. americanum* and *D. variabilis* were used for osmolarity testing over winter. All ticks were either procured from colonies maintained at The Ohio State University on laboratory rabbits or field collected from local sites. Overwintering laboratory ticks were fed as described in Chapter 2; however, nymphs were fed 2-4 months before low temperature water absorption testing. Only females were used for the
rehydration/temperature experiments. Unless otherwise noted, all pharate and ecdysed females were kept at 23°C, 93% R.H. (Winston and Bates 1960) under a 12:12 photoperiod. Osmolarities were determined from ticks exposed to 1997 winter conditions (Fig 2.3).

*Hydration status during winter.* While ticks were being assessed for survival in Chapter 2, they appeared turgid. That is, they were somewhat restricted in their movement because the legs were extended by hydrostatic pressure, and the body dorso-ventrally was thicker than normal. To determine the mole fraction of water, body water solute concentration, within tick hemolymph during winter: osmotic pressures were taken on a monthly basis. Hemolymph osmolarity was measured from two or three individual ticks during each sampling period using a nanoliter osmometer (Clifton Technical Physics™, Hartford, NY) as described by Frick and Sauer (1973), which measures melting points of a frozen sample. Hemolymph samples were collected from each tick by placing the distal leg segments of a single leg under hydrated immersion oil (water/oil mixture used to reduce water loss), breaking the cuticle of these segments and collecting a single drop of hemolymph within the oil. Nanoliter samples were then transferred to receptacles in the base of the instrument with finely drawn capillary tubes. Oil was drawn up into the capillary in front of and behind the sample to ensure no contact with air. Using known osmolarity standards (Fisher Scientific), melting points were determined. The melting point is the temperature at which a single ice crystal does not change in size.

*Low temperature sorption.* To predesiccate females, ticks were placed in aluminum boats. Boats were made by rolling a strip of aluminum foil (approximately
2x4 cm) around a pencil and crimping one end to form a cylinder. Holes were punched in the aluminum with an insect pin to allow air movement between the environment and the tick. A single tick was then placed in the open end of each boat and that end was crimped to seal the tick inside. These ticks, now in boats, were then reweighed and placed over Drierite™ (0% R.H.) for 24 hrs. Ticks were weighed after this 24-hr period to assure loss and establish new desiccated water pool baselines (see below). Ticks were then placed at different temperatures under saturated conditions. Boats were always handled with forceps to ensure no transfer of oil or moisture from hands.

Low temperatures were attained by submerging 15 boats (for each species), sealed in a Rubbermaid™ container under saturation conditions, in water baths. Saturated environments were made by filling the bottom of the container with distilled water and floating the boats inside a separate container on the water. Weights were subsequently taken every 48 hrs for 8 days after removing each boat from the container and placing it on Drierite™ for 10 min to remove any condensation. Exposure temperatures used were 0, 2, 4, 6, 8, 10, 12, 14, 16, 17, 18 and 23°C for both species.

To determine the amount of water within each tick, dry weights were taken at the conclusion of each trial by placing the boat at 55°C over Drierite™ (0% R.H.), then weighing the tick and boat together, then the boat separately. Boat weights were then subtracted from boat and ticks weight combinations. Numbers were expressed as percent change in water content from predesiccated weights.
RESULTS

*Hydration status during winter.* On average, hemolymph osmolarity decreased during winter from 340 in October to 220 mOsm in January; after this period osmolarities increased and in March were 249 mOsm. A slight increase was observed between November and December in females; however, their osmolarities decreased between December and January. In this study an average decrease of 21% between October and November and a decrease of 35% was observed between October and January for *D. variabilis*. Average increases of nine and two percent were seen between January - February and February - March respectively (Table 4.1). Osmolarity values differed markedly between sexes. Males had lower osmolarities than females as their osmolarity dropped an average of 31% between October and December, while females dropped only eight percent. Osmolarity values were lower in *A. americanum* during January than at the end of winter (Table 4.1).

*Low temperature sorption.* *Amblyomma americanum* adults gained water, above their predesiccated condition, at the same temperature as *D. variabilis* (Figs. 4.2, 4.4). At no temperature below 10°C was any weight gain observed for either species (Figs. 4.1, 4.3). Both species gained water at 16° but not 14°C. *Amblyomma americanum* weight gain was seen at 48 hrs and maintained above the predesiccated weight, while *D. variabilis* weight gain was more ephemeral, seen only at 48 hrs. *Dermacentor variabilis* continually retained and gained water to a greater degree than *A. americanum*. Survival of ticks was also different as 0, 20, and 0.3 % *D. variabilis* and 0.7, 23, and 15 % *A. americanum* adults died at temperatures 0-6, 8-14 and 10-23, respectively.
Figure 4.1. Change in weight from initially desiccated female *Dermacentor variabilis* over a 192 h rehydration period at 0, 2, 4, 6, 8 and 10°C. Sample size was 15 females for each temperature and results are presented as the mean change in weight.
Figure 4.2. Change in weight from initially desiccated female *Dermacentor variabilis* over a 192 h rehydration period at 12, 14, 16, 18, and 23°C. Sample size was 15 females for each temperature and results are presented as the mean change in weight.
Figure 4.3. Change in weight from initially desiccated female *Amblyomma americanum* over a 192 h rehydration period at 0, 2, 4, 6, 8 and 10°C. Sample size was 15 females for each temperature and results are presented as the mean change in weight.
Figure 4.4. Change in weight from initially desiccated female *Amblyomma americanum* over a 192 h rehydration period at 12, 14, 16, 18, and 23°C. Sample size was 15 females for each temperature and results are presented as the mean change in weight.
Table 4.1. Hemolymph osmolarities measured from overwintering *Dermacentor variabilis* throughout winter. Numbers indicate individual tick osmolarities and parenthesis denote averages. Laboratory originating *Amblyomma americanum* osmolarities were measured during January and March and were 308.5:262 and 314:285.5 (male:female), respectively (averages of 2 ticks for each sex).

<table>
<thead>
<tr>
<th>Origin/sex</th>
<th>October</th>
<th>November</th>
<th>December</th>
<th>January</th>
<th>February</th>
<th>March</th>
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<tbody>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>330,313</td>
<td>288,287</td>
<td>300,284</td>
<td>190,242</td>
<td>212,256</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>(321.5)</td>
<td>(287.5)</td>
<td>(292)</td>
<td>(212.7)</td>
<td>(234)</td>
<td></td>
</tr>
<tr>
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<td>300,255</td>
<td>281,235</td>
<td>257,230</td>
<td>...</td>
<td>239,240</td>
<td>220,268</td>
</tr>
<tr>
<td></td>
<td>(377.5)</td>
<td>(258)</td>
<td>(243.5)</td>
<td></td>
<td>(239.5)</td>
<td>(244)</td>
</tr>
<tr>
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<td>355,307</td>
<td>267,237</td>
<td>315,290</td>
<td>235,230</td>
<td>269,244</td>
<td>257,243</td>
</tr>
<tr>
<td>Female</td>
<td>(331)</td>
<td>(252)</td>
<td>(302.5)</td>
<td>(232.5)</td>
<td>(256.5)</td>
<td>(250)</td>
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<tr>
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<td>290,265</td>
<td>255,225</td>
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<td>250,235</td>
<td>268,240</td>
</tr>
<tr>
<td></td>
<td>(333)</td>
<td>(277.5)</td>
<td>(240)</td>
<td></td>
<td>(242.5)</td>
<td>(254)</td>
</tr>
<tr>
<td>Average</td>
<td>(340.75)</td>
<td>(268.8)</td>
<td>(269.5)</td>
<td>(220.6)</td>
<td>(243.1)</td>
<td>(249.5)</td>
</tr>
</tbody>
</table>
DISCUSSION

Although no ticks were collected from temporary aquatic environments (formed from melting ice and snow) overwintering microhabitats are saturated and occasionally frozen. During the winter of 1996 *D. variabilis* adults removed from these microhabitats appeared turgid. Osmolarity values, collected during the winter of 1997 reflect these observations as melting points decreased throughout the winter until February. The average osmolarity for all *D. variabilis* measured during October was 340 mOsm. which decreased by January to 220 mOsm and increased again by March to 249. Osmolarity values below 250 mOsm are very uncommon, except in millipedes (Hadley 1994). Low osmolarities are indicative of low solute/solvent mole ratios; therefore less solute or more water was present in the hemolymph. *Amblyomma americanum* adults exhibited a similar trend when osmolarities were taken in January and March (Table 4.1). These trends are the opposite of what Needham et al. (1996) found with pre-exposed *A. americanum* at 0°C, 93% R.H. for 2 h. Because the same osmometer was used and the same osmometer operator conducted the experiments, it is unlikely that error was introduced during the procedure. These differences likely reflect true differences between extended cold exposure to saturated environments and brief exposures, or are species specific differences between *A. americanum* and *D. variabilis*. Needham et al. (1996) observed an osmolarity increase of 5.7 and 8.2% in females and males, respectively. In this study much greater average decreases were observed between October and November as well as October and January. Small increases were also
observed between January and February as well as February and March. Male osmotic pressure was consistently lower than that for females of both species. Male and female *A. americanum* levels differed from 348.6 (Needham et al. 1996) under laboratory conditions to 308.5 mOsm in females overwintering during January, and from laboratory maintained males (364.8. Needham et al. 1996) to January overwintering males (262). The largest osmolarity change in this study as well as in Needham et al. (1996) was observed for males. This is potentially due to water kinetics. Smaller bodies of water (found in males) have greater surface/volume ratios and can pick up or lose water at faster rates. Lower osmolarities, seen during winter in both species and sexes, do not suggest the use of cryoprotectants and support the observations by Lee and Baust (1987) where only cryoprotectant traces were present within the hemolymph.

