UNIVERSITY OF ALBERTA

ATTEMPT TO DETERMINE THE INFLUENCE OF PARASITISM ON A SNOWSHOE HARE POPULATION DURING THE PEAK AND INITIAL DECLINE PHASES OF A HARE CYCLE.

BY

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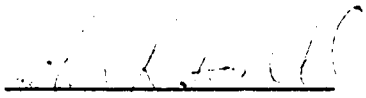

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled "Impact of parasitism on a snowshoe hare population during the peak and initial decline phases of a hare cycle" submitted by John R. Sovell in partial fulfillment of the requirements for the degree of Master of Science.

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ABSTRACT

Few naturally-occurring host-parasite systems have been experimentally manipulated. Consequently the dynamics of such systems in field are poorly understood. I surveyed the parasites of snowshoe hares, *Lepus americanus*, at Kluane Lake, southwestern Yukon and examined the potential of two pathogenic nematodes (*Protostrongylus boughtoni* and *Nematodirus triangularis*) to affect their population dynamics. Nematode numbers were manipulated in hares by subcutaneous injection (0.4mg/kg) of ivermectin. Two weeks post-treatment, nematode numbers of both species were reduced by approximately 80%. However, beyond two weeks ivermectin did not affect the total number of worms, the maturation of dormant larvae or reinfection by newly acquired larvae. Thus, repeated treatment of individual hares is necessary for successful reduction of worm numbers in the field. Turnover of the hare population was high on both treated and untreated study areas; 70% of the hares were trapped only once. This high turnover reduced the proportion of hares receiving repeated treatments of ivermectin; consequently the manipulation had no effect on overall hare density, recruitment or survival. However, at the level of individual hosts, ivermectin did have an effect. Repeatedly treated hosts were in better body condition and females produced twice times as many offspring as untreated females. In addition, untreated females lost more weight after parturition, and had 45% more of their young die of starvation and exposure. Overall survival did not differ between offspring of treated and untreated hares. My data suggest that the effects of parasites in this population of hares is compensatory to other forms of mortality, that in this population is primarily determined by predation.

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Chapter 1: General Introduction

Recently effects of parasites and disease on animal populations have received a great deal of attention. This attention has been stimulated by theoretical models showing that parasites can, given certain assumptions, regulate the size of host populations (Anderson and May 1979, May and Anderson 1979, Anderson 1982). Assumptions of these models include that: 1) infection within the host population is persistent due to continual reinfection of individual hosts, 2) infected hosts harbor parasites for long periods of time and 3) a full probability distribution of parasites within the host population can be defined. In order to simplify the models it is assumed that the parasite distribution can be reasonably modeled as a negative binomial. The negative binomial defines a distribution where more individuals are infected with a high number of parasites, or are lightly infected, and fewer have intermediate numbers of parasites than in a random (Poisson) distribution. The degree of clumping of parasites within individual hosts is defined by the inverse of the parameter k; the limit $k \rightarrow \infty$ corresponds to a Poisson distribution and a very small k corresponds to very high clumping.

Other assumptions of the model include that: 1) the host population has intrinsic rates of birth and death, 2) mortality due to the parasite or parasite induced depression of the host birth rate is a function of the number of parasites infecting a given host, 3) parasite transmission is determined by the number of contacts between hosts and parasite infective stages (as host density increases parasite transmission increases), 4) there is a natural mortality rate of adult parasites, 5) adult parasites produce infective stages at a specific rate and 6) these infective stages are subject to a natural rate of mortality. From these assumptions a model can be developed that describes the

dynamics in terms of three linked differential equations, one each for the host population, for the parasite population in individual hosts and for the free-living infective stages of the parasite.

In this model the net reproductive rate of the parasite defines whether or not host population size is regulated. If the net reproductive output of the parasite is negative, the parasite obviously can never become established. Also important is the degree of clumping of parasites within the host population. If clumping of parasites is extreme and the mortality of hosts infected with many worms is high, the parasite will be unable to establish in the host population. If both clumping and net reproductive rate of the parasite are low, regulation of the host population is unlikely. However, if clumping of parasites falls between these two extremes and net reproductive output of the parasite is moderate or high, then some regulation of the host population is likely.

Other results of the model are that: 1) parasite induced mortality must be higher than the intrinsic growth rate in the uninfected population for regulation to occur, and 2) parasites compromising host reproduction have greater potential to regulate host population size. These models have stimulated discussion of how parasites and diseases may have important consequences for animal conservation, and how biologists must consider the significance of disease when developing management programs (Scott and Dobson 1989, Scott 1988).

The dynamics of these models are supported by laboratory experiments conducted on parasites in vertebrate hosts (Scott and Anderson 1984, Scott 1987, Scott 1990). An elaborate study was conducted with the nematode *Heligmosomoides polygyrus*, naturally transmitted within laboratory mouse colonies (Scott 1987, Scott 1990). Infections of *H. polygyrus* had no effect on

mouse reproduction, but had both acute and chronic effects on survival of mice. Mouse colonies subjected to infection with *H. polygyrus*, at high rates of parasite transmission, reached lower densities than did uninfected colonies. Upon introduction of an anthelminthic the size of infected mouse colonies increased. Furthermore, the effects of the parasite on mouse survival and population size increased as colony size and parasite density per infected mouse increased, suggesting that laboratory colonies of mice can be regulated in a density-dependent manner by infection with this nematode.

Theory provides clear predictions about when and how parasites can regulate host population size, and laboratory studies on vertebrate hosts support the dynamics of the models. However, the idea that parasites might contribute to regulation of wild populations is still controversial. The main controversy centers on whether parasite-induced mortality is additive or compensatory to losses to other factors (Holmes 1982). The models outlined above do not consider intraspecific (behavioral and physiological) or interspecific (competition and predation) density-dependent factors that might affect the host population in concurrence with parasites. How these factors interact, and their relative importance in regulation of the host population, are difficult to know. Consequently, many researchers view these models with caution, noting that more information from natural field populations of hosts is required before the issue can be resolved (Holmes 1982, Scott and Lewis 1987, Scott and Dobson 1989).

In the field, populations of animals whose densities fluctuate temporally, such as cyclic species, offer good opportunities for examining how parasites influence host population size. The influence that any one factor has on the population cycle can be examined by comparing populations in the presence and absence of that factor. For parasites this could be accomplished

by either introducing or removing parasites from one or more naturally cycling populations of the host and comparing these manipulated populations to similar, but unmanipulated host populations.

This technique has been used to implicate parasites as the agents responsible for the naturally occurring population cycles of red grouse, *Lagopus lagopus scoticus*, in Scotland (Hudson 1986, Hudson and Dobson 1989, Dobson and Hudson 1992, Hudson *et al.* 1992a, Hudson *et al.* 1992b). Cyclic populations of wild red grouse were monitored over a 10 year period and in certain populations of grouse, treatments with an anthelminthic were used to control infections with the parasitic nematode *Trichostrongylus tenuis*. It was determined that both winter losses and breeding losses were responsible for changes in grouse numbers, and that recruitment of parasites and their numbers in individual grouse were correlated with the size of the grouse population. Both winter losses and breeding losses were correlated with the number of parasites per infected grouse and experimental elimination of worms increased both overwinter survival of grouse and hen reproductive output. However, during years of high grouse density, the anthelminthic treatment had no effect on grouse reproduction and the effects on overwinter survival were reduced. It was suggested that this lack of success was due to higher rates of parasite transmission in high density grouse populations that were overriding the effects of treatment. Alternatively, resistance to parasites might be lower in high density grouse populations because of interaction with other environmental factors such as nutritional or social stress. Given this situation, chemotherapuetic reduction of worm numbers was not able to prevent red grouse from cycling; however, prevention of the cycle was not the purpose of Hudson and his coworkers research.

It was also found that numbers of adult worms in grouse continued to

increase late into winter, long after transmission of *T. tenuis* had ceased. The increase in numbers of worms in late winter was explained by a temporary delay in worm development in autumn, which was resumed in the following February and March; delays in the development of parasites are common in hosts of temporal regions and are referred to as arrested development (Shad 1977). Arrested development allowed winter recruitment of *T. tenuis* to be consistently greater than summer recruitment and numbers of worms in infected grouse to be high during winter when grouse mortality was high.

Dobson and Hudson (1992) explored models of the *T. tenuis*-red grouse system and found that grouse numbers tended to cycle if parasite-induced reductions in fecundity were greater than parasite-induced mortality. Furthermore, cycle period was dependent upon the intrinsic growth rate of the grouse population, and either the life expectancy of larval parasites or the length of the temporary delay in worm development. Interestingly, model simulations and the field studies indicate that maximum worm numbers per infected host do not peak until after the grouse population has begun its decline.

Empirical evidence from this system supports the contention that parasites can influence the outcome of predator-prey interactions. Hudson *et al.* (1992b) found that on their main study area grouse taken by predators were consistently infected with higher numbers of *T. tenuis* than were birds collected by shooting. In addition, grouse found dead on the area were infected with greater numbers of *T. tenuis* than were grouse taken by predators. Furthermore, the degree of predator control varied among all of the moors studied by Hudson and his coworkers and on areas with intense predator control programs there was an increase in the number of grouse infected with high numbers of worms. This suggests that predators do not take

all grouse infected with high numbers of worms, but that they will more frequently prey upon such grouse. Modified models of the *T. tenuis* grouse system, which included both selective and random predation, show that an interesting consequence of selective predation on individuals infected with high numbers of worms was an increase in the size of the prey (grouse) population. Thus, selective predation effectively reduced the regulatory affect that parasitism had upon the grouse population, demonstrating just how complex parasite-host-predator interactions can be.

In the boreal forests of North America the populations of a number of vertebrates, including those of snowshoe hares, *Lepus americanus*, and their predators, fluctuate in a 9-10 year cycle (Keith 1963). The fluctuation of other boreal forest vertebrate populations are closely coupled to fluctuations of snowshoe hare populations, and hares have even been referred to as the keystone species in this community (Keith 1963, Krebs *et al.* 1992). For these reasons, anything influencing populations of snowshoe hares is probably important to this system.

Populations that undergo cyclic fluctuations in size, like those of snowshoe hares, offer opportunities for identifying factors that influence the dynamics of a wider variety of vertebrate populations. In an attempt to identify factors regulating cyclic populations, studies involving field manipulations have been conducted on snowshoe hares (Vaughan and Keith 1981, Boutin 1984, Boutin *et al.* 1986, Krebs *et al.* 1986 a and b, Krebs *et al.* 1992). These studies, along with others (Green and Evans 1940 a, b, c; Keith and Windberg 1978; Keith *et al.* 1984), have provided evidence that food resources and predation are important factors in the hare cycle.

Some of these studies have been part of the Kluane Boreal Forest Ecosystem Project (KBFEP). This program, conducted at Kluane Lake,

southwestern Yukon territory, Canada, began in the 1970's, consists of an investigation into the dynamics of a second snowshoe hare cycle. KBFEP is broadly focused and involves nine faculty members from three Canadian universities, along with 15 graduate students and technicians. They are providing a detailed description of the vertebrate community at Kluane, and through field manipulations, they are attempting to identify how predation, food supply, and plant nutrient levels influence the dynamics of the hare cycle.

Knowledge accumulated through this long term study includes identification of a 141-fold increase in spring hare density between the years of low and peak hare numbers, a predator assemblage that is an important cause of adult mortality throughout the cycle, a 9-fold increase in starvation deaths during the initial year of decline, and a decline in juvenile recruitment that is thought to be a major force driving the cycle (Boutin *et al.* 1986, Krebs *et al.* 1986a, b, Krebs *et al.* 1992). Interestingly, Hudson *et al.* (1992b) also found an increase in nonpredator deaths and a decline in juvenile recruitment to be important factors contributing to the demise of red grouse populations. Hudson *et al.* (1992b), found that these changes in the grouse population correlated with an increase in the numbers of parasites per host, and they attributed these changes to parasitism. The grouse that Hudson *et al* (1992b) said died of parasitism were also probably malnourished and it is possible that hares whose deaths are attributed to starvation are also infected with high numbers of parasites. However, there are no data about changes in mean intensity of parasitism during the hare cycle at Kluane.

Parasites have been dismissed as a factor of importance in other cyclic populations of snowshoe hares (Keith *et al.* 1985). This is interesting because work on red grouse by Hudson's group suggests that parasites can account for

the naturally observed cycles of a vertebrate (note however that Scottish workers have different explanations: Watson 1985). In addition, intestinal trichostrongylids are known to have negative effects on lagomorph fecundity (Dunsmore 1981), and it is reasonable to assume that trichostrongylids of snowshoe hares might also have similar effects on hare reproduction (Dobson 1988).

Surveys of snowshoe hares from throughout eastern Canada, Alberta, and Alaska have demonstrated that hares have large populations of a variety of helminths (see Chapter 2). However, information on parasites from snowshoe hares occupying the region between Alberta and Alaska, a region that includes some of the best snowshoe hare habitat in North America, is lacking. The Kluane hare population offered the opportunity to study parasites and their affect on host population size in an unsurveyed population of hares with the logistical, technical, and personnel support of an already existing research project, which had already generated a great deal of information on the dynamics of snowshoe hare cycles.

In addition to helminths, hares are host to several species of arthropods (Keith and Carey 1990; Green *et al.* 1939; Philip 1939), protozoans (Samoil and Samuel 1977), and microorganisms (Green *et al.* 1939; Spalatin and Iverson 1970). The present survey focuses on the helminths because parasites within this group, such as the trichostrongylids mentioned above and the lungworm, *Protostrongylus boughtoni*, are known to be pathogenic to this host (Goble and Dougherty 1943, Tobon 1973), and are more easily manipulated in field populations.

I conducted this study over a two year period encompassing the peak and initial decline years of the present hare cycle at Kluane. In Chapter 2 of this thesis, I report the patterns of infection of helminth parasites in hares at

Kluane. The purposes of this chapter are to determine: 1) the species of helminths present at Kluane, 2) which helminths at Kluane occur in high enough numbers to affect hare abundance, 3) which helminths show seasonal, age related or sex related patterns of infection that would increase their potential to influence hare populations through reproduction and survival, and 4) whether any of the helminths infecting these hares show significant increases in worm numbers between the peak and initial years of decline. The mathematical models of the *T. tenuis*-red grouse system suggest that peak numbers of worms in the host population should be reached after initiation of decline of the host population.

In Chapter 3, I examine whether chemotherapeutic treatment using the anthelminthic ivermectin successfully reduces numbers of those helminths having the greatest potential to influence reproduction and survival in this population of hares. I conducted three experiments designed to determine: 1) the degree of worm loss associated with a single administration of ivermectin, 2) the length of time ivermectin was effective in reducing worm numbers, and 3) the effect of repeated ivermectin administration in reducing worm numbers.

In Chapter 4, I report the results of a field manipulation using repeated ivermectin treatments to reduce numbers of nematodes in certain populations of free-ranging hares. With this manipulation I test the hypotheses that 1) female hares treated with ivermectin will have higher rates of fecundity and their offspring will have higher rates of survival, than do untreated females; and 2) treated populations of adult hares will be maintained at higher densities and have higher rates of survival than untreated populations.

In addition, I had planned to estimate parasite numbers, through examination of hare fecal pellets, in radio collared snowshoe hares on all of

KBFEP study areas. With this information I had planned to look at the effects that each of KBFEP manipulations had on parasite numbers and to examine the numbers of parasites in animals dying, taken by predators or surviving. Acquiring a large enough number of samples to perform this analysis would have required a major change in the protocol of other researchers and technicians working on KBFEP. Also, it would have required more personnel than was available through KBFEP. The number of fecal samples actually collected were inadequate to evaluate these questions, therefore these samples were not examined.

