

THE TICKS OF INSULAR NEWFOUNDLAND AND
THEIR POTENTIAL FOR TRANSMITTING DISEASE

CENTRE FOR NEWFOUNDLAND STUDIES

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The ticks of insular Newfoundland and their potential for transmitting disease.

by

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Abstract

A two – year study was undertaken to determine the tick fauna of insular Newfoundland and explore their potential as vectors of pathogens to both humans and animals. During 2002 and 2003 seven species of ixodid ticks were collected from nine hosts (snowshoe hare, Lincoln's sparrow, domestic cat, domestic dog, domestic rabbit, Atlantic puffin, common murre, red fox and human) on the island portion of the province: *Haemaphysalis leporispalustris*, *Ixodes uriae*, *I. muris*, *I. scapularis*, *I. ricinus*, *Dermacentor variabilis* and *Rhipicephalus sanguineus*. The most common species was *H. leporispalustris* with July, August and September being the peak period of activity. Although higher numbers of *H. leporispalustris* were collected from male hares than female hares, the difference between the number of ticks of each life stage found on each sex was not statistically significant, except for male ticks in 2002. All life stages of *H. leporispalustris* were capable of surviving temperatures as low as 0°C but none lower than -5°C. Another species that showed a seasonal distribution for the females was *I. scapularis*. This tick had a bimodal temporal distribution with no ticks recorded during August and September. Four pathogens were tested for: *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, *Francisella tularensis* and vector – borne viruses, but only *B. burgdorferi* was detected in 16 % of the 14 *I. scapularis* tested. The overall conclusion was that Newfoundland has relatively few tick species most of which are introduced. Although the threat of acquiring pathogens from ticks is low, the detection of *B. burgdorferi* in this study and viruses from *I. uriae* in previous studies, means ongoing monitoring of tick populations and the pathogens they vector, in the province is prudent.

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Introduction

Tick Characteristics

Ticks belong to the Class Arachnida, Subclass Acari, Order Parasitiformes, Suborder Ixodida and are thought to have evolved as early as the Cambrian and as late as the Devonian period (Barker and Murrell 2002, Krantz 1978, Roberts and Janovy 1996, Sonenshine 1991a). The Suborder Ixodida can be divided into three Families: Ixodidae (the hard ticks - 12 genera with 683 species), Argasidae (the soft ticks - 4 genera with 183 species), and Nuttalliellidae (just one species - *Nuttalliella namaqua* of which only females have been found) (Horak *et al.* 2002).

The hard ticks and the soft ticks may be distinguished by several characteristics. The hard ticks possess a scutum on the dorsal body surface whereas the dorsum of a soft tick is composed a leathery cuticle which is folded to expand during feeding (Gregson 1956, Roberts and Janovy 1996, Sonenshine 1991a). The scutum that covers the dorsal surface of the ixodid ticks is limited to the anterior dorsal body region in larvae, nymphs and adult females while the males have a complete scutum dorsally (Gregson 1956, Sonenshine 1991a). In addition, hard ticks have a capitulum (which bears the mouthparts) that is terminal and can be seen in the dorsal view. The capitulum of a soft tick, however, is subterminal and cannot be seen when the tick is viewed dorsally (Gregson 1956, Roberts and Janovy 1996, Sonenshine 1991a). The capitulum of a soft tick is actually located in a groove called the camerostome. The dorsal portion of the camerostome, known as the hood, can sometimes be seen from the dorsal view (Roberts and Janovy 1996).

The life cycles of all ticks consist of four stages: egg, larva, nymph and adult (Martin 1978, Roberts and Janovy 1996, Sonenshine 1991a). Larvae have three pairs of legs while nymphs and adults have four pairs (Gregson 1956, Krantz 1978, Sonenshine 1991a). Nymphal ticks lack a genital opening and are generally smaller than adults. Ventrally male ixodid ticks have a set of small chitinized sclerites which are absent in the female (Gregson 1956, Krantz 1978, Roberts and Janovy 1996, Sonenshine 1991a).

Life Cycle

Most Ixodidae have a three - host life cycle with larva, nymph and adult each feeding on separate individual hosts. The hosts that are parasitized by a tick with a three - host life cycle may be the same species for each life stage or they may be different species for each life stage. After each blood meal they drop from the host, digest the blood and develop to the next stage or lay eggs in the case of the adult female. Species that have a two - host life cycle remain on the host between the larval and nymphal stage to undergo development to the next stage on the same host. In species that have a one - host life cycle a larva seeks out a host but neither larva nor nymph leaves the host after the blood meal and thus only adults drop from the host (Roberts and Janovy 1996, Sonenshine 1991a). In contrast, argasid ticks feed rapidly and then leave the host. They may feed more than once in one life stage and may lay more than one batch of eggs (Sonenshine 1991a).

All ixodid tick species require a blood meal before oviposition and mating usually occurs on the host except for members of the genus *Ixodes*. The male completes mating by depositing a spermatophore under the genital operculum of the female (Roberts and

Janovy 1996). The fed females then drop off the host and oviposit in a sheltered environment, e.g. under leaf litter or some crevice within a man - made shelter. Once in the environment, the females lay thousands of eggs within a single oviposit. Most of the eggs are laid within the first ten days after mating (about 90% of the egg mass) and then small numbers of eggs are laid after the tenth day until the fatigued female dies (Sonenshine 1991a). Members of the genus *Ixodes* have a different pattern of development than other ixodid ticks. In this genus, when the nymphs molt into adults, gametogenesis begins and mating can occur before the adults feed. Vitellogenesis and egg maturation are then delayed until the blood meal (Sonenshine 1991a).

The life cycle of members of the Family Argasidae differs from those of the Ixodidae in that argasid species can have two or more nymphal stages therefore making the life cycle of a soft tick longer than that of a hard tick (i.e. up to several years for a soft tick compared to one to three years for a hard tick) (Roberts and Janovy 1996, Sonenshine 1991a). The female soft ticks do not necessarily die after oviposition but can continue to take blood meals and oviposit several times (Roberts and Janovy 1996, Sonenshine 1991a). In most species, the larvae search for a host, take a blood meal in approximately fifteen minutes and drop off the host. The emerging nymphs also search for a host, rapidly take a blood meal and drop off the host. Soft ticks can have up to eight nymphal stages depending on certain factors. The number of nymphal stages is not constant between or within species. Adult males usually appear before the females (Sonenshine 1991a). Due to the repeated feeding of soft ticks they tend to be associated with nest sites, burrows or places where hosts return for a period of time.

Reproduction in argasid ticks is quite similar to that of *Ixodes* spp. Argasid ticks

are sexually mature as soon as the nymphs molt into the adults and mating usually occurs before the female feeds (Sonenshine 1991a). Once fed and blood digested a female can oviposit hundreds of eggs. The females may feed and oviposit up to six times in her life (Sonenshine 1991a). Some argasid ticks even display brooding behaviour. They remain with the egg mass until the eggs hatch and one species, *Argas boueti*, has been known to transfer the larvae to a place which will increase their chance of finding a suitable host (usually bats) (Estrada-Pena and Jongejan 1999, Sonenshine 1991a).

Locating Hosts

Most Ixodidae search for their hosts by waiting on vegetation rather than waiting in the nests and burrows of their hosts. All ticks use the very specialized Haller's organ to detect the presence of hosts (Sonenshine 1991a). This sensory organ is located on the first tarsus of the forelegs and ticks use their forelegs like an insect uses its antennae. The Haller's organ is responsive to carbon dioxide, ammonia, lactic acid as well as vibrations and the body temperature of warm - blooded animals (Sonenshine 1991a). Carbon dioxide is a well - known attractant for ticks and may very well be one of the ways that ticks locate their hosts (Carroll 2002, Falco and Fish 1989, Schulze *et al.* 2001). Some ixodid ticks may also use deer urine and squalene, a naturally occurring mammalian skin secretion, to locate their hosts (Carroll 2000, Schulze *et al.* 2001). In a recent study conducted by Carroll (2002) the behavioural response of *Ixodes scapularis*, *Dermacentor variabilis*, and *Amblyomma americanum* to residues from the body of their primary hosts was examined. It was shown that after 24 hours host – seeking adults of all three tick species displayed arrestment behaviour to glass rods rubbed with substances from the

tarsal glands of white – tailed deer and *D. variabilis* also showed this arrestment behaviour to glass rods rubbed with substances from the dorsal surface of dogs ears (Carroll 2002). This arrestment behaviour to substances secreted by their hosts greatly increases the chance of that tick finding a suitable host as presence of the substance indicates that the hosts once passed this spot (e.g., edge of run or trail) and may do so again.

Vector Competence and Pathogen Transmission

Vector competence may be defined as the ability of an arthropod to become infected, to perpetuate and to transmit a disease agent (Brown and Lane 1992). Hard ticks are successful pathogen vectors not only in the number of pathogens they transmit but also in the variety (i.e. fungi, viruses, bacteria, filariae and protozoa) (Randolph 2000, Roberts and Janovy 1996, Sonenshine 1991a). Some of the reasons for their success as vectors of disease include: long life spans, long feeding times and slow digestive processes (Randolph 2000, Sonenshine 1991a).

The life span of a hard tick is very long when compared to other blood sucking arthropods. Tick life spans are usually measured in years as opposed to days or months (Roberts and Janovy 1996, Sonenshine 1991a). Because of these long life spans ticks are able to carry and transmit pathogens for longer periods of time than other arthropods. In addition to the long life span of a hard tick, they also feed on the host for a much longer time (often feeding for hours or days as opposed to seconds or minutes) than most other arthropods (Ribeiro *et al.* 1985). Most ixodid species produce a cement – like substance that attaches them closer to the skin of the host (Roberts and Janovy 1996, Sonenshine

1991a). This prolonged contact with the host also contributes to the tick's success as a vector (Randolph 2000).

Finally, the digestive processes of ticks are extremely slow (Sonenshine 1991a). Once blood is taken as a meal it sits in the midgut and remains undigested for a long period of time (being consumed gradually over months and in some cases years). The blood meal remains as a food reserve and is consumed little by little except during oviposition or ecdysis when it is consumed faster. There is no great influx of digestive enzymes, which allows pathogens that may be present to survive. By the time the blood is digested the pathogens have had time to migrate into the tick's body tissues and remain unharmed (Sonenshine 1991a). Sonenshine *et al.* (2002) stated that *Ixodes scapularis*, the primary vector of Lyme disease (caused by *Borrelia burgdorferi*) in North America, did not produce defensins (i.e. small antimicrobial peptides) in response to the bacteria therefore allowing it to survive. This bacterium is also capable of surviving within the tick during molting which means that any bacteria obtained by a larva will survive the molt to a nymph and to an adult (Brown and Burgess 2001).

Ticks subsist by sucking the blood of other animals including birds, reptiles, amphibians and mammals. They are found worldwide in practically all terrestrial environments and are second only to mosquitoes as vectors of numerous human and animal disease causing agents (Randolph 2000, Sonenshine 1991a). Just a few of the pathogens that ticks are capable of transmitting include: Lyme borreliosis (also known as Lyme disease - a multi - order disease caused by a bacterium - *Borrelia burgdorferi*) (Brown and Burgess 2001, Dolan *et al.* 2000, Falco and Fish 1989, Greene 1990, Kahn and Line 2005, Pelczar *et al.* 1993, Randolph 2000, Randolph 2001, Roberts and Janovy

1996, Sonenshine 1991b), human granulocytic ehrlichiosis (caused by *Anaplasma phagocytophilum* – a bacterium that infects white blood cells) (Daniels *et al.* 2002, Drebot *et al.* 2001, Ramsey *et al.* 2002, Sonenshine 1991b), tularemia (a bacterial disease caused by *Francisella tularensis*) (Gregson 1956, Kahn and Line 2005, Sonenshine 1991b), Rocky Mountain spotted fever (a severe bacterial disease caused by *Rickettsia rickettsia*) (Gregson 1956, Kahn and Line 2005, Sonenshine 1991b), heartwater (a bacterial disease of ruminants caused by *Cowdria ruminatum*) (Kahn and Line 2005, Sonenshine 1991b), Q fever (caused by the bacterium *Coxiella burnetii*) (Gregson 1956, Kahn and Line 2005, Sonenshine 1991b), babesiosis (a disease of humans caused by a protozoan, *Babesia* spp.) (dos Santos and Kain 1998, Kahn and Line 2005, Lindsay *et al.* 1998, Lindsay *et al.* 1999, Sonenshine 1991b), East Coast fever (caused by the protozoan *Theileria parva*) (Kahn and Line 2005, Sonenshine 1991b), tick – borne encephalitis (a hazardous viral disease) (Kahn and Line 2005, Randolph 2000, Randolph 2001, Sonenshine 1991b), several other viral diseases of birds and mammals, three of which have been identified from seabirds in Newfoundland previously – Avalon, Bauline and Great Island viruses (Chastel 1988, Nuttall 1984) and tick paralysis (caused by a neurotoxin that is released from the salivary glands of ticks while feeding) (Beyer and Grossman 1997, Gregson 1956, Kahn and Line 2005, Sonenshine 1991b).

Tick – Borne Pathogens in Insular Newfoundland

Lyme disease was first discovered in humans in 1975 while several hundred cases of a mysterious infection were being investigated in Old Lyme, Connecticut (Anon. 1988, Pelczar *et al.* 1993, Spielman *et al.* 1985). However, museum specimens of ticks from

Long Island were found to be carrying *Borrelia burgdorferi* as early as 1945 (Romoser and Stoffolano 1998, Pelczar *et al.* 1993). The disease occurs mainly in the northeastern states from Massachusetts to North Carolina but has also been reported in the midwestern and western states of the United States in addition to Germany, Switzerland, France and Australia (Brown and Burgess 2001, Falco and Fish 1989, Pelczar *et al.* 1993). Cases of human Lyme disease diagnosed in Canada have been relatively low (Lindsay *et al.* 1998). Those provinces affected include British Columbia, Alberta, Manitoba, Ontario, Quebec, New Brunswick and Nova Scotia (Anon. 1988, Anon. 1991a, Anon. 1991b, Anon. 1997, Anon. 2001c, Anon. 2002, Barker *et al.* 1992, dos Santos and Kain 1998, Greenwood 2004). Continued surveillance for the tick vectors of Lyme disease in Canada is taking place in all provinces to determine the spread of the vector as the ticks are known to be found in the Maritimes and have also been documented to use migratory birds as hosts thereby spreading the ticks over long distances (Scott *et al.* 2001).

Lyme disease is caused by the bacterium *Borrelia burgdorferi* and is transmitted by infected three – host ticks, namely certain *Ixodes* spp. (Anon. 1988, Brown and Burgess 2001, Falco and Fish 1989, Pelczar *et al.* 1993). The bacterium is not usually transmitted transovarially and so an infected tick (either a nymph or an adult) must transfer the bacterium to the host when taking a blood meal after becoming infected from an infected host during the taking of the previous blood meal (Brown and Burgess 2001, Greene 1990, Pelczar *et al.* 1993).

The ticks that serve as competent vectors of the Lyme disease bacterium to man and other larger vertebrates are those within the *Ixodes ricinus* species complex (Anon. 1991a, Brown and Burgess 2001, Brown and Lane 1992). These are a group of ticks

which are capable of supporting the pathogen within the midgut and/or the salivary glands and transmitting the Lyme disease spirochete while blood feeding. The *I. ricinus* complex includes *Ixodes ricinus* (in Europe), *I. persulcatus* (eastern Europe and northern Asia), *I. pacificus* (western North America), and *I. scapularis* (eastern United States) (Brown and Burgess 2001, Dolan *et al.* 2000, Greene 1990). These ticks are generalist feeders and the nymphal stages of these ticks are active earlier in the year than are the larval stages. The larvae then acquire infections from animals that were previously infected with the bacteria by the nymphs (Brown and Burgess 2001, Greene 1990, Sonenshine 1991b, Spielman *et al.* 1985, Yuval and Spielman 1990). It normally takes up to 24 hours of attachment to a host before *I. scapularis* can transmit *Borrelia burgdorferi* (Greene 1990, Peavey and Lane 1995, Piesman *et al.* 1987). This delay in transmission is influenced by characteristics of how ticks feed and by the distribution of the bacterium within the body of the tick. There are pharmacological constituents in the saliva of *I. scapularis* that increase the transmission of *B. burgdorferi* (Peavey and Lane 1995, Ribeiro *et al.* 1985). In addition, before the tick feeds the bacteria are mainly found in the midgut of the tick (Burgdorfer *et al.* 1982) but as the tick takes a blood meal the bacteria multiply, cross the midgut wall, and circulate to the salivary glands (Benach *et al.* 1987, Peavey and Lane 1995, Ribeiro *et al.* 1987, Zung *et al.* 1989). *Borrelia burgdorferi* is maintained in rodent reservoir hosts (white – footed mice (*Peromyscus leucopus*), cotton mice (*P. gossypinus*), deer mice (*P. maniculatus*), European dormice (*Glis glis* and *Eliomys quercinus*), *Apodemus* spp., dusky – footed wood rats (*Neotoma fuscipes*), California kangaroo rats (*Dipodomus californicus*) and other rats (*Rattus norvegicus* and *R. rattus*) and not in large vertebrate reservoirs (white – tailed deer

(*Odocoileus virginianus*) and roe deer (*Capreolus capreolus*)) (Donahue *et al.* 1987, Jaenson and Talleklint 1992, Lane and Brown 1991, Oliver *et al.* 2000, Rand *et al.* 1993, Richter *et al.* 2000, Spielman *et al.* 1985, Telford *et al.* 1988). The cycles of the bacteria in the small mammal population can be maintained by other tick species such as *Ixodes muris* (Dolan *et al.* 2000, Randolph 2001), *I. neotomae* (Brown and Lane 1992) or *I. dentatus* (Telford and Spielman 1989).

Human granulocytic ehrlichiosis (HGE) was first described in Minnesota and Wisconsin in 1994 (Daniels *et al.* 2002, Drebot *et al.* 2001, Ramsey *et al.* 2002, Walker and Dumler 1996) and was considered the second most common tick – borne disease in the United States (Daniels *et al.* 2002). Within Canada an HGE – like agent was isolated from *Ixodes scapularis* in Ontario but there have been no reports of the development of the disease in humans (Drebot *et al.* 2001). The HGE agent is a bacterium known as *Anaplasma* (formerly *Ehrlichia*) *phagocytophilum* and is transmitted by *I. scapularis* (Daniels *et al.* 2002, Drebot *et al.* 2001, Ramsey *et al.* 2002). These bacteria infect the white blood cells, more specifically neutrophils (Chen *et al.* 1994, Ramsey *et al.* 2002, Walker and Dumler 1996). While details of the events leading to the development of the disease are not fully understood it is suspected that the bacteria infect myeloid precursors in the bone marrow rather than mature neutrophils and subsequently repress immune defenses (Walker and Dumler 1996).