Vapor pressure gradients determine the rate and net water flux between arthropods and the environment. Since vapor pressure in equilibrium with supercooled water is greater than the equilibrium pressure with ice at the same temperature, water should slowly move from the arthropod to the environment. If the environmental water remains in a liquid state, water should be accumulated via osmosis. If tick water content increases, their hemolymph would be more prone to freezing (Zachariassen 1991).

How ticks acquire water during winter is an interesting question. The water vapor pump, normally used by ticks to acquire water from subsaturated environments (Lees 1946, Knülle 1966, Needham and Teel 1986, Yoder and Spielman 1992), does not function during the majority of winter when temperature are below a certain point. Figures 4.1-4.4 show that both species cannot consistently gain water below 17 or 18°C (*D. variabilis*, *A. americanum*, respectively) although some gain was seen as low as
16°C for both species. Because temperatures are seldom consistently above 16°C during Ohio winters (Figs. 2.1-2.3), overwintering ticks are most likely not acquiring water through this mechanism. Metabolic water may have contributed to some of these decreased osmolarities; however, metabolism throughout winter should be severely depressed. Another alternative is suggested based on my observation of tick overwintering microhabitats. Microhabitats during the winter of 1997 were consistently saturated and never froze (Fig. 2.3); allowing ticks to acquired water via osmosis. Submerged one-host larval ticks can increase water volume via osmosis or drinking (Schuntner and Tatchell 1970, Kemp and Tatchell 1971). However, *Boophilus microplus*, likely increased water content through drinking (Wildenson 1953). Three host ticks, however, are not known to drink water and even when offered water droplets, they desiccated in subsaturated humidities (Kahl and Alidousti 1997). It is unlikely that *D. variabilis* or *A. americanum* increased water as 100% of 18 submerged *D. variabilis* females gained weight when their mouthparts were sealed with wax over a seven day period (Stewart, Personal Observation).

Within these two species, active uptake does not take place below 16°C at near saturation. This suggests that low temperatures may incapacitate the vapor uptake mechanism proposed by (Gede and Knülle 1997). Potentially, the thin membrane between the hypostome and chelicerae that they identified as a collection site cannot condense water or that condensed water cannot be imbibed. Another possibility is that ticks do not maintain high enough metabolic rates (below 16°C) to support active secretion of dilute salts. If this is the case, temperatures below 16°C may place ticks in vulnerable situations where they are unable to regulate body water. Although these
possibilities are highly speculative, the lack of information detailing water vapor uptake does not easily lend itself to many other probable methods.

Since the threshold temperature for active vapor absorption at near saturation is the same between species, it is unlikely that water absorption during winter influences their geographical ranges (Figs. 1.1. 1.2). Potentially, prolonged exposure to temperatures below the threshold may contribute to different geographical ranges. Fewer ticks exposed to temperatures between 0-6°C died throughout the hydration portion of this study and slightly less mortality was observed at these temperatures in *D. variabilis*. This additional small difference in low temperature tolerance may help extend the northern distribution of *D. variabilis*. Temperatures between 8-14°C do not promote survival as 20% of *D. variabilis* and 23% of *A. americanum* died. This also may help explain the conclusions of Chapter 2 where temperatures near 0°C (experienced during the winter of 1996) promoted survival while higher temperatures (winter of 1997) decreased survival.

Tick water balance during winter may be different from that of other arthropods. By losing water, and therefore concentrating hemolymph solutes, insects increase freeze tolerance and improve survival. Unlike most insects, ticks in this study gained water during winter, which should decrease cold-hardiness. How ticks obtain water during winter is unresolved as colder temperatures hamper active absorption. Because submerged ticks readily gain water with their mouthparts sealed, passive water uptake through the cuticle appears to be the most logical explanation. Temperatures below 16°C impair the function of the water vapor uptake mechanism. Although metabolism may play a role in this inability, it is more likely that water kinetics are affected by lower
temperatures thereby decreasing the ability of structures within this pump. It is difficult to speculate what specific structures or mechanisms performed by the vapor pump are affected; however, the thin membrane between the hypostome and chelicerae appears to be a vital component of absorption. This structure may not be able to collect water or may have difficulties transferring water to the oral cavity. Future studies should be focused around the role of this structure in vapor uptake at low temperatures.

REFERENCES


CHAPTER 5

PHYSIOLOGICAL AND BEHAVIORAL EVIDENCE AGAINST WINTER DIAPAUSE IN *DERMACENTOR VARIABILIS* (ACARI: IXODIDAE)

ABSTRACT

* Dermacentor variabilis* adults do not exhibit characteristics associated with behavioral diapause during winter in Ohio. Using lab-reared (1996) and field-caught (1997) specimens in the vivarium and outdoors I evaluated their willingness to quest (search for hosts, attach and feed), and their metabolism based on respiration. Regardless of whether they were lab-reared or field-caught, ticks removed from outdoor arenas in the fall and winter readily quested (70%) and attached/fed to repletion. Females returned to outdoor winter conditions failed to oviposit, while those in the lab oviposited viable eggs. The results suggest that overwintering locally-collected field and laboratory strain *D. variabilis* are not entering behavioral diapause; rather they quest, feed and successfully mate throughout winter in Ohio.
Many arthropods in temperate areas undergo a period of diapause during adverse weather conditions by entering an alternative dormant physiological condition. Decreased metabolism and/or arrested development characterize dormancy and it ranges from quiescence, an immediate response to inclement conditions, to diapause. Insect diapause is a developmental (Tauber et al. 1986) and cell cycle arrest (Tammariello and Denlinger 1998) under neuroendocrine control (Denlinger 1985, Shimizu et al. 1997). It may be mediated by a unique developmental pathway (Flanagan et al. 1998). Diapause is divided into two types based on physiological and behavioral responses to environmental cues. Obligatory diapause, as the name suggests, occurs during the same period of development irrespective of environmental condition. Some environmental factor, usually photoperiod and temperature, alternatively, must preprogram facultative diapause (Denlinger 1991). Arguably, facultative diapause is much more beneficial for arthropod populations, permitting certain species to survive in extremely varied conditions. Diapause is not a static developmental condition. It follows an alternate pathway of regulatory mechanisms that ultimately returns the arthropod to a non-diapausing condition (Denlinger et al. 1988). Unlike arthropods undergoing quiescence, diapausing arthropods cannot respond to short periods of favorable weather until the alternate pathway has been completed. This is advantageous as uncharacteristically mild periods could falsely alert arthropods that favorable conditions have arrived.

In ticks two types diapause are generally reported, and both appear to be facultative. Behavioral diapause is the suppression of host-seeking activity and refusal to

Although behavioral diapause is apparently much more common than is morphogenetic diapause (Belozerov 1982), few detailed physiological studies have been performed on behaviorally diapausing ticks. Belozerov (1982) points out that decreased metabolism during diapause contributes to a more economical utilization of food reserves and in behaviorally diapausing ticks this is also enhanced by a decline in locomotor activity. Oxygen consumption in morphogenetically diapausing *Ixodes ricinus* and *Dermacentor marginatus* decreased to levels as low as 10 fold lower than those of non-diapausing individuals (Belozerov 1964, 1966). Ticks in behavioral diapause also exhibit decreased respiratory rates as shown by *D. marginatus*, which decreased oxygen consumption by as much as four fold when compared to active ticks (Belozerov 1968).

Photoperiod has been implicated in providing initial cues for tick behavioral diapause (Wright 1969, 1971). However, feeding effectiveness, as measured in attachment, for adult *Dermacentor variabilis* placed under different photoperiods was unaffected (Smith and Cole 1941). Behavioral diapause has been reported in adult *D. variabilis* (Belozerov 1982): however, only a single behavioral (Smith and Cole 1941) and no physiological studies have been conducted to quantitatively describe this condition in *D. variabilis*. In this study, during the winter of 1996, questing activity was
observed during warm periods in January (Fig. 2.2). Since adults were subjected to natural overwintering conditions, no activity was expected. To test the ability of adult *D. variabilis* to behaviorally diapause, I used the defining characters of Belozerov (1982) and Sonenshine (1993), namely: suppression of host-seeking activity, refusal to feed and reduction in metabolic rates. The hypothesis is: during winter *D. variabilis* goes into behavioral diapause. To test this hypothesis, I analyzed questing, feeding and metabolic rates in overwintering ticks and compared them to lab ticks. This hypothesis would be supported if few ticks quested or fed as well as low metabolic rates were observed in overwintering ticks in comparison to lab ticks.