In the concluding chapter (Chapter 5), I will discuss the effectiveness of this study in clarifying the role that parasitism has in the Kluane hare cycle. Specifically, I will discuss how the chemotherapeutic treatment was only partially successful at providing a manipulation through which the effects of parasitism on hare survival and reproduction could be identified. Where the effects are identified, I will discuss what those effects were and where the manipulation failed; I will discuss the reasons for that failure. Finally, I will discuss how uncontrollable factors outside of the manipulation led to unexpected results.

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Chapter 2: Helminths infecting snowshoe hares, *Lepus americanus*, at Kluane Lake, southwest Yukon, Canada

Introduction

In this chapter I examine the general characteristics of infection with helminths in the population of snowshoe hares at Kluane. The basic purpose for this study is to identify those helminths that have the potential to affect hare survival (by affecting energy budgets or by making hares more susceptible to predators) or productivity (by negatively affecting survival of young hares). In this study, I attempt to determine if the population dynamics of snowshoe hare cycles are affected by parasitism. The ability of parasites to regulate host populations is a topic of much debate, and recent field experiments indicate that the potential for regulation does exist (see Chapter 1).

Seasonality of helminth infections is very well documented in the parasitological literature, especially among helminths of vertebrates of temperate climates (Keymer and Dobson 1987, Haukisalmi *et al.* 1988, Montgomery and Montgomery 1988, Gregory *et al.* 1992). The mechanisms driving seasonality are complex and result from interactions between host reproduction, changes in host immunity, parasite life history traits including arrested development and, especially, the influence of environmental conditions on parasite generation time and development (Blitz and Gibbs 1972a, b, Schad 1977). It is probable that helminths of snowshoe hares also show strong seasonal dynamics based on these complex interactions.

It is also possible that seasonal fluctuations in mean intensity and prevalence of infection can influence host populations, particularly if worm

abundance peaks during periods that are important for host reproduction or survival. For example, if numbers of parasites in females peak during the breeding season, as is the case with *Obeliscoides cuniculi* (Erickson 1944, Gibbs *et al.* 1977, Keith *et al.* 1985) and *P. boughtoni* (Kralka and Samuel 1989) in hares, reproductive output may be compromised. Hudson *et al.* (1992) found that the yearly mean number of nesting red grouse, *Lagopus lagopus scoticus*, was negatively correlated with the yearly mean intensity of infection with *Trichostrongylus tenuis*, and that chemotherapeutic reduction of worm numbers increased grouse reproduction.

Alternatively, if potentially pathogenic parasites reach high mean intensities of infection during periods when the host suffers increased predation or food resource limitation (such as during winter for snowshoe hares) then the hosts physical condition might be further compromised. Hudson *et al.* (1992) found that chemotherapy significantly increased over-winter survival of red grouse. In hares, peak mean intensity of infection with *Nematodirus aspinosus* and *Protostrongylus* spp. in *Lepus timidus* from the former Soviet Republics occur in winter (Kontrimavicus and Popov 1960), and in snowshoe hares from Minnesota, prevalences of *Trichostrongylus* spp. and *Nematodirus triangularis* also peaked during the winter months (Erickson 1944). If parasites infecting snowshoe hares at Kluane exhibit similar seasonal variations, they could potentially affect the population dynamics of the hares. Therefore, one objective of this study was to determine the seasonal dynamics of the helminth populations in snowshoe hares at Kluane.

The potential of helminths to influence host population dynamics is directly related to the numbers of worms within the host (Hudson and Dobson 1992). If parasites are to regulate host populations, then worm populations should be highest during periods of host population decline (e.g. Dobson and

Hudson 1992). Indeed, in snowshoe hares in Minnesota, Erickson (1944) found that mean intensities of nematodes peaked during the initial year of hare decline and remained high for an additional 2 years, declining only after the hare population had approached its lowest density. Any parasites of hares at Kluane that have high densities of infection in peak and declining populations of hares may be important in regulating this host population. A further objective of this study was therefore to compare worm populations between the year of peak hare density and the year of initial hare decline.

Materials and Methods

Collection Site

Hares were collected from an area of approximately 400km^2 within the boreal forest at Kluane Lake, Yukon Territory, Canada (61° North, 138° West). The vegetation on this area varied from dense closed spruce forest, to a patchwork of dense forest and shrub, to very open spruce (Krebs *et al.* 1986). *Salix glauca* and *Betula glandulosa* are the dominant winter food plants and their abundance varied throughout the study area. Important herbaceous food plants included *Lupinus arcticus*, *Arctostaphylus uva-ursi* and *Arctostaphylus rubra* (Krebs *et al.* 1986).

Common predators of snowshoe hares in the study area included goshawks (*Accipiter latrans*), great horned owls (*Bubo virginianus*), coyotes (*Canis latrans*), and lynx (*Lynx canadensis*). Other predators in the area include Harlan's hawks (*Buteo harlani*), golden eagles (*Aquila chrysaetos*), foxes (*Vulpes vulpes*), wolverines (*Gulo gulo*) and wolves (*Canis lupus*) (Boutin *et al.* 1986). Predators accounted for 78% of all mortalities of radio-collared hares at Kluane, between 1986 and 1990 (Krebs *et al.* 1992). Forty percent of

these predator kills were by mammals, 27% were by raptors and 36% by mammals or a raptor.

From 1989 to 1991 the mean monthly temperature in spring - summer was 8.4° C ± 4.7, and in autumn - winter, -16.2° C ± 7.0. Corresponding precipitation in spring-summer was 31.95mm ± 21.10, and in autumn - winter, 13.9mm ± 5.9 . The mean snow depth in winter from 1978 to 1984 was 54cm and snow cover persisted on the study area from approximately mid October to May (Krebs *et al.* 1986).

Collection of hares

I obtained hares for necropsy using a variety of methods. KBFEP personnel collected fresh road-killed hares throughout the year from the Alaska Highway which bisects the study area. Road killed hares accounted for the majority of hares that I examined. I personally collected and examined the carcasses of an additional 114 hares while performing experiments associated with other aspects of my thesis work. In addition, approximately 100 hares, most of which were used in my research, were periodically collected for various purposes off KBFEP study grids. Also, during KBFEP bimonthly trapping sessions all trap-dead hares from their study grids were collected. Lastly, KBFEP tracked, at all times and throughout the study area, approximately 150 radio-collared hares. These radio-collars changed pulse rate whenever the hare had not moved for 4 hours; this indicated mortality. Dead radio-collared hares were collected as soon as possible. Approximately 250 radio-collared hares were collected between winter 1989 and winter 1991, of which approximately 20 were complete carcasses that could be examined for parasites. All complete carcasses of hares were necropsied to determine the species of helminths present, and their numbers. Cysticercus larvae of both *Taenia macrocystis* and *T. pisiformis* were present, but were pooled together

because they occurred in the same locations in the hares, and their larvae can only be identified by microscopic examination of their hooks, which was not feasible.

Examination of hares

From December 1989 to December 1991 approximately 1063 snowshoe hares were collected; I examined 489. Most of the 489 were processed by KBFEP personnel at the time of collection. After weight and sex were determined, the hares were processed using either one of two procedures. Most hares were eviscerated and gastrointestinal tracts and lungs were frozen for examination at a later date. Occasionally, carcasses were frozen whole and I examined these at a later date.

KBFEP necropsy protocol called for the examination of all four ankle joints of all hares for the presence of *Dirofilaria scapiceps*; none were found. Legs were skinned and subcutaneous tissues, intramuscular fascia, and connective tissues around tendons of each leg were examined for nematodes (Bartlett 1983). I personally examined 20 hares collected in December 1989 and 60 hares collected between June and October of 1990 for the presence of *D. scapiceps*. None were found.

Examinations for metacestodes of *Taenia* spp. and cursory examinations for those of *Teania serialis* were performed only on hare carcasses that were complete at the time I examined them. This means that for most hares eviscerated by KBFEP personnel, there was no information on infection with these metacestodes. To access the thoracic and abdominal cavities, hares were skinned from their ventral surface, to the lateral sides of their body and along the four limbs to the ankles. During the performance of this skinning procedure no *Teania serialis* were ever found. The abdominal cavity was opened ventrally from the pubic symphysis to the diaphragm and the

gastrointestinal tract and its associated mesenteries were removed. The mesenteries associated with the gastrointestinal tract and spleen, the sub-lumbar musculature, and the area dorsal to each displaced kidney were examined for cysticerci (Bursey and Burt 1970). The liver was removed and examined for flukes, and for cysticerci, which can be deeply embedded within the tissue (Keith *et al.* 1985). The pubic symphysis was severed, the vertebral column broken at the sacrum, and the pelvic cavity examined for cysticerci (Yuill, unpublished). The cysticerci of all *Taenia* spp. were recovered and counted.

Gastrointestinal tracts were divided into three parts; the stomach, the small intestine, and the large intestine/caecum. The stomach contents from 20 hares collected in December 1989 and from 60 hares collected between June and October 1990 were examined for the presence of *O. cuniculi.* The stomachs from 50 additional hares representing collections from throughout 1991 were also examined for *O. cuniculi.* The stomach was opened, its contents collected, the mucosa scraped, and all accumulated debris washed first through an 850 micron sieve and then through a 425 micron sieve. The material collected in each sieve was partitioned into glass petri dishes, diluted with tap water, and examined using a dissecting microscope. In all, 130 stomachs from hares collected throughout 1990 and 1991 were examined for *O. cuniculi*, but none were ever found.

The intestinal tracts of 337 hares were examined for the presence of helminths. The small intestine and the large intestine/caecum were each flushed with tap water, opened along their length and scraped with forceps. Debris collected was washed through two sieves (850 micron and 250 micron (small intestine) or 850 micron and 425 micron (large intestine/caecum)). The material collected in each sieve was examined as for the stomach material. All

nematodes were removed and counted, but on occasion, when the number of *Passalurus* sp. present was too great for complete direct enumeration, a 20% aliquot was used for estimating numbers. All adult tapeworms from the small intestine were recovered and counted.

The intact lungs were removed from the thoracic cavity by severing the trachea at the point of the pharynx and separating the lungs from the associated pleural mesenteries. A piece of tissue 6 mm or less was removed from the tip of the apical, cardiac, and diaphragmatic lobes of each half of the lung, and the bronchi flushed with water passed through a hose to a pipette inserted down the trachea; the effluent was collected and examined using a dissecting microscope for the presence of any *P. boughtoni*. Then, under a dissecting microscope, the primary bronchi, secondary bronchi, and bronchioles of both the flushed lung and the tissue pieces were opened with scissors and examined. All *P. boughtoni* were removed and counted.

Preparation of parasites

Samples of each nematode species recovered were periodically collected and fixed in cold glycerin alcohol, and identified in temporary glycerin mounts. Adult tapeworms were fixed in (aceto-formol-alcohol), stained with Semichon's acetocarmine, dehydrated in alcohols, cleared in xylene and mounted in Permount. On occasion, aquamount was used to prepare "*en face*" mounts of hooks of fresh *Taenia* spp. cysticerci for identification.

Statistical analysis

The tendency of parasites to be clumped or aggregated within the host population has been known for many years (Williams 1964). Two accepted measures of the degree of clumping are variance to mean ratios and the parameter k. It is recognized that both of these methods are to some extent

problematical in their measurement of clumping; however, variance to mean ratios have been shown to be good measures of clumping when prevalence and/or mean intensity of infection is changing and when the tail of the distribution is of interest (Scott 1987). Hurlbert (1990) demonstrated that variance to mean ratios can erroneously detect randomness in distributions that are not normal. However, variance to mean ratios significantly >1.0 are legitimate measures of clumped or aggregated distributions and for this reason I used variance to mean ratios to measure clumping in parasite distribution among hosts. Because all five helminth species were non-normally distributed (aggregated) within the hare population, I used log-transformed mean intensities or median values of worm numbers (zeros included) when analyzing for seasonal and age-related patterns of infection. Abundance of infection is defined as the number of worms found in all necropsied hares and includes both infected and non-infected hares; mean intensity is defined as the mean number of worms per infected hare; and prevalence is defined as the proportion of infected hares in the sample (Margolis *et al.* 1982).

I compared mean intensities of infection between years and among seasons with a two-way ANOVA (SYSTAT, Inc., Evanston Illinois) on log-transformed values of mean intensities for all adult hares necropsied. For this purpose I defined the 2 years of the study as December 1989 through November 1990 and December 1990 through November 1991. Each year was then divided into four seasons (winter = December - February, spring = March - May, summer = June - August, and autumn = September - November).

I further examined seasonal patterns of infection by fitting values to a sine function. A simple form of a seasonal cycle is one that reoccurs every 12 months. If the patterns in median abundance of infection that I observed were seasonally determined, reoccurred annually and were gradual, then a

cyclic function with a 12 month phase should explain a significant portion of the variance. I tested this possibility by fitting the median values of abundance for each year divided into four periods to a standard sine function:

$y = A+b(\sin(3.14x/2-C))$, where

y = median abundance by period,

A = y-intercept,

b = slope of the sine wave = the magnitude of the wave,

x = month,

3.14/2 = converts to radians for a 12 month sine wave with four periods/year, and

C = constant determining initial position on the sine wave.

The effect of age on infection was examined by separating the samples into four body weight classes (≤ 600g, 601g - 1000, 1001g -1200g and > 1200g). Analyses for patterns in abundance of infection by age were performed only on hares collected between June and September of both years of the study. During this period female hares produce two to four litters of young and it is during this time that there are leverets in the population that weigh less then 1200g. By the end of September, young of the year have reached 1200g in weight, and it is impossible to distinguish them from adult hares that are > 1 year old.

Results

Seven species of helminths, including four species of nematodes and three species of cestodes, were identified in the hares from Kluane (Table 2.1). Prevalences of three, *Protostrongylus boughtoni*, *Nematodirus triangularis*, *Taenia leporis* were high; numbers of parasites were extremely variable for all

Table 2.1. Helminth parasites in snowshoe hares from Kluane Lake.

Nematodes:	Prevalence	N	Intensity (Mean±S)	Range	Status[e]	References
Nematodirus triangularis[a]	90	344	164.0 ± 254.9	0 - 1524	s	Yamaguti (1961)
Protostrongylus boughtoni[b]	73	332	10.3 ± 1.5	0 - 89	s	Boev (1975); Kralka and Samuel (1990)
Trichurus leporis[a]	43	344	12.8 ± 22.8	0 - 125	g	Yamaguti (1961)
Passalurus sp.[a]	24	344	1274.7 ± 3907.3	0 - 25,389	l	Skrjabin *et al.* (1974)
Cestodes:						
Taenia spp. (*T. macrocystis* & *T. pisiformis*)[c]	31	269	5.8 ± 7.1	0 - 32	g	Verster (1969)
Mosgovoyia pectinata[d]	13	425	9.3 ± 16.7	0 - 92	l	Skrjabin (1951)

a Life cycle is direct.
b Snowshoe hares are definitive hosts and terrestrial snails of the families Zonitidae and Pupillidae are intermediate hosts.
c Snowshoe hares are intermediate hosts and felids and canids, respectively, are definitive hosts.
d Snowshoe hares are definitive hostsd and oribatid mites are intermediate hosts.
e s = These parasites are specialists, reported only in *Lepus americanus* and *Sylvilagus floridanus*.
l = This parasite infects members from a number of different genera in the family Leporidae.
g = These parasites are generalists infecting mammals other than leporids

species. Only *Passalurus* sp. and, perhaps, *Nematodirus triangularis* could be considered numerous.

Variance to mean ratios were high for all helminths indicating that parasites were aggregated with a small number of hosts carrying high proportions of parasites. Variance to mean ratios for each species were generally higher than 5 at all times; never was the ratio below unity for any parasite. For example, fewer than 1% of hares had over 75 *T. leporis* (500+ *T. leporis* is thought to result in reduced host vitality and death; Mascarenhas 1989). Infections with *Passalurus* sp. were rare at Kluane in 1990 with only 5 of 147 hares infected. In 1991 79 of 197 hares were infected with this parasite, however, only 38 had over 3000 *Passalurus*, numbers known to cause mortality in lagomorphs (Stimenov 1987).