Symptoms can occur anywhere from five to twenty – one days after exposure to a tick bite and are normally nonspecific but most often they include: fever, muscle pain, severe headache and shaking chills (Ramsey *et al.* 2002, Walker and Dumler 1996). Less frequently, a person suffering from HGE experiences nausea, vomiting, weight loss,

mental confusion, cough and skin rash (Walker and Dumler 1996). Some people that have been treated with antibiotics for HGE still experience fevers, chills, sweats and fatigue one to three years after the onset of the disease which has been attributed to a postinfectious syndrome (Ramsey *et al.* 2002). In rare cases the disease can be fatal (Daniels *et al.* 1997, Ramsey *et al.* 2002).

Tularemia, more commonly known as rabbit fever, was described as early as 1820 in Japan as a disease in people who had eaten rabbit meat, however, it was not until the early 1900s before the causative agent was discovered and the disease was recognized as tularemia (Morner and Addison 2001, Sonenshine 1991b). The disease is widespread throughout the United States, Canada, Mexico, almost all parts of the former USSR and most countries in central Europe (Arthur 1961, Hornick 2001, Morner and Addison 2001). Tularemia is caused by the gram – negative bacillus *Francisella tularensis* (Eliasson *et al.* 2002, Farlow *et al.* 2001, Feldman *et al.* 2001, Feldman *et al.* 2003, Friedrich 2000, Hornick 2001, Morner and Addison 2001, Sonenshine 1991b). There are two subspecies of the bacterium: Type A (*F. tularensis* subsp. *tularensis*) which is highly virulent and occurs mainly in North America and Type B (*F. tularensis* subsp. *holarctica*) which causes only a mild form of the disease and occurs throughout the Northern Hemisphere (Eliasson *et al.* 2002, Farlow *et al.* 2001, Feldman *et al.* 2001, Hornick 2001, Morner and Addison 2001). Type A is usually tick – borne but can also be contracted by direct contact whereas Type B is most often water – borne (Farlow *et al.* 2001, Sonenshine 1991b). The bacteria can be transmitted in a variety of ways. These include: the bites of ticks, deerflies, mosquitoes and other blood sucking arthropods; contact with blood or tissue of infected animals (hundreds of wild animals are capable of serving as a

reservoir for the bacteria and bacteria can be contracted through unbroken skin); the ingestion of contaminated food or water; and the inhalation of contaminated aerosols (Feldman *et al.* 2001, Feldman *et al.* 2003, Friedrich 2000, Hornick 2001, Morner and Addison 2001, Sonenshine 1991b). It is not directly transmitted from person to person (Feldman *et al.* 2001).

In general, tularemia has an incubation period of about two to seven days in humans and people suffering from tularemia have swollen lymph nodes and are plagued by chills, fever, pain and rapid weight loss (Feldman *et al.* 2003, Gregson 1956, Morner and Addison 2001, Sonenshine 1991b). If the bacterium is inhaled, the person may develop pneumonia. Inhalation of the bacterium is the most severe form of tularemia (Feldman *et al.* 2001, Feldman *et al.* 2003, Morner and Addison 2001). If the bacterium is contracted by a tick bite, most people develop papulae that become pustular and ulcerated at the site of the tick bite (Morner and Addison 2001, Sonenshine 1991b).

Animals that have contracted tularemia in the wild (Type A usually infects rabbits while Type B usually infects muskrats, beavers and hares) (Morner and Addison 2001) usually have gross lesions in the form of white spots throughout the liver, spleen and lymph nodes (Friedrich 2000). Diagnosis in humans is made by serologic testing but antibody titers take approximately a week to become positive (Hornick 2001). The bacteria can also be cultured from sputum, gastric lavage fluid and sometimes blood (Hornick 2001).

Vector – borne viruses are viruses of several types or variants that can be transmitted by different species of ticks. About 50 viruses or variants have been isolated from ticks and the main vector of these viruses in sea birds is *Ixodes uriae* – a hard tick

that usually feeds on seabirds making viral infections common in the seabird populations infested with *I. uriae* (Chastel 1988). These viruses are considered to be arboviruses, or arthropod – borne viruses, which means that they replicate in a blood – sucking arthropod and are transmitted to a vertebrate host when that arthropod takes a blood meal from it. The virus is then capable of replicating within the blood and/or tissues of the infected vertebrate and can then be contracted by another arthropod when it feeds on the infected host (Nuttall 1984).

Three variants (i.e. Avalon, Bauline and Great Island) have been isolated from *Ixodes uriae* in Newfoundland (Chastel 1988). Avalon virus (*Nairovirus*, Bunyaviridae) belongs to the Sakhalin serogroup while Bauline virus and Great Island virus both belong to the Kemerovo serogroup (*Orbivirus*, Reoviridae) (Chastel 1988). Oprandy *et al.* (1988) reported that 18 *Orbivirus* isolates in total have been recorded from Great Island and Yunker (1975) reported these two plus Avalon virus from Great Island. Despite the fact that infections with these viruses seem to be quite common among seabirds, only minor problems among the birds have been noted (Chastel 1988). These problems are more associated with the infestation of the ticks themselves (i.e. blood loss, irritation, pruritus, etc.) than the viruses they transmit (Chastel 1988). While these viruses can also be transmitted to humans, infections are rare and only mild when they do occur (Chastel 1988).

In addition to being vectors of disease, hard ticks are also considered pests of livestock, wildlife, and humans, causing painful and irritating bite wounds that often lead to secondary infections by microbial agents (Bishopp and Trembley 1945, Randolph 2000, Sonenshine 1991a). Also, these bites are usually of long duration (Roberts and

Janovy 1996, Sonenshine 1991a), cause immune responses (Roberts and Janovy 1996, Tragar 1939), and severe blood loss (Bishopp and Trembley 1945, Roberts and Janovy 1996, Sonenshine 1991a). The blood loss from tick bites is much more serious than that of other arthropods since ticks feed for longer periods of time and they consume much larger blood meals (Roberts and Janovy 1996, Sonenshine 1991a).

Tick Studies in Canada

Little research on the distribution of ticks in Canada and particularly Newfoundland has been carried out for several years. Bishopp and Trembley (1945) completed a list of North American tick species and included the following species in their list for Canada: *Amblyomma americanum* (only one unfed female specimen from Labrador), *Argas miniatus* (four nymphs collected in Vancouver), *Dermacentor albipictus* (most common and widespread Canadian species), *D. andersoni* (British Columbia and Manitoba), *D. variabilis* (southern parts of provinces – specific provinces not given), *Haemaphysalis chordeilis* (east of the Rocky Mountains in Canada), *H. leporispalustris* (widely distributed throughout Canada), *Ixodes angustus* (British Columbia and Ontario), *I. auritulus* (one nymph collected in Ontario), *I. brunneus* (no locality given), *I. cookei* (one specimen from Quebec), *I. diversifossus* (southwestern Canada – no specific locality given), *I. kingi* (Medicine Hat, Alberta), *I. marxi* (Guelph, Ontario and British Columbia), *I. ricinus scapularis* (Ontario), *I. sculptus* (no locality given), *I. texanus* (southern parts of Canada), and *Otobius megnini* (Bryant Creek, British Columbia).

Roberts and Janovy (1996) also included a list of some North American species in

their work but never specified where exactly the species had been found and the list they provided was not nearly as detailed as that provided by Bishopp and Trembley (1945). Roberts and Janovy (1996) simply stated that nearly 40 species of *Ixodes* and only 2 species of *Haemaphysalis* and *Dermacentor albipictus* have been found in North America.

Gregson (1956) wrote the first comprehensive survey of the ticks of Canada which included brief descriptions, illustrations, identification keys, common hosts and distribution. The following species were recorded by Gregson (1956): *Argas persicus* (British Columbia), *A. reflexus* (British Columbia), *Ornithodoros hermsi* (British Columbia), *Otobius lagophilus* (Alberta), *O. megnini* (British Columbia), *Dermacentor albipictus* (British Columbia, Alberta, Saskatchewan, Ontario, Nova Scotia and New Brunswick), *D. andersoni* (British Columbia, Alberta and Saskatchewan), *D. variabilis* (Saskatchewan, Manitoba, Ontario and Nova Scotia), *Haemaphysalis chordeilis* (British Columbia, Alberta, Saskatchewan, Manitoba, Ontario and New Brunswick), *H. leporispalustris* (British Columbia, Alberta, Saskatchewan, Manitoba, Ontario, New Brunswick, Nova Scotia and Newfoundland), *Ixodes angustus* (British Columbia, Alberta, Saskatchewan, Manitoba, Ontario, Quebec and Nova Scotia), *I. auritulus* (British Columbia and Ontario), *I. banksi* (Ontario), *I. cookei* (Manitoba, Ontario, Quebec, New Brunswick, Nova Scotia and Prince Edward Island), *I. hearlei* (British Columbia), *I. kingi* (Alberta and Saskatchewan), *I. marmotae* (British Columbia), *I. marxi* (Ontario), *I. muris* (Nova Scotia), *I. ochotona* (British Columbia), *I. pacificus* (British Columbia), *I. rugosus* (British Columbia), *I. sculptus* (British Columbia, Alberta and Saskatchewan), *I. signatus* (British Columbia), *I. soricis* (British Columbia), *I. spinipalpis* (British

Columbia and Alberta), *I. texanus* (British Columbia and Ontario), *I. uriae* (British Columbia and Nova Scotia), and *Rhipicephalus sanguineus* (Ontario, Nova Scotia and Quebec). Gregson (1956) also mentioned a few other tick species that may occur in Canada given the country's close proximity to the United States and also those tick species which may be introduced to Canada by way of hosts. The ticks listed that may occur as a result of close proximity included: *I. dentatus*, *I. scapularis*, *I. brunneus*, *O. parkeri* and *O. kelleyi* and those that may be introduced via hosts included: *D. occidentalis*, *D. parumapertus*, *O. concanensis*, *O. dyeri*, *O. hermsi*, *O. kelleyi*, *O. stageri*, *O. yumatensis* and *Antricola coprophilus* (Gregson 1956).

Lindsay *et al.* (1999) stated that the distribution of *Ixodes scapularis* in Canada is uneven and focal with low numbers of adults collected in southern Manitoba, Ontario, the eastern townships of Quebec, Nova Scotia, New Brunswick, Prince Edward Island and Newfoundland. Despite the various collections from different parts of Canada, Lindsay *et al.* (1999) declared that the only endemic area in Canada for this species of tick is on the Long Point Peninsula and Point Pelee National Park on Lake Erie, Ontario. The explanation given for the collection of *I. scapularis* in non – endemic areas of Canada is that they were transported there as larvae or nymphs by migrating birds (Artsob *et al.* 1992, Banerjee *et al.* 1995, Banerjee *et al.* 1996, Barker *et al.* 1992, Bjoersdorff *et al.* 2001, Klich *et al.* 1996, Lindsay *et al.* 1998, Lindsay *et al.* 1999, Morshed *et al.* 1999, Scott *et al.* 2001). The ticks arriving via migrating birds, however, do have the potential to establish populations as was illustrated recently in Nova Scotia when *I. scapularis* was found to be widespread throughout the province and was presumed to be established within the province (Anon. 2004).

Scott *et al.* (2001) studied ixodid ticks of Canada, which could be dispersed throughout the country via birds. This study is of importance since birds migrating both into and out of Canada have the potential to transport ticks to areas that the tick would never have otherwise been introduced to. The list of bird dispersed ixodid species for Canada includes: *Amblyomma americanum*, *A. longirostre*, *A. maculatum*, *A. sabanerae*, *Haemaphysalis leporispalustris*, *H. chordeilis*, *Ixodes baergi*, *I. brunneus*, *I. muris*, and *I. scapularis* (Scott *et al.* 2001).

In Nova Scotia, Campbell and MacKay (1979) and Garvie *et al.* (1978) have both reported that *Dermacentor variabilis* has well established populations and Davis and Campbell (1979) stated that *Haemaphysalis leporispalustris* was also well established there.

There have been few studies done on the ticks of insular Newfoundland. Usually the presence of ticks in the province is mentioned in works dedicated to other topics and so a complete list does not appear in any one place. Dodds and Mackiewicz (1961) and Gregson (1956) both agreed that *Haemaphysalis leporispalustris* had established populations on the island of Newfoundland and was usually found on snowshoe hares. The presence of *Ixodes uriae* on seabirds in Newfoundland has been well documented (Eveleigh 1974, Eveleigh and Threlfall 1974, McCoy *et al.* 1999, and Threlfall 1968). In addition, Colbo (2001 pers. comm.) had identified *Ixodes angustus* and *I. muris*, from a meadow vole and house mouse respectively, on the Avalon Peninsula of Newfoundland and *I. cookei* from pets that have traveled off the island. Finally, Whitney (2001 pers. comm.) has reported periodic instances of *Dermacentor variabilis* and *Rhipicephalus sanguineus* on family pets that have traveled off the island. Recently also *I. scapularis*

was recovered from pets that have had no history of travel off the island of Newfoundland.

In July 2001, the first record of a tick, *Ixodes scapularis*, carrying *Borrelia burgdorferi* was documented in Newfoundland (Whitney 2001). *Ixodes scapularis* had previously been collected in Newfoundland (in 1994 and in 2000) from dogs, but *B. burgdorferi* was never isolated from these ticks (Artsob *et al.* 2000). The tick that was collected in 2001 was taken from a dog, in the Cape Broyle area, that did not have any symptoms of Lyme disease. *Ixodes scapularis* is not known to be a resident tick of Newfoundland and it is not known how or when the tick first entered the province (Whitney 2001). Due to public health concerns the current study was undertaken to determine the tick fauna of insular Newfoundland and explore the role that these ticks play in transmitting pathogens to both humans and animals. The ticks collected in this study were tested for four pathogens: *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, *Francisella tularensis* and vector – borne viruses (Avalon, Bauline and Great Island).

Materials and Methods

Location of Study

The island of Newfoundland, which lies approximately between 46 and 51 degrees north latitude and approximately 53 and 60 degrees west longitude (Figure 1) in the northwest Atlantic Ocean, represents an extension of the boreal forest of eastern Canada. The island, as a result of the Wisconsin glaciation, was stripped of productive topsoils and was left with shallow glacial till. The natural flora and fauna were eliminated with possibly a few species surviving in isolated refugia. The present diversity stemmed from widely dispersing mainland taxa and accidental and deliberate exotic introductions (Macpherson and Macpherson 1981).

The climate of Newfoundland is largely dependent on latitude, its geographic location on the eastern side of a continent and the cold water that surrounds it. The cold western Atlantic Ocean generally keeps winter air temperatures a little higher and summer air temperatures a little lower than comparable mainland latitudes (Phillips 1990). The marine influence on climate results in large amounts of precipitation in various forms, high humidity, more cloud cover, reduced sunshine and stronger winds than similar latitudes in a continental zone (Phillips 1990). Prevailing wind directions are west – southwest in summer and west in winter and Newfoundland is well known for its strong winds (Banfield 1983, Macpherson and Macpherson 1981, Phillips 1990). Temperatures vary seasonally as well as with distance from the ocean. Inland winter temperatures are on average between -6 °C and -10 °C while coastal winter temperatures are on average between -2 °C and -4 °C. Summer temperatures are relatively cool with an

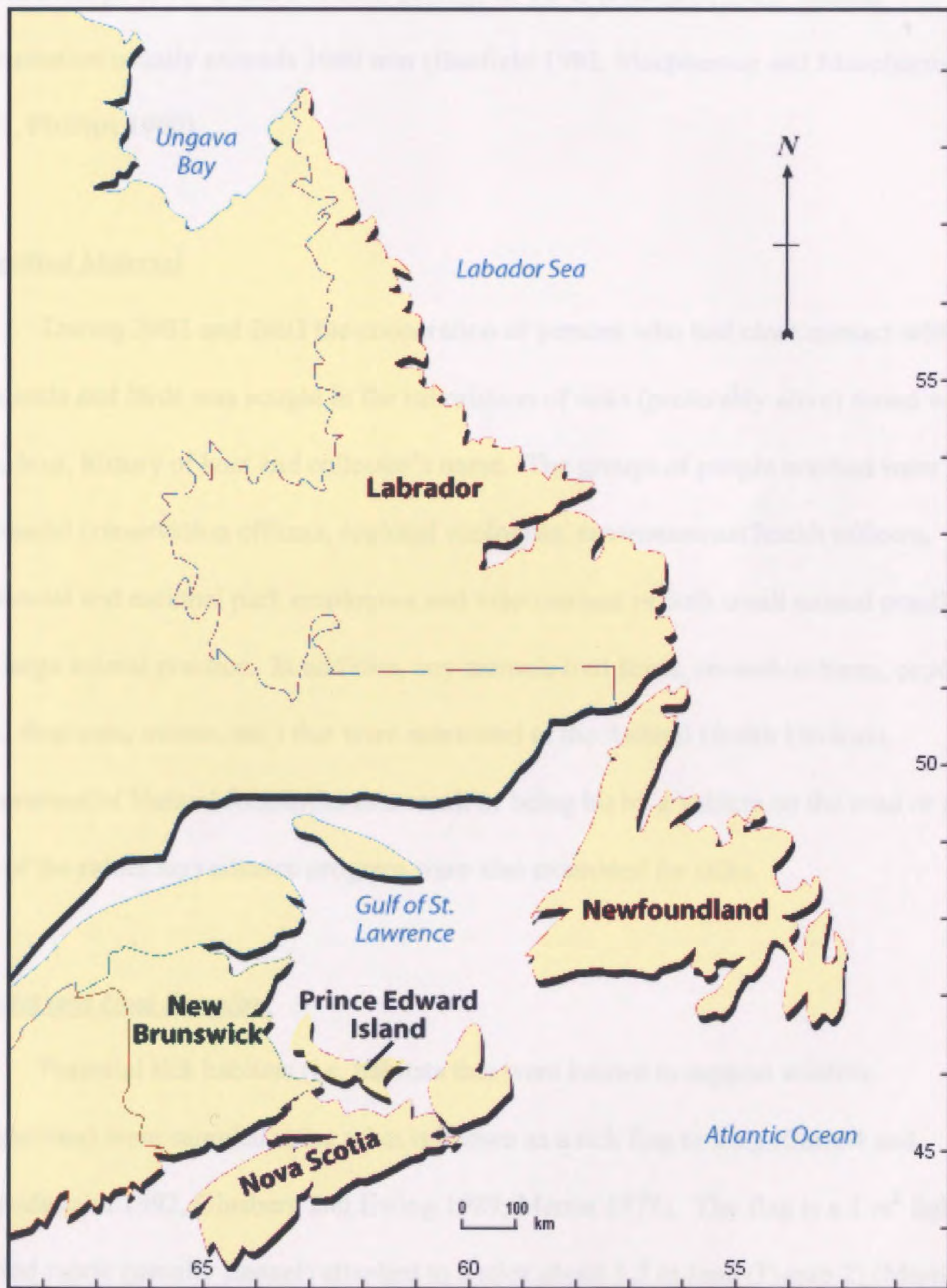


Figure 1: Map of Atlantic Canada showing the proximity of the island of Newfoundland to mainland Canada.

inland average of 16 °C and a coastal average of 14 °C (Phillips 1990). Annual precipitation usually exceeds 1000 mm (Banfield 1983, Macpherson and Macpherson 1981, Phillips 1990).