**MATERIALS AND METHODS**

*Dermacentor variabilis* adults were either reared from laboratory cultures originating from Oklahoma, or field collected from local sites (Ohio wild-type). Laboratory ticks were fed as nymphs on New Zealand (NZW) white rabbits and placed in molting chambers (93% R.H., Winston and Bates, 1960) under 14:10 (L:D) conditions. Field originating adults were collected on July 19 and 20, 1996 from Alum Creek and Delaware State Parks (Delaware Co., Ohio) and placed nearby at a local area outside the Columbus city limits (Franklin Co., Ohio) where *D. variabilis* normally occurs on 20 July. Care was taken to maintain natural light conditions during transport.

*First winter (1996).* While completing the experiments detailed in chapter 2, I observed *Amblyomma americanum* and *D. variabilis* adults clinging to the underside of screening used to secure them into arenas. Arenas were constructed and utilized within
the field as described in chapter 2 and by Stewart et al. (1998). When these ticks were
stimulated by human breath, they would move in an excited manner, presumably in
response to the presence of carbon dioxide (Chapter 6). *Dermacentor variabilis* were
removed from arenas during January 1997 to assay feeding ability. Feeding success was
determined by placing males and females in a secured translucent cell on the dorsum of
laboratory rabbits. Using an adjustable dog trimmer (Oster), hair from an area
approximately 20 X 15 cm was removed and a feeding cell (constructed from the top of
a clear plastic 2 L bottle) was secured using masking tape. Feedings were performed
under vivarium conditions (14:10 L:D/range 18-22°C) or outdoor conditions (Figure 2.2)
inside an unheated building with windows. Attachment success was monitored every 48
hrs for 16 days. One half of the resulting engorged replete females from both vivarium
and outdoor feeding locations were returned outside. The remaining half were placed
within rearing chambers (14:10 L:D, 23°C, 93% R.H.) to evaluate viability.

*Second winter.* Previous experiments (Chapter 2) revealed that adult *D. variabilis*
remain on the soil surface and there was no evidence of decreased survival between ticks
choosing overwintering sites or being sealed in screen squares at the soil surface.
Therefore, when ticks were placed in field situations during the second year, they were
sewn in window screen (10 cm²) and placed on the soil surface under available litter.
Litter usually consisted of a mix of dried grasses and leaves from native flora.
Throughout the winter, pools of water collected in several naturally occurring low spots.
Although ticks are able to remain submerged for more than 100 days (Honzakova 1971),
these samples were excluded from diapause assays. Ticks were removed from field
situations on 9, 14 October. 20 November. 22 December. 2 February and 23 March
1997-98 and transported to the laboratory to evaluate diapause status, or were mailed overnight to Berry College, Mt. Berry, Georgia (L. J. Fielden) for respiration assays. Care was taken throughout transport to maintain natural light conditions until ticks were sealed in Zip Lock™ plastic bags inside cardboard boxes for transport to Georgia. During transport, high humidity was maintained by including several leaves from naturally occurring vegetation or with a moistened paper towel when leaves were unavailable.

Ticks were removed from overwintering screen squares for immediate use with an in vitro questing assay, modified from Madder and Berkvens (1997), to characterize an individual tick's willingness to commence host-seeking activity (approx. 23°C). The assay consisted of ten 30 cm lengths of clear plastic tubes (1.5 cm in diameter) mounted on a flat piece of cardboard. This apparatus was then secured on edge at approximately a 5° angle on the desktop. Once this apparatus was positioned, ticks were placed on top of moistened crumpled sheets of individual Kimwipes™. With the ticks on top, these tissue paper balls were secured in the bottom of the plastic tubes with two individuals placed within each tube. The result of this exercise assured that ticks were exposed to a hydration gradient inside where a host could be detected from one direction (opposite direction from hydration source). The hydration gradient was necessary because under field situations, questing involves migration vertically away from moist leaf litter. To assure host presence, a person remained within 4 m of the questing assay throughout the experiment and to assure the ticks were not affected by visual cues, the apparatus was covered with black cloth during each assay period. Questing heights were recorded every 15 min. for an hour. A tick was considered questing if it had moved a minimum of 15
cm (half of the available length) away from the humidity source during the assay. No laboratory-maintained ticks were used in this experiment as they readily move when hosts are detected.

Placement of ticks on the rabbit during the second winter was the same as the first winter (1996) only feeding was performed exclusively under vivarium conditions. Photoperiod and temperature during the second winter feedings were 14:10 L:D/20 to 22°C since no significant attachment differences were observed between locations during the first winter (Stewart et al. 1998). Attachment success was observed every 2 d for 12 days. Replete females were returned to tick rearing chambers to evaluate egg viability. Each egg mass was checked weekly for hatchability over 6 weeks [14:10 (L:D), 93% R.H., and approximately 23°C]. No ticks were returned outdoors as egg viability was absent during the winter of 1996.

Respirometry was performed on female ticks within 4 days of removal from the field. Measurements of metabolic rate expressed as the rate of CO₂ production (\( V_{CO_2} \)) were calculated using a computerized flow-through respirometry system similar to that described by Fielden et al. (1998). All measurements were performed at 25 ± 1°C. Scrubbed air (H₂O and CO₂ removed with a drierite-ascarite column) was pumped into the system and regulated at 50 ml/min using a mass flow controller (range 10-370 ml/min). This air was channeled past a single tick, held in a 5 ml glass tube, and then passed through a CO₂ analyzer (LiCor, Lincoln, NE). The analyzer was a differential non-dispersive infrared gas analyzer and detector with an accuracy of ± 1 ppm CO₂ concentration in air sampled at 8 s intervals for the duration of the recording (usually 16 hrs). Sixteen hours was chosen because of long inter-burst periods previously reported
To account for drift during recording, the CO₂ baseline was monitored before and after the recording period. Temperature and pressure standards were used to correct CO₂ production rate determinations, using a data analysis program (Sable Systems). Confidence interval testing was conducted on mean rates of CO₂ emission, discontinuous ventilation cycle frequencies, burst lengths and interburst lengths for each specified month to compare differences at α = 0.05 (Steel and Torrie 1960).

RESULTS

First winter. Sixteen D. variabilis were placed on a rabbit inside the climate-controlled vivarium and all but one attached during the 19 day feeding period. On the rabbit held outdoors, fourteen of the 15 ticks attached during a 20 day feeding period (Table 5.1). Several of the attached males were later found unattached inside the feeding-cell as male D. variabilis commonly attach and detach to search for females (Sonenshine 1991). Attachment successes between these two environments were nearly identical; however, longer roaming periods (approximately one day) were observed for ticks feeding on the rabbit held outside. Repletion was similarly protracted when ticks were on rabbits held outdoors. Two-three day lag periods were observed in repletion times, partially due to longer roaming. One half (6) of the replete females fed under vivarium and outdoor conditions were returned to outdoor conditions while the other half was maintained under rearing chamber conditions. All females that were maintained
Table 5.1. Attachment efficiency of overwintering laboratory strain *Dermacentor variabilis* at Reynoldsburg, Ohio. Ticks were placed in arena during October and removed during January. They were then placed on naive rabbits held under vivarium conditions or outdoor conditions during the same day as removal.

<table>
<thead>
<tr>
<th>Location</th>
<th>Sex</th>
<th>Number infested</th>
<th>Maximum Number (%) attached</th>
<th>Replete females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vivarium</td>
<td>Female</td>
<td>7</td>
<td>7 (100)</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>9</td>
<td>8 (89)</td>
<td></td>
</tr>
<tr>
<td>Outside</td>
<td>Female</td>
<td>8</td>
<td>8 (100)</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>7</td>
<td>6 (86)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>31</td>
<td>29 (94)</td>
<td>13</td>
</tr>
</tbody>
</table>
under vivarium conditions laid viable eggs while no females returned to the outdoors survived to lay eggs.

Second winter. Seventy percent of assayed ticks quested throughout winter. Table 5.2 depicts tick numbers (percentages) by source and sex that successfully quested when removed from overwintering sites during different winter months. Significantly more ticks quested during the entire winter sampling period than remained sessile ($t = 3.5$, d.f. = 8, $P < 0.05$) and no significant differences were observed between questing ability and month sampled ($\chi^2 = 9.43$, d.f. = 4, $P > 0.05$). Statistics were not performed between sexes as too few individuals were assayed; however, both sexes quested during each month and no differences in pattern between males or females is apparent. Several ticks remained with the moistened substrate at the entry point and did not walk from placement during the assay. During October, for instance, four of the 25 ticks (two field-originating females and two laboratory originating males) did not move from initial placement. During November, only one of the 28 ticks (female, lab origin) remained static for the entire hour. All of the 89 assayed ticks during December, February and March moved vertically during the one-hour assays.

Ticks were fed under vivarium conditions and replete females were not returned to the environment during the winter of 1997. Ticks were not fed and placed in under the same vivarium conditions of 1996 during 1997 because no significant differences were observed between feeding sites during the winter of 1996 (Stewart et al. 1997). When they were fed under vivarium conditions, number of attached ticks was evaluated at two-day post inoculation intervals. A mean attachment of 65% was observed throughout the study (Table 5.3).
Table 5.2. Questing activity in overwintering laboratory on local *Dermacentor variabilis* using and *in vitro* assay during October – December. February and March.