Infection with 3 helminths, *Trichuris leporis* and *Taenia* spp., did not differ either between the 2 years of the study or by season. Both prevalence and mean intensity of infection with *Taenia* spp. were low. Only 29% of the hares were infected with numbers of *Taenia* spp. that were greater than mean intensities.

The adult cestode, *Moscovoyia pectinata*, occurred in only 12% of the examined hares (Table 2.1). However, 92% of these infections occurred during the summer period, June - August, and 90% of these summer infections occurred in leverets.

Because *Protostrongylus boughtoni* and *Nematodirus triangularis* were the only parasites found to be potential pathogens for snowshoe hares, and both show patterns of infection associated with season of collection and host age, the rest of this section will focus on them.

Seasonal patterns of infection

Infections of *P. boughtoni* varied seasonally, but there was no significant variation between years, and no interaction between season and year (Table 2.2). For both years, prevalence of infection and median values of abundance peaked in the summer to autumn periods (Fig. 2.1a). Further evidence of a seasonal pattern is provided by the sine regression with an amplitude of 0.5 log units that explained 95% of the variance in median values of abundance in adult hares (F_S = 18.178, $p < 0.001$; y = 4.8 - 3.2(sine(3.14x/2 - 0.7))). For most seasons, the actual median values of abundance were well predicted by the sine wave; however, the curve consistently under-estimated median abundance's for 1990 and over-estimated median abundance's for 1991.

Nematodirus triangularis also varied seasonally. Both prevalence of infection and median values of abundance of *N. triangularis* were lowest during spring of each year (Fig. 2.1b). In every season of 1991 median values of abundance were higher than that for the same season in 1990. A sine curve with an amplitude of 0.7 log units explained 69% of the variance in median values of abundance in adult hares (F_S = 18.235, $p < 0.001$; y = 78.3 + 44.0(sine(3.14x/2 - 0.3))). Mean intensities of *N. triangularis* differed significantly between seasons, and also between years (Table 2.2), but there was no interaction between season and year. The sine wave over-estimated median abundances for 1990 and under-estimated most median abundance's for 1991. Seasonal patterns of infection were not affected by sex of the host for either species (Table 2.3).

Patterns of infection by host age

Prevalence of infection of *P. boughtoni* rose sharply as hare weight increased to 1200g and then declined in older hares weighing in excess of 1200g (Fig 2.2a). Adults used in this comparison were collected from

Table 2.2. Summary statistics of two-way ANOVA testing for effect of season (winter, spring, summer, autumn) and year (1990 and 1991) on mean intensity of infection of *Protostrongylus boughtoni* and *Nematodirus triangularis*.

P. boughtoni Source of Variation	DF	SS	F
Season	3	10.365	3.402 *
Year	1	1.372	1.351 NS
Interaction	3	4.379	1.437 NS
Error	156	158.443	
Total	163	174.559	

N. triangularis	DF	SS	F
Season	3	44.996	8.195 ***
Year	1	19.705	10.766 ***
Interaction	3	5.183	0.944 NS
Error	201	367.878	
Total	208	437.762	

a * = $p < 0.05$
*** = $p < 0.001$
NS = not significant

Fig. 2.1. Prevalence of infection (top), and observed and predicted (sine function) values of abundance for*Protostrongylus boughtoni* (a) and *Nematodirus triangularis* (b) in snowshoe hares at Kluane. Sample sizes are in parentheses.

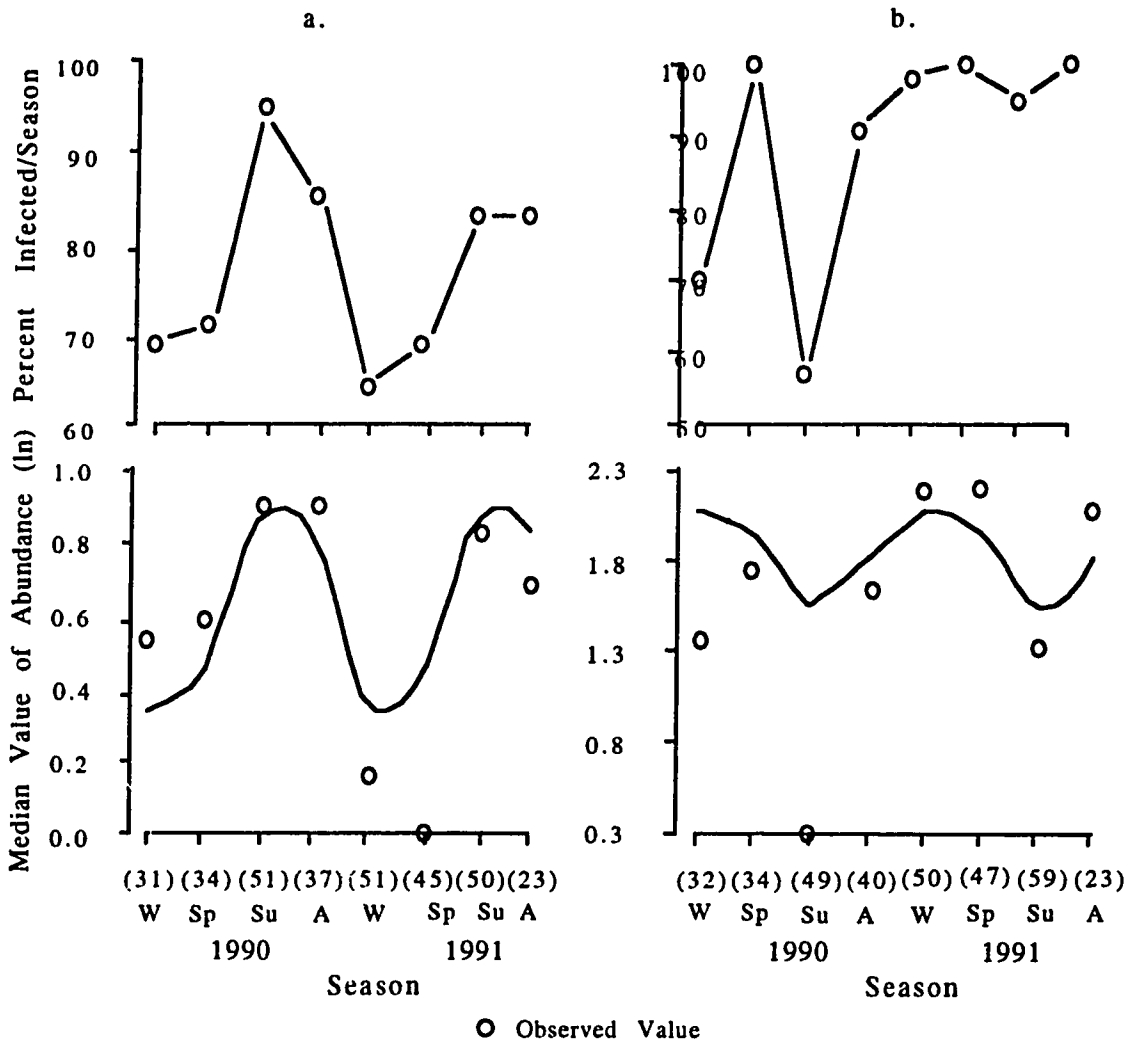


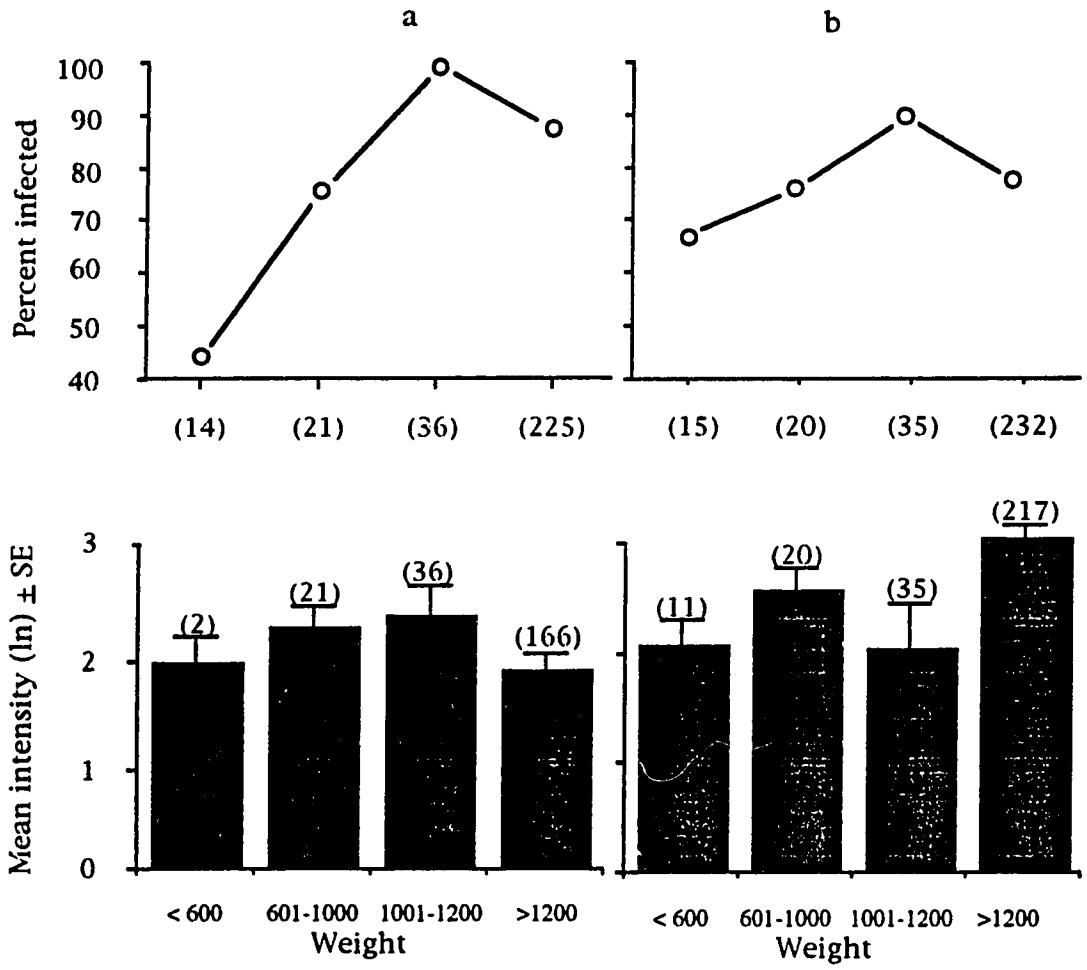
Table 2.3. Summary statistics of two-way ANOVA testing for effect of season (winter, spring, summer, autumn) and sex on mean intensity of infection of *Protostrongylus boughtoni* and *Nematodirus triangularis*.

P. boughtoni Source of Variation	DF	SS	F
Season	3	8.088	2.626 *
Sex	1	1.988	1.936 NS
Interaction	3	2.498	0.811 NS
Error	145	148.879	
Total	152	161.444	

N. triangularis	DF	SS	F
Season	3	62.137	10.994 ***
Sex	1	0.414	0.220 NS
Interaction	3	15.397	2.724 NS
Error	195	367.374	
Total	202	445.322	

a * = p = 0.05
*** = p < 0.001
NS = not significant

Fig. 2.2. Prevalence (top) and intensity of infection in snowshoe hares at Kluane by weight class (grams) for *Protostrongylus boughtoni* (a) and *Nematodirus triangularis* (b). Sample sizes are in parentheses.



throughout the summer while juveniles from the heaviest weight class occur only in late summer. However, the differences in prevalence of infection were still present after eliminating adults from early summer from the data. Mean intensity of infection with *P. boughtoni* increased as hare weight increased to 1200g (Fig 2.2a). In hares weighing in excess of 1200g mean intensity of infection with *P. boughtoni* appeared to decline but the decline in mean intensity was not significant (ANOVA; $F_S = 1.459$, $p = 0.232$). Eliminating adults from early summer from the data did not change the patterns.

Similarly, prevalence of infection with *N. triangularis* increased in hares weighing up to 1200g and then decreased in hares weighing in excess of 1200g (Fig. 2.2b). Also, mean intensity of infection with *N. triangularis* differed significantly between age classes (Fig. 2.2b). Mean intensities were significantly higher in adults weighing in excess of 1200g ($F_{(S)} = 8.184$, $P = 0.005$); differences of infection among the three lighter weight classes were of marginal significance (post hoc test; $F_{(S)} = 2.922$, $P = 0.059$).

Discussion

All seven of the helminths infecting the snowshoe hares at Kluane are commonly found in other parts of the North American range of this mammal (Table 2.4). *Trichostrongylus* spp. that commonly infect hares elsewhere are absent from this hare population, as are *Obeliscoides cuniculi* and *Dirofilaria scapiceps*. The absence of these nematodes in hares from Kluane is important because they are all potential pathogens that could influence host survival and productivity (growth and reproduction) (Russell *et al.* 1966, Dunsmore 1981, Bartlett 1984).

Table 2.4. Helminth parasites of snowshoe hares identified from various localities of North America.

Location	N	Helminth Species[a]											References
		Nematodes							Cestodes			Trem-atode	
		Pb	Nt	Tl	P	Oc	T	Ds	Ta	Mp	M	Dd	
Alaska	165+				√	√		√	√	√			Philip (1939), Barret & Dau (1981)
Yukon	425	√	√	√	√				√	√			Present study
Alberta	346	√	√	√	√b	√	√	√	√	√c	√c		Keith *et al.* (1985/86)
Manitoba	420	√	√	√	√	√			√	√	√		Boughton (1932)
Minnesota	1000	√	√	√	√	√	√	√	√	√	√		Erickson (1944)
Michigan	285	√	√	√	√	√	√	√	√		√		Bookhout (1971)
Newfoundland	630		√			√			√		√	√	Dodds & Mackiewics (1961), Smith & Threlfall (1972)

a Pb = *Protostrongylus boughtoni*, Nt = *Nematodirus triangularis*, Tl = *Trichuris leporis*, P = *Passalurus* sp., Oc = *Obeliscoides cuniculi*, T = *Trichostrongylus* sp., Ds = *Dirofilaria scapiceps*, Ta = *Taenia* spp., Mp = *Mosgovoyia pectinata*, M = *Multiceps* sp .and Dd = *Dicrocoelium dendriticum*.
b Personal observation of the author.
c Samual, unpublished.

Parasitic nematodes in general tend to be under-represented in populations of hares occupying the northern and eastern boundaries of the range of this mammal (Table 2.4). Nematodes may be under-represented in hares from Newfoundland because dispersal to the island from the mainland of North America is restricted. The absence of common hare nematodes from Alaska and the Yukon may be related to climatic conditions. The length of time that snow cover persists in these regions might disrupt transmission making it impossible for viable populations of *O. cuniculi* to become established. Alternatively, Measures (1983) noted that eggs of *O. cuniculi* were not viable at 4°C. The duration of winter coupled with extremely low winter temperatures might explain the absence of *O. cuniculi* in the southwestern Yukon. Also, Bartlett (1984) suggests that in the absence of *Sylvilagus floridanus*, the normal host of *D. scapiceps*, establishment of this parasite depends on the population density of both snowshoe hares and mosquitoes (the vector responsible for transmission of *D. scapiceps.*). In the southwestern Yukon densities of snowshoe hare populations at the cycles low might be too low to support viable populations of *D. scapiceps.*

Only the lungworm *Protostrongylus boughtoni*, and the small intestine worm, *Nematodirus triangularis*, appear to have the potential to adversely affect populations of these hares. Infections with *Passalurus* sp. can cause pathology in lagomorphs (Romero-Rodriquez *et al.* 1987) and high mean intensities of infection can lead to mortality (Stoimenov 1987). However, few of the hares examined from Kluane were infected with high numbers of *Passalurus* sp. Infections with pinworms are usually controlled by host immune responses (Jacobson and Reed 1974). The marked increase in prevalence of infection with *Passalurus* sp. during the initial year of decline

may result from reduction in immune response possibly indicating a general decline in condition of the hares at Kluane. Thus, pinworms could have an effect on some hares, but so few as to have little influence on the hare population.