Submitted Material

During 2002 and 2003 the cooperation of persons who had close contact with land mammals and birds was sought in the submission of ticks (preferably alive) found with date, host, history of host and collector's name. The groups of people notified were provincial conservation officers, regional ecologists, environmental health officers, provincial and national park employees and veterinarians in both small animal practice and large animal practice. In addition, any animals (red foxes, snowshoe hares, coyotes, lynx, feral cats, moose, etc.) that were submitted to the Animal Health Division, Department of Natural Resources as a result of being hit by a vehicle on the road or as part of the rabies surveillance program were also examined for ticks.

Habitat and Host Sampling

Potential tick habitats (i.e. habitats that were known to support wildlife populations) were sampled using what is known as a tick flag or drag (Carroll and Schmidtman 1992, Ginsberg and Ewing 1989, Martin 1978). The flag is a 1 m² light colored fabric (usually flannel) attached to a stick about 1.5 m long (Figure 2) (Martin 1978). Hard ticks usually climb up vegetation and wait for an animal to brush past in order to gain a host. The tick flag is used to simulate an animal as it is slowly brushed over the vegetation. Ticks which grasp or fall onto the flag are then collected.

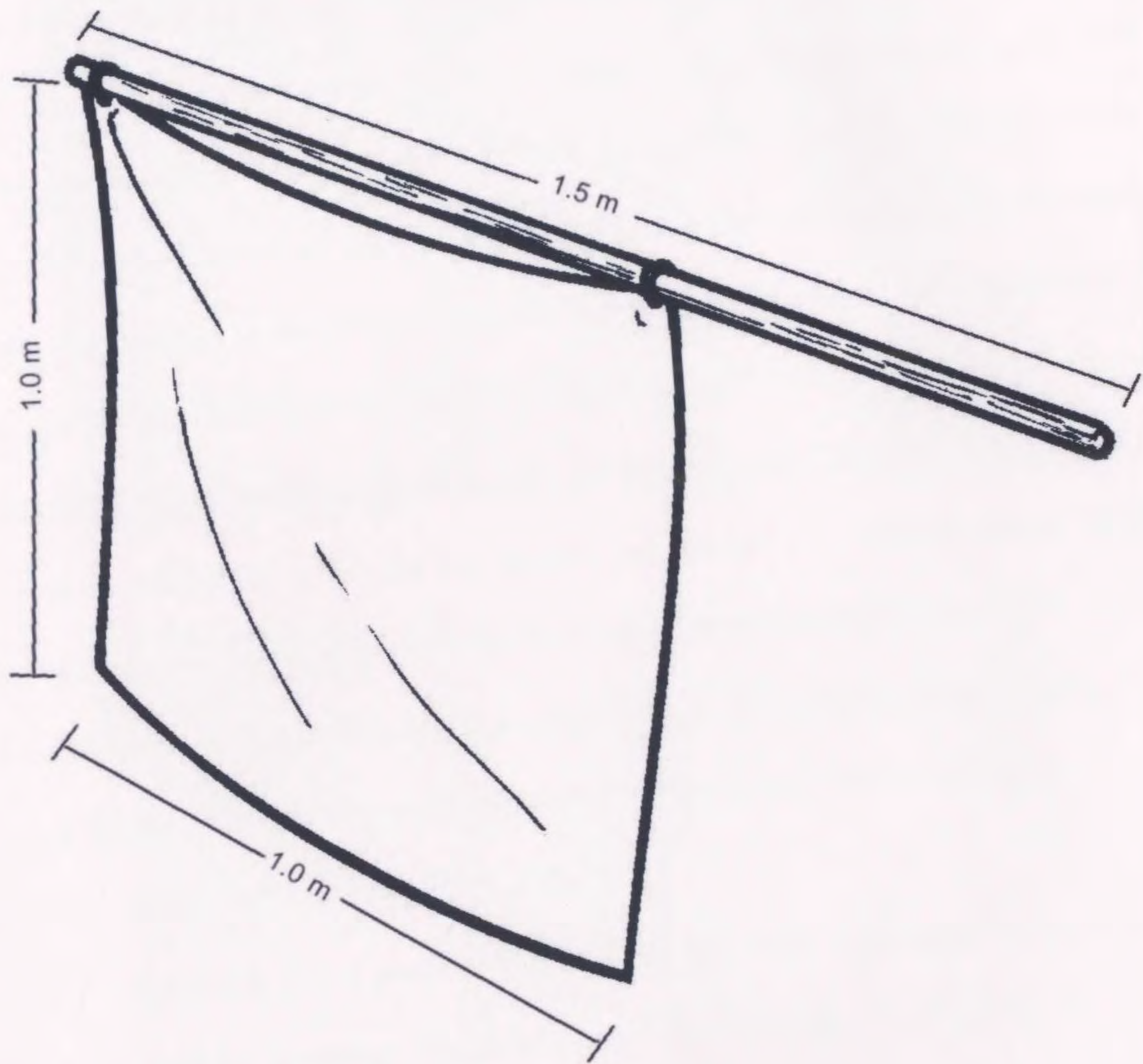


Figure 2: Conventional flag (Martin 1978).

Flags were used by personnel in four provincial parks (La Manche, Butterpot, Lockston Path and Sandbanks) to sample the vegetation for any ticks that may have been present. Each park was sampled once between the months of July and September 2003 with the exception of La Manche where the procedure was carried out in both July and August. The procedure was to run five 50 m transects each separated by 10 m. Each 50 meter transect was then flagged, flags examined for ticks and any ticks collected placed in appropriately labelled bottles. Transects were laid out using a 50 meter measuring tape with one end attached to the person doing the flagging. The tick flag was then held to one side of the collector who walked slowly (about one step per second) in a straight line to the end of the 50 meter transect (Figure 3A). At the end of each transect the flag was examined for ticks (Figure 3B) and any present were placed in a collection bottle for that particular transect. The next transect line was run at least 10 meters to the left or right of the preceding transect according to the transect design. Data sheets along with any ticks collected were then submitted to the laboratory of the Animal Health Division, Department of Natural Resources in St. John's.

In cooperation with Canadian Wildlife Services, ticks were collected from the Witless Bay Seabird Sanctuary, Newfoundland. This collection involved checking chicks of Atlantic Puffins (*Fratercula arctica*) in their burrows for ticks (Figure 4A) in 2002 (approximately 10 burrows were checked). Ticks were also collected (in both 2002 and 2003) by having the collector sitting next to puffin burrows and picking up ticks moving towards the collector (Figure 4B).

In Salmonier Nature Park (SNP) snowshoe hares (*Lepus americanus*) were live

A



B



Figure 3: Flagging vegetation for ticks.

A



B



Figure 4: Collecting seabird ticks from puffins (A) and environment (B).

trapped using 12 – 24 Tomahawk single door live traps (Model 205 – 66.04 X 22.86 X 22.86 cm) approximately every 2 weeks from June to November in 2002 and from April to November in 2003. Weather permitting (i.e. the live traps were not set if the forecast called for rain, drizzle or low temperatures, e.g. snow or overnight temperatures below - 12 °C) the traps were baited with apples and alfalfa late in the evening (just before dark) and then checked just after dawn the following morning (Figure 5A). A syringe of dextrose was brought during each trapping procedure to be used in the case of a snowshoe hare experiencing shock. Any hares that were caught in the traps (Figure 5B) were released from the trap into a burlap bag. While in the bag the hare was weighed, tagged with an ear tag and checked for ticks. Any hare less than 700 g was let go due to the fact that the ear tags would not usually remain in place on a hare this small. Ticks were removed by grasping the tick as close to the skin as possible with a pair of fine forceps and gently moving the tick in a back and forth motion while pulling outward (Anon. 2001b, Brown and Burgess 2001). Ticks were only collected from the ears of the hares and only five minutes was spent collecting ticks from each ear. This was done to minimize sampling error due to more ticks being collected as a result of more time spent collecting ticks from heavily infested hares. After the tick collection, if the hare was not too stressed (tested by hare's willingness to apply pressure to the researcher's hand with its hind foot) a small sample of blood (approximately 0.5 – 2.0 mls) was taken from the central ear artery. Finally, the hare was then turned onto its back (still inside the bag) to be sexed. Once sexed, the hare was let out of the burlap bag and released back into the environment.

In SNP a series of Sherman live traps were also used in an attempt to trap small

A



B



Figure 5: Live trapping snowshoe hares; (A) trap set in Salmonier Nature Park; (B) snowshoe hare in trap.

rodents. The live traps were supplied with a sample of sheep's wool to keep any potential rodents warm and were baited with a combination of peanut butter, rolled oats and honey. Victor snap traps were also used to sample small rodents. The snap traps were set in 25 pairs along a single transect measuring about 150 m in length. These traps were baited (using the same bait used in the live traps) about once a month from June to September in 2002 and 2003 and were set for three consecutive nights. In addition, a similar small rodent survey using snap traps was carried out by the staff at SNP each fall (2002 and 2003) and the rodent carcasses were submitted for this study. Once the small rodents had been collected they were individually bagged in Ziploc bags and labeled with an ID number for future examination. This examination (which was done on fresh specimens when collected by the researcher and on previously frozen specimens when submitted from SNP) included a search for ticks either still attached to the body or in the Ziploc bag containing the animal.

Identification Methods and Submission for Pathogen Testing

The general body structure of adult ticks is shown in Figure 6 (Kierans and Litwak 1989). The body structure of nymphal and larval ticks is essentially the same as the adults with a few differences. Both nymphal and larval ticks lack the genital aperture and the porose areas found on the adults and larval ticks have only six legs. The ticks collected in this study were examined and identified to species and life stage using the identification keys of Gregson (1956), Kierans and Clifford (1978) and Kierans and Litwak (1989). The identification was then reported to the submitter and the ticks then frozen for future pathogen testing (unless there was a diagnostic need in the case of

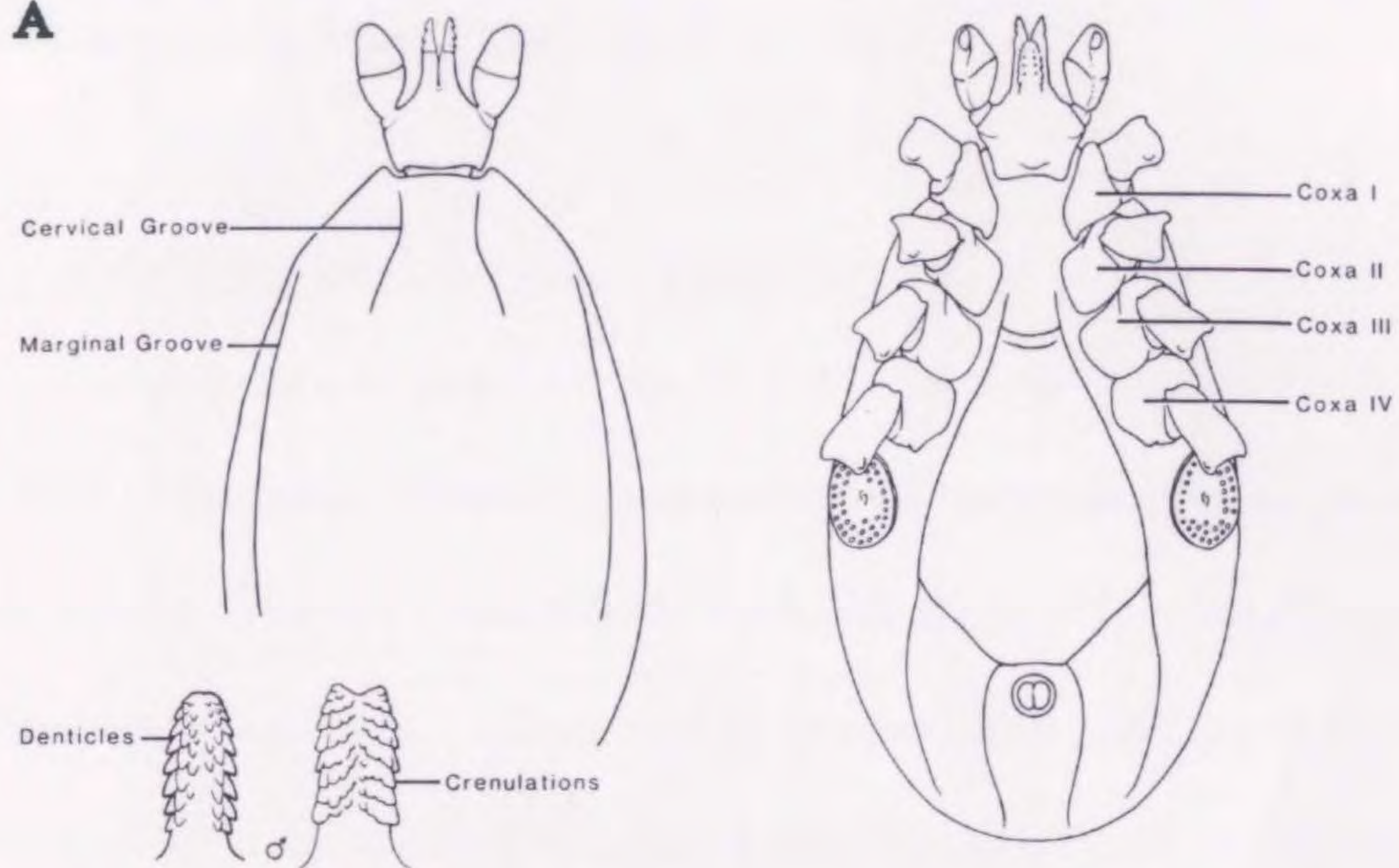
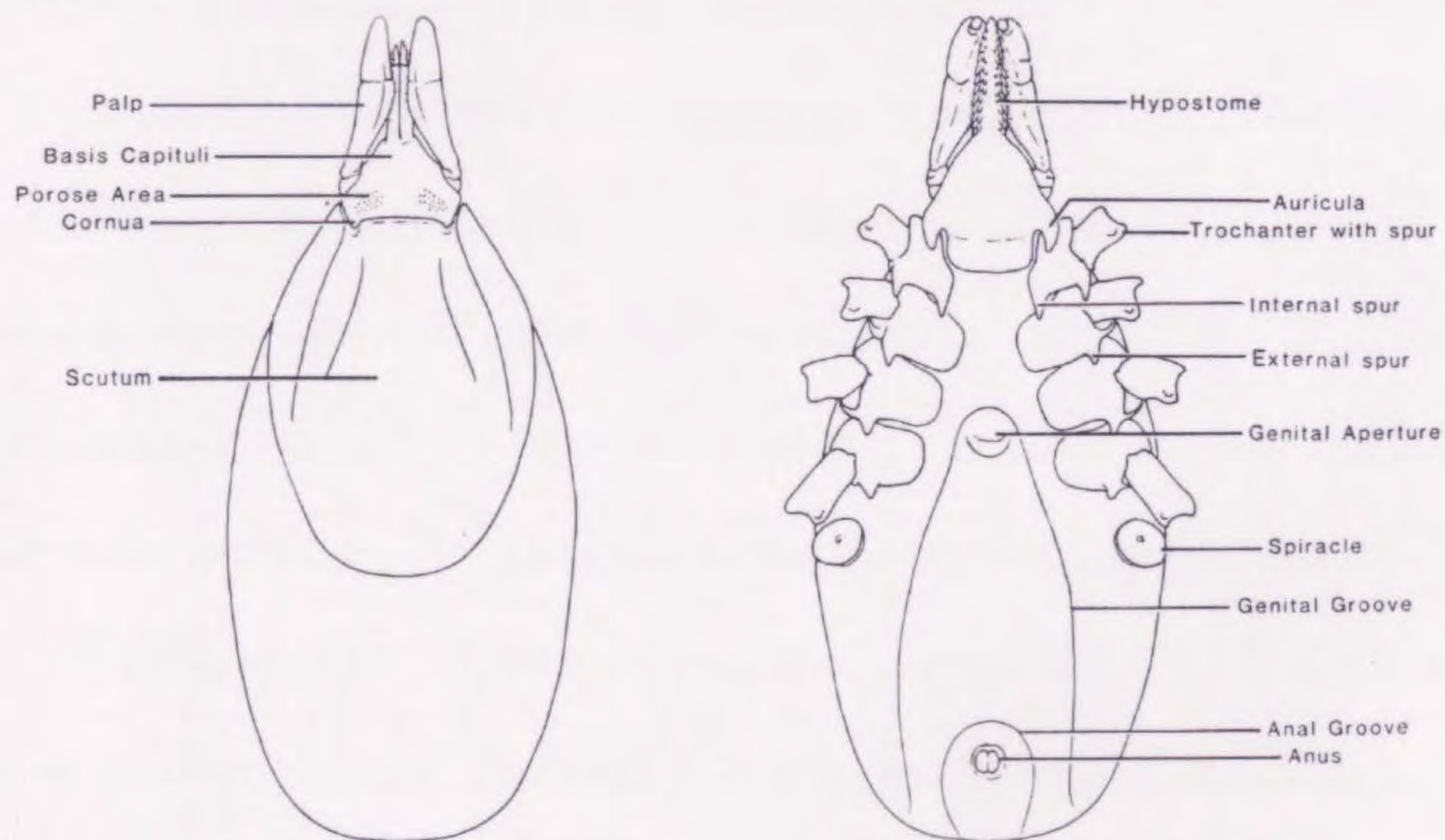
A**B**

Figure 6: General body structure of (A) male and (B) female Ixodid ticks (Kierans and Litwak 1989).

domestic animals who would require treatment, in which case the ticks were submitted for pathogen testing while still alive).

Experimental Trials on Activity and Temperature

The collection of large numbers of *Haemaphysalis leporispalustris* permitted an evaluation of the range of temperatures over which the various life stages of the tick were able to survive. The ticks were separated into life stages (i.e. larvae (l), nymphs (n), females (f) and males (ma)). They were further separated into engorged (e) or unengorged (u) and placed in five separate glass test tubes in equal groups (i.e. five groups of five ticks or five groups of four ticks, etc.). The test tubes contained a moistened strip of gauze, were plugged with cotton balls, covered with gauze and placed in a Tupperware container lined with moist paper towels. The Tupperware container was then kept at room temperature (20 – 25 °C) until the ticks were placed into the experimental incubator. Once a week the incubator was set at a particular temperature and the five test tubes of each life stage were placed in a separate Tupperware container and placed in the incubator. The temperatures that the incubator was set at were 20°C, 15°C, 10°C, 5°C, 0°C, and -5°C. Ticks were examined every 24 hours for five days and the level of activity was noted. The level of activity was based on a subjective score assigned by the observer (Table 1). If an activity level below 4 (i.e. displaying what was considered normal tick behaviour based on observations made at room temperature 24 hours prior to the ticks being placed in the experimental setting) was noted for any of the ticks when they were removed from the incubator, the ticks were left at room temperature and examined every hour for 12 hours. If after 12 hours all 5 ticks scored a 0 activity

Table 1: Activity level index for *Haemaphysalis leporispalustris*.