<table>
<thead>
<tr>
<th>Date</th>
<th>Sex/Origin</th>
<th>Number assayed</th>
<th>Number (%)* questing</th>
</tr>
</thead>
<tbody>
<tr>
<td>October</td>
<td>female/field</td>
<td>5</td>
<td>3 (60)</td>
</tr>
<tr>
<td></td>
<td>female/laboratory</td>
<td>8</td>
<td>6 (75)</td>
</tr>
<tr>
<td></td>
<td>male/field</td>
<td>4</td>
<td>4 (100)</td>
</tr>
<tr>
<td></td>
<td>male/laboratory</td>
<td>8</td>
<td>5 (63)</td>
</tr>
<tr>
<td></td>
<td>subtotal</td>
<td>25</td>
<td>18 (72)</td>
</tr>
<tr>
<td>November</td>
<td>female/field</td>
<td>9</td>
<td>9 (100)</td>
</tr>
<tr>
<td></td>
<td>female/laboratory</td>
<td>5</td>
<td>3 (60)</td>
</tr>
<tr>
<td></td>
<td>male/field</td>
<td>9</td>
<td>8 (89)</td>
</tr>
<tr>
<td></td>
<td>male/laboratory</td>
<td>5</td>
<td>5 (100)</td>
</tr>
<tr>
<td></td>
<td>subtotal</td>
<td>28</td>
<td>25 (89)</td>
</tr>
<tr>
<td>December</td>
<td>female/field</td>
<td>6</td>
<td>3 (50)</td>
</tr>
<tr>
<td></td>
<td>female/laboratory</td>
<td>10</td>
<td>9 (90)</td>
</tr>
<tr>
<td></td>
<td>male/field</td>
<td>5</td>
<td>5 (100)</td>
</tr>
<tr>
<td></td>
<td>male/laboratory</td>
<td>6</td>
<td>2 (33)</td>
</tr>
<tr>
<td></td>
<td>subtotal</td>
<td>27</td>
<td>19 (70)</td>
</tr>
<tr>
<td>February</td>
<td>female/field</td>
<td>9</td>
<td>4 (44)</td>
</tr>
<tr>
<td></td>
<td>female/laboratory</td>
<td>15</td>
<td>6 (40)</td>
</tr>
<tr>
<td></td>
<td>male/field</td>
<td>6</td>
<td>3 (50)</td>
</tr>
<tr>
<td></td>
<td>male/laboratory</td>
<td>4</td>
<td>2 (50)</td>
</tr>
<tr>
<td></td>
<td>subtotal</td>
<td>34</td>
<td>15 (44)</td>
</tr>
<tr>
<td>March</td>
<td>female/field</td>
<td>6</td>
<td>4 (67)</td>
</tr>
<tr>
<td></td>
<td>female/laboratory</td>
<td>8</td>
<td>6 (75)</td>
</tr>
<tr>
<td></td>
<td>male/field</td>
<td>6</td>
<td>4 (67)</td>
</tr>
<tr>
<td></td>
<td>male/laboratory</td>
<td>8</td>
<td>8 (100)</td>
</tr>
<tr>
<td></td>
<td>subtotal</td>
<td>28</td>
<td>22 (79)</td>
</tr>
<tr>
<td>Total</td>
<td>142</td>
<td>99</td>
<td>70 (70)</td>
</tr>
</tbody>
</table>

*Number (%) questing successfully walked vertically at least half the measured distance (15 cm). *Origin refers to location where they were reared or collected, not where they were held during winter.*
Table 5.3. Attachment efficiency of field-collected *Dermacentor variabilis* from central Ohio. Ticks were placed in arena during July, removed monthly throughout winter and placed on naive rabbits maintained in a vivarium the same day as removal.

<table>
<thead>
<tr>
<th>Date</th>
<th>Sex</th>
<th>Number Infested</th>
<th>Maximum Number (%) Attached</th>
<th>Replete Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>October</td>
<td>female</td>
<td>12</td>
<td>4 (33)*</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>male</td>
<td>10</td>
<td>5 (50)*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>subtotal</td>
<td>22</td>
<td>9 (41)</td>
<td></td>
</tr>
<tr>
<td>November</td>
<td>female</td>
<td>14</td>
<td>11 (79)</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>male</td>
<td>4</td>
<td>3 (75)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>subtotal</td>
<td>18</td>
<td>14 (78)</td>
<td></td>
</tr>
<tr>
<td>December</td>
<td>female</td>
<td>8</td>
<td>5 (63)</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>male</td>
<td>8</td>
<td>4 (50)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>subtotal</td>
<td>16</td>
<td>9 (56)</td>
<td></td>
</tr>
<tr>
<td>February</td>
<td>female</td>
<td>11</td>
<td>9 (82)</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>male</td>
<td>6</td>
<td>5 (83)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>subtotal</td>
<td>17</td>
<td>14 (82)</td>
<td></td>
</tr>
<tr>
<td>March</td>
<td>female</td>
<td>12</td>
<td>8 (67)</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>male</td>
<td>7</td>
<td>6 (86)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>subtotal</td>
<td>19</td>
<td>14 (74)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>92</td>
<td>60 (65)</td>
<td>23</td>
</tr>
</tbody>
</table>

* Five females and three males were found dead attached to tape used to secure feeding cell, which effectively removed them from the experiment. January was not assayed during the second year due to low tick availability.
Significantly more ticks attached than remained unattached throughout the entire winter (t = 2.59, d.f. = 8, P< 0.05) (Table 5.3). While the proportion attaching during each month was not equal (χ² = 10.15, d.f. = 4, P> 0.05), some ticks attached during each infestation period. Attached female numbers are not indicative of repletion although repletion also occurred during each infestation period. The number of attached males was determined by examining maximum concurrent attachment. Replete females were returned to laboratory rearing chambers and all subsequent eggs hatched within 2 months.

Overwintered adult *D. variabilis* exhibited a regular discontinuous ventilation (CO₂ release) cycle (Figs. 5.1 A&B) where characteristically short CO₂ burst periods (spiracular opening events) were followed by longer interburst periods (spiracles closed or slightly open). Carbon dioxide levels during interburst periods were so low as to be indistinguishable from baseline levels. The average CO₂ emission rates (μl/h) measured for ticks during the different months of winter varied greatly (from 0.2048 ± 0.0617 to 0.5019 ± 4.41). These differences in individual CO₂ rates normalized when metabolic rates were expressed on a mass specific basis (μl/mg/h). However, significantly more CO₂ (μl/h) was discerned using confidence intervals to evaluate data during October, November and February. The mass specific CO₂ emission (μl/mg/h) was always higher for overwintering than laboratory maintained ticks and no significantly lower rates were detected for any month. The cycle of CO₂ bursts (DVC-discontinuous ventilation cycle frequency, one cycle equals the end of one burst phase to the end of the following burst phase) ranged from 1.37 ± 0.445 to 3.55 ± 0.18 bursts/h with ticks in February.
Figure 5.1. Graphs illustrating discontinuous CO$_2$ release in adult female *Dermacentor variabilis* respiration at room temperature (approx. 25°C). Both overwintering (A) and control (B) *D. variabilis* exhibited similar patterns.
Table 5.4. Summary of respiration data collected from overwintering field collected *Dermacentor variabilis*. \( \dot{V}CO_2 \ l/h = \) volume of measured \( CO_2 \) from a single tick, \( \dot{V}CO_2 \ l/mg/h = \) volume of measured \( CO_2 \) divided by tick the weight of the specimen, DVC frequency/h = discontinuous ventilation cycle frequency, Blen = length of burst phase, IBlen = length of interburst phase, \( n = \) number of female ticks measured, *male ticks. (S.D.). * indicate numbers that were significantly greater and \( ^\gamma \) indicates numbers that were significantly less than controls. m = minutes.

<table>
<thead>
<tr>
<th>Date</th>
<th>n</th>
<th>mass (mg)</th>
<th>( \dot{V}CO_2 \ (l/h) )</th>
<th>( \dot{V}CO_2 \ (l/mg/h) )</th>
<th>DVC (h)</th>
<th>Blen (m)</th>
<th>IBlen (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oct. 1997</td>
<td>5</td>
<td>3.89</td>
<td>0.2221</td>
<td>0.05932*</td>
<td>2.48</td>
<td>4.12</td>
<td>29.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.67)</td>
<td>(0.0401)</td>
<td>(0.0174)</td>
<td>(1.53)</td>
<td>(0.58)</td>
<td>(17.92)</td>
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<td>Nov. 1997</td>
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<td>0.2722</td>
<td>0.05427*</td>
<td>2.61</td>
<td>4.33</td>
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<td>(0.74)</td>
<td>(0.0932)</td>
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<td>(1.16)</td>
<td>(12.57)</td>
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<td>3.77</td>
<td>39.94</td>
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<td></td>
<td>(0.91)</td>
<td>(0.0594)</td>
<td>(0.00668)</td>
<td>(0.54)</td>
<td>(0.45)</td>
<td>(14.64)</td>
</tr>
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<td>Feb. 1998</td>
<td>2</td>
<td>6.14</td>
<td>0.5019</td>
<td>0.0861*</td>
<td>3.55*</td>
<td>3.23</td>
<td>13.69(\gamma)</td>
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<td></td>
<td></td>
<td>(0.33)</td>
<td>(4.41)</td>
<td>(0.0075)</td>
<td>(0.18)</td>
<td>(0.21)</td>
<td>(0.84)</td>
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<td>March 1998</td>
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<td>0.2048</td>
<td>0.0401</td>
<td>1.37</td>
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<td>(0.06)</td>
<td>(0.0617)</td>
<td>(0.0118)</td>
<td>(0.445)</td>
<td>(1.05)</td>
<td>(10.26)</td>
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<td>Control</td>
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<td>(0.030)</td>
<td>(0.008)</td>
<td>(0.61)</td>
<td>(0.84)</td>
<td>(17.92)</td>
</tr>
</tbody>
</table>
displaying significantly more bursts/h than the control. Tick DVCs during December and March were less frequent than laboratory maintained ticks although no significant differences were detected. The length of the burst phase (Blen) ranged from $3.23 \pm 0.21$ to $4.81 \pm 1.05\text{min}$ with ticks in March displaying the longest burst length. The Blen was shorter only for ticks in February, however, no significant differences were detected during this or any other month. Interburst length (IBlen) fluctuated greatly from $13.69 \pm 0.85$ to $43.28 \pm 14.98\text{ min}$ with ticks in March displaying the longest and ticks in February displaying the shortest burst length. None of these deviations was significantly different than the control except during February (Table 5.4).