Infections of short-lived intestinal cestodes induce relatively low pathology and have little effect on host energy budgets (Munger and Karasov 1989). In hares at the Kluane, infections with the intestinal cestode *Mosgovoyia pectinata* were highest in summer when food resources are not limited and occur, for the most part, in rapidly growing, but non-reproducing leverets. By the time leverets reach 4 months of age prevalence declines to 4%, the same prevalence in adult hares. Infected leverets probably respond immunologically to *M. pectinata* and are then mostly immune to reinfection, making it unlikely that this parasite has any influence on the hare population.

Trichuris spp. can cause severe pathology in infected hosts, affecting host productivity (Nooruddin *et al.* 1987) and high mean intensities of infection can lead to reductions in host vitality resulting in death (Mascarenhas 1989). In this study few of the examined hares were infected with high numbers of *T. leporis*. In addition, this parasite was not concentrated in hares in any of the critical time periods. Thus *T. leporis* could have an effect on some hares, but so few as to have little influence on the hare population.

Taenia spp. can also cause severe pathology in infected hosts, affecting host survival and productivity (De Aluja and Vargas 1988, Langham *et al.* 1990, and Lin *et al.* 1990). Larval stages of tapeworms are known to affect host ability to avoid predators and this increased risk to predation enhances transmission rates of the parasite (Leiby and Dyer 1971). However, those

larval tapeworms that increase host risk to predation usually encyst within the limb musculature or the lungs. The larval taeniids infecting snowshoe hares at Kluane encyst only within the mesenteries of the abdominal cavity, the cavity of the pubic symphysis and within the liver. *Taenia taeniaeformis* larvae encysting in rat liver can reduce reproductive output (Lin *et al.* 1990). However, numbers of larvae resulting in reductions in reproduction were 50% higher than the highest mean intensity infection found in any hare examined from Kluane. To summarize, taeniids infecting hares at Kluane do not encyst in organs important for escape, occur in low numbers and exhibit no seasonal patterns of infection. Although they could have an effect on some hares, they should have little influence on the hare population.

Infection with gastrointestinal nematodes can change host physiology and reduce host survival and productivity (Dunsmore 1981, Rau 1985, Holmes 1987). Holmes (1987) reviewed the effects of gastrointestinal nematodes on host physiology and he states that gastrointestinal nematodes reduce food-intake, gastrointestinal motility, digestion, absorption, and energy and mineral metabolism, while increasing protein catabolism. All of these factors limit energy stores, causing reduced productivity in infected hosts. Furthermore these energy-limited hosts should have a greater need for food and the trade-off between feeding and avoiding predators should shift to feeding, making infected individuals more susceptible to predation (Milinski 1990).

There have been no studies of the physiological effects that infections with *N. triangularis* have on snowshoe hares. However, infections of this nematode reach highest mean intensities in late winter when food resources are limited and when predation is a significant cause of hare mortality at Kluane. In the last cycle of hares there, predation accounted for 58% of all

mortalities during the initial winter of decline (Boutin 1986). In the winter of 1990-1991, when the hare population at Kluane began to decline, mean intensities of *N. triangularis* were significantly higher than they were during winter 1989-1990. Bookhout (1971) and Keith *et al.* (1986) reported mean worm numbers that were only 1/2 and 1/7, respectively, of the numbers I found in hares from Kluane. All studies involved hares examined from the peak of a cycle, and between year differences in worm numbers resulting from differing hare densities at different points in the cycle probably does not explain these differences. These authors, however, found more species of gastrointestinal nematodes in the hares they examined. The less diverse nematode community in hares at Kluane may lessen competition for gastrointestinal resources and numbers of *N. triangularis* may be higher in hares at Kluane for this reason. It is also possible that resource-stressed animals are simply unable to limit infection.

Furthermore, adult hares are infected with the highest numbers of *N. triangularis*, suggesting that they are unable to limit reinfections with this worm. This inability to avoid reinfection may further reduce energy stores or increase risk of predation during winter when infections of *N. triangularis* are highest. The high abundances, the seasonal and age-related dynamics of infection, the significant increase in mean intensity of infection in the winter of initial decline and the effects that gastrointestinal nematodes have on host physiology all suggest that *N. triangularis* could be an important factor affecting hare survival during the initial winter of hare decline.

Nematodirus triangularis may also affect fecundity in adult female hares. Female European rabbits, *Oryctolagus cuniculi*, infected with the small intestinal nematode, *Trichostrongylus retortaeformis*, produced fewer and smaller kittens than did uninfected does (Dunsmore 1981). This reduction in

fecundity was associated with parasite-induced atrophy of the does' intestinal mucosa. The small intestinal nematode *N. triangularis* may affect female hares in a similar manner.

Lungworms are also known to affect host survival and productivity. Lung tissue damage associated with *Protostrongylus* spp. infecting bighorn sheep, *Ovis canadensis*, affect the sheeps' ability to run (Giest 1971, pp. 214 - 215), potentially making infected sheep more susceptible to predation (Uhazy and Holmes 1973). Also, the lungworm *Rhabdias bufonis* infecting juvenile toads, *Bufo bufo*, negatively affect toad growth and survival (Goater *et al.* 1992). These negative effects result from a decrease in food-intake that leads to a decline in locomotory performance, particularly stamina. This negative effect on stamina of juveniles may then compromise the toads' overwinter survival or ability to avoid predators (Goater *et al.* 1993).

In hares at Kluane, infections of *P. boughtoni* reach highest mean intensities in summer during the hares' reproductive season. Infection with lungworms damages hare lung tissue and may lead to decreased lung function (Goble and Dougherty 1943, Tobon 1973). This could affect hare stamina. Hares that are infected with high numbers of worms, already stressed by high mean intensities of *N. triangularis* in spring and by the lactational demands of their young, may not be able to meet both their own energy demands and those of their young, compromising the survival of both mother and offspring. This is true of bighorn ewes infected with high numbers of *Protostrongylus* spp. Lambs born to ewes infected with high numbers of worms, suckle less frequently and suffer higher rates of mortality then do lambs born to ewes infected with low numbers of worms of worms (Fiesta-Bianchet 1988).

Infections of *Protostrongylus boughtoni* in snowshoe hares from Kluane fall within the mid-range of mean intensities reported for hares in

North America (Bookhout 1971, Keith *et al.* 1986, Kralka and Samuel 1990). Infections of *P. boughtoni* reach their highest prevalences and mean intensities in leverets between 600g and 1200g in weight. Infections increase rapidly in young hares and infections are not significantly different between neonates (≤ 300g) and older leverets weighing in excess of 1000g. Infections of *P. boughtoni* are high in summer, when females are lactating and juvenile hares are undergoing rapid rates of growth. These infections damage lung tissue potentially affecting host stamina suggesting that *P. boughtoni* may significantly affect hare survival and productivity during the summer of initial decline. Alternatively resource stressed hares may simply be unable to limit infestation.

Conclusions

Of the parasites infecting snowshoe hares at Kluane Lake, Yukon there are two species, *Protostrongylus boughtoni* and *Nematodirus triangularis*, that could be affecting the dynamics of the hare cycle. Prevalence, mean intensity and seasonal dynamics of these two parasites suggest that they have the potential to influence both hare reproduction and survival.

Ultimately it is not possible to assess the effect that these parasites are having on the dynamics of the Kluane hare cycle without manipulating parasite loads in selected populations of hares. Selected population features, such as survival and reproduction, could then be compared between parasite manipulated groups and control groups of hares, and a more convincing assessment of the influence of parasitism on hare population dynamics could then be made.

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Chapter 3: Efficacy of Ivermectin against nematodes of snowshoe hares, *Lepus americanus*, with special consideration of those nematodes most likely to affect hare reproduction and survival

Introduction

The use of anthelminthics in wild hosts, while recent, has proved promising. Examples include reduction in numbers of: 1) *Trichostrongylus tenuis* in free ranging populations of red grouse *Lagopus lagopus scoticus* following treatment with levamisole hydrochloride (Hudson 1986); 2) larvae of lungworms in the feces of free ranging mountain sheep, *Ovis canadensis*, following treatment with ivermectin (Miller *et al.* 1987); 3) *Parelaphostrongylus andersoni* larvae in feces of captive white-tailed deer, *Odocoileus virginianus*, after treating with ivermectin (Samuel and Gray 1988); and 4) nematodes in naturally infected raccoons, *Procyon lotor*, that were maintained in captivity following treatment with ivermectin (Hill *et al.* 1991). The objective of this study was to assess the efficacy of ivermectin against nematode parasites in free ranging populations of snowshoe hares, *Lepus americanus*, from Kluane Lake (see Chapter 1).

Ivermectin was chosen for use in this study for several reasons. First, in domestic animals it has proven effective against a broad range of nematodes (Campbell 1989), including ones that are taxonomically related to those of snowshoe hares (Stewart *et al.* 1981, Schillhorn and Gibson 1983, Ostlind *et al.* 1985, Ferguson 1986, and Flynn *et al.* 1989). Second, ivermectin is relatively non-toxic (Wright 1986, Lankas and Gordan 1989) so free-ranging hares can be treated without dangerous side effects that might make them less competitive or more susceptible to predators. Third, ivermectin can have

persistent, long-lasting effects in treated animals (Tolan 1980, Campbell and Benz 1984, Lo *et al.* 1985). In lagomorphs, for example, high concentrations of ivermectin were sustained in tissues and body fluids for at least 13 days following treatment (McKellar *et al.* 1992). Treated animals should, therefore, be protected for a long time. Last, ivermectin can be easily administered under field conditions by subcutaneous injection (Campbell 1989), making it easy to determine which animals were treated and how much ivermectin they received is known.

The purpose of this study was to determine the efficacy of ivermectin against parasites of snowshoe hares. My experiments were designed to determine: 1) the degree of worm loss associated with a single administration of ivermectin; 2) the length of time that ivermectin was effective in reducing worm numbers; and 3) the effect of repeated ivermectin administration in reducing worm numbers.

Materials and Methods

Study Site

This research was conducted in the boreal forest at Kluane Lake, Yukon Territory, Canada (61° North, 138° West) (see Chapter 1). Three experiments testing the efficacy of ivermectin were performed. Two of these were conducted on a 5.4 ha grid that contained 40 trap sites arranged in a uniform, 10 x 4 pattern with 30m between sites. The third experiment was conducted on two 13 ha grids. On these grids, 140 trap sites were arranged within a 10 x 14 grid system with 30m between sites.

For a description of the vegetation on the study grids and climate within the study area, see Chapter 2.

Trapping and handling of hares

On each grid, hares were live-trapped with 20 - 60 single or double-door Tomahawk live-traps (20cm x 20cm x 60cm). Traps were usually placed at alternate trap sites on each grid; however, due to a shortage of traps, some of the inner lanes on the 13ha grids were not trapped. During trapping sessions, traps were baited with alfalfa and apples, set prior to nightfall, and checked the following morning at first light.

Hares were removed from traps directly to a burlap sack in order to minimize handling stress. They were moved to the bottom of the sack and their heads held firmly between the handler's left elbow and body. This effectively immobilized and quieted the animal. Each hare was sexed, weighed and tagged in its right ear with a numbered monel eartag (National Band and Tag Company, Newport, KY; size 3).

Trapped hares destined for necropsy were removed from the traps to burlap sacks as described previously. They were grasped firmly by the neck with both hands and killed by cervical dislocation. The sex, weight and eartag number of each collected hare was recorded at the time of its collection. Hare necropsies were performed as described in Chapter 2.

Injection of hares

Each treated hare was injected subcutaneously with a nonaqueous solution of ivermectin in propylene glycol (ASD AGVET, Merck Frosst Canada, and Merck Frosst U. S. A.). A 1 ml syringe with a 20G 1.5in needle was used to deliver 0.4 mg/kg (McKellar *et al.* 1992) of ivermectin beneath the skin of the right hip of each hare. The eartag number of each treated hare was recorded.

Single dose of ivermectin: long-term effect (Experiment 1)

The first experiment, designed to determine the long-term influence of ivermectin on subsequent numbers of nematodes, was conducted on the small grid between 3 July and 24 August 1990. Sixteen hares were killed and examined for parasites to assess numbers of worms present at the start of the experiment. Another 60 hares were trapped, 40 were treated with a single dose of ivermectin and released, the remaining were released as untreated controls. Seven weeks later 10 treated and eight control hares were trapped, killed and examined for nematodes.

Single dose of ivermectin: short-term effect (Experiment 2)

This experiment, designed to determine the short-term effects of a single dose of ivermectin on subsequent numbers of nematodes, was conducted on the small grid between 7 July and 8 August 1991. Thirty-one hares were trapped, six were examined for numbers of worms present at the start of the experiment, 15 hares were treated and released, and 10 hares were released as untreated controls. At 2 weeks post-treatment, six treated and five control hares were trapped, killed and examined for parasites. At 4 weeks, seven treated and five controls were trapped, killed and examined for parasites.

Effect of multiple doses of ivermectin (Experiment 3)

This experiment, designed to assessed the long-term effects that multiple treatments with ivermectin have on subsequent numbers of hare nematodes, was conducted on the large grids between April and November of 1991. This experiment was conducted as part of the major manipulation (reported in Chapter 4), which examined the density, survival and reproduction of treated hares. It involved repeatedly collecting and treating

known individuals, allowing me to examine the effect that multiple treatments had on subsequent numbers of nematodes.

Between April and July of 1991 I trapped and treated hares on a monthly basis on the two larger grids. Trap success was variable between months, and there was some immigration and emigration on and off these grids during the 4 months. However, during this period, monthly treatments were maintained in a large group of hares from these two grids with each hare receiving three to four treatments of ivermectin. Between August and November of 1991 some of these previously treated hares were not recaptured and did not receive treatments. In addition, during this period newly arriving hares on these two grids were not treated with ivermectin. During the November trapping session, six hares that during summer 1991 had received treatments at each capture, were trapped, killed and the carcasses frozen for examination by me at a later date. At the same time, nine untreated control hares that had arrived on the grids after August were also trapped, killed and the carcasses frozen. Numbers of worms in these two groups of hares were compared.

Ivermectin pen-trial

Between 21 August and 13 September of 1990 18 hares were trapped on the 5.4ha grid. Eight of these hares were examined for worm numbers present at the start of the experiment and 10 were treated with a single dose of ivermectin. The 10 treated hares were kept in five pens (1.8m x 4.6m x 1.0m) with one male and one female or two females in each pen. The pens were constructed from chicken wire and the base of each wall was buried 15cm into the ground. The hares were fed rabbit chow once a day and were given willow, *Salix glauca*, and bog birch, *Betula glandulosa*, in the morning and evening of each day. At 3 weeks I killed and froze the carcasses of the nine

treated hares (1 escaped) from the holding pens and 10 hares, which had not been treated, from the 5.4ha grid were trapped, killed and the carcasses frozen.

Statistical analysis

The effect of treatment with ivermectin on numbers of *Protostrongylus boughtoni* and *Nematodirus triangularis* was determined by a one-way ANOVA (SYSTAT, Inc. Evanston Illinois) with treatment analyzed as a fixed effect. For the experiment in which hares were collected at two different times the effect of treatment and time was analyzed by a two-way ANOVA with treatment and weeks analyzed as fixed effects. Because the size (age) of hares is known to affect nematode numbers, the weight of hares at collection was analyzed as a covariate in all three experiments. Nematode numbers were log-transformed prior to analysis. The control hares used for the analyses were those animals that were collected in the same week as treated hares.