Activity Level	Description
0	No activity; all legs curled underneath body
1	Very low; rearmost legs curled under body, front 2 legs moving slightly
2	Low; all legs extended with front 2 legs moving
3	Moderate (approaching normal behaviour); exploring environment slowly/cautiously
4	Normal; exploring environment and displaying normal tick behaviour*

* Normal tick behaviour based on observations made at room temperature 24 hours prior to the ticks being placed in the experimental incubator.

level they were assumed dead. Each individual tick was only placed in the experimental incubator once and only ticks collected from the live trapped snowshoe hares from the beginning of June 2003 to the end of August 2003 were tested.

Pathogen Survey

Once the ticks had been identified they were tested for various pathogens. All pathogen testing was carried out at the National Microbiology Laboratory of Health Canada in Winnipeg with the exception of some tularemia testing which was carried out at the Public Health laboratories of the Medical Services Branch, Department of Health and Community Services in St. John's.

The particular pathogen tested for depended on the sample being tested. All *Haemaphysalis leporispalustris* ticks were tested for tularemia using Polymerase Chain Reaction (PCR) at Health Canada (Long *et al.* 1993) and cultured on Blood agar, Chocolate agar, MacConkey agar, Cysteine Heart agar and Cysteine Heart agar with penicillin at the Public Health lab (Murray *et al.* 2003); all *Ixodes uriae* ticks were tested for vector – borne viruses (eg. Avalon, Bauline and Great Island) (cultured in Vero cells) (Dibernardo 2004, pers. comm.) and all *Ixodes scapularis*, *I. ricinus*, *I. muris*, and three of the five *Dermacentor variabilis* ticks were tested for Lyme disease (using PCR) (Wise and Weaver 1991) and Human Granulocytic Ehrlichiosis (HGE) (using PCR) (Persing *et al.* 1993). The remaining *D. variabilis* and *Rhipicephalus sanguineus* were not tested for the four pathogens of interest. The serum collected from the snowshoe hares at SNP was also tested for tularemia (using microagglutination) (Chu *et al.* 2001).

Statistical Analysis

Descriptive statistical analysis of tick data was accomplished using the Microsoft Office Excel 2003 statistical package as was the comparison of host species examined. The comparison of the number of *Haemaphysalis leporispalustris* ticks per hare based on the sex of the hare was carried out using the Mann-Whitney non-parametric test in Minitab for Windows version 13.20.

Results

Tick Species Record

In the period from the Fall of 2001 to the end of 2003 seven species of hard ticks, Ixodidae, were identified (Table 2) on nine hosts (Table 3) but no soft ticks, Argasidae, were found. A summary of the raw data for these seven species is given in Appendix 1. Figure 7 shows the distribution of the ticks collected during this study. A synoptic description of the nine species of ticks known from Newfoundland, which includes the seven collected in this study, is provided in Appendix 2.

Observations on Abundance and Temporal Distributions of Ticks

Haemaphysalis leporispalustris was by far the most numerous tick with 2349 specimens collected in 66 samples. Approximately 80 % of the *H. leporispalustris* collected were taken from the live trapped snowshoe hares at SNP between April and November. The prevalence, intensity, mean intensity and relative density of the different life stages of *H. leporispalustris* of SNP collections were calculated based on the definitions of the terms as stated by Margolis *et al.* (1982) (Table 4). The SNP samples were further analyzed to determine the seasonal pattern of occurrence (Figure 8 and 9). The analysis showed that for most months there were more ticks found per hare in 2003 than in 2002 and July, August and September are the peak months (Figure 8). The breakdown by life history stage (Figure 9) shows that July is the peak month for adults while the peak months for immature stages are August and September.

The number of *Haemaphysalis leporispalustris* ticks per hare was also calculated

Table 2: Ixodid tick species collected from insular Newfoundland during 2002 and 2003.

Tick Species	# of Collections from all Sites/Animals	# of Ticks
<i>Haemaphysalis leporispalustris</i>	66	2349
<i>Ixodes uriae</i>	8	91
<i>Ixodes muris</i>	1	1
<i>Ixodes scapularis</i>	14	14
<i>Ixodes ricinus</i>	1	1
<i>Dermacentor variabilis</i>	5	5
<i>Rhipicephalus sanguineus</i>	1	1

Table 3: Comparison of numbers of hosts examined during 2002 and 2003 and the tick species collected from those hosts (number of hosts with ticks attached in parentheses).

Collection Site or Host Species	# in 2002	# in 2003	Tick Species
Puffin Nesting Sites	2 (2)	0 (0)	<i>Ixodes uriae</i>
Cabin	1 (1)	0 (0)	<i>Ixodes uriae</i>
Flagging	0 (0)	5 (0)	None
House	0 (0)	1 (1)	<i>Dermacentor variabilis</i>
American Crow	0 (0)	8 (0)	None
Atlantic Puffin	1 (1)	1 (1)	<i>Ixodes uriae</i>
Boreal Owl	0 (0)	1 (0)	None
Common Murre	20 (20)	0 (0)	<i>Ixodes uriae</i>
Great Horned Owl	0 (0)	2 (0)	None
Lincoln's Sparrow	0 (0)	1 (1)	<i>Haemaphysalis leporispalustris</i>
House Mouse	4 (0)	0 (0)	None
Masked Shrew	22 (0)	2 (0)	None
Meadow Vole	18 (0)	6 (0)	None
Red Squirrel	1 (0)	0 (0)	None
American Mink	0 (0)	1 (0)	None
Coyote	0 (0)	9 (0)	None
Feral Cat	1 (0)	26 (0)	None
Lynx	0 (0)	5 (0)	None
Red Fox	0 (0)	483 (1)	<i>Ixodes scapularis</i>
Snowshoe Hare	32 (32)	16 (15)	<i>Haemaphysalis leporispalustris</i>
Woodchuck	1 (0)	22 (0)	None
Black Bear	0 (0)	1 (0)	None
Moose	1 (0)	1 (0)	None
Domestic Cat	4 (4)	2 (2)	<i>Haemaphysalis leporispalustris</i> <i>Ixodes scapularis</i>
Domestic Dog	5 (5)	13 (13)	<i>Ixodes scapularis</i> <i>Ixodes ricinus</i> <i>Dermacentor variabilis</i> <i>Rhipicephalus sanguineus</i> <i>Ixodes muris</i>
Domestic Rabbit	1 (1)	0 (0)	<i>Haemaphysalis leporispalustris</i>
Human	1 (1)	0 (0)	<i>Dermacentor variabilis</i>

Figure 7: Distribution of ixodid ticks collected from *Ixodes ricinus* Newfoundlands.

- A. *Ixodes ricinus* collected from Mount Pearl.
- B. *Ixodes ricinus* collected from (east to west) St. John's, Chamberlain Bay, Roberts and Port Antonio.
- C. *Ixodes ricinus* collected from St. John's.
- D. *Ixodes ricinus* and I. *sp.* collected from St. John's, Chamberlain Bay, Roberts, Goobies, Sweet Bay, Bungee, Corner Brook, Cow Head, L. *sp.* collected from (east to west) St. John's, Salmonier Line, Mount Pearl.
- E. *Ixodes ricinus* collected from the White Bay Seabird Sanctuary.
- F. *Ixodes ricinus* National Park and Cow Head.
- G. *Ixodes ricinus* Port Antonio, Terra Nova National Park, Appleton, Springdale, Paradise, Bannock Provincial Park, Salmonier, Mount Park, Bay Roberts.
- H. *Ixodes ricinus* collected from (east to west) St. John's, Upper Goobies.

Figure 7: Distribution of ixodid ticks collected from insular Newfoundland.

- H. leporispalustris* collected from (east to west) St. John's, Upper Gullies, Paradise, Butterpot Provincial Park, Salmonier Nature Park, Bay Roberts, Clarenville, Port Blandford, Terra Nova National Park, Appleton, Springdale, Gros Morne National Park and Cow Head.
- I. uriae* collected from the Witless Bay Seabird Sanctuary.
- I. muris* collected from Parson's Pond.
- I. scapularis* collected from (east to west) St. John's, Salmonier Line, Mount Carmel, Bay Roberts, Goobies, Sweet Bay, Burgeo, Corner Brook, Cow Head, St. Theresa's and Lark Harbour.
- I. ricinus* collected from St. John's.
- D. variabilis* collected from (east to west) St. Michael's, St. John's, Chamberlains, Bay Roberts and Port Rexton.
- R. sanguineus* collected from Mount Pearl.

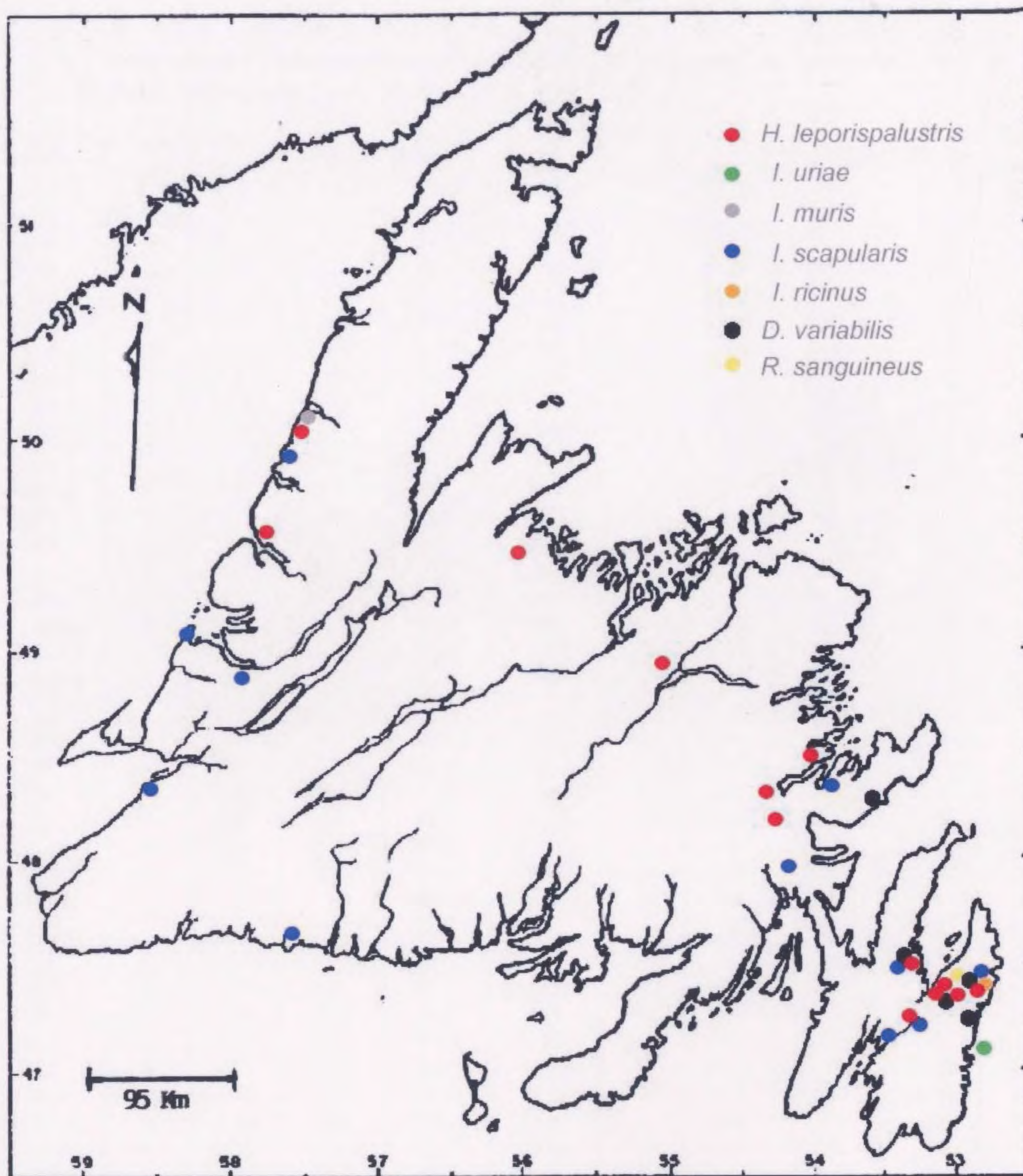


Table 4: A monthly breakdown of the prevalence, intensity and mean intensity of *Haemaphysalis leporispalustris* (by life stage) on snowshoe hares collected at Salmonier Nature Park during 2002 and 2003.

Month	Prevalence 2002					Prevalence 2003				
	# of hares	Males	Females	Larvae	Nymphs	# of hares	Males	Females	Larvae	Nymphs
April	0	NA	NA	NA	NA	5	0	0	0	0.80
May	0	NA	NA	NA	NA	1	1.00	1.00	1.00	1.00
June	3	0.67	1.00	0.33	0.33	0	NA	NA	NA	NA
July	4	1.00	1.00	0.25	1.00	1	1.00	1.00	1.00	1.00
August	12	0.75	0.92	0.92	1.00	1	1.00	1.00	1.00	1.00
September	5	0.60	0.40	1.00	1.00	4	0.75	1.00	1.00	1.00
October	4	0.25	0.25	1.00	1.00	0	NA	NA	NA	NA
November	4	0.25	0	0.25	0.75	1	0	0	0	1.00
Intensity 2002						Intensity 2003				
April	0	NA	NA	NA	NA	5	0	0	0	0-5
May	0	NA	NA	NA	NA	1	5	12	1	35
June	3	0-1	3-6	0-1	0-2	0	NA	NA	NA	NA
July	4	1-7	4-17	0-1	1-8	1	24	17	17	27
August	12	0-18	0-19	0-218	1-100	1	6	6	39	41
September	5	0-5	0-4	10-65	5-77	4	0-7	1-4	7-35	39-68
October	4	0-1	0-1	1-39	16-83	0	NA	NA	NA	NA
November	4	0-1	0	0-2	0-6	1	0	0	0	5
Mean Intensity 2002						Mean Intensity 2003				
April	0	NA	NA	NA	NA	5	NA	NA	NA	2.75
May	0	NA	NA	NA	NA	1	5.00	12.00	1.00	35.00
June	3	1.00	4.33	1.00	2.00	0	NA	NA	NA	NA
July	4	4.25	10	1.00	4.50	1	24.00	17.00	17.00	27.00
August	12	5.00	3.8	29.09	21.08	1	6.00	6.00	39.00	41.00
September	5	3.00	2.5	29.40	39.00	4	5.33	2.25	19.25	51.50
October	4	1.00	1	12.25	34.50	0	NA	NA	NA	NA
November	4	1.00	NA	2.00	2.67	1	NA	NA	NA	5.00
Relative Density 2002						Relative Density 2003				
April	0	NA	NA	NA	NA	5	0	0	0	2.20
May	0	NA	NA	NA	NA	1	5.00	12.00	1.00	35.00
June	3	0.67	4.33	0.33	0.67	0	NA	NA	NA	NA
July	4	4.25	10.00	0.25	4.50	1	24.00	17.00	17.00	27.00
August	12	3.75	3.50	26.76	21.08	1	6.00	6.00	39.00	41.00
September	5	1.80	1.00	29.40	39.00	4	4.00	2.25	19.25	51.50
October	4	0.25	0.25	12.25	34.50	0	NA	NA	NA	NA
November	4	0.25	0	0.50	2.00	1	0	0	0	5.00