DISCUSSION

Overwintering *D. variabilis* adults quested, attached, fed to repletion (females) and laid viable eggs under laboratory conditions during both winters, and displayed metabolic rates similar to laboratory maintained ticks. These behavioral and physiological attributes are directly opposite of what has been reported for behaviorally diapausing *D. variabilis* adults (Belozerov 1982, Sonenshine 1993) and contradict my hypothesis. Therefore, when field collected *D. variabilis* are confined in arenas during July or October and evaluated for diapause characters throughout winter (October – March) they are not in behavioral diapause as defined.

Significantly more ticks quested and attached, than did not quest or attach throughout the entire winter, indicating that they maintain temperature-dependent activity. Although the number of ticks was significantly different for attachment during
each month, successful attachment also was observed for each month. During October of
the second year, 42 and 30% of the female and male ticks, respectively, were attached to
tape used to secure the feeding cell. These ticks were not available for attachment and
appeared to be responsible for the difference in attachment between winter months. If
they are removed from the sample, no significant differences are observed between the
months ($\chi^2 = 7.59, \text{ d.f.} = 4, P > 0.05$). In this study, gas exchange patterns for
overwintering ticks were typical of the discontinuous CO$_2$ release already described for
other ixodid ticks (Fielden et al. 1994). All CO$_2$ levels ($\mu l/mg/h$) for overwintering ticks
were equivalent to or higher than levels for ticks maintained under laboratory conditions.
Only during one month were any significantly lower differences detected. Interburst
length (IBlen) during February was significantly less than the control. Overwintering
ticks were apparently opening and closing their spiracles for gas exchange more
frequently than the controls. A decrease in IBlen is indicative of a higher metabolic rate
(Fielden et al. 1998, Fielden and Lighton 1996). From the table (5.4), one may conclude
that the respiration characteristics of overwintering ticks are indicative of an increased
metabolic rate when measured at room temperature (23°C) throughout winter. Increased
temperature probably did not cause the ticks to break diapause as Lighton et al. (1993)
have shown lower metabolic rates in $A. \text{maculatum}$ at high temperatures. This increase in
metabolic rate was not activity induced since periods of locomotory activity were
excluded from calculations of rates of CO$_2$ emission. Ongoing studies suggest that this
increase in metabolic expenditure may be related to dehydration stress (Fielden and
Lighton 1996).
In Ohio, the adult *D. variabilis* is seasonal with activity beginning in March/April and ending in August (Micher and Rockett 1993). Potentially there is a brief period of diapause in August-early September. This period may be associated with abiotic parameters, such as daylength, initially placing ticks in physiologically mediated dormancy that was missed in the present study. However, the questing activity (Table 5.2) and respiration rates (Table 5.4) for ticks assayed in October do not reflect diapause during fall. If ticks do experience a brief period of diapause, they may remain inactive throughout winter and begin questing when temperatures permit activity in spring. There is precedence for this occurring in flesh fly pupae and stem borers (Denlinger 1972, Kfir 1991). However, Clark (1995) has demonstrated that lower temperature thresholds for activity in *D. variabilis* are exceeded during several extended periods of most Ohio winters (Tables 2.1, 2.2, 2.3). Parasitism by *D. variabilis* should occur during these warm periods of Ohio winters since the ticks are not in diapause. Tick submissions to the Ohio Department of Health (from people and their pets) during nine consecutive years (1988-96) showed no evidence of *D. variabilis* activity beyond October (Ohio Department of Health, Personal Communication). Potentially *D. variabilis* activity does occur during warm periods in winter but humans infrequently enter tick habitats. Certainly fewer people visit the outdoors during winter than summer. If ticks were to continue feeding during warmer winter periods, successful feeding and oviposition would be disadvantageous. If both sexes secure a host in the middle of winter, the female will not be able to lay eggs that will hatch in winter environments. Therefore, ticks may be active and able to move throughout winter, but their host acquisition behaviors are apparently limited. Snow cover potentially also plays a role in host acquisition. Ticks are
able to quest when placed in warm (23°C) conditions; however these conditions occur infrequently on the soil surface where they are likely to be found (Chapter 2).

Why the *D. variabilis* population in Ohio ceases questing in mid-August when behavioral diapause is not a factor in overwintering remains to be determined.

Photoperiod appears to be the dominant abiotic factor inducing facultative (behavioral) diapause in insects and other arachnids (Wright 1969, 1971, Evans and Krafsur 1990, Pan et al. 1993, Kroon et al. 1997, Gadenne et al. 1997); however, other factors such as host energy quality have recently emerged (Feder et al. 1997, Hunter and McNeil 1997). Potentially, many *D. variabilis* adults secure a host or die from exposure or lack of reserves in late summer. The remaining ticks within the population may enter a period of quiescence initiated by factors other than photoperiod like lack of water (as surface soil humidity usually decreases as summer progresses) or appetence. Although these explanations are highly speculative, no details correlating the physiological condition of ticks during punctuated activity periods exist.

Tick diapause has permitted host exploitation in environments that are extremely harsh as well as synchronize their activity periods with host availability. Among the physiological and behavioral characteristics of ticks, decreased metabolic rate is one of the most important factors regulating radiation (speciation) (Hoogstraal 1978). It is clear that Ohio *D. variabilis* are not utilizing behavioral diapause, as characterized in insects (Denlinger 1991) to circumvent harsh winter conditions. Since behavioral diapause appears to be absent in overwintering *D. variabilis* in Ohio, I suggest that these ticks are utilizing a more ephemeral form of dormancy, that is more like quiescence. Ticks unable to secure a host, or recently molted adults, may terminate questing in August in Ohio.
through quiescence. The controlling environmental and/or physiological cues remain undiscovered. As the soil surface temperature remains low throughout much of winter and snow covers the ground, ticks seldom have the opportunity to initiate questing behaviors. Additional studies, such as energy and water content, need to be performed to understand the physiological condition of ticks during the period when they cease questing (August in Ohio). Illuminating the abiotic and biotic factors governing this period of inactivity is paramount in understanding the physiological mechanisms utilized by overwintering ticks.

LITERATURE CITED


CHAPTER 6

ACCUMULATION OF *DERMACENTOR VARIABILIS* ALONG ROADSIDES
PERMITS TIMELY CONTROL THROUGH MOWING

ABSTRACT

Accumulation of adult *Dermacentor variabilis* Say was documented in three locations in central Ohio throughout the summer in 1996 and 97. Significantly more ticks were recovered within one meter of the road edge than between 1-2 m while tick numbers remained constant in areas that were not influenced by the characteristics associated with roads. Few ticks were collected along the road edge during April and the largest number of ticks along the road edge occurs in late June. Evidence to support auto emissions as the stimulant for attraction is presented as more ticks were recruited towards more heavily traveled roads. Roads did not act as barriers as a similar number of marked ticks were discovered on both sides of the road after release on one side.

Mowing road edges during peak abundance significantly reduces the number of ticks questing along roadsides and may serve as an economically viable method to reduce numbers in areas that have high human traffic. Adults were collected from April 6 until August 21. Significantly more males (80%) were collected during April, but the proportion equalized to near 50% for the remaining months of activity. Accumulation of
ticks within 2 meters should provide a "trap crop" for their control. The results of this experiment indicate that mowing roadside vegetation during late June or early July in Ohio dramatically reduces American dog tick numbers in high use areas.

INTRODUCTION

Hard ticks (Acari: Ixodidae) generally use passive host acquisition that is characterized by ticks' assuming a position on vegetation and waiting. Adults usually become sessile with their anterior pointing toward the ground, holding on with the last three pair of legs. The first pair remains folded until a host is detected, then the legs are extended into a grasping position. Stimulants include vibrations, odors, heat and shadows (Sonenshine, 1993). During this host acquisition period horizontal movement by adult *Dermacentor variabilis* in open fields is minimal. Sonenshine et al. (1966) showed that the average movement distance is less than a meter when ticks were placed in a field. When attractants were present near ticks in the field, adults moved greater distances. Carbon dioxide traps attracted *I. ricinus* from a maximum distance of 3.5 m. *Amblyomma americanum* from several meters (Sonenshine 1993) and several *A. variegatum* from 6 m (Barre et al. 1997). When CO₂ plus a tick pheromone were combined, *A. hebraeum* traversed 11 m in 1.5 hrs (Norval et al. 1989).