Analysis of the pen-trial experiment was complicated by the fact that I was unable, for practical reasons, to maintain 10 untreated hares in similar pens. Moreover, maintaining hares in non-individual pens means that individual worm numbers are non-independent, making interpretation of ANOVA results difficult. However, the purpose of this trial was to determine the broad effects of ivermectin under extremely controlled conditions, where the loss of treated hares due to predation or dispersal would be minimized.

Results

Parasite loads at the initiation of Experiments 1 and 2, and the ivermectin pen-trial are given Table 3.1. Four species of nematodes were found, only *Passalurus* sp. occurred infrequently. Infections with *Passalurus* p. were infrequent in these hares, and prevalences of infection and numbers

Table 3.1. Numbers of parasites in free-ranging snowshoe hares at the initiation of experiments testing the efficacy of ivermectin.

	Experiment 1	Experiment 2	Pen Trial
Date	July-Aug. 1990	July-Aug. 1991	Aug.-Sept. 1990
N	16	6	8
Protostrongylus boughtoni			
No. Infected (%)	10 (63)	5 (83)	8 (100)
Mean ± S.D.	11.2 ± 7.1	10.4 ± 13.7	13.8 ± 8.1
Nematodirus triangularis			
No. Infected (%)	9 (56)	6 (100)	4 (50)
Mean ± S.D.	10.0 ± 8.0	37.3 ± 34.5	5.3 ± 4.6
Passalurus sp.			
No. Infected (%)	0	1 (17)	0
Mean ± S. D.	-	16.0	-
Trichuris leporis			
No. Infected (%)	6 (38)	4 (67)	4 (50)
Mean ± S.D.	11.0 ± 12.1	30.8 ± 33.8	10.8 ± 24.4

of *T. leporis* were low. These two worms have little potential to affect survival and reproduction of hares (see Chapter 2) and treatments with ivermectin had no effect on their numbers (Table 3.2). They will not be discussed further.

The experiments were conducted at different periods in the season. Because of differences in seasonal cycle relative to timing of experiments the prevalences and mean intensities of *P. boughtoni* and *N. triangularis* varied at the initiation of the different experiments. However, both were found in at least one-half of the hares, and, in most experiments, in reasonable numbers.

In the ivermectin pen-trial, where opportunities for reinfection were very low, *P. boughtoni* was completely eliminated from treated hares, and both prevalence of infection and numbers of *N. triangularis* were markedly reduced (Table 3.3).

In the field trials, where hares had ample opportunities for reinfection, a single dose of ivermectin significantly reduced the numbers of both *P. boughtoni* and *N. triangularis* for up to 4 weeks following treatment (Fig. 3.1, Table 3.4). Numbers of worms in both treated and control hares significantly increased between the second and fourth weeks of Experiment 2. Although there were differences between the means of the treated and control groups at 4 weeks there was no significant interaction between treatment and week (Table 3.4), indicating that after 2 weeks the rates of reinfection did not differ between the treated and control groups. Seven weeks after treatment there were no significant differences in numbers of these species of worms in the treated and control groups (Fig. 3.2, Table 3.5).

In Experiment 3, where the long-term effects of multiple treatments with ivermectin were assessed, numbers of both species of worms appeared to be reduced in the treated groups at 12 weeks post-treatment (Fig. 3.3), but the

Table 3.2. Percent prevalence and mean intensities of *Passalurus* sp. and *Trichuris leporis* in snowshoe hares treated with ivermectin, compared to those in untreated hares collected at the same time.

Species	Experiment 1	Experiment 2 Week 2	Experiment 2 Week 4	Experiment 3	Pen Trial
			Controls		
Passalurus sp.					
No. Infected (%)	0	1 (20)	2 (40)	2 (22)	1 (10)
Mean ± S. D.	-	5.0	2.0	228.0 ± 53.7	1.0
T. leporis					
No. Infected (%)	4 (50)	2 (40)	1 (20)	3 (33)	8 (80)
Mean ± S.D.	21.5 ± 30.9	1.5 ± 0.7	1.0	3.7 ± 2.3	15.2 ± 20.8
			Treated		
Passalurus sp.					
No. Infected (%)	1 (10)	1 (14)	4 (67)	2 (33)	0
Mean ± S.D.	1.0	1.0	1248.3 ± 2216.4	16.0 ± 11.3	-
T. leporis					
No. Infected (%)	8 (80)	4 (57)	4 (67)	3 (50)	5 (56)
Mean ± S.D.	37.4 ± 47.2	3.5 ± 1.9	14.2 ± 9.2	4.7 ± 6.4	14.8 ± 9.9

Table 3.3. Percent prevalence and mean intensity of *Protostrongylus boughtoni* and *Nematodirus triangularis* in snowshoe hares given one subcutaneous injection of ivermectin (0.4mg/kg) and kept in pens until examination 3 weeks after treating, compared with those in free-ranging untreated hares collected at the same time.

Species	N	Prevalence	Mean No. Parasites (±S.D.)
	Controls		
P. boughtoni	10	100	9.4 (± 5.9)
N. triangularis	10	70	109.0 (± 141.2)
	Treated[a]		
P. boughtoni	9	0.0	0.0
N. triangularis	9	11	1.0

a one hare escaped

Fig. 3.1 Effect of ivermectin on mean (+ SE) numbers of *Protostrongylus boughtoni* (a) and *Nematodirus triangularis* (b) at 2 and 4 weeks after a single subcutaneous injection with 0.4 mg ivermectin per kg body weight.

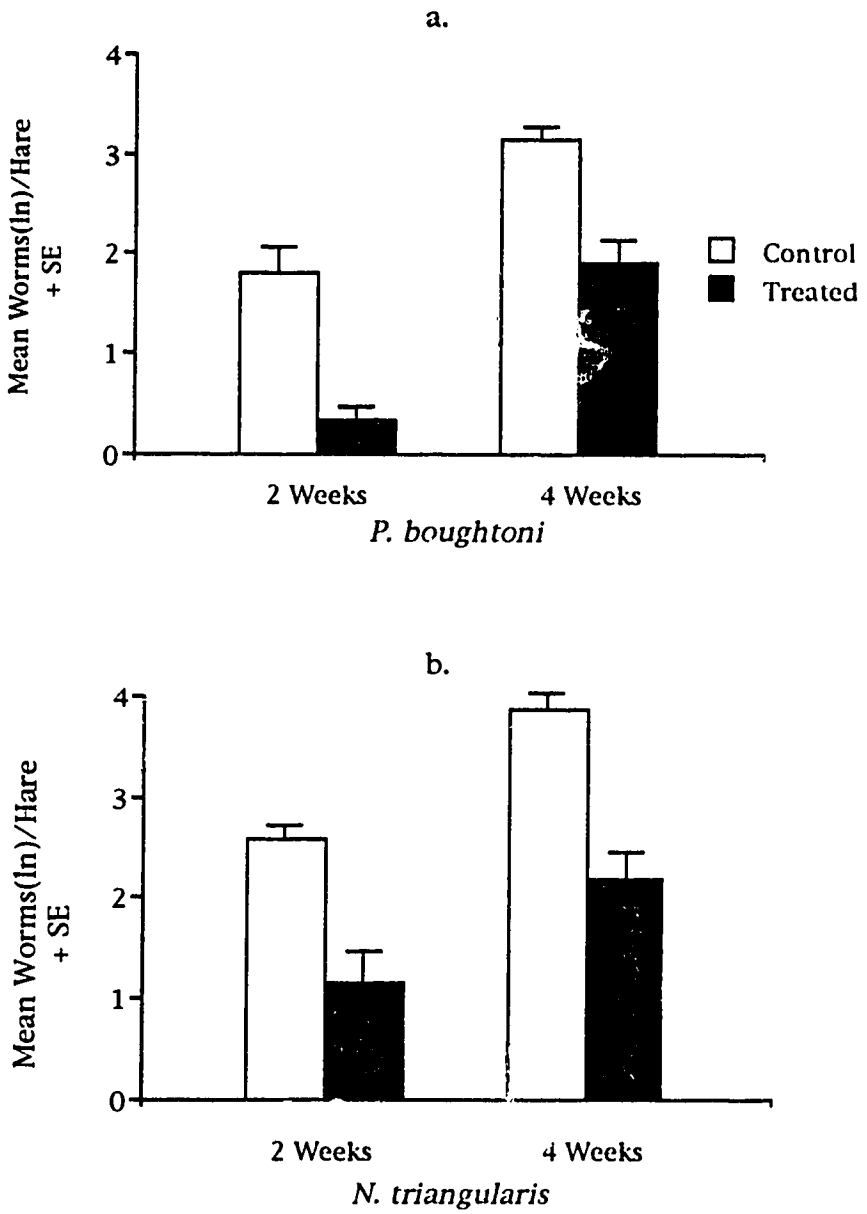


Table 3.4. Summary statistics of two-way ANCOVA testing for effect of a single dose of ivermectin (0.4mg/kg) at 2 and 4 weeks (Experiment 2) on numbers of *Protostrongylus boughtoni* and *Nematodirus triangularis* in free-ranging snowshoe hares. Data were log transformed and hare body size was analyzed as a covariate.

	Source	DF	SS	MS	F-Ratio	P
P. boughtoni	Covariate	1	1.972	1.972	1.873	0.188
	Treatment	1	10.367	10.367	9.846	0.006
	Week	1	11.509	11.509	10.931	0.004
	Treatment * Week	1	0.286	0.286	0.271	0.609
	Error	18	18.952	1.053		
N. triangularis	Covariate	1	12.783	12.783	29.374	0.000
	Treatment	1	13.293	13.293	30.546	0.000
	Week	1	7.117	7.117	16.355	0.001
	Treatment * Week	1	0.192	0.192	0.441	0.515
	Error	18	7.833	0.435		

Fig. 3.2. Effect of ivermectin on mean (+ SE) numbers of *Protostrongylus boughtoni* and *Nematodirus triangularis* at 7 weeks after a single subcutaneous injection with 0.4 mg ivermectin per kg body weight.

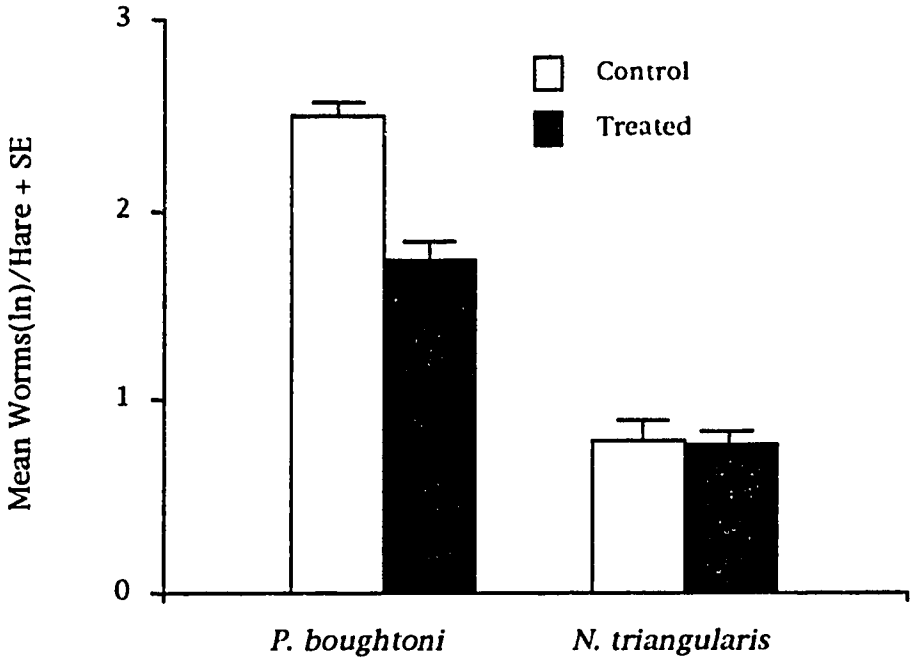
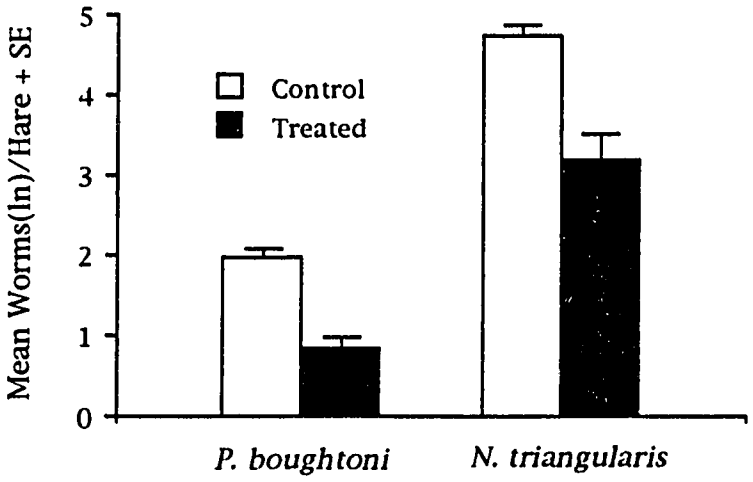


Table 3.5. Summary statistics of one-way ANCOVA testing for effect of a single dose of ivermectin (0.4mg/kg) at 7 weeks (Experiment 1) on numbers of *Protostrongylus boughtoni* and *Nematodirus triangularis* in free-ranging snowshoe hares. Data were log transformed and hare body size was analyzed as a covariate.

	Source	DF	SS	MS	F-Ratio	P
P. boughtoni	Covariate	1	1.203	1.203	1.532	0.236
	Treatment	1	1.650	1.650	2.101	0.169
	Error	14	10.990	0.785		
N. triangularis	Covariate	1	0.300	0.300	0.359	0.732
	Treatment	1	0.102	0.102	0.122	0.559
	Error	14	11.688	0.835		

Fig. 3.3. Effect of ivermectin on mean (+ SE) numbers of *Protostrongylus boughtoni* and *Nematodirus triangularis* at 12 (± 4.7) weeks after multiple subcutaneous injections with 0.4 mg ivermectin per kg body weight.



differences between treated and control groups were not significant (Table 3.6).

Discussion

The results of this study suggest that the therapeutic effects of ivermectin against these two parasites would be maximized if treatments were administered every 2 to 4 weeks. A single injection of ivermectin reduced the numbers of *Protostrongylus boughtoni* and *Nematodirus triangularis* in treated hares. However, some adult worms are present by 2 weeks post-treatment, and infections are reacquired at approximately the normal rate after 2 weeks. Given the developmental period of these two parasites (Kralka and Samuel 1983), it is not possible for new infections to enter hares and reach maturity in the 2 week period between lapsing of the drug's active period and the initiation of worm recovery at 4 weeks.

In some hosts, ivermectin is effective against larval worm stages within the tissues (Bisset *et al.* 1990) and in others it is not (Kim *et al.* 1988). In snowshoe hares fourth stage larvae within the lung parenchyma (*P. boughtoni*) or within the intestinal mucosa (*N. triangularis*) are apparently not affected by the drug, although they may be prevented from emerging or killed if they do emerge. These immature stages may then reach maturity and enter the bronchi or small intestine when concentrations of the drug in the hare decrease to ineffective levels. This would account for the decline in efficacy noted in hares collected 4 weeks post-treatment. The further decline in efficacy 7 weeks after treatment is probably due not only to maturing of larval stages present at the time of treatment, but also to development of

Table 3.6. Summary statistics of one-way ANCOVA testing for effect of multiple treatments of ivermectin (0.4mg/kg) at 14 (± 4.7) weeks (Experiment 3) on numbers of *Protostrongylus boughtoni* and *Nematodirus triangularis* in free-ranging snowshoe hares. Data were log transformed and hare body size was analyzed as a covariate.