NA – not applicable

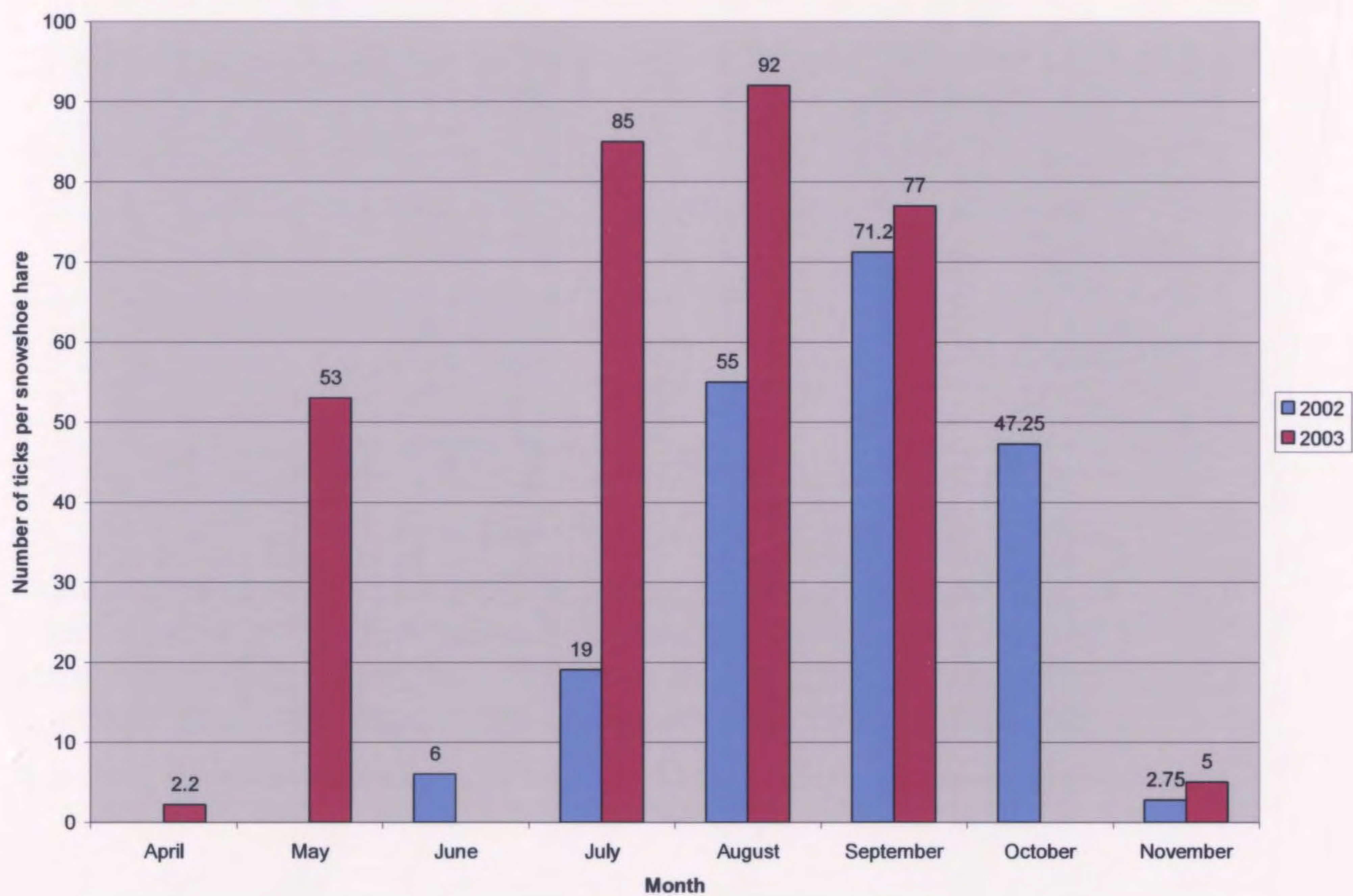
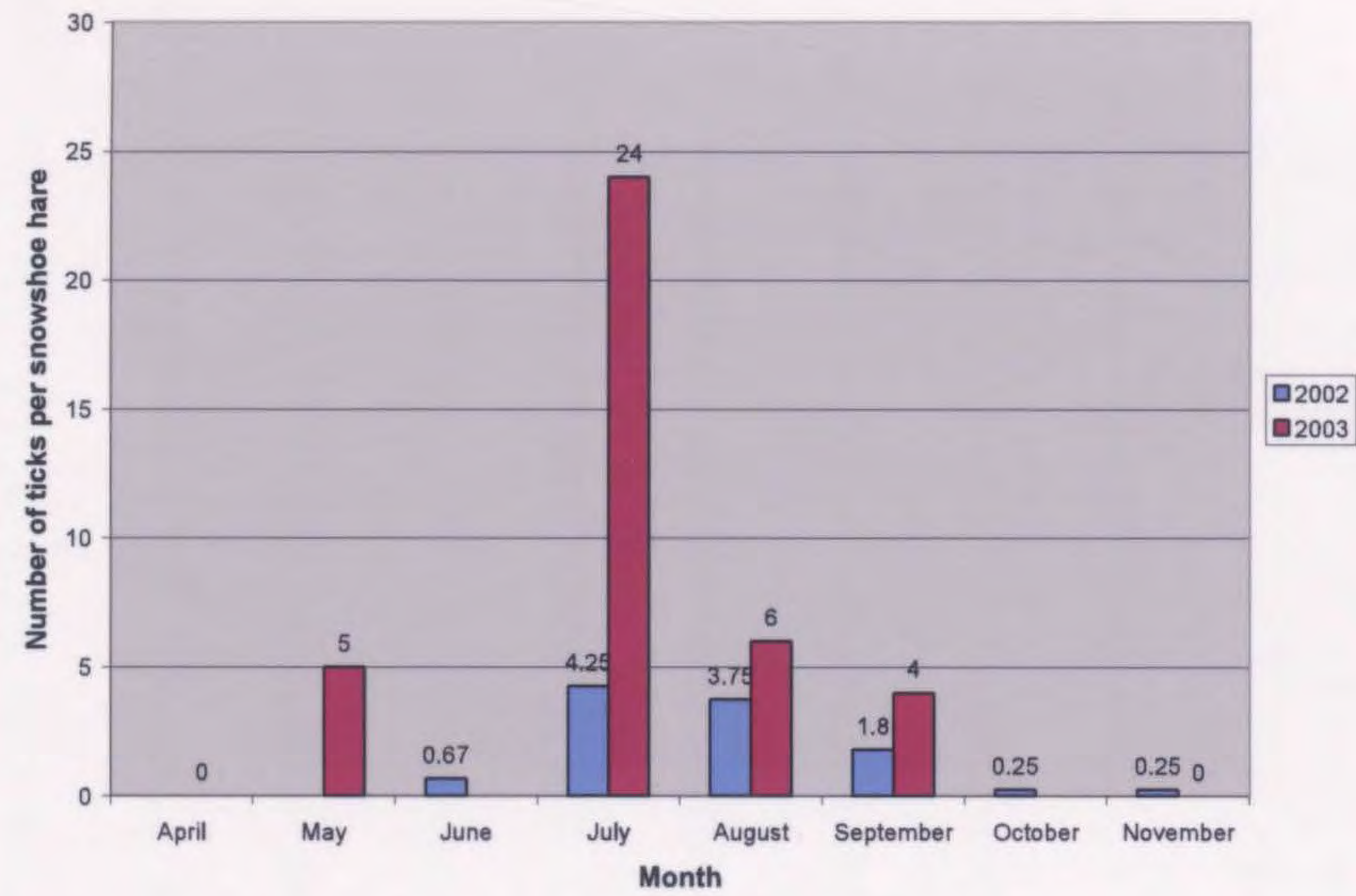
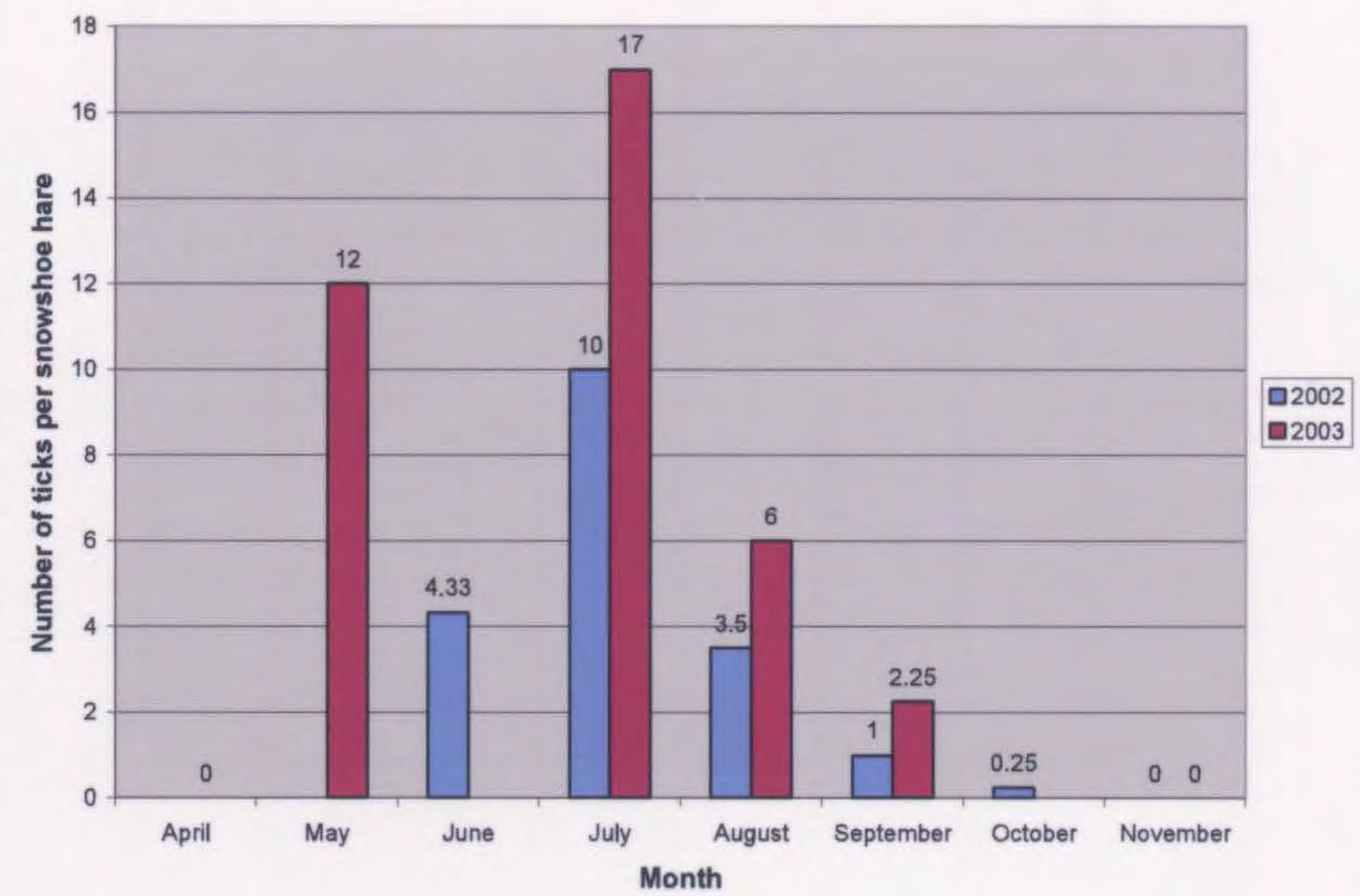
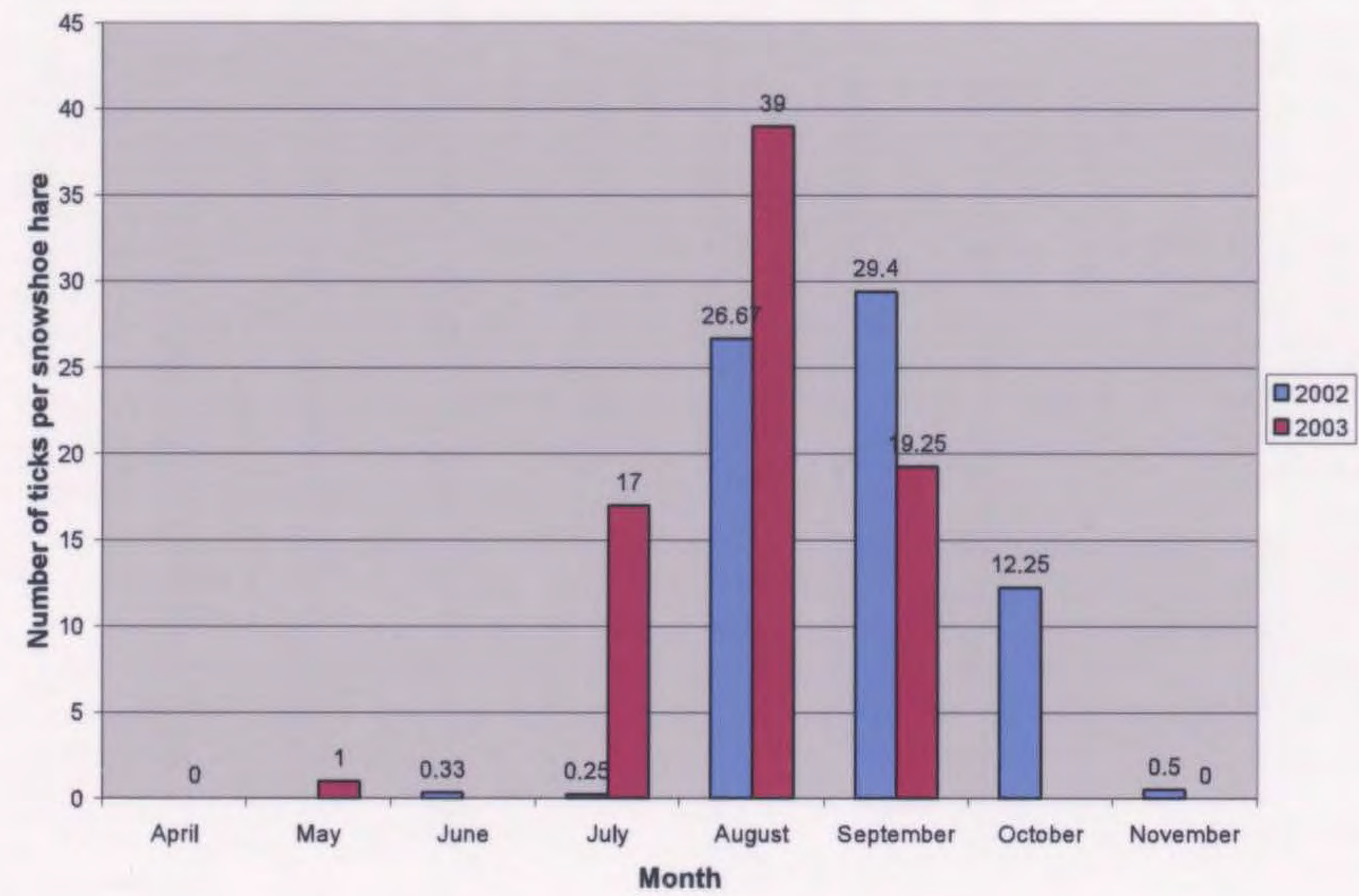
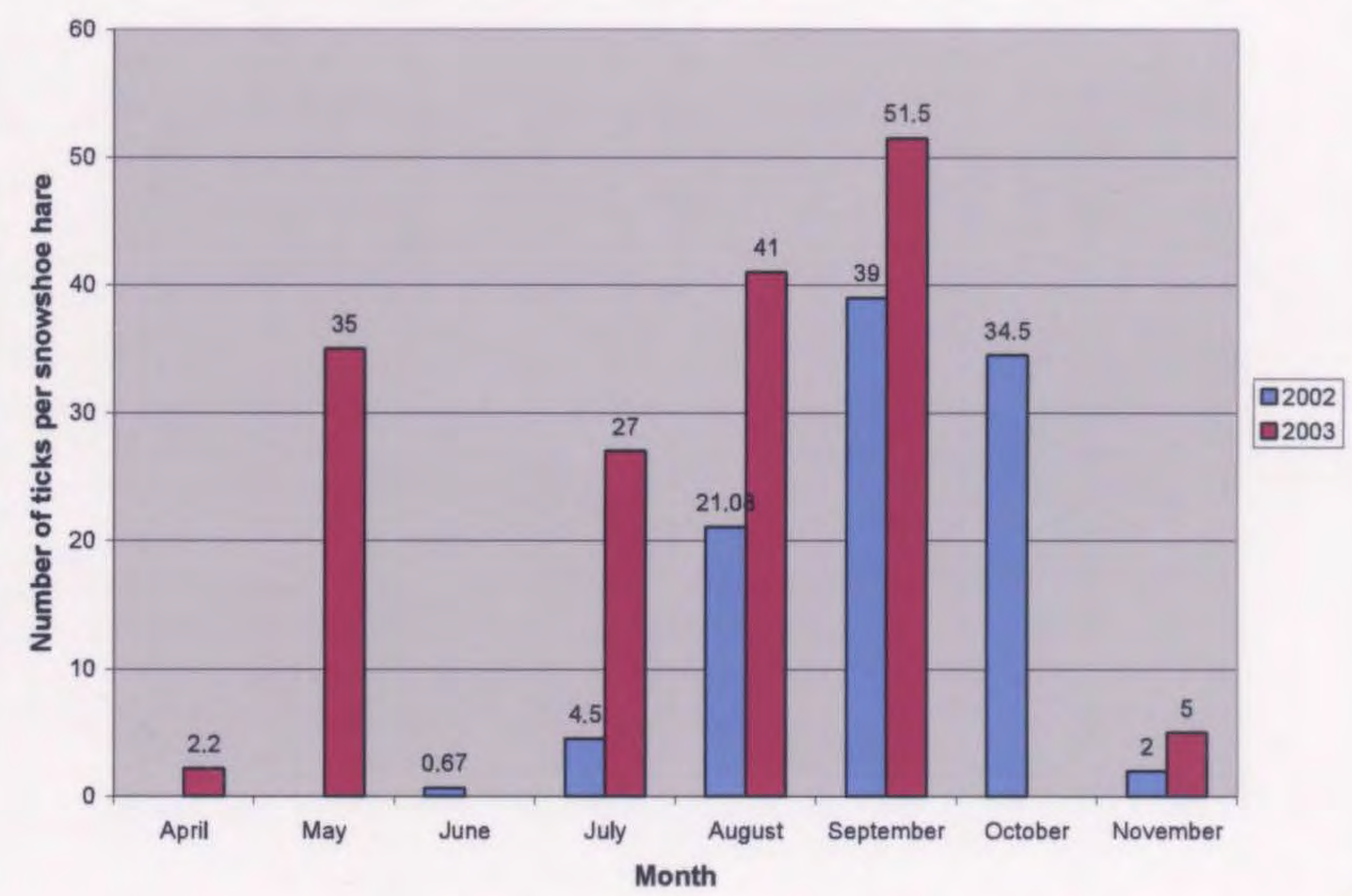


Figure 8: Total number of *Haemaphysalis leporispalustris* (all life stages combined) collected per snowshoe hare at Salmonier Nature Park during 2002 and 2003.

Figure 9: A distribution of *Phrynosoma* specimens collected at Natural History Park during 1992 and 1993 by the stage (A) Male ticks per specimen; (B) Female ticks per specimen; (C) Larval ticks per specimen; and (D) Hypodermic ticks per specimen.

Figure 9: A breakdown of *Haemaphysalis leporispalustris* collected at Salmonier Nature Park during 2002 and 2003 by life stage. (A) Male ticks per snowshoe hare; (B) Female ticks per snowshoe hare; (C) Larval ticks per snowshoe hare; and (D) Nymphal ticks per snowshoe hare.

A**B****C****D**

based on the sex of the hare. However, since it was not possible to sex all of the hares captured, due to stress on the hares, the sample size for this analysis is much smaller (31 hares compared to 45 hares captured in total). Figure 10 shows male snowshoe hares were more heavily burdened with all life stages of *H. leporispalustris* than female snowshoe hares in both 2002 and 2003. A Mann – Whitney analysis (95 % confidence interval), however, showed that this difference was significant only for the male ticks in 2002 (i.e. there were significantly more male ticks on male hares than on female hares in 2002). All other differences were not statistically significant.

The effect of temperature on the survival of *Haemaphysalis leporispalustris* ticks was examined by stage. The results for larvae, nymphs and adults taken from wild caught hares at SNP are shown in Table 5. The results showed all life stages survived temperatures as low as 0°C but none lived longer than 24 hours at -5°C. All ticks that were still alive (activity level 1 – 3) but did not exhibit “normal” tick activity (activity level 4) were examined at room temperature until this level of activity was displayed. In no case did this take longer than one hour.

During the temperature experiments it was also noted that engorged female *H. leporispalustris* laid eggs as early as 24 hours after detaching from the host at temperatures as low as 15 °C and the eggs would hatch at room temperature. The length of time taken for the eggs to hatch was not recorded during this observation.

Ixodes uriae was collected from puffins and murres and the majority of these ticks were collected from Gull Island in 2002 and 2003. There were also some specimens collected from the environment around puffin burrows. Only females and nymphs of this species were collected during the July and August collection period.