*Dermacentor variabilis* has moved 121 m towards a road (Smith et al. 1946), possibly stimulated by canine and human presence along the road. They speculated that ticks remained at the road edge in response to canine and human attractants or that bare ground formed a barrier. McEnroe (1971) later noted this phenomenon of moving
towards and accumulating along roadsides. He speculated that CO₂ emissions from vehicles, not people, were attracting adult American dog ticks to the road. He noted that adult dog ticks failed to move toward roads when placed 165 m away but did from 33, 10 and 5 m. The first tick, from these release points, arrived at the road edge the same day of release and at least one crossed the road within 5 days. Those released at the road edge did not cross, rather they moved parallel to the road at a rate of 10 m/week. McEnroe (1971) also reported that tick survivorship along roadsides was closely linked to habitat condition.

Using acaricides is currently the most reliable method to control ticks (Sonenshine 1993). Resistance to several key pyrethroids and other compounds has occurred on a regional basis (Nolan et al. 1989, Regassa and De Castro 1993, Beugnet and Chardonnet 1995). In addition to resistance, many acaricides are nonspecific and cause adverse effects to the environment, and Schulze and Jordan (1995) have questioned their use for controlling ticks altogether. Controlling ticks requires an integrated approach and several authors have begun examining models using acaricides and a variety of non-acaricidal methods especially for the control of Ixodes scapularis (Wilson and Deblinger 1993, Mount et al. 1997a,b). These methods include environmental modification, limit host availability, developing host immunity against ticks and using predators, parasitoids and pathogens to decrease the population. Many of these tactics target periods when ticks are off the host.

The present study documents accumulation of an adult American dog tick population along roadsides in central Ohio and evaluates a tactic that may safely and economically decrease local tick abundance. The specific hypotheses tested during this
study are that: 1) adult Ohio *D. variabilis* accumulate along roadsides during the summer, 2) timely mowing decreases the number of roadside ticks, 3) the road acts as a barrier that facilitates roadside accumulation, and 4) road usage influences roadside abundance.

**MATERIALS AND METHODS**

All ticks were collected during the summers of 1996-97 using a standard 1m x 1m white flannel drag for a sampling period of 1 h in each area of the site. Dragging was always done simultaneously by two or more individuals. When performed next to the road, two individuals would flag side by side at the same rate, and when performed at distances away from the road, flagging was done in a more random fashion with drags being checked every 5 steps. Ticks were obtained from four areas located near Alum Creek and Delaware State Parks, Delaware County, Ohio. Site 1 is located along Africa Rd, approximately 2 miles south of Alum Creek Dam. It is a Christmas tree farm surrounded by fallow fields and deciduous forest approximately >400 m from any paved road. Site 2 (Fig. 6.1) is also located along Africa Rd north of site 1 on the east of Alum Creek dam. Both of these areas have an east/west profile and southern exposure is abundant. Site 3 is located along State Route 229 north of Delaware Reservoir, approximately 15 miles northwest of Alum Creek. It begins several meters east of a bridge over the Olentangy River and continues for approximately 500 m. This site has a southern exposure and is protected on the west by a mixed mesophytic forest composed primarily of maple.
Figure 6.1. Diagram, not drawn to scale, of tick collection site at Alum Creek State Park. Mowed = mowed grass, and distances along the left margin indicate approximate distances away from the road edge. Ten ticks each were placed at the release sites, indicated by dots, during the summer.
(Acer sp) trees. Perpendicular to SR229 is Gearhiser Rd. (Twp. Rd. 142) running north and south. The edges of this seldom-traveled township road and surrounding fields are site 4. Collected ticks were not returned to any area within the sites except during mark-recapture experiments in 1996. All collection was done in the late afternoon or evening and by various individuals.

First summer To confirm previous studies (Smith et al. 1946, McEnroe 1971) in other geographical areas, ticks were collected, marked, released and sampled throughout the summer in Ohio. Two-hundred and eighteen ticks were collected on May 9 & 10, 1996 from the fallow field adjacent to site 2 at Alum Creek (Fig. 6.1). Of these 200 we marked, using a dot of Testors™ paint on the dorsum, and released 9 (n=100) or 7.5 m (n=100) away from the road edge. Ticks were released in groups of 10, 1.5 m away from each other. An additional 140 ticks were collected from an adjacent field on May 18 and released as previously described 7.5 m away from a cement culvert (Fig. 6.1). Six collections each were made from the roadside, culvert area, behind the release site ranging from 12-40 m and in the fallow field during the next three months to monitor tick movement as indicated in Figure 6.1.

Second summer Collection began on April 6, 1997 and continued weekly until August 31 throughout the summer at site 2 (Alum Creek). Supplemental collections were made at sites 1, 3 and 4 throughout the summer and began April 24, June 14 and June 14, respectively. Only site 3 was monitored for the entire summer. Samples were taken within 1 m of the road edge, between 1-2 m of the road edge and in the fallow fields >150 m away from the road for one hour at each site during the majority of collection periods.
Grass along the road at site 2 was mowed to approximately 4 cm on June 4, 1997. Because this mowing took place throughout Alum Creek State Park, site 3 was chosen to monitor tick abundance along the road edges. The roadside grass remained high throughout the summer at sites three and four, which provided opportunities to evaluate the effects of mowing.

The roadsides at sites 3 and 4 drew ticks from the same specimen pool. Both roads within these sites were composed of asphalt and had similar flora (primarily grasses). Traffic counts were done to assess the relative number of vehicles using these avenues. Statistics were performed using Systat™.

RESULTS

Of 796 adult *D. variabilis* collected from May to August 1996 at site 2, 301 were within 1 m of the road, 38 were between 1-2 m, and two were collected between 12-40 m behind the release point from the road. All other ticks were >50 m from the road. Twenty-four of the 200 marked ticks released 10 or 7.5 m away from the road edge, and no marked ticks released 7.5 m from the concrete culvert were recovered during the summer. No marked ticks were recovered further than the release point away from the road. Thirteen of the 24 ticks along the road were recovered on the same release side, while the others were recovered on the opposite side. The average minimum distance traveled by these ticks was 19.9 m ranging from 9 to 65.5 m. The tick that traveled 65.5 m did so over 20 days, potentially averaging 3.3 m/day assuming movement in a straight
line from release to recapture. Only one unmarked tick was recovered from the area
surrounding the culvert during the entire summer.

Of the 2,020 ticks collected during the summer of 1997, 912 were located within
1 m of the road edge, 117 were within 1-2 m and 991 were collected >70 m away from
the road. Significantly more ticks were obtained within 1m of the road than between 1-
2m (paired t-test, p=0.037, d.f.=3) (Fig. 6.2). Sample sizes from each site varied greatly.
Fifteen ticks were collected at site 1, 1,214 from site 2, 591 from site 3 and 200 were
collected from site 4. Ticks were discovered from April 6 until August 21 (Fig. 6.2). No
collection was done prior to April 6; however, sites 2 and 3 were visited 2 additional
times after August 21.

Significantly fewer ticks were recovered within 1 and 1-2 m from the road
immediately after mowing (ANOVA, p=<0.001, d.f.=9) (Fig. 6.3). Numbers of both
males and females dropped from 31 and 39 to 3 and 1, respectively, between June 3 and
June 9 after mowing. Tick numbers remained low throughout the summer at site 2 along
the road edge after mowing. As a positive control, tick numbers collected within 1 m of
the road at site 3 on June 14 (36 male, 37 female) were comparable to those found prior
to mowing at site 2. These numbers remained high throughout the remainder of the tick
season.
Figure 6.2. Number of ticks collected at each site during the summer of 1997 and at Alum Creek during 1996 within one meter and between 1-2 m of the road edge.
Throughout the summer...

Before and after mowing, compared to number of ticks collected at all other sites.

Figure 6.3: Number of ticks collected within one meter of the road edge at Alum Creek.

---

Average # Ticks Collected (All Sites)

# Ticks Collected (<1 meter)

<table>
<thead>
<tr>
<th>Month</th>
<th>April 6</th>
<th>May 1</th>
<th>May 7</th>
<th>May 11</th>
<th>May 21</th>
<th>May 31</th>
<th>June 9</th>
<th>June 12</th>
<th>June 14</th>
<th>June 22</th>
<th>July 6</th>
<th>July 14</th>
<th>July 21</th>
<th>Aug 21</th>
<th>Aug 24</th>
<th>Aug 31</th>
</tr>
</thead>
</table>
To evaluate vehicular road usage as an attractant for ticks, paired samples were made between high- and low-use roads around the same tick population. Although no statistical differences were observed between the two roads, the trend supports there being more ticks along the more heavily traveled road (Fig. 6.4). Vehicle traffic counts were performed for the two roads demonstrating that SR229 has much more traffic than Gearhiser Rd. Total vehicle counts during the late afternoon were 163/hr for SR229 and 10/hr for Gearhiser Rd.