	Source	DF	SS	MS	F-Ratio	P
P. boughtoni	Covariate	1	2.040	2.040	1.286	0.279
	Treatment	1	2.804	2.804	1.768	0.208
	Error	12	19.033	1.586		
N. triangularis	Covariate	1	3.740	13.433	5.702	0.034
	Treatment	1	13.433	3.740	1.587	0.232
	Error	12	28.272	2.356		

parasites that were acquired after the drug had been cleared from the tissues and body fluids of the h e.

All treatment groups included one or more treated hares in which numbers of *P. boughtoni* or *N. triangularis* were not reduced. The number of worms in these outlier individuals was similar to, or higher than, numbers occurring in control hares. There are at least two potential explanations for this apparent lack of efficacy in these hares. First, in sheep dosed subcutaneously with ivermectin there is sometimes a local tissue reaction at the point of injection that restricts the drug to the site of inoculation (Shoop, pers. com.). In such cases the treatment fails because the drug is not taken into the blood stream and, therefore, not delivered to the target parasites. This explanation may not account for all of the outliers, as both parasites were high in the same animal in only 50% of the cases. Second, ivermectin itself will paralyze and kill many helminth parasites without assistance from the immune system (Preston 1984, Casado *et al.* 1989). However, there are instances in which the immune system plays an important role in elimination of the worm (Rao *et al.* 1987). If the immune system is important in hares, outliers may be immunocompromised individuals. Either or both explanations could account for the presence of treated hares infected with high numbers of *P. boughtoni* or *N. triangularis*.

Numbers of *P. boughtoni* and *N. triangularis* were not significantly lower at 12 weeks post-treatment in hares receiving multiple doses of ivermectin. Even after eliminating one hare infected with many worms from the treated data set, the number of *P. boughtoni* and *N. triangularis* in treated hares was not significantly lower than in controls.

Single and multiple injections of ivermectin also had no effect on numbers of *Trichurus leporis* or *Passalurus* sp.. The results with *Passalurus* sp.

are difficult to explain because previous studies have shown the high therapeutic value of ivermectin against oxyurid worms (Ostlind *et al.* 1985, Battles 1987, Baumans *et al.* 1988, and Flynn *et al.* 1989). The results with *T. leporis*, however, are less surprising. Studies addressing the efficacy of ivermectin show that its therapeutic effect against trichurids depends upon the host in which the drug is being used (Bisset *et al.* 1990, Lyons *et al.* 1986, Ogunusi *et al.* 1986, Schillhorn and Gibson 1983, Stewart *et al.* 1981). However, this inability of ivermectin to affect these 2 species is unlikely to be important to the success of my field manipulation (see Chapter 4), because they occur at low numbers and probably are not having a significant impact on the dynamics of this population of hares (see Chapter 2).

As previously stated, the therapeutic effects of ivermectin against *P. boughtoni* and *N. triangularis* would be maximized if treatments were administered every 2 to 4 weeks. Efficacy could probably be further enhanced if a sustained-release bolus was used to deliver the drug (Alva-Valdes *et al.* 1988, Zimmerman *et al.* 1988). Use of a sustained-release bolus would alleviate the need to repeatedly capture and treat the host, and it would also maximize the potential therapeutic effects of the treatment.

If turnover of hosts in the treated population is high, however, then even the use of a sustained-release bolus would not be effective. In such a situation, food treatments might be more effective, even if some hosts went untreated, dosages received could not be determined, and treated and untreated individuals could not be distinguished. In-feed formulations of ivermectin have proven effective in other field settings (Forcyt 1993). However, a high proportion of the hosts must be attracted to the treated food. If hosts cannot be attracted to treated food, or if it is important to identify treated animals individually, injection may be the only choice.

Overall, ivermectin was not as effective against the nematodes of snowshoe hares as has been reported for nematodes of domestic animals. However, treatment significantly reduced the numbers of nematodes suggesting the potential for ivermectin to influence reproduction and survival of snowshoe hares. These results provide some of the first evidence to show that ivermectin can be used to control numbers of worms in the helminth/snowshoe hare system under natural conditions. Ivermectin's potential for use in manipulative experiments involving snowshoe hares is therefore confirmed.

Conclusions

The success of drug chemotherapy in a wild host population requires considerable knowledge of the host and the parasites that infect it. The parasites present will dictate the choice of a drug that is both efficacious against those parasites most pathogenic to the host, and non-toxic to the host in question. The choice of a treatment protocol will be determined by the length of time the drug is active in the host, and by seasonal fluctuations in mean intensity and prevalence of infection for all parasites that infect the host.

In an experimental manipulation, one factor important to the ultimate success of chemotherapy is the ability to treat hosts repeatedly. If treatments in the host population can be maintained on a regular basis, then the potential benefits associated with that treatment are maximized. In essence, the researcher desires a host study group exhibiting low population turnover. Ultimately researchers cannot assume that a selected drug will be as effective in the wild as it is in domestic animals.

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Chapter 4: Influence of Parasites on a Cyclic Population of Snowshoe Hares, *Lepus americanus*, During the Peak and Initial Decline Phases of a Hare Cycle

Introduction

The cycling of snowshoe hare populations, *Lepus americanus*, with a 10 year period is a phenomenon that has been well studied (Green and Evans 1940 a, b, c; Keith and Windberg 1978; Vaughan and Keith 1981; Keith *et al.* 1984; Boutin *et al.* 1986; Krebs *et al.* 1986; Krebs *et al.* 1992). Research on the hare cycle indicates that: 1) hare density between years of low and peak hare numbers can change anywhere from 50 to over 100 fold, 2) predation is a significant cause of adult mortality throughout the cycle and 3) predation, starvation and reduced juvenile recruitment all contribute to the reduction of hare populations during the decline phase of the cycle. In particular, starvation and reduced juvenile recruitment appear to be important factors contributing to hare population decline (Keith and Windberg 1978, Boutin *et al.* 1986).

Much information, including some of that listed above, on the demographics of snowshoe hare cycles has been accumulated through the efforts of the Kluane Boreal Forest Ecosystem Project (KBFEP). This project, conducted at Kluane Lake, southwest Yukon, Canada, began in the 1970's and is presently studying a second hare cycle. KBFEP is using field manipulations to assess the effects that food resources, nutrient levels and predation (Krebs *et al.* 1992) have on the dynamics of the hare cycle. On grids of approximately 40ha KBFEP is either supplying hares with food *ad libitum*, excluding large terrestrial predators through the use of electric fences, fertilizing the natural vegetation with one application of nitrogen in spring or both supplying food

and excluding predators on the same grid. In addition, two unmanipulated control grids were maintained for comparison.

One factor not initially covered by the multifactorial KBFEP was the influence of parasites on the hare cycle. Keith *et al.* (1985) dismissed parasites as a factor of potential importance in cyclic hare populations in Alberta. This dismissal was based on analyses of prevalences of parasites, not on their numbers or any manipulative experiments.

Three lines of evidence suggest that parasites may be of importance in the dynamics of cyclic species, such as snowshoe hares: mathematical models of interacting host and parasite populations, laboratory studies of such populations, and field studies, including field manipulations. The models (see Chapter 1 for more detailed coverage) identify several features that allow parasites to regulate host densities:

1) a net reproductive rate (which includes transmission) high enough to assure substantial numbers of parasites.

2) a moderate degree of aggregation, so that high numbers of parasites are found in more than just a few animals.

3) pathogenicity that increases with parasite numbers, so that mortality is concentrated on those individuals with high numbers of parasites.

4) a moderate degree of pathogenicity, high enough so that parasite-induced mortality is greater than the host's net population growth rate, but low enough so that selective mortality does not regulate the parasite population at levels too low to affect host populations.

5) parasite-induced reduction in host fecundity.

Field and laboratory studies covered in Chapter 1 have demonstrated that parasites with these features can affect host survival and reproduction, regulating host population size. In addition, the field studies of Hudson and

coworkers (Hudson 1986, Hudson and Dobson 1989, Dobson and Hudson 1992, Hudson *et al.* 1992a, Hudson *et al.* 1992b), and others reviewed in Chapter 3, have demonstrated that parasite populations in free-ranging hosts can be reduced by treating with anthelminthics.

I have shown that a moderate proportion of hares at Kluane are infected with many lungworms, *Protostrongylus boughtoni* and many intestinal trichostrongylid, *Nematodirus triangularis*, that these parasites are clumped in the hare population and that they can be reduced in numbers by an intramuscular injection of ivermectin (Chapter 3). The net reproductive rates of these two parasites in this hare population are unknown. However, the period between elimination of worms through anthelminthic treatment and reinfection of hares with these two parasites is short, suggesting a high net reproductive output (Chapter 3).

The rates of parasite-induced mortality or reductions in reproduction in this hare population are also unknown; however, both lungworms and trichostrongilids are known to affect mortality and reproduction in other hosts (Chapter 2), and it is reasonable to assume similar effects on snowshoe hares (Dobson 1988). I have also shown that a high proportion of hares are infected with these two parasites at the peak and initial stages of hare decline (Chapter 2), when reproductive output and the intrinsic growth rate in the hare population are low (Keith and Windberg 1978, Boutin *et al.* 1986).

As previously mentioned, an increase in starvation deaths and declines in juvenile recruitment are important factors associated with the decline of snowshoe hare populations. Interestingly, Hudson *et al.* (1992b) also found increases in nonpredator deaths and declines in juvenile recruitment to be important factors contributing to the demise of red grouse populations; both were ascribed to the effects of parasites. Given these features, particularly the

parallels in the dynamics of the population cycles of red grouse and snowshoe hares, I designed a manipulative test of the importance of these two species of parasites in the snowshoe hare cycle.

Although a single treatment of the nematocide ivermectin significantly reduced numbers of these two nematodes in hares for up to 2 weeks after treatment, the treatment did not prevent or affect the rate of reinfection in hares after 4 weeks post-treatment (Chapter 3). Thus, a successful treatment program requires retreating the same hares every 4 weeks during the non-snow months and periodically throughout the winter, when snow cover prevents reinfection.

In this chapter I report the results of the ivermectin manipulation. Specifically I address two questions: 1) Will treated populations of adult hares monitored by mark-and-recapture have higher rates of survival and be maintained at higher densities than untreated populations? 2) Do female hares continuously retreated with ivermectin every 4 to 8 weeks have higher rates of fecundity than untreated females? 3) Do offspring born to treated females have higher rates of survival in their first 14 days of life?

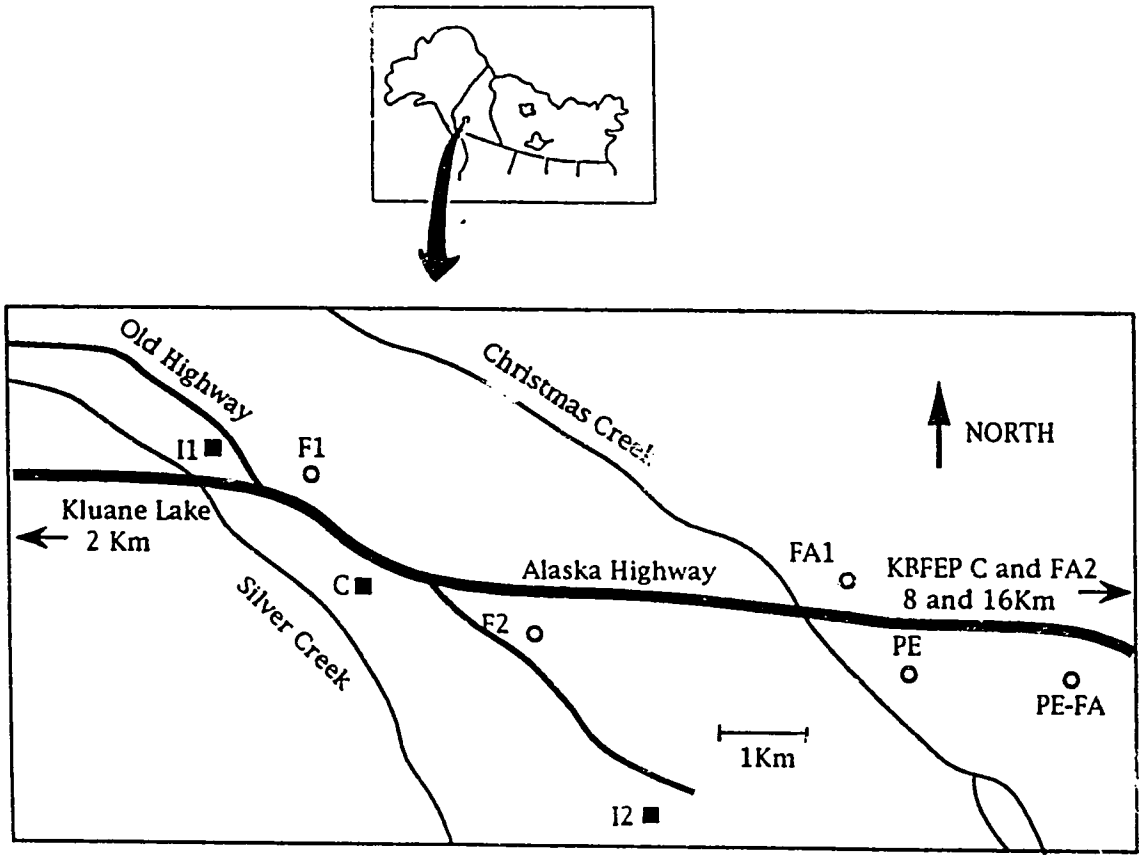
Materials and Methods

Monitoring study

Study Site

This study was conducted at Kluane Lake in southwestern Yukon Territory, Canada (61° N, 138° W). Hares were trapped on 3 three grids. The location of these grids, along with others of KBFEP, are shown in Fig. 4.1. Two of these grids (Treatment 1 and Treatment 2) were 13ha in size; on these

Fig 4.1. Relative position of study grids along the Alaska Highway near Kluane Lake Yukon, Canada. ■ grids used in this study; o KBFEP grids. The experimental treatments are also shown. I=ivermectin; F=fertilizer; C=controls; FA=food addition; PE=predator exclosure; PE-FA=predator exclosure, food addition.



grids hares were trapped and treated with ivermectin (see below). The vegetation on these areas consisted of open spruce forest with an understory of open shrub willow, *Salix glauca*, with intermittent patches of bog birch, *Betula glandulosa*. Shrubs were absent from 10% of the trapping stations on these two grids, and deadfall was present at 68% of the trapping stations. On these grids 140 trap sites were arranged within a 10 x 14 grid system 30m between sites. On each of these grids Tomahawk live-traps were placed at trapping sites 60m apart. Limited numbers of traps made it impossible to trap three lanes, and these untrapped lanes were distributed uniformly across the grid.

The third grid used in this study (control grid) was 36ha in size. Hares on this grid were trapped and treated with drug-free carrier (see below). Vegetation on this grid varied from closed spruce forest to dense spruce forest. A closed shrub understory dominated by *S. glauca* occurred at 77% of the trapping stations on this grid. Shrubs were absent from only 1% of the trapping stations and deadfall was present at 75% of the stations. Trapping stations and placement of traps on this grid were similar to the treated grids. Limited availability of traps made it impossible to trap 10 of the lanes on this grid.

Handling and trapping of hares

Hares on the treatment grids were trapped and treated at 4 week intervals between May and September of each year. Between October and May of each year, snow cover persisted on the study area, and at this time, rates of transmission of parasites are probably near zero but recruitment from previously acquired arrested larvae continues. During this period hares were

trapped and treated in mid-December and at the end of February. Trapping of hares on the 36ha grid was conducted in the same manner as on treated grids. On all grids the setting, baiting and checking of traps were as described in Chapter 3, Methods.