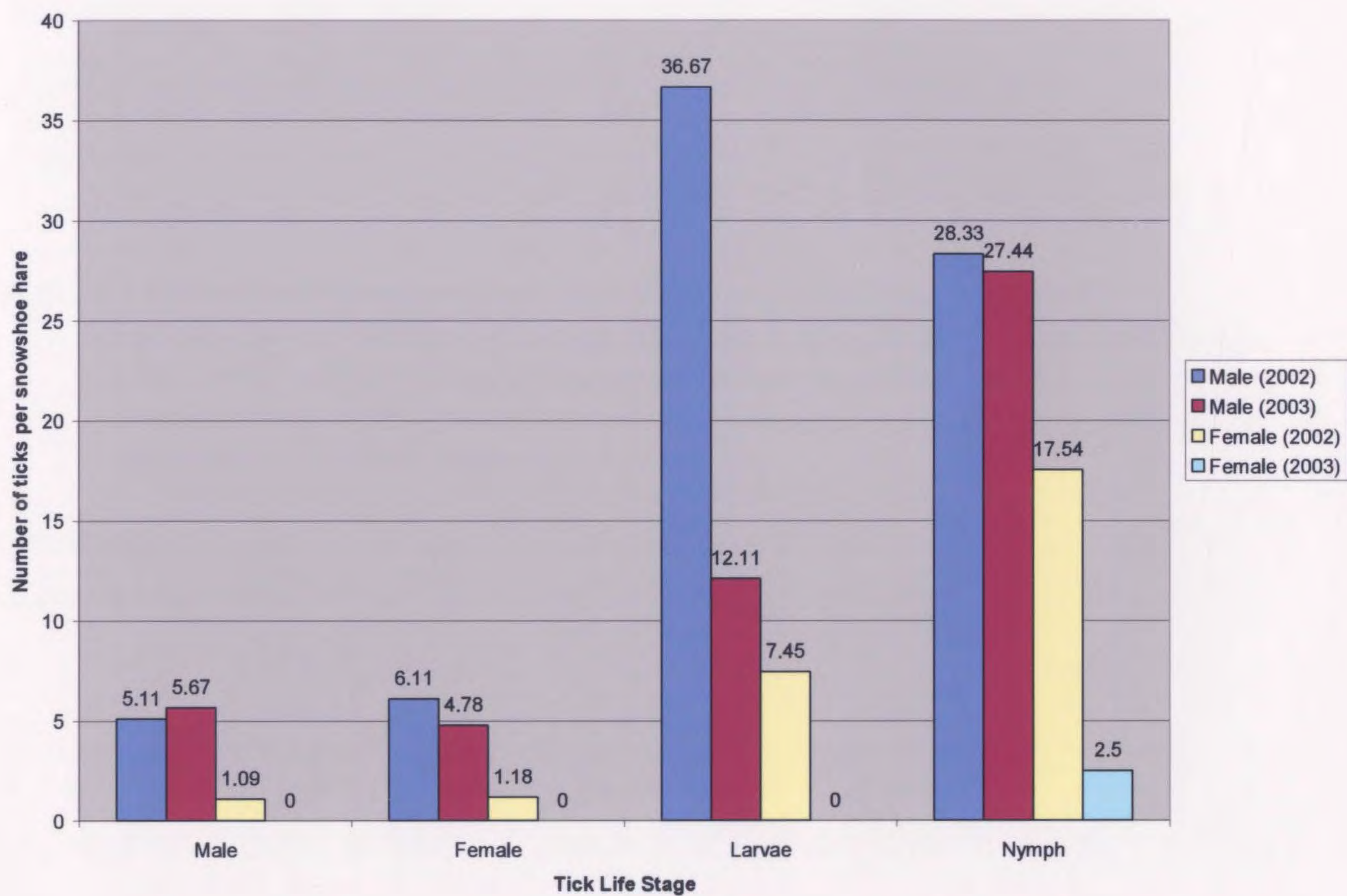


Figure 10: Total number of *Haemaphysalis leporispalustris* ticks per snowshoe hare in 2002 and 2003 based on the sex of the hare (Male 2002 (n=9), Male 2003 (n=9), Female 2002 (n=11), Female 2003 (n=2)).

Table 5: Activity level of engorged (e) and unengorged (u) *Haemaphysalis leporispalustris* life stages (larvae (l), nymph (n), female (f) and male (ma)) after being held for five days at six different temperatures. The first number is the activity level from Table 1 and the number in parentheses is the number of ticks exposed.

Temperature (°C)	ul (150)	el (50)	un (115)	en (55)	uf (35)	ef (65)	ma (85)
20	4 (25)	2 (5)	4 (25)	4 (10)	4 (5)	3 (15)	4 (15)
15	4 (25)	2 (5)	4 (20)	4 (5)	3 (5)	3 (10)	4 (10)
10	3 (25)	2 (5)	4 (15)	4 (5)	3 (5)	3 (5)	3 (5)
5	2 (25)	1 (5)	3 (10)	3 (5)	2 (5)	2 (5)	3 (10)
0	1 (25)	1 (5)	3 (20)	2 (5)	2 (10)	1 (5)	1 (20)
-5	0 (25)	0 (25)	0 (25)	0 (25)	0 (5)	0 (25)	0 (25)

All other tick species that were collected (i.e. *Ixodes muris*, *I. scapularis*, *I. ricinus*, *Dermacentor variabilis* and *Rhipicephalus sanguineus*) during this study were submitted by veterinarians in small animal clinics across the province with the exception of one *I. scapularis* that was submitted from a hunter. Most of these ticks were females and all were taken from domestic dogs with the exception of two specimens of *D. variabilis* (one from a person and one found in a house) and two specimens of *I. scapularis* (one from a domestic cat and one from a red fox). Of the ticks submitted over the two years *I. scapularis* with fourteen specimens and *D. variabilis* with five specimens were the most common. The distribution of *I. scapularis* collections in 2002 and 2003 are given in Figure 11. With reference to Figure 11 two points are of interest: 1) a bimodal distribution of collections with more ticks in 2003 than in 2002 and 2) no ticks submitted during the months of August and September. A very significant observation was that one *I. scapularis* tick collected in December laid a small batch of eggs and three larvae hatched from those eggs. These larvae were kept within the glass test tubes with moistened gauze but none survived longer than a week.

No ticks were obtained by the flagging procedures carried out at the provincial parks. Personnel from four provincial parks (La Manche, Butterpot, Lockston Path and Sandbanks) flagged vegetation once between the months of July and September 2003 with the exception of La Manche where the procedure was carried out in both July and August. The vegetation that was flagged included: forested areas (one with mainly spruce (*Picea* sp.), fir (*Abies balsamea*) and larch (*Larix laricina*) and another with spruce (*Picea* sp.), fir (*Abies balsamea*), birch (*Betula* sp.) and alder (*Alnus* sp.)), marshy areas

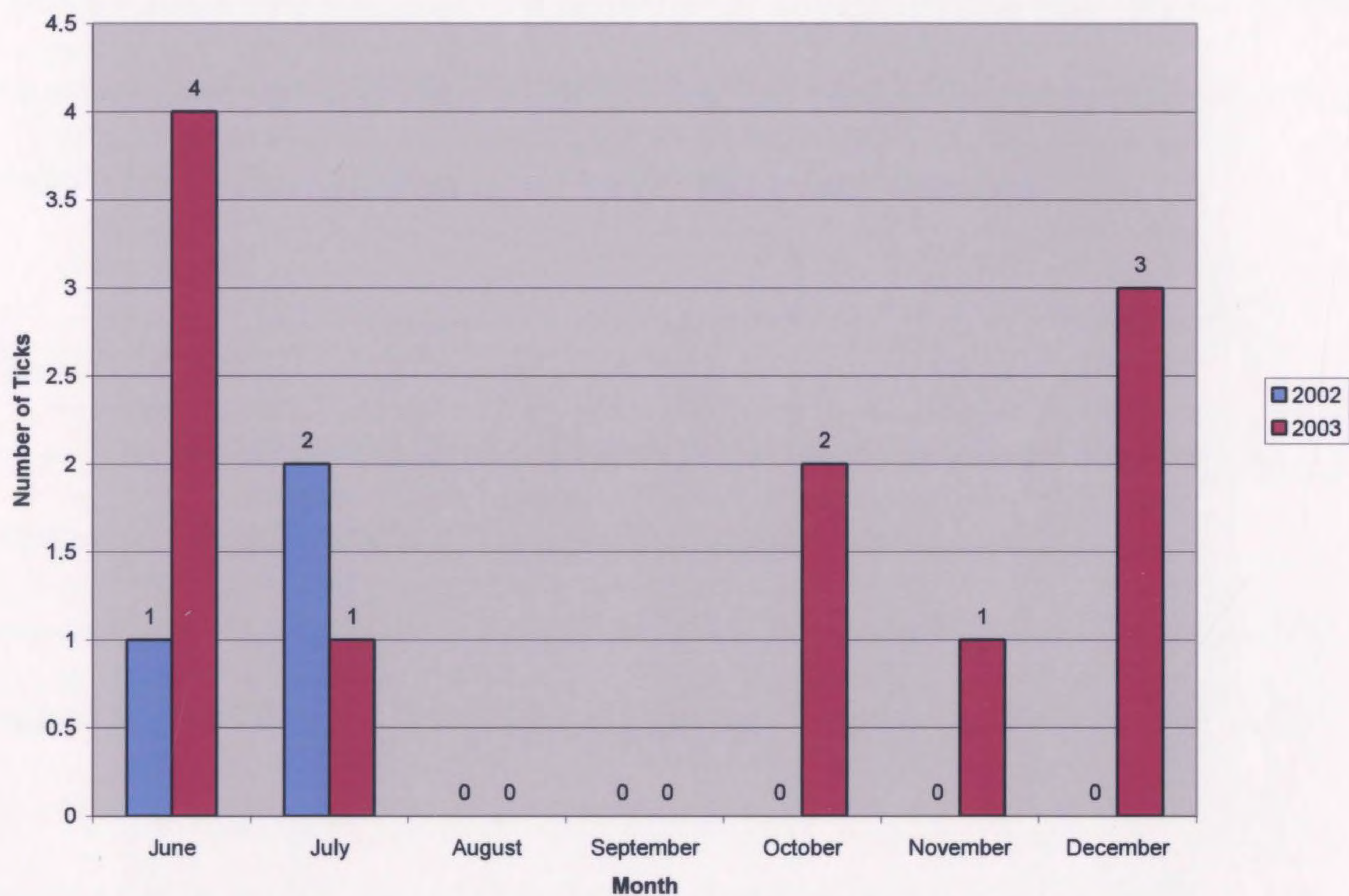


Figure 11: *Ixodes scapularis* collected from insular Newfoundland during 2002 and 2003.

(with blueberry plants (*Vaccinium* sp.), bakeapple plants (*Rubus* sp.) and pitcher plants (*Sarracenia purpurea*)) and a grassy sand dune area near a sandy beach.

No rodents were collected using the Sherman live traps and no ticks were obtained from the rodents snap trapped by the researcher or from the rodents snap trapped at SNP. The rodents that were collected included 21 meadow voles (*Microtus pennsylvanicus*), 20 masked shrews (*Sorex cinereus*) and 4 house mice (*Mus musculus*).

Pathogen Testing

Representative samples of ticks were submitted to the national microbiology laboratory of Health Canada in Winnipeg for pathogen testing and some of the *Haemaphysalis leporispalustris* were submitted to the Public Health laboratories of the Medical Services Branch, Department of Health and Community Services in St. John's for tularemia testing. For the purpose of testing *H. leporispalustris* and *Ixodes uriae* specimens were pooled and tested as groups rather than each tick being tested individually. Table 6 shows that all ticks tested negative for *Anaplasma phagocytophilum*, *Francisella tularensis* and vector – borne viruses. The only pathogen that was detected among those tested was *Borrelia burgdorferi* in *Ixodes scapularis* (Table 6). This pathogen was detected in 3 of 19 submissions over 2 years.

Table 6: Results of testing for pathogens in ixodid ticks collected from insular Newfoundland during 2002 and 2003.

Pathogen	2002			2003		
	Tick species tested	Positive	Negative	Tick species tested	Positive	Negative
<i>Borrelia burgdorferi</i>	<i>I. scapularis</i> (n=3), <i>I. ricinus</i> (n=1), <i>D. variabilis</i> (n=1)	1	4	<i>I. scapularis</i> (n=11), <i>I. muris</i> (n=1), <i>D. variabilis</i> (n=2)	2	12
<i>Anaplasma phagocytophilum</i>	<i>I. scapularis</i> (n=3), <i>I. ricinus</i> (n=1), <i>D. variabilis</i> (n=1)	0	5	<i>I. scapularis</i> (n=11), <i>I. muris</i> (n=1), <i>D. variabilis</i> (n=2)	0	14
<i>Francisella tularensis</i>	<i>H. leporispalustris</i> (n=650)	0	23	<i>H. leporispalustris</i> (n=731)	0	17
Vector-Borne viruses	<i>I. uriae</i> (n=90)	0	16	<i>I. uriae</i> (n=1)	0	1

Discussion

The first record of *Borrelia burgdorferi*, the causative agent of Lyme disease, in a dog in Cape Broyle, Newfoundland in July 2001 initiated a multitude of questions regarding the tick fauna of the province. The threat of this zoonosis had never before been a concern to the people of Newfoundland and the tick fauna of the island had not been thoroughly examined. The current survey of the ticks of the province recorded seven species (*Haemaphysalis leporispalustris*, *Ixodes uriae*, *I. muris*, *I. scapularis*, *I. ricinus*, *Dermacentor variabilis* and *Rhipicephalus sanguineus*) and evaluated the threat of new zoonoses by four tick vectored pathogens. Of the seven species, only three have been recorded in previously published works, i.e. *H. leporispalustris* (Bishopp and Trembley 1945, Dodds and Mackiewicz 1961, Gregson 1956), *I. uriae* (Eveleigh 1974, Eveleigh and Threlfall 1974, McCoy *et al.* 1999, and Threlfall 1968) and *I. scapularis* (Lindsay *et al.* 1999). *Dermacentor variabilis* and *R. sanguineus* had been submitted to the Animal Health Division, Department of Natural Resources from animals that had traveled elsewhere and *I. muris* had been previously collected from a house mouse by Colbo (pers. comm.). *Ixodes ricinus*, on the other hand, had never been collected or recorded in insular Newfoundland prior to the current study.

Newfoundland is an island surrounded by sea water and this geographical isolation would be expected to reduce the tick diversity in relation to the mainland. Gregson (1956) has recorded 29 species of ticks in Canada and only five of the species were recorded in Newfoundland during this study. Gregson (1956) did not record either *Ixodes scapularis* or *I. ricinus*. Three of the seven species, *I. ricinus*, *Dermacentor*

variabilis and *Rhipicephalus sanguineus*, were collected from animals that had spent time elsewhere in Canada, and in the case of *I. ricinus* record, Germany. In order for a tick species to be considered endemic to an area all life stages of the tick must be collected from the area either on resident animals or in the environment for a minimum of two consecutive years (Artsob *et al.* 1992). All life stages of *Haemaphysalis leporispalustris* and *I. uriae* have been found on insular Newfoundland in previous studies which suggests that these two species are endemic to the island (Dodds and Mackiewicz 1961, Eveleigh 1974, Eveleigh and Threlfall 1974, Eveleigh and Threlfall 1975, Gregson 1956, McCoy *et al.* 1999, Muzaffar 2000, Threlfall 1968). *Ixodes muris* could potentially have an established population on the island as well considering its geographical distribution elsewhere in Canada (Gregson 1956) but all life stages have not been reported so its endemicity cannot be confirmed. Likewise, only adult female specimens of *I. scapularis* have been collected in Newfoundland which also leaves questions to be answered regarding its population status.

Tick biodiversity is related to the biodiversity of vertebrate hosts as well as climate. The native mammalian fauna of the island is depauperate with only 14 species recorded. The native mammalian species include two bats, one hare, three rodents, two canids (wolf – now extinct), one bear, three mustelids, one felid and one cervid (Dodds 1983). Newfoundland mammal diversity has markedly increased through the introductions of a shrew, a hare, two scuriids, five rodents, a mustelid, one wild canid, cats, dogs, cattle, sheep, goats, horses, alpacas, llamas, bison (now extinct) and moose since the European colonization (Table 7 and public records of domestic stock). Rats, mice, dogs, cats, cattle, horses and sheep were all early arrivals with people. The wild

Table 7: Wild mammals that have been introduced to the island of Newfoundland.

Date	Mammal	Accidental/Intentional (A/I)
Unknown	Norway Rat	A-first sailing ships ¹
Unknown	House Mouse	A-first sailing ships ¹
1864	Snowshoe Hare	I-from Nova Scotia to provide fresh meat ²
1878 & 1904	Moose	I-from Nova Scotia and New Brunswick to provide meat ³
1934	American Mink	I-distributed to fur farmers ¹
1958	Masked Shrew	I-predator for larch sawfly and hemlock looper ^{1,4}
1962	Eastern Chipmunk	I-enhance small mammal prey base ⁵
1963	Red Squirrel	I-enhance small mammal prey base ⁶
1967	Bank Vole	I-only on Notre Dame Bay islands for scientific study ¹
1968	Deer Mouse	A-inside imported bales of hay ^{1,7}
1987	Coyote	A-Extension of habitat over land and frozen ice ⁸
1997	Red Backed Vole	A-possibly imported with logs ⁹

¹ Snow 1996

² Dodds 1957

³ Dodds 1983

⁴ Warren 1970

⁵ Northcott *et al.* 1973

⁶ Payne 1976

⁷ Gould and Pruitt 1969

⁸ Parker 1995

⁹ Baggs, pers. comm.

mammals were directly introduced to the island both officially by governments and either accidentally or illegally by individuals (Table 7). Therefore 21 species have been added to the original 14 native mammals for a total of 35 species. The native wolf and the introduced bison have gone extinct leaving 33 species which is over the number of native taxa. These introductions are still continuing and are contributing to an increased diversity of mammals and also to the potential diversity of hosts for ticks. This consequently may alter the diversity of the established tick fauna and the potential opportunity for additional species to be introduced as well as increased risk for the establishment of pathogens vectored by them.

Introductions of ticks can occur in a variety of ways. Ticks are introduced by migrating birds which have been shown to transport them over long distances. This may be the mechanism responsible for introducing species such as *Ixodes scapularis* to the island (Artsob *et al.* 1992, Banerjee *et al.* 1995, Banerjee *et al.* 1996, Barker *et al.* 1992, Bjoersdorff *et al.* 2001, Klich *et al.* 1996, Lindsay *et al.* 1998, Lindsay *et al.* 1999, Morshed *et al.* 1999, Scott *et al.* 2001). The bulk importation of hay, pulp wood, timber, cattle and domestic dogs is another route (Whitney, pers. comm.). This latter is particularly relevant since ticks can survive away from the host for long periods of time (Sonenshine 1991a).

Domestic animals being taken on and off the island, e.g. dogs, are known to bring in ticks. In this study the American dog tick (*Dermacentor variabilis*) and the brown dog tick (*Rhipicephalus sanguineus*) were found on the family pet taken off the island during family vacations. In addition, *Ixodes ricinus*, during the course of this research was recovered from a dog brought in from Germany.