While monitoring tick populations we noticed an overt difference in the sex ratio beginning in the early spring but normalizing as the tick season progressed. The sex ratio over the entire summer of 1997 was 50:50; however, significantly more (ANOVA, \( p<0.001, \text{d.f.}=14 \)) males than females were collected during the month of April (Fig. 6.5). This trend was observed during the early collections in May 1996; however, no significant differences were observed between May and the rest of the summer that year.
Figure 6.4. Tick number comparisons within one meter of the road edge between a heavily traveled state highway and a seldom traveled county road.
Figure 6.5. Percentage of male *Dermacentor variabilis* collected throughout the spring and summer at different locations in central Ohio.
DISCUSSION

Although this study reflects only two years of observations and ticks were sampled at irregular intervals, the data strongly suggests that adult *D. variabilis* accumulate along roadsides in central Ohio, and this accumulation may be closely correlated with vehicular road usage. Forty-three and 51% of the total specimens collected during the summer of 1996 and 97 were collected within 2 m of the road and several individuals had traversed great distances toward the road. When areas ranging 12-40 m from the road were sampled in 1996, <1% of the total was discovered. The remainder came from habitat located several hundred meters distant from any road and their distribution did not appear to be influenced by roads. Ticks discovered along the road throughout the summer were likely recruited from more hospitable overwintering and host-securing habitats since many of the roadside habitats were very harsh (scant thatch, rocky terrain and little moisture). *Dermacentor variabilis* adults apparently assume a more active host searching strategy when a host is detected from immediate or remote locations than previously recognized. Because no marked ticks moved away from the road at a distance of 9 or 7.5 m we suggest that roads or road usage is greatly affecting tick location within a given habitat. When the attractiveness of two roads was compared at nearby Delaware State Park in Ohio, more ticks were detected within 2 m of the more heavily traveled road. Although no significant differences were detected between tick numbers along these two roads, increasing sample size would likely have yielded similar results permitting statistical differences.
The attraction of *D. variabilis* to roads may be partially explained by numerous experiments on tick behavior confirming their response to CO₂. Ticks from the families Ixodidae and Argasidae have demonstrated attraction and stimulation in response to high levels of CO₂ (Table 6.1). Certainly higher levels of CO₂ exist along roads since it is the primary emission from vehicles (Schaefer et al. 1994). Although the attractiveness of CO₂ to ticks listed in Table 1 is not the same, accumulation along roadsides seems possible; however, no published records exist demonstrating this in any other species.

While CO₂ serves as an exceptional attractant and/or stimulant for ticks, infrared radiation may also influence movement towards roads. The IR-dielectric waveguide theory of insect olfaction has been proposed by Callahan et al. (1985) as an explanation for lovebugs (*Plecia nearctica*) accumulating along roads. As described in his companion paper (Callahan 1985) a sensillum on the antennae is able to detect wavelengths of light in the infrared spectrum. A recent article (Bruce 1997) describes a comparable sensillum on the tarsus of *Varroa jacobsoni* (Acari: Varroidae) that may serve as an infrared detector. Although this hypothesis is speculative in ticks, it shows that they may be utilizing this sensillum as a detection device. If this is so, heated road surfaces should stand out from the surrounding environment during cooler night hours and act like a beacon for directed tick movement. There is some data to support increased tick activity during periods of low ambient temperature. *Dermacentor occidentalis* and *I. pacificus* quest more avidly at night or in the morning hours than during the afternoon or evening (Lane et al. 1995) and *I. scapularis* has been observed questing more actively during the night than day (Durdan et al. 1996).
Table 6.1. Documented tick species that are excited by or attracted to the presence of carbon dioxide.

<table>
<thead>
<tr>
<th>Species</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ixodidae</strong></td>
<td></td>
</tr>
<tr>
<td><em>A. maculatum</em> Koch</td>
<td>Semtner and Hair 1975, Holscher et al. 1980</td>
</tr>
<tr>
<td><em>Dermacentor albipictus</em> Packard</td>
<td>Garcia 1969</td>
</tr>
<tr>
<td><em>D. andersoni</em> Stiles</td>
<td>Garcia 1965</td>
</tr>
<tr>
<td><em>D. occidentalis</em> Marx</td>
<td>Garcia 1962</td>
</tr>
<tr>
<td><em>D. variabilis</em> Say</td>
<td>Semtner and Hair 1975, Holscher et al. 1980, Perritt et al. 1993</td>
</tr>
<tr>
<td><em>Ixodes pacificus</em> Cooley</td>
<td>Garcia 1962</td>
</tr>
<tr>
<td><em>I. ricinus</em> L.</td>
<td>Gray 1985</td>
</tr>
<tr>
<td><em>I. scapularis</em> Say</td>
<td>Falco and Fish 1989</td>
</tr>
<tr>
<td><strong>Argasidae</strong></td>
<td></td>
</tr>
<tr>
<td><em>Ornithodoros coriaceus</em> Koch</td>
<td>Garcia 1962</td>
</tr>
<tr>
<td><em>O. parkeri</em> Cooley</td>
<td>Miles 1968</td>
</tr>
<tr>
<td><em>O. savignyi</em> Audouin</td>
<td>Nevill 1964</td>
</tr>
</tbody>
</table>
Despite recovering only 12% of the marked ticks, far fewer than other studies (Kramer et al. 1993), some information can be gained because none was recovered farther away from the road than the release point. Because an equal number of marked ticks (13 or 11) was recovered from both sides of the road during 1996, roads do not appear to be acting as significant barriers to tick movement. Although an equal number of marked ticks was collected from each side, more unmarked ticks were collected within 2 m on the side of the road with more suitable habitat (seceding fields) than within 2 m on the opposite side (annual grasses). This observation supports an earlier report (McEnroe 1971) that local roadside habitat can be correlated with tick survival. The tick in our study that traveled 65.5 m was recovered north of the release point on the same side of the road. This directional movement may aid in colonizing of new areas once an initial population has been established, potentially making man-made roads corridors for colonization. All unaccounted marked ticks may have dehydrated, secured a host, moved beyond the areas being sampled or were killed on the road by the heat or crushed during crossing. Attempts were made to recover marked ticks from all settings by expanding the range of collection on numerous occasions, examining road-killed mammals and looking for road-killed ticks. Road-killed invertebrate sampling is indeed possible (Seibert and Conover 1991). Despite these efforts, no additional marked ticks were recovered.

Ticks are not randomly distributed within any given habitat and an unusually high number of adults can be collected using standard dragging techniques during the months of May and June along the road edge. Most ticks on the road edge are located within a single meter (see Fig. 6.2) of the road, and can be observed with regularity.
grasping grass blades that extend over the road surface. This unnatural accumulation of ticks in an area highly accessible to pedestrian and vehicle usage should increase the probability that humans and their domestic animals will be at greater risk to encounter ticks. It also places a large number of ticks, attracted from great distances from either side of the road, in a highly vulnerable position at a specific time, much like a trap crop in field crop pest management. The grassy vegetation and small amount of decaying litter makes the application of acaricides in this area during peak abundance a financially and environmentally friendly alternative to widely distributed acaricides. Simply modifying the habitat by mowing during early June in central Ohio increases mortality. To increase the potential for killing the majority of ticks along the road edge, we suggest blowing the grass clippings on the hot road surface instead of throwing them into the remaining tall grass. Lowering the average ambient humidity by mowing has long been prescribed for tick control and our findings add the element of timing to this tactic. Waiting for this accumulation would add to the effectiveness. Utilizing strategic road-edge mowing as the sole means of tick control may not suppress the entire population but it appears to considerably reduce tick numbers in high-use areas. Mowing or applying acaricides to vegetation within 2 m of the road edge appears to be a highly effective method that can supplement other control strategies of *D. variabilis*.

A byproduct of our sampling was seeing significantly more males early in the tick season (April). This phenomenon has been seen before (Sonenshine and Staut 1971). One potential explanation for males emerging from their overwintering sites before females is to provide an advantage for mating, as they would be partially fed and attached to hosts when the females arrive. This hypothesis is supported by observations
of male *D. andersoni* being placed on a cow in fall and remaining attached until March 28 (Wilkinson, 1968). *Dermacentor variabilis* are not questing during September or October (Fig. 6.4) and presumably are not presented with the opportunity to remain on the host throughout winter. It is unclear why these ticks cannot be collected in late August as they can feed during the extremely harsh periods of midwinter (Stewart et al. 1998).