On each grid, hares were trapped with single and double-door Tomahawk live-traps (20cm x 20cm x 60cm). Hares were removed from traps directly to a burlap sack in order to minimize handling stress. Each hare was injected subcutaneously with a nonaqueous solution of ivermectin in propylene glycol (ASD AGVET, Merck Frosst Canada, and Merck Frosst U. S. A.). A 1ml syringe with a 20G 1.5in needle was used to inject 0.4mg/kg (McKellar *et al.* 1992) of ivermectin beneath the skin of the right hip of each hare. Handling of hares on the 36ha grid was conducted in the same manner as on treated grids except that hares on this grid received treatments of a placebo (propylene glycol, the carrier for the ivermectin, without the drug). A measurement of weight (to the nearest gram) and of the length of right hind foot (to the nearest millimeter) was taken from every trapped hare.

At the time of my last trapping session in November of 1991 all hares trapped on the two treated grids were collected. This included both resident animals on the two grids that had received between 3 and 10 treatments of ivermectin and hares not previously treated with ivermectin. The bone marrow of the right tibia was collected from these hares during examination of their carcasses. The weight of the marrow was measured at the time of its removal from the bone and after drying in a conventional drying oven. An index of body condition was calculated as marrow dry weight/marrow wet weight (Keith 1984). Other procedures used and information collected from each animal were the same as for Chapter 3.

I compared mean intensities of infection between treated and untreated hares from the treated grids with a one-way ANOVA on log-transformed values of mean intensity. Because the size (age) of hares is known to affect nematode numbers, hare weight at collection was analyzed as a covariate. I tested the effects of ivermectin on bone marrow index among treated and untreated hares from the treated grids with a Kruskal-Wallis test. I tested for the effects of ivermectin on overwinter weight loss and body condition among adult resident male hares of the treated and control grids using repeated measures ANOVAs. Females were eliminated from the analysis because the weight of any pregnant females in May would be biased by litter size. The weight and condition of resident males trapped in September and October of 1990 were compared to the same measures of those same males trapped in May of 1991. I calculated indices of autumn and spring body condition of resident adult males using the relationship between hare body weight and right hind foot length (Bailey 1968). A regression was fitted to log-transformed values of weight and right hind foot length (MGLH module, SYSTAT, [illegible] Evanston Illinois) from trapping data for all males on the control grid in autumn 1990 and spring 1991. The resulting relationships WT(g)=-2.354+1.940RHF(mm) (autumn) and WT(g)=-7.750+3.067RHF(mm) (spring), were used to calculate predicted weight for a hare with a given right hind foot length. The condition index for that hare was then calculated as observed weight/predicted weight.

Hares violate the assumptions of equal trappability (Krebs *et al.* 1986) assumed by most methods for estimating population size for mark-and-recapture data. I estimated population sizes on each grid using the Capture Model (Otis *et al.* 1978) because it does not assume equal probabilities of capture for each animal. The model assumes that the trapped population is closed treating each trapping week separately. Precise estimation of population size

requires that at least three days of trapping be undertaken in each trapping week. During any trapping session hares could be affected by past trapping experience in that session and this model assumes that 1) there can be heterogeneity of capture probabilities in the population, 2) capture probabilities can change due to behavioral responses to the first capture and 3) there can be time specific changes in capture probabilities. An appropriate estimator is selected from a set of eight models. The series of eight models includes all combinations of the three possible assumptions including all of them and none of them.

I estimated survival and the arrival of new recruits to the three study grids using the Jolly-Seber model (Seber 1982) as implemented in a program developed by C. J. Krebs (University of British Columbia, Vancouver, BC, Canada). These estimates are biased to some degree because hares do violate the assumption of equal trappability; however, they supply useful information on relative rates of both survival and recruitment among the grids. Recruitment was estimated for both adult and juvenile hares. Hare body weight was used to determine hare age with hares in excess of 949g being classified as adults. In addition, the number of new residents among recruits arriving during a single trapping period was determined, with "residents" defined as hares remaining on the grids for $\geq$ 10 weeks.

Reproductive study

In the summer of 1991, I examined the effects of treatment on female reproduction. In order to compare litter size, weight of neonates at birth, stillborn rates and survival of offspring between treated and control females, it is necessary to locate newly born litters in the field. Snowshoe hares do not build nests or use burrows (Severaid 1942, Graf and Sinclair 1987), making location of litters in the field difficult. In order to measure the reproductive

parameters associated with females I used methods developed and used by O'Donaghue (1992) in a previous study of hare reproduction at Kluane. Near-term pregnant females are trapped, kept in a cage in the field until birth of the litter, then both mother and litter are marked and released. In 1991, prior to parturition of each litter, hares were trapped every week; distention of nipples and hare body weight were recorded and used to estimate the dates of parturition of litter 1. Hares were treated with ivermectin only in the first week of each month. Near-term pregnant females were trapped shortly before parturition and placed in 60 x 60 x 120cm chicken wire cages covered with burlap and located at the site of capture. Each cage was provisioned with straw, spruce branches and grasses for natural cover. However, I had a disturbance by bears on Treatment 1 during litter 1 and subsequently pregnant females on half of this grid were placed in cages within a 20 x 20 x 15m enclosure surrounded by an electric fence. I moved pregnant females to this enclosure upon capture before parturition. Twice per day each caged female was supplied with 150ml of water, 170g of rabbit chow and approximately 5g of fresh herbaceous plants. In addition females were given one apple per day.

Upon parturition females were moved from the cages to Tomahawk live-traps that were covered with burlap. Each neonate was then counted, weighed, sexed and tagged in the right ear with a numbered monel eartag (National Band and Tag Co., Newport, Kentucky; size 1). I affixed 2g radio transmitters (Model SR-1, Biotrack, Dorset, England) with glue to the back of half of the neonates in each litter. The entire litter was then moved beneath dead-fall or a bush that afforded natural cover in the immediate area (those in the electrified enclosure were released near the original trapping location). The door of the Tomahawk live-trap was then locked open and the trap was

placed such that the female upon exiting the trap was forced to step over her litter. I then left the area and returned in approximately 15 minutes to remove the vacated trap.

Radio-collared neonates were located by telemetry every day. Located neonates were listed as either alive, predated (with the predator species recorded, where known) or dead from exposure or starvation. Neonates whose radio transmissions were not detected were listed as " signal lost".

Snowshoe hares are synchronous breeders, producing between 2 to 4 litters per year. Neonates are born in distinct litter groups with approximately 35 to 40 days between litters (Severaid 1942). I calculated pregnancy rates, litter sizes, stillborn rates, parturition death rates and mean litter weight for each litter group separately.

Weights of pregnant females at the time of placement into cages for all litters were recorded. Subsequently, weights of pregnant females after parturition of litter 2 were recorded prior to their release from the Tomahawk live-trap. These weights were compared between treated and control females. In 1990 and 1991 changes in weight of females during gestation of litter 2 were compiled from trapping data. Comparative data on dates of parturition for litters 1 and 2 in 1990 were taken from O' Donaghue (1992).

I compared weight gained and lost by pregnant females during gestation and after parturition of litter 2 between treated and control females with a one-way ANOVA (MGLH module, Systat, Inc., Evanston Illinois). I also used a one-way ANOVA to compare mean size of litters and mean dates of parturition for litters 1 and 2 among the three grids. Weight of neonates at birth for litters 1 and 2, among the three grids, were compared with a nested ANOVA, with offspring of each female nested within grid. Neonate body weight at birth was log-transformed before analyses.

Differences in factors causing mortality of neonates born to control and treated females were tested with a Chi-Square contingency analysis.

Results

Monitoring study

Prevalence and mean intensity of infection with *Protostrongylus boughtoni* and *Nematodirus triangularis* was significantly lower in treated hares collected from the treated grids in November of 1991 (Table 4.1). These treated hares were also in significantly better condition than untreated hares as determined by the bone marrow index (Table 4.2).

Resident male hares on the three study grids lost a great deal of weight over the winter of 1990 - 1991. Although there were differences in body size between the treated and control males the amount of overwinter weight loss was the same (Table 4.3). Body condition of resident male hares did not differ between treatments and body condition did not decline overwinter 1990 - 1991 (Table 4.4).

Very few hares remained on the grids for any extended length of time. Only 35% of the study animals could be classified as residents remaining on the area for 10 or more weeks (Fig. 4.2). The other 65% of the trapped hares either died within 10 weeks of their appearance, were transients, or were residents that could not be recaptured (for purposes of this thesis these animals will be referred to as transients). In addition, another 15% of the hares were recognized as remaining on the study grids for less than 24 weeks and were trapped only two or three times. An important consequence of this is that about 80% of the treated hare population received only one to three doses of ivermectin before disappearing from the treated grids. Figure 4.3 shows

Table 4.1. Summary statistics of one-way ANOVA testing for effect of ivermectin (0.4mg/kg) on numbers of *Protostrongylus boughtoni* and *Nematodirus triangularis* in treated and untreated snowshoe hares taken from the treated grids. Data were log-transformed and hare body size was analyzed as a covariate. The number of hares examined, prevalence and mean intensity (± SD) (calculated from non-transformed data) are also shown.

	Source	DF	SS	MS	F-Ratio	P
P. boughtoni	Covariate	1	1.574	1.574	1.745	0.201
	Treatment	1	9.429	9.429	10.442	0.004
	Error	21	18.962	0.903		
N. triangularius	Covariate	1	5.365	5.365	1.885	0.180
	Treatment	1	58.635	58.635	20.596	0.000
	Error	30	85.409	2.847		

		Grid	
		Control	Treated
P. boughtoni	N	8	16
	Prevalence	75	31
	Mean Intensity	14.3±16.7	4.75±4.8
N. triangularis	N	13	20
	Prevalence	100	65
	Mean Intensity	250±381	37.7±55.2

Table 4.2. Summary statistics for Kruskal-Wallis test for effect of ivermectin (0.4mg/kg) on percent-bone-marrow for treated and untreated hares collected from the treatment grids in November, 1991. The number of hares examined and the mean percent-bone-marrow (±SD) are also shown.

Group	Count	Rank Sum
Treated	12	109.000
Control	15	269.000

Mann-Whitney U test statistic = 31.000
P = 0.004
Chi-Square approximation 8.288 with 1 df

	Grid	
	Control	Treated
N	12	15
Percent-Bone-Marrow	37.5±19.5	59.8±7.4

Table 4.3. Summary statistics for repeated measures ANOVA testing for effect of ivermectin (0.4mg/kg) on overwinter weight loss in snowshoe hares. Only male weights were analyzed, because spring body weights of pregnant females were affected by litter size. Data were log-transformed before analysis. The number of hares examined, and the mean autumn (September - October) and spring (May) body weight (±SD) are also shown.

	Source	DF	SS	MS	F-Ratio	P
Between subjects	Treatment	1	0.0507	0.0507	4.376	0.054
	Error	16	0.1853	0.0116		
Within subjects	Time	1	0.1800	0.1800	39.731	0.000
	Interaction	1	0.0001	0.0001	0.027	0.878
	Error	16	0.0725	0.0045		

	Grid	
	Control	Treated
N	9	9
Autumn Weight	1513±114	1308±80.2
Spring Weight	1404±113	1218±79.6

Table 4.4. Summary statistics for repeated measures ANOVA testing for effect of ivermectin (0.4mg/kg) on changes in overwinter body condition in snowshoe hares. Only data from males were analyzed as spring body weights of pregnant females are affected by litter size. Body weights and RHF measurements were log transformed before analysis. The number of hares examined, and autumn (September - October) and spring (May) body condition (± SD) are also shown.

	Source	DF	SS	MS	F-Ratio	P
Between subjects	Treatment	1	0.001	0.001	2.650	0.123
	Error	16	0.003	0.000		
Within subjects	Time	1	0.000	0.000	0.096	0.760
	Interaction	1	0.000	0.000	0.118	0.736
	Error	16	0.002	0.000		

	Grid	
	Control	Treated
N	9	9
Autumn Condition	0.998±0.018	1.00±0.010
Spring Condition	0.995±0.083	1.00±0.007

Fig. 4.2. The percent of study animals remaining for 10, 10 to 24 or for more than 24 weeks on the treated and control grids from May 1990 to October 1991.

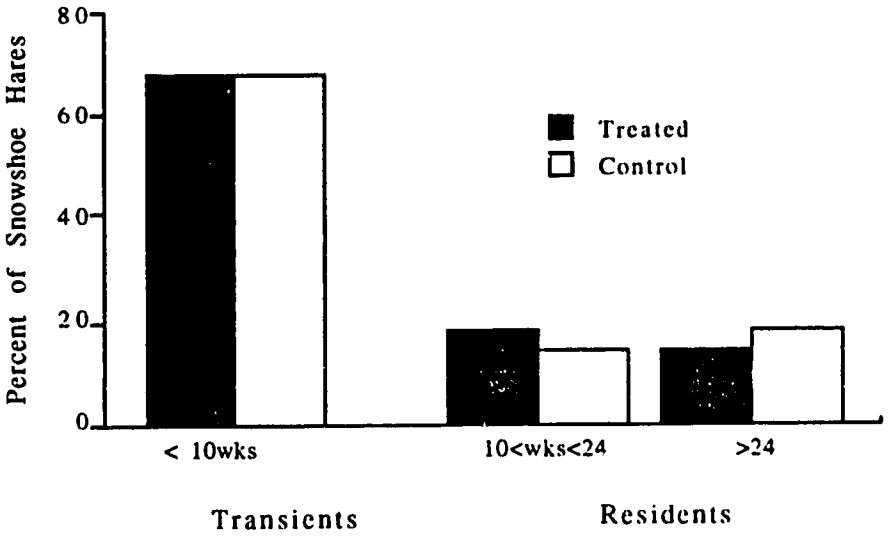
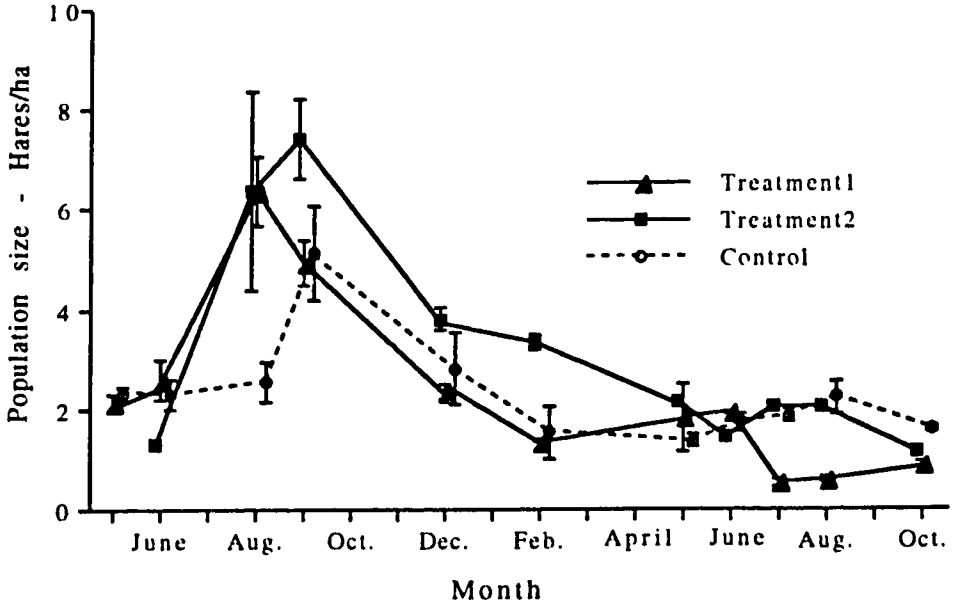


Fig. 4.3. Changes in population size (± SE) on treated and control grids from May 1990 to October 1991, as estimated by the Capture method (Otis *et al.* 1978). See text for details



changes in population sizes of hares on the treated and control grids as estimated by the Capture model (Otis *et al.* 1978). On all three grids, peak hare numbers were reached in August and September of 1990. The population declined through winter 1990 - 1991 so that by spring 1991 numbers were similar to one year earlier. Population size on Treatment 2 and the control grid remained essentially constant during summer 1991, but declined further on Treatment 1. Peak numbers of hares on Treatment 1 and Treatment 2 were 1.2 and 1.5 times higher, respectively, than on the control grid.

Figure 4.4 shows changes in survival of hares on the two treated grids and the control grid as estimated by the Jolly-Seber model (Seber 1982). On all three grids, hare survival was highest between June and August. Hare survival ranged between values of 0.58 and 1.0 during the summer of 1990 to values of between 0.4 and 0.7 during the winter of 1990 - 1991. Hare survival rebounded in summer, 1991 on all study grids, reaching rates of survival similar to those of the previous summer. Differences in survival did not differ significantly among the grids. The peak rate of survival on the Control grid was 1.1 times higher, respectively, than on the treated grids during the summer of 1990, and they were 1.2 times higher during the summer of 1991.

The trend in new juvenile and new adult recruits was similar among grids (Table 4.5). The percent of new recruits establishing as permanent residents was highest during the first trapping month on the control grid. The number of residents establishing from the new recruits on the treated grids was high for the first five months of the study. However, the decline in the establishment of residents occurred at the same time on all three study areas.

Fig. 4.4. Changes in the rates of survival (±SE) on treated and control grids from May 1990 to August 1991, as estimated by the Jolly-Seber model (Seber 1892). See text for details.

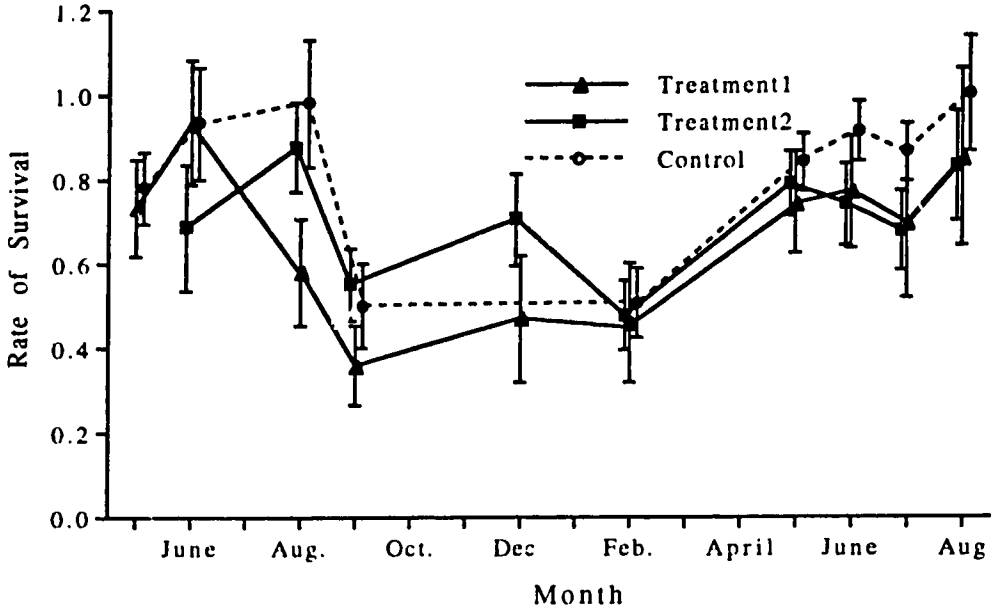


Table 4.5. Number of new adult and juvenile recruits on the treated grids (combined) and the control grid at each trapping period in 1990 and 1991. The percent of new recruits remaining as permanent residents (≥ 10 weeks residency) are also shown.

	Control			Treated		
Month	Adults	Juveniles	Permanent Residents	Adults	Juveniles	Permanent Residents
May	41	10	41	26	8	11
June	9	83	8	8	80	16
July	15	29	12	20	27	21
Aug.	17	7	7	30	3	15
Dec.	-	-	-	23	0	15
Feb.	7	0	9	12	0	7
May	8	3	5	1	1	6
June	5	8	1	3	18	2
July	0	16	4	0	12	4
Aug.	2	13	13	0	8	3
Sept.	13	4	-	6	2	-
Oct.	8	0	-	4	0	-

Reproductive study

On average each treated female produced approximately two more live young than did a female from the control group (Table 4.6). This discrepancy in fecundity between treated and controls was caused by slightly higher rates of pregnancy in treated groups, and by slightly higher numbers of females dying during parturition and slightly higher stillborn rates in the control group (Table 4.6). There was no difference in mean number of offspring per litter, or in mean parturition date (Table 4.7). However, it should be noted that, in general, survival of juveniles to 14 days in 1991 was much lower on all grids than in 1989 or 1990 (Figs. 4.5 and 4.7). The absence of a third litter in 1991 was a major factor contributing to this decline.

This apparent decline in female reproductive output is coincided with the inability of females in 1991 to increase and maintain their body weight during gestation and parturition of the second litter (Fig. 4.6). Weight gain was higher in treated females prior to parturition but these differences were not significant (Table 4.8). However, weight loss after parturition was significantly lower in the treated females.

One might suspect that these light untreated females would be less capable of caring for their offspring, leading to lower rates of neonate survival. However, overall neonate survival was very low and did not differ between treated and control females (Fig. 4.7). The factors responsible for neonate mortality, however, differed significantly between treated and control grids (Table 4.9). Neonates born to control females died more frequently of exposure, while neonates of treated females fell prey more frequently to predators. Treated neonates were also more likely to disappear ("lost signal") than were controls. Body weight at parturition of neonates for litter 1 differed significantly among the three study grids (Table 4.10), but

Table 4.6. Total estimated reproductive output[a] per surviving female on study grids during 1991, and factors causing the differences in fecundity observed between treated and control grids. Sample sizes are in parentheses.

	Grid		
	Control	Treatment 1	Treatment 2
Litter	Percent Pregnant		
1	100 (7)	100 (5)	100 (2)
2	85 (13)	100 (7)	100 (5)
3	0 (13)	0 (4)	20 (5)
	Percent Dying at Parturition		
1	0 (7)	0 (5)	0 (2)
2	18 (11)	0 (7)	0 (5)
3	-	-	0 (1)
	Percent Stillborn		
1	0 (20)	0 (7)	0 (24)
2	30 (46)	28 (29)	4 (26)
3	-	-	0 (2)
	Average Litter Size		
1	3.4	4.0	3.5
2	4.2	4.3	3.4
3	-	-	2.0
	Median Parturition Date		
1	29 May	1 June	28 May
2	6 July	2 July	7 July
3	-	August 11	-
	Total Reproductive Output[a] (Juveniles/Female)		
	5.9	8.3	7.3

a (Mean litter size) x (Pregnancy rate) x (1 - stillborn rate) x (1 - parturition death rate) summed over the 3 litters.

Table 4.7. Mean litter size (± SD) of hare litters born in cages on study grids during 1991 and mean date of parturition (± SD) for litters 1 and 2, (number of litters sampled in parentheses). F_s and P values are summarized from one-way ANOVA testing for effect of treatment. Data in Table 4.6.

	Mean Litter Sizes		
	Treatment 1	Treatment 2	Control
Litter 1[a]	4.0 ± 0.0 (5)	3.5 ± 0.71 (2)	3.4 ± 1.27 (7)
Litter 2[b]	4.3 ± 1.3 (7)	3.4 ± 1.5 (5)	4.2 ± 1.8 (11)
	Median Parturition Date		
Litter 1[c]	29 May	1 June	28 May
Litter 2[d]	6 July	2 July	7 July

a $F_{(2,11)} = 0.831$, P = 0.461
b $F_{(2,20)} = 0.594$, P = 0.562
c $F_{(2,11)} = 0.095$, P = 0.910
d $F_{(2,20)} = 0.487$, P = 0.621

Fig. 4.5. Reproductive output, as calculated for Table 4.6, for 1989 and 1990 on food addition and control grids combined (from O'Donaghue 1992), and for 1991 on treated grids combined and the control grid.

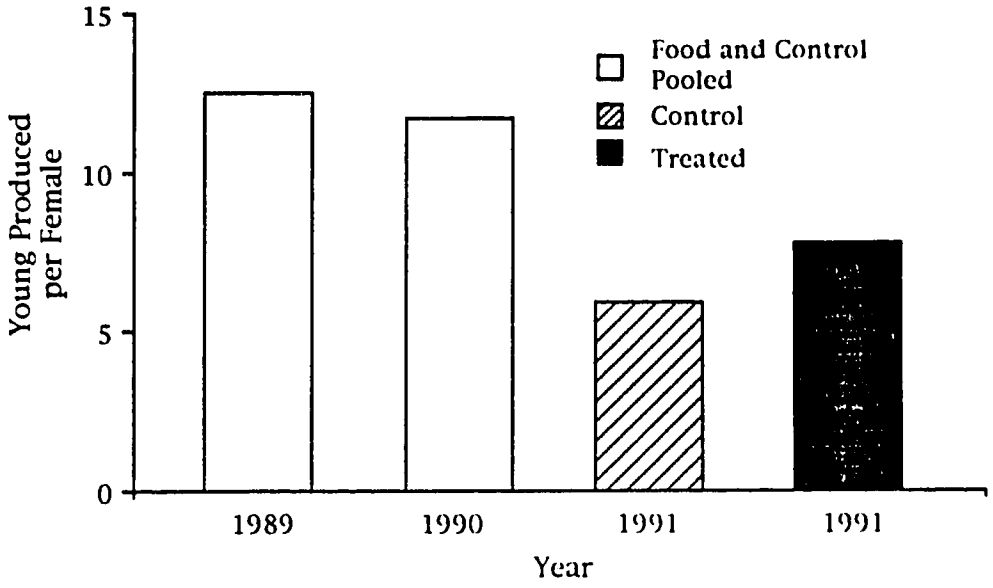


Fig. 4.6. Weight change (+SE) in reproducing females during gestation of second litter for pre-parturition weight 1990, and both pre and post-parturition weight changes in 1991.

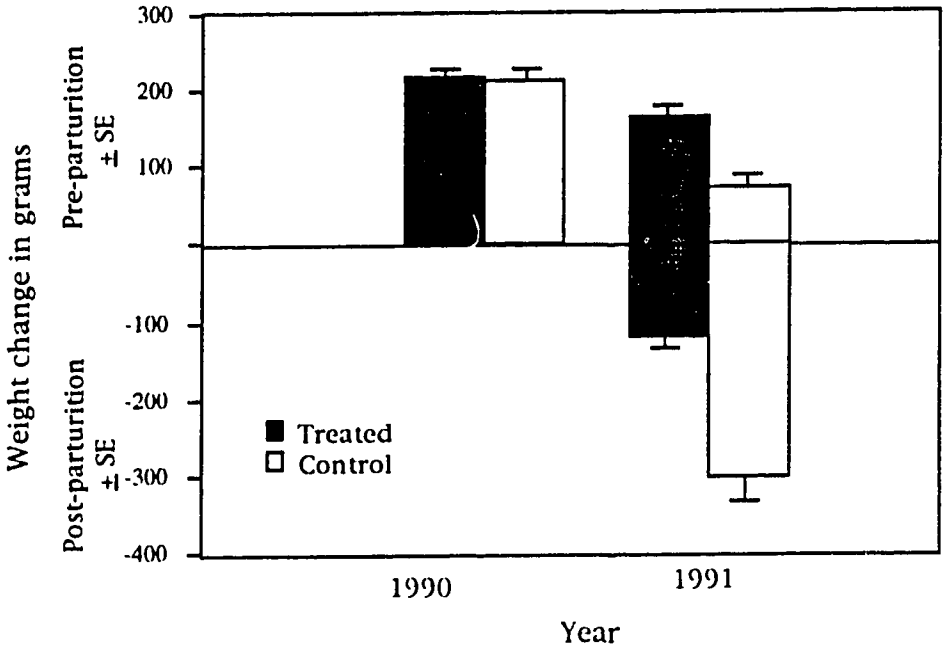


Fig. 4.7. Survival and mortality factors in percent for collared neonates from treated grids combined, the separate control grid (litters 1 and 2 combined), and from O'Donaghue (1992), food and control grids pooled (litters 1 - 3 combined).

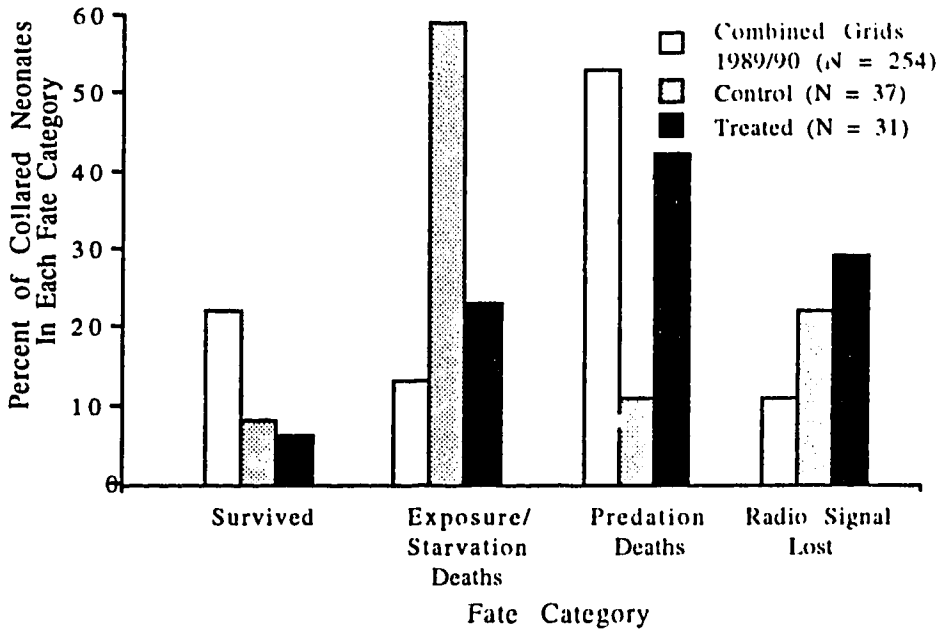


Table 4.8. Summary statistics of one-way ANOVA testing for effect of treatment on pre-parturition weight gain during gestation of litter 2 and post-parturition weight loss after birth of litter 2 for females on treated (combined) and control grids, 1991 only. Data in Table 4.6.

	Source	DF	SS	MS	F-Ratio	P
Pre-Parturition Weight Gain	Treatment	1	53,141	53,141	2.514	0.134
	Error	15	317,086	21,139		
Post-Parturition Weight Loss	Treatment	1	134,067	134,067	4.810	0.049
	Error	12	334,437	27,870		

Table 4.9. Chi-Square contingency analysis testing for the effect of treatment on factors responsible for neonate mortality between treated grids combined and the control grid, for litters 1 and 2 combined. Number of neonates from a particular grid(s) dying of a particular mortality factor are in box.

	Exposure Deaths	Predation Deaths	Radio Signal Lost	Total
Treated Grids	7	15	11	33
Control Grid	23	4	8	35
Total	30	19	19	49

Pearson Chi-Square: DF = 2; X^2 = 14.589; p = 0.001

Table 4.10. Mean neonate birth weight (± SD) and sample sizes (in parentheses) of neonates born in cages on study grids during 1991 for litters 1 and 2. F values and probabilities are summarized from a nested ANOVA testing for effect of treatment