The finding of *Ixodes ricinus* also illustrates the range of potential introduction sources and potential for pathogens to be introduced here as this species is the European vector of Lyme disease. Also this species occurrence is relevant to possible effects of climate change on Newfoundland's fauna. Randolph (2000, 2001) examined how the changing climate in Europe is affecting the distribution of *I. ricinus* there. Her work is also relevant to the North American Lyme disease vector, *I. scapularis*, which is closely related to *I. ricinus*. Randolph (2000) stated that since some ticks develop from one life stage to the next off the host they are quite sensitive to the environment around them. For instance, while off the host, ticks require a certain level of moisture to avoid drying out. Furthermore, for the tick to transmit the tick – borne encephalitis virus (TBEV), *I. ricinus* requires a certain temperature regime to coordinate the time of feeding of infected nymphs with that of infectible larvae in order for transmission to occur. Thus the distribution and pathogen transmission can be predicted from climate which can be determined by satellite imagery particularly the relation between vegetation cover and tick population density (Randolph 2000, 2001). Randolph (2001) showed that the current distribution of *I. ricinus* may change based on computer models of how climate change will modify the current ecosystems. Randolph (2001) predicted that the distribution of tick – borne encephalitis would move into higher latitude and higher altitude regions throughout the 2020's, 2050's and 2080's. Therefore, in Newfoundland, climate change coupled with new host introductions could affect the distribution and diversity of ticks and the pathogens they transmit in the province.

Life Cycle of *Haemaphysalis leporispalustris*

Haemaphysalis leporispalustris data from 2002 and 2003 live trapping of snowshoe hares at SNP showed similar seasonal patterns of feeding activity of all stages. Although the hare trapping season was longer in 2003 starting in April it did not alter the pattern (Figure 9). The earliest date that ticks were found on a snowshoe hare was April 27 and only nymphs were found at this time. Larvae appeared in low numbers in May and June with high numbers from July to October dropping to very low numbers in November. Adults were not found on the hares until May but were present through to November with very low numbers beyond September. Nymphs and larvae were found on snowshoe hares as late as November 26. The patterns observed suggested all stages overwintered in Newfoundland. The low numbers of larvae in the early part of the year may, however, suggest that the engorged females overwintered or laid eggs the previous fall that had survived the winter. The study of Keith and Cary (1990) near Rochester, Alberta showed that *H. leporispalustris* was not found on adult snowshoe hares after mid-November and the ticks, life stage not specified, were found on the hares in early April. Keith and Cary (1990) also note, however, that all stages may overwinter but it is the nymphs and adults that more commonly do so.

There was no statistically significant difference in infection rates between male and female hares with respect to all life stages of *Haemaphysalis leporispalustris* with the exception of male ticks in 2002. In 2002 there were significantly more male ticks on male hares than female hares. Female hares have been shown previously to have fewer ticks than male hares (Campbell *et al.* 1980, Keith and Cary 1990). Campbell *et al.* (1980) and Keith and Cary (1990) suggested this may be due to the greater mobility and

larger home range of the male hare which increases the risk of contact with ticks.

The experiment to test the survival of all stages of *Haemaphysalis leporispalustris* over a range of temperatures showed all were capable of surviving to 0°C but no life stage survived at -5°C or below. This seems to suggest that while overwintering in Newfoundland the ticks must exist *within* the leaf litter or below an insulating layer of snow where the temperature would not drop below -5°C as *H. leporispalustris* does not remain on a host during the winter. However, it should be noted that during the course of this experiment the ticks were held at room temperature and then placed directly into the experimental temperature environment with no chance to adjust to colder temperatures. In addition, all ticks tested were those taken from hares during the summer months. In the natural seasonal cycle ticks that drop off the host would slowly cool in the fall and thus may develop a physiological state more capable of being frozen without damage.

Ixodes uriae Data

The life cycle, diversity, ecology and distribution of *Ixodes uriae* in Newfoundland has already been extensively studied and well documented (Eveleigh 1974, Eveleigh and Threlfall 1974, Eveleigh and Threlfall 1975, McCoy *et al.* 1999, Muzaffar 2000, Threlfall 1968). The collections made during the current study were used solely to test for pathogens. Eveleigh and Threlfall (1975) showed *I. uriae* is most abundant in early July and occurs less frequently in June and August. The sampling for *I. uriae* was done in July and August in 2002 but only in July in 2003. Only females and nymphs of this species were collected from known hosts and their environment and the testing for vector – borne viruses were negative for the Avalon, Bauline or Great Island

strain which were originally described from these populations (Chastel 1988, Nuttall 1984). The lack of vector – borne viruses in the ticks collected now may be because they were collected from puffin chicks that may not have come into contact with the viruses as Eveleigh and Threlfall (1975) stated that puffins likely play a small role in the life cycle of *I. uriae* in Newfoundland. Also, those collected from around the puffin burrows (i.e. the unengorged ticks) may never have come into contact with the viruses even if present in puffins.

Ticks from Other Hosts

Five other tick species, *Ixodes muris*, *I. scapularis*, *I. ricinus*, *Dermacentor variabilis* and *Rhipicephalus sanguineus* were only observed in collections submitted by veterinarians from small animal clinics except for one *I. scapularis* submitted by a hunter. All were female ticks except for three male *D. variabilis*. Females being highly visible when engorged are more likely to be found by owners and taken to the veterinarian than are hosts infected with other stages and may partly account for this bias. Comparatively also the male *D. variabilis* are large and more visible than the immature stages.

Ixodes muris was collected from a dog from Parson's Pond and Colbo (pers. comm.) had previously identified *I. muris* from a sample sent for identification in November 1999 from a dog in Cape Spear thus this species has been recovered from both east and west coasts of the island. Based on the distribution of this tick in Canada (Gregson 1956) and the fact that it is normally on rodents it is likely that it could establish a resident population here and in fact may have already done so. This is more possible with the increased diversity of rodents noted above. The occurrence of this three – host

tick coupled with the increased rodent populations needs to be carefully evaluated as *I. muris* is considered to be the prime vector responsible for maintaining *Borrelia burgdorferi* in rodents in the absence of *I. scapularis* (Dolan *et al.* 2000, Randolph 2001). Should a rodent reservoir of *B. burgdorferi* become established as a result of *I. scapularis* being continuously introduced to Newfoundland then a resident population of *I. muris* could perpetuate the bacteria within the rodent population.

The current records of *Ixodes scapularis* on the island are very important considering the fact that all of the ticks submitted came from animals with no known history of travel outside of the province. Figure 11 shows that these submissions begin in June of each year and continue through to December with no submissions at all in August and September. This bimodal peak of adult *I. scapularis* activity has been well documented (Daniels *et al.* 1989, Klich *et al.* 1996, Lindsay *et al.* 1995, Sonenshine 1991b, Yuval and Spielman 1990). According to these studies, adult *I. scapularis* become active in the fall and if they do not find a host before temperatures become too cold then they overwinter as unfed ticks. The following spring these same ticks become active again and if they do not find a host by mid – June then they die. Therefore, adults are not present in late summer.

Research done by Bjoersdorff *et al.* (2001), Klich *et al.* (1996) and Scott *et al.* (2001) has demonstrated that migrating birds are capable of dispersing ticks over long distances and so it is indeed possible that migrating birds bring *Ixodes scapularis* and other ticks to Newfoundland each spring. American robins, of which Newfoundland has an abundance, are known to transport *I. scapularis* and are capable of serving as reservoir hosts for *Borrelia burgdorferi* (Banerjee *et al.* 1995, Banerjee *et al.* 1996, Richter *et al.*

2000, Scott *et al.* 2001) and *Anaplasma phagocytophilum* (Daniels *et al.* 2002).

Therefore, it is possible that nymphs brought into Newfoundland by migrating birds have had a chance to feed on the birds, drop off into the environment and molt into adults which are seen in October, November and December. These adults feed on larger mammalian hosts in the fall or overwinter as unfed ticks. The following spring those ticks that overwintered would again become active and would account for the ticks submitted during June and July. The absence of adult submissions in August and September is due to the seasonality of this particular species. Those adults that do not find a host by mid – June die, so no ticks would be found on animals during August and September.

The fact that viable eggs were laid by one *I. scapularis* female collected in late fall suggests that the female had mated with a male prior to being collected since parthenogenesis is relatively rare in ticks (Sonenshine 1991a). This suggests that foci of these ticks have a high enough population for males and females to occur together and mate in Newfoundland. This is of considerable significance and requires further surveillance to determine if there are viable established populations here.

Whether *Ixodes scapularis* can actually become a resident tick in Newfoundland is in question. These ticks feed on a variety of hosts ranging from lizards, birds, shrews and rodents as larvae and nymphs while adults most often feed on larger mammals, mainly white – tailed deer (Main *et al.* 1982, Parker and White 1992, Rand *et al.* 1993, Sonenshine 1991b, Wilson *et al.* 1988). In order for *I. scapularis* to establish populations on the island a number of criteria must be met including the number of introduced specimens, the physiogeography, the climatic attributes and the wealth of suitable hosts

(Spielman *et al.* 1985). Of primary importance is the existence of white – tailed deer since this animal is the preferred host of adult *I. scapularis* and its distribution has been strongly correlated with the presence of the deer (Spielman *et al.* 1985, Wilson *et al.* 1988). Newfoundland's mammalian fauna does not include the white – tailed deer, however, it does include foxes and domestic dogs which *I. scapularis* adults have been known to parasitize though not in large numbers (Spielman *et al.* 1985). In addition, Newfoundland's fauna includes moose and caribou which could serve as potential cervid hosts in the absence of white – tailed deer, however, this relationship needs further investigation. Due to the nonspecific host selection of the immature stages of *I. scapularis*, Newfoundland's fauna may include some rodent species that can serve as hosts for the tick. Whether these species are capable of maintaining the tick population, however, also remains to be verified. The lack of white – tailed deer on the island though may serve as a formidable barrier to the establishment of this species. In addition, the winter climate of Newfoundland does tend to be harsh which will also hinder the establishment of a stable population of *I. scapularis* (Spielman *et al.* 1985). Some portions of the island, however, do have consistent snow cover throughout the winter which would allow the adults to overwinter and therefore allow their survival (Lindsay *et al.* 1995). The data collected in this study does seem to suggest that adult *I. scapularis* are capable of overwintering on insular Newfoundland. In light of this observation, further research on the seasonality and distribution of *I. scapularis* on insular Newfoundland is warranted.

Ixodes ricinus, *Dermacentor variabilis* and *Rhipicephalus sanguineus* are considered to be only introductions to Newfoundland since these ticks were all submitted

from dogs with a history of travel, with the exception of two specimens of *D. variabilis* – one from a person who had just returned from the prairie provinces and one found in a house where the family dog had traveled off the island. *Ixodes cookei* has also been found in the province, in May of 2000, in a house and on domestic pets in that house that had come in from the Maritimes.

Flagging and Snap Trapping

The flagging procedures carried out by the staff at some of the provincial parks did not recover any ticks from the vegetation. This does not mean ticks were absent nor that the flagging procedures would not collect ticks as there is no independent evidence for the presence or absence of ticks in these locations. Elsewhere in North America flagging has been a successful method of collecting ticks (Barker *et al.* 1992, Benach *et al.* 1987, Burgdorfer *et al.* 1982, Carroll *et al.* 1992, Daniels *et al.* 1989, Dodds *et al.* 1969, Garvie *et al.* 1978, Ginsberg and Ewing 1989, Goddard 1992, Kocan *et al.* 1992, Lindsay *et al.* 1995, Ribeiro *et al.* 1987, Yuval and Spielman 1990). Also, since most of the parks only carried out the procedure once, sampling effort may be considered insufficient to detect populations of ticks.

The use of the Sherman live traps for small rodents was unsuccessful in capturing any rodents. The Victor snap traps, however, were successful in capturing small rodents but the rodents that were snap trapped did not yield any ticks. Larval and nymphal ticks would have been expected to be found on rodents but low numbers caught, time between catching and inspection and inexperienced persons checking them may have all contributed to the lack of ticks collected.

Pathogen Testing with Special Emphasis on the Potential Establishment of Lyme Disease in Newfoundland

All ticks tested for *Anaplasma phagocytophilum*, *Francisella tularensis* and vector – borne viruses were negative and the serum collected from the snowshoe hares at SNP also tested negative for *F. tularensis*. This suggests that either the pathogens were not present in the province during 2002 and 2003 or sample size was insufficient to detect it. In addition, there are two subspecies of *F. tularensis* so the type that is found in hares was not detected in this study. There has been a case of tularemia in the province in a person that had contact with an infected beaver (the water – borne Type B) (Peacock 1989). Human tularemia has also been reported from New Brunswick (Anon. 2001d) and the bacterium has been isolated from snowshoe hares in Nova Scotia and Prince Edward Island (Anon. 1996) and from muskrats and a beaver in Prince Edward Island (Anon. 2001a). There were no beavers or muskrats sampled throughout the course of this study so if it is *F. tularensis* subsp. *holarctica* (Type B) that is present in the province it would not have been detected in the *H. leporispalustris* ticks tested or the hare serum samples.

The only ticks that have tested positive for *Borrelia burgdorferi* were *Ixodes scapularis* collected in June and July. These ticks could have arrived on migrating birds and could have been infected as larvae elsewhere then as nymphs picked up and brought in on migrating birds as noted by Bjoersdorff *et al.* (2001), Klich *et al.* (1996) and Scott *et al.* (2001) and even infected by some of these birds which can also harbor the pathogens (Bjoersdorff *et al.* 2001, Klich *et al.* 1996, Scott *et al.* 2001).

As *Borrelia burgdorferi* is not usually transmitted transovarially (Daniels *et al.* 1997, Greene 1990, Sharon *et al.* 1992), any eggs and subsequent larvae arising from

positive adults brought into Newfoundland and feeding here on a large mammal will be negative for the bacteria. They would only be positive if there was an indigenous infection in the vertebrate hosts of the province. Previously, birds have not been considered to be extremely important in the transmission of *Borrelia burgdorferi* but the bacteria have been isolated from the liver of a veery (*Catharus fuscescens*) and in the blood of seven different songbirds (one northern mockingbird (*Mimus polyglottos*), one gray catbird (*Dumetella carolinensis*), two prairie warblers (*Dendroica discolor*), one orchard oriole (*Icterus spurius*), one common yellowthroat (*Geothlypus trichas*), and one American robin (*Turdus migratorius*)) (Anderson and Magnarelli 1984, Anderson *et al.* 1990). Ticks that feed on birds and small rodents can transmit *B. burgdorferi* to other vertebrates and American robins and house wrens have actually been confirmed to be competent reservoir hosts for the bacteria (Anderson *et al.* 1986, Anderson *et al.* 1990, Richter *et al.* 2000). Richter *et al.* (2000) showed that infected American robins were capable of infecting about 88% of ticks that attached to them over a three week period. After two months of being infected with *B. burgdorferi*, however, the robin's infectivity diminished and eventually disappeared after six months (Richter *et al.* 2000). Nevertheless, if reinfected with the bacterium, American robins are again capable of transmitting the bacteria to uninfected ticks (Richter *et al.* 2000).

If *Ixodes scapularis* is able to establish an endemic population on the island of Newfoundland, due to increased diversity and density of hosts resulting from human introductions and land use changes, there still needs to be a viable circulation of *Borrelia burgdorferi* in these hosts. Many factors are involved in the transmission and persistence of *B. burgdorferi* (Anderson *et al.* 1986, Anderson *et al.* 1990, Dolan *et al.* 2000, Richter

et al. 2000, Scott *et al.* 2001, Tragar 1939). Whether there was a resident population is uncertain as too few rodent collections were made leaving this question unanswered. Dolan *et al.* (2000) do not list *I. muris* as a competent vector for the Lyme disease spirochete, although they did illustrate that in a laboratory setting *I. muris* had a 66% infection rate in larvae and a 38% infection rate in nymphs. Dolan *et al.* (2000) and Randolph (2001) stated that *I. muris* is a poor vector when compared to *I. scapularis* but may serve as a competent enough vector to maintain *B. burgdorferi* numbers in a population in the absence of *I. scapularis*. Thus, a better understanding of *I. muris* biology and its vector competence is required. Similarly, *I. neotomae* has maintained a population of *B. burgdorferi* in California where the usual vector of *B. burgdorferi*, *I. pacificus*, has not maintained the spirochete population (Brown and Lane 1992).

Conclusion

A two – year study was carried out on insular Newfoundland to determine the diversity of tick fauna on the island and to further understand the potential role that they may play in transmitting pathogens to both humans and animals. Seven species of ixodid ticks were identified on nine hosts. *Haemaphysalis leporispalustris* was the most common tick collected with peak activity in July, August and September. The *H. leporispalustris* adults peaked in July and immature stages in August and September. Male hares were more heavily burdened by *H. leporispalustris* than females but this difference was not statistically significant among life stages except for male ticks in 2002. All stages of *H. leporispalustris* were able to survive temperatures as low as 0°C but none lived longer than 24 hours at -5°C or below.

The second most common tick collected was *Ixodes uriae* but due to the extensive work previously done on this tick little effort was made to collect additional data on this species.

The other five tick species (i.e. *Ixodes muris*, *I. scapularis*, *I. ricinus*, *Dermacentor variabilis* and *Rhipicephalus sanguineus*) were submitted by other individuals whose work involved contact with animals. Out of these five species, *I. scapularis* was the most commonly submitted species. The seasonal distribution of the females of *I. scapularis* indicated a bimodal temporal distribution, with no adults being submitted in August and September.

The flagging procedures and the trapping of rodents by Sherman live traps and by Victor snap traps yielded no ticks in this study.

Representative samples of the ticks collected were tested for the pathogens, *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, *Francisella tularensis* and vector – borne viruses. The only organism identified in the ticks tested was *B. burgdorferi* with a 16 % infection rate, all from adult *Ixodes scapularis*.

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Appendix 1: Summary of raw data for ixodid ticks recorded in insular Newfoundland during 2002 and 2003.

Tick Species	Life Stage	Engorged or Unengorged (E or U)	Date Collected	Host Species	Probable Localities of Acquisition
<i>Haemaphysalis leporispalustris</i>	All	E and U	April to November (2002 and 2003)	Snowshoe hare, Lincoln's sparrow, Domestic cat, Domestic rabbit	Widespread throughout NL
<i>Ixodes uriae</i>	Females and nymphs	E and U	July and August 2002	Atlantic puffin, Common murre, Environment	Groswater Bay, Witless Bay, Gull Island, Funk Island
	Female	E	July 19/03	Atlantic puffin	Coleman Island, Notre Dame Bay
<i>Ixodes muris</i>	Female	E	Sept. 6/03	Domestic dog	Parson's Pond, NL
<i>Ixodes scapularis</i>	Female	E	June 7/02	Domestic dog	Unknown
	Female	E	July 3/02	Domestic dog	Pennywell Road, St. John's
	Female	E	July 27/02	Domestic cat	Unknown
	Female	E	June 5/03	Domestic dog	Pinchgut Lake
	Female	E	June 10/03	Domestic dog	Sweet Bay, NL
	Female	E	June 15/03	Domestic dog	Goobies, NL
	Female	E	June 17/03	Domestic dog	Farm near Bay Roberts, NL
	Female	E	July 25/03	Domestic dog	St. John's, NL
	Female	E	Oct. 28/03	Domestic dog	St. Theresa's, NL
	Female	E	Oct. 29/03	Domestic dog	Cow Head, NL
	Female	E	Nov. 28/03	Domestic dog	St. John's, NL
	Female	E	Dec. 3/03	Domestic dog	Burgeo, NL
	Female	E	Dec. 17/03	Red fox	Unknown
	Female	E	Dec. 17/03	Domestic dog	Lark Harbour, NL
<i>Ixodes ricinus</i>	Female	E	June 26/02	Domestic dog	Germany
<i>Dermacentor variabilis</i>	Female	E	June 2/02	Human	Alberta
	Male	E	July 25/02	Domestic dog	Tourist's dogs
	Female	E	Aug. 20/02	Domestic dog	Nova Scotia
	Male	E	June 20/03	Environment	Unknown
	Male	E	July 22/03	Domestic dog	Nova Scotia
<i>Rhipicephalus sanguineus</i>	Female	E	Aug. 2/03	Domestic dog	Bridgeport, Connecticut

Appendix 2: Identifications keys and species descriptions of ixodid ticks recorded in insular Newfoundland during 2002 and 2003.

The identification keys provided herein were adapted by the present author from other keys to allow the identification of only those species that have been recorded in insular Newfoundland either in this study or previous studies. The keys are meant to be used as a preliminary tool and the reader is directed to the identification keys and descriptions of Gregson (1956), Kierans and Clifford (1978) and Kierans and Litwak (1989) if the tick being identified does not conform to characteristics in the key below.

Key to the Ixodid Genera for all Life Stages

- 1) Six legs.....larva.....2
Eight legs.....5
- 2) Eyes present.....3
Eyes absent.....4
- 3) Basis capituli hexagonal.....*Rhipicephalus*
Basis capituli not hexagonal.....*Dermacentor*
- 4) Anal groove posterior to anus.....*Haemaphysalis*
Anal groove anterior to anus.....*Ixodes*
- 5) Genital pore absent.....nymph.....6
Genital pore present.....adult.....see key below
- 6) Eyes present.....7
Eyes absent.....8
- 7) Basis capituli hexagonal.....*Rhipicephalus*
Basis capituli rectangular with or without basal spurs.....*Dermacentor*
- 8) Palps conical and distinctly flared at the base.....*Haemaphysalis*
Palps elongate without flaring at the base.....*Ixodes*

Key to the Adult Ixodid Ticks Recorded in Newfoundland

- 1) Scutum covering only anterior portion of dorsal surface.....**female**.....2
Scutum covering entire dorsal surface.....**male**.....10
- 2) Anal groove anterior to anus; festoons absent.....*Ixodes*.....3
Anal groove posterior to anus or indistinct or absent; festoons present.....8
- 3) Cornua absent; auriculae absent; spurs absent on all coxae; body noticeably hairy.....*uriae*
Without this combination of characteristics.....4
- 4) Internal and external spurs on coxa I about equal in length; hypostome long, narrow, 3/3 dentition throughout length and denticles that flare laterally.....*angustus*
Internal spur longer than external spur on coxa I.....5
- 5) Auriculae absent; scutum angular.....*cookei*
Auriculae present.....6
- 6) Auriculae present as posteriorly directed spurlike protuberances.....*muris*
Auriculae ridgelike.....7
- 7) Cornua present but small.....*scapularis*
Cornua absent; widely distributed in Europe.....*ricinus*
- 8) Eyes absent; distinct flaring at the base of the conical palps; ventral cornua present.....*Haemaphysalis leporispalustris*
Eyes present; no distinct flaring at the base of palps.....9
- 9) Ornate tick; rectangular basis capituli; spiracular plate with dorsal prolongations and numerous small goblets.....*Dermacentor variabilis*
Inornate tick; hexagonal basis capituli.....*Rhipicephalus sanguineus*
- 10) Anal groove anterior to anus; festoons absent.....*Ixodes*.....11
Anal groove posterior to anus or indistinct or absent; festoons present.....16
- 11) Cornua absent; auriculae absent; hypostome lacking dentition or dentition faint.....*uriae*
Without this combination of characteristics.....12
- 12) Hypostomal dentition present as crenulations.....13
Hypostomal dentition present as distinct denticles.....15
- 13) Auriculae present; hypostome deeply notched apically.....*muris*

- Auriculae absent.....14
- 14) Internal and external spurs on coxa I about equal in length; hypostome with long regular crenulations that overlap the base of the preceding row.....*angustus*
Internal spur longer than external spur on coxa I; hypostome with diagonal crenulations that do not overlap the preceding row.....*cookei*
- 15) Cornua present but small; large lateral denticles on hypostome.....*scapularis*
Cornua absent; large lateral denticles on hypostome; widely distributed in Europe.....*ricinus*
- 16) Eyes absent; distinct flaring at the base of the conical palps; ventral cornua present.....*Haemaphysalis leporispalustris*
Eyes present; no distinct flaring at the base of palps.....17
- 17) Ornate tick; rectangular basis capituli; spiracular plate with dorsal prolongations and numerous small goblets.....*Dermacentor variabilis*
Inornate tick; hexagonal basis capituli.....*Rhipicephalus sanguineus*

Species Descriptions

Genus *Ixodes*

All species of *Ixodes* have an anal groove that is anterior to the anus (Plate 1: A) and all species lack festoons.

Ixodes angustus

Ixodes angustus has internal and external spurs on coxa I that are about equal in length and a scutum that is longer than broad. This species also lacks auriculae. The females of the species have a hypostome that is long, narrow, 3/3 dentition throughout the length and denticles that flare laterally while the hypostome of the male has long regular crenulations that overlap the base of the preceding row. This tick is most often found on tree squirrels, voles, mice and other rodents (Gregson 1956, Kierans and Clifford 1978, Kierans and Litwak 1989).

Ixodes cookei

Like *Ixodes angustus*, *Ixodes cookei* also has a scutum that is longer than broad and also lacks auriculae, however, unlike *I. angustus*, *I. cookei* has an internal spur on coxa I that is considerably longer than the external spur. *Ixodes cookei* also has small cornua. *Ixodes cookei* females also have a scutum that is angular and has punctuations. The males of the species also have denticles present as crenulations like *I. angustus*, the difference being that in *I. cookei* the median denticles are present as diagonal crenulations and they do not overlap the preceding row. The males also have a spiracular plate that is almost oval in shape. This tick is usually found on skunks, raccoons, dogs and marmots (Gregson 1956, Kierans and Clifford 1978, Kierans and Litwak 1989).

Ixodes muris

Ixodes muris has an internal spur on coxa I that is longer than the external spur on coxa I (Plate 1: B), a rounded plate on palpal segment I and a scutum that is relatively impunctate and about as broad as it is long. This species also has very distinct auriculae; they are described as being posteriorly directed, spurlike protuberances (Plate 1: C). Males of the species have an apically notched hypostome with denticles present as crenulations and a large spiracular plate. This tick is usually found on mice and other small rodents (Gregson 1956, Kierans and Clifford 1978, Kierans and Litwak 1989).

Ixodes ricinus

In addition to the characteristic anal groove anterior to the anus and lack of festoons, *Ixodes ricinus* also has a distinct internal spur on coxa I (Plate 1: D) and auriculae present as distinct ridges. Cornua are absent in this species. Females of the species have a rounded scutum and a hypostome with dentition that decreases from 4/4 at

the apex to 3/3 and 2/2 at the base. *Ixodes ricinus* males have a hypostome with large lateral denticles and a large rounded denticle postero – ventrally directed. The geographic distribution of *I. ricinus* is widespread throughout Europe and the most common hosts of *I. ricinus* are sheep, cattle and other domestic animals (Arthur 1963).

Ixodes scapularis

Ixodes scapularis is considered to be the North American equivalent to *Ixodes ricinus* and therefore closely resembles *I. ricinus*. This species has a smooth scutum that is nearly circular. *Ixodes scapularis* has small but definite cornua (however the males lack these) and small but distinct auriculae. The internal spur of coxa I is longer than the external spur reaching to about the midlength of coxa II. This tick also has palps that are generally longer than wide and a hypostome with 4/4 dentition apically. There are external spurs present on all coxa but the trochanters lack spurs. *Ixodes scapularis* males have elongate spiracular plates and a hypostome with dentition that is larger laterally. *I. scapularis* can be found on a wide range of hosts with the adults most often being found on white – tailed deer, domestic cattle, dogs and other large mammals and the immature stages usually being found on white – footed mice, red – backed voles, grey squirrels, raccoons, eastern chipmunks, ovenbirds, blue jays, house wrens, American robins and numerous other mammals, birds and lizards (Kierans and Clifford 1978, Kierans and Litwak 1989, Kierans *et al.* 1996).

Ixodes uriae

Ixodes uriae is characterized by the absence of cornua, auriculae and internal and external spurs on the coxae. The females of the species are noticeably hairy while the males have a fringe of spines terminally. The hypostome of the male has very faint

dentition or is lacking dentition completely. This tick is almost exclusively a parasite of marine birds but does occasionally attach to humans (Gregson 1956, Kierans and Clifford 1978).

Haemaphysalis leporispalustris

Haemaphysalis leporispalustris is described as an inornate tick with an anal groove posterior to the anus. Like all members of the genus, *H. leporispalustris* lacks eyes but does have festoons (Plate 1: E) and distinct flaring at the base of the conical palps (Plate 1: F). More specific to the species is the presence of ventral cornua, small internal spurs on coxa IV and 3/3 hypostomal dentition. The basis capituli of the adult is rectangular dorsally whereas the basis capituli of nymphs and larvae is quadrangular dorsally. *Haemaphysalis leporispalustris* is most often found on snowshoe hares and rabbits but it can also be found on numerous species of small birds (Gregson 1956, Kierans and Litwak 1989).

Dermacentor variabilis

Dermacentor variabilis is described as an ornate tick that has eleven festoons and an anal groove that is posterior to the anus. This tick is characterized by a rectangular basis capituli (triangular in immature stages), palps that are about as long as the basis capituli and the presence of eyes. One of the most distinguishing features of this tick is the spiracular plate; it has dorsal prolongations and numerous small goblets. The main host for *Dermacentor variabilis* is the domestic dog, however, it can also be found on coyotes, deer, sheep and humans (Gregson 1956, Kierans and Litwak 1989).

Rhipicephalus sanguineus

Rhipicephalus sanguineus is another inornate tick that has festoons and an anal groove that is posterior to the anus. The basis capituli of this tick is hexagonal and the palps are dome – shaped and as long as or longer than the basis capituli. *Rhipicephalus sanguineus* is also characterized by the presence of eyes and is described as a large tick. The main host for *Rhipicephalus sanguineus* is also the domestic dog but humans may also become an accidental host and less commonly the tick can be found on rabbits, deer and mules (Gregson 1956, Kierans and Litwak 1989).

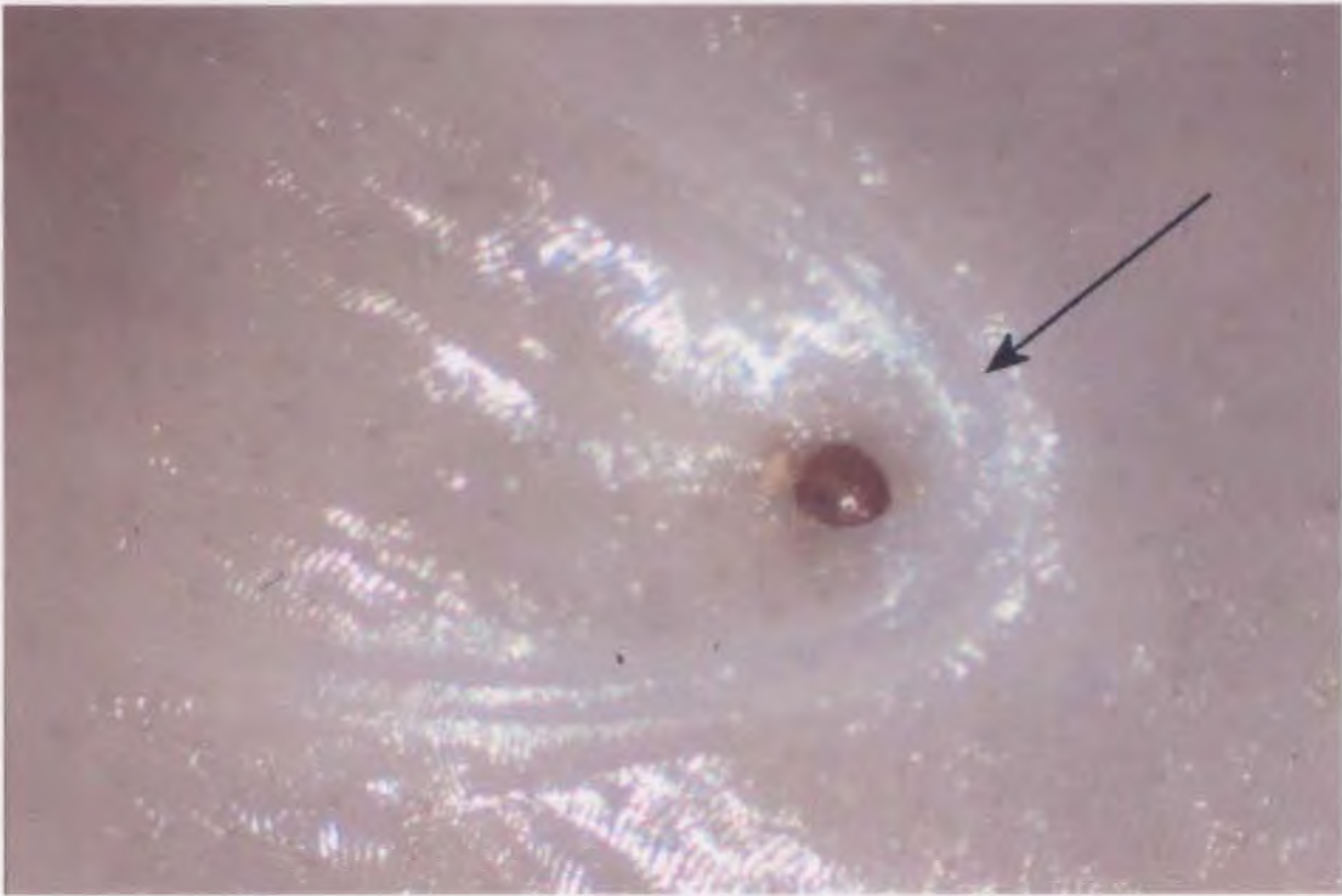
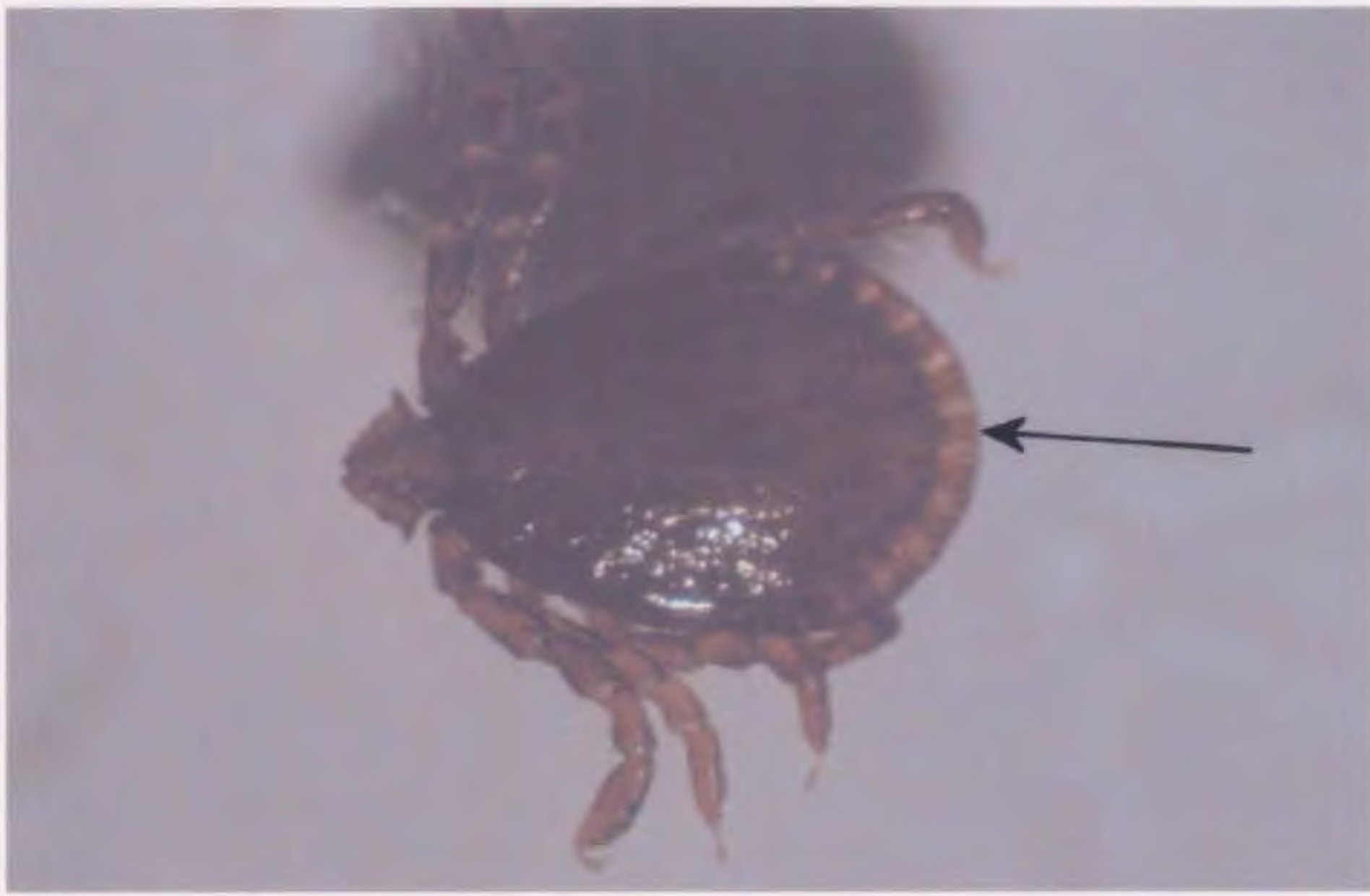
A**B****C****D****E****F**

Plate 1: Ixodid ticks collected in Newfoundland during 2002 and 2003; A) Anal groove of *Ixodes* sp.; B) Internal spurs of *Ixodes muris*; C) Auriculae of *Ixodes muris*; D) Internal spurs of *Ixodes ricinus*; E) Festoons of *Haemaphysalis leporispalustris*; and F) Palps of *Haemaphysalis leporispalustris*.