Adult *D. variabilis* accumulate along roadsides and vehicular road usage appears to be the primary attractant but this needs to be tested within a controlled environment. Local, single lane bi-directional roads do not represent significant barriers to tick movement. Mowing roadsides increases mortality in high use areas and should provide an economical method of tick control in selected locations. Although it does not appear to decrease the local tick population on the short term, road edges serve as a "trap crop" and if strategically mowed over consecutive years may decrease the local tick population. This tactic should be implemented into regionalized tick control programs for *D. variabilis*. Research should be implemented to assess the attractiveness of roads for other species as roadside modification or acaricidal application should be one of the most economical methods to control ticks in areas that humans tend to frequent. Mowing roadsides should be encouraged especially in high-use areas rather than letting them grow wild. The Departments of Transportation at the county and state levels should be aware of the benefits of timely mowing and should use this method to protect their workers if their schedules are flexible enough to permit mowing during regionalized American dog tick peak abundance.
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CHAPTER 7

CONCLUSIONS

As early as the 1940's researchers were actively questioning the role of the environment on ticks' seasonality. Although these reports were completed decades ago, few studies been conducted to verify, contradict or add to their conclusions about survival throughout winter. In some cases, these seminal studies represent the known information on tick survival while off the host. Papers by Lee, Lees, McEnroe, Needham and Sonenshine have directed me to ponder many points concerning off-host survival, which led me to conduct studies on tick overwintering. Several advances have been made in specific off-host areas such as water balance. Whenever new information has been complied, it often focuses around ticks enduring a particular season (usually summer in North America). Even today, little research exists dealing with the ecological and physiological factors governing tick survival during winter and the role of winter in temperate areas in regulating distribution. Therefore, I endeavored to characterize overwintering strategies for two of the most important ticks in North America. My experiments focused on D. variabilis, the principle vector of Rocky Mountain spotted fever in the east and A. americanum an extremely efficient vector of the causative agent for Ehlichiosis and Anaplasmosis. Better understanding of the physiological and
ecological mechanisms utilized by these two species should aid in the development of novel control strategies during periods when ticks may be vulnerable.

Using *D. variabilis* maintained under laboratory conditions for multiple generations and field-collected adults, overwintering experiments were conducted to compare *A. americanum*: 1) survival, 2) overwintering microhabitat, 3) evaluate metabolic reserves during winter, 4) determine hydration status and comparative water absorption characteristics and 5) attempt to place overwintering *D. variabilis* in previously described diapause. Additionally, non-acaricidal control of ticks was evaluated during summer as ticks localize into a narrow zone along roadsides. Survival between species was monitored during three very different winters (Figs. 2.1-2.3). Survival of *D. variabilis* was consistently higher than *A. americanum* except during extremely mild winters when survival was not significantly different. Survival was expected to be higher for *D. variabilis* as its distribution extends much farther north (Figs. 1.1, 1.2). Nymphal survival between years was unexpected as the majority of both species survived during milder conditions. No *A. americanum* and less than one percent *D. variabilis* nymphs survived colder winters. This indicates that *D. variabilis* nymphal survival is minimal in northern temperate areas and that cold temperatures restrict the range of *A. americanum*. Microhabitat selection for adults of these two species was very similar. Ticks remained on the soil surface and overwintered between the litter layer and the substrate. No ticks were encountered deep within the soil except where naturally occurring cracks occurred. Microhabitats between ecologically similar areas, however, appeared to differ substantially as degree warming days significantly affected survival. Overwintering microhabitats are underrated in modeling systems that attempt to predict
where different species could occur. They not only dictate survival but also appear to be highly variable within ecologically similar habitats located within close proximity. Predictions concerning spring abundance can be made but are different from what one would normally predict. The most successful overwintering survival for both species was when temperatures were moderately mild. Extremely harsh or mild winters (as seen during the winters of 1995 and 97, respectively) produced greater mortality, which resulted in an average population the following season. So to answer the question: is it going to be a good or bad tick year? one must consider numerous factors within a specific locality but rely heavily on degree warming days.

Chapter 2 represents the first comparative overwintering study conducted with these two species. Many species have been compared to A. americanum and future comparisons to it should provide a benchmark to evaluate overwintering characters for numerous other species. Comparing species within a given geographical area is paramount if efficient progress is to be completed for overwintering. The primary objective of this study was to compare survival of a southern and northern tick species in Ohio and determine if winter conditions have historically eliminated populations of A. americanum. Specifically, is winter mortality regulated by naturally occurring low temperatures? It is apparent that winter temperatures regulate survival in both species and that lower winter temperatures may have historically excluded A. americanum from Ohio. Populations may have been present in Ohio in the past during warmer winters but have gone extinct during colder ones. It will be interesting to see what happens to the newly established population of A. americanum near Cincinnati. OH.
Metabolic reserves during tick overwintering may play a significant role in survival. Generally, lipid levels increase as ticks digest blood meals obtained while feeding as nymphs. This occurs irrespective of environmental conditions. The rate at which lipids are accumulated or used appears to be highly dependent on temperature. When ticks are exposed to winter temperatures, they seem to store much more lipid than if they are exposed to warm indoor conditions. Production of storage compounds did not appear to be different between species and most likely does not contribute to differential survival. This increased level of lipid within overwintering ticks appears to convey some cold protection as more ticks with higher lipid levels survived than ticks with low lipid levels when exposed to cold temperatures. This connection between lipid levels and cold-tolerance maybe presumptuous as levels of other biologically active compounds were not assayed. Ticks appear to utilize lipid as their primary source of energy during winter as glycogen levels remained relatively consistent. Cryoprotectant production, such as glycerol, is highly unlikely because no significant increases of glycogen were observed toward the end of winter. If glycogen were being utilized, it would have been broken down into its primary building blocks and glycogen levels would have dropped.

The objective for chapter 3 was to observe lipid levels in ticks throughout winter, specifically to examine lipid and glycogen reserves and determine if differences in production or utilization are contributing to differential survival. This objective was met.

Since more cold temperature tolerance was observed in *D. variabilis* stored product comparisons were conducted. When they demonstrated that little observable difference between the two species was evident, overwintering hydration states and water absorption was compared. The effective temperature for successful water vapor
absorption between the two species is identical. Sixteen degrees appears to be the
threshold for successful water vapor absorption. No sustained absorption between the
two species was observed at temperatures below this point. Since this is the normal
method by which ticks acquire water, observations of ticks gaining water throughout
winter were surprising. Ticks of both species, appeared to increase hemolymph water as
the winter progressed and then during the later portions of winter, decreased hemolymph
water. Potentially, during winter ticks are unable to regulate water content and only
reacquire this mechanism when temperatures remain high enough to maintain essential
metabolic rates. Water acquisition throughout winter is likely passive as submerged ticks
are able to absorb water in vitro. If ticks loose the ability to regulate water throughout
winter, freeze induced mortality may be increased. Fewer osmolarity values were
obtained for overwintering A. americanum than D. variabilis so comparisons were
difficult to make. However, the hypotheses for this chapter, that the critical temperature
for water vapor absorption in D. variabilis is lower than A. americanum and that D.
variabilis remains partially desiccated throughout winter to increase cold-hardiness.
were met.

Diapause is utilized by numerous species of arthropods to endure inclement
temperatures. Despite the absence of experiments where diapause characteristics were
observed for D. variabilis, the literature describes the characteristics associated with
behavioral diapause in D. variabilis. Ticks are reported to cease questing and feeding
activity and decrease metabolic rates during the dormant state. In chapter 5, I present
data that strongly suggest overwintering D. variabilis are not utilizing diapause.
Overwintering, local strain adults, exhibited similar metabolic rates throughout winter to
laboratory maintained adults. Questing ability during winter was slightly diminished; however, the majority of adults quested in vitro throughout most winter months. Successful attachment and repletion was observed during each feeding period under indoor or outdoor conditions. Although these characters are not indicative of diapausing ticks, the window where they diapause may have been missed. Since *D. variabilis* activity in the summer ceases during mid August, assays conducted in the middle of October observed ticks that had completed diapause and appeared dormant because temperatures were not consistently warm enough to sustain activity. Similarly, adults may not enter a physiologically preprogrammed state of behavioral diapause but instead utilize a lesser form of dormancy. While the information detailing diapause in a variety of insects is reaching critical mass, behavioral diapause in ticks is in its infancy. Potentially alternative characteristics indicate tick diapause and these characters were not assayed during this study. While these characters remain to be studied, the specific objectives of this chapter, overwintering diapausing ticks do not quest or feed and have significantly decreased metabolic rates, were completely met.

While ticks were being collected for overwintering studies, I realized that significantly more could be collected along the roadside than in adjacent fields. The specific objectives for chapter 6 were to test if adult Ohio *D. variabilis* accumulate along roadsides during the summer, if timely mowing decreases the number of roadside ticks, does the road acts as a barrier that facilitates roadside accumulation and does road usage influences roadside abundance. Marked ticks moved toward roadsides and no ticks were observed further away from the road than the release point. Accumulation was minimal in the spring and intensified during mid June. Timely mowing, during mid June.
significantly reduced roadside populations and initially appears to be able to provide
effective relief from ticks in areas highly frequented by people. Ticks do not appear to be
accumulating along roadside because they are unable to cross as similar numbers of
marked ticks were found on either side of the road. Ticks are most likely drawn to
roadsides by vehicular usage. Carbon dioxide has been shown to excite *D. variabilis* as
well as numerous other species. The effect of roads on other species has not been studied
but if other species do accumulate along roads, this may provide opportunities to
economically repress populations in high human use areas.

Tick overwintering deserves additional attention by researchers. Little
information exists describing the ecological and physiological implications of surviving
winter. This dissertation surveys numerous physiological responses of winter in two of
the most economically important tick species in North America; however it is only the
beginning. It lays initial groundwork where little information was present in the three
areas relevant for tick survival during off host periods: temperature, bioenergetics and
water balance. Many of the specific details described within should be further analyzed
and may be important for overwintering of other arthropod species. Future directions
should be focused on temperature tolerance differences as many similarities were
observed between species.
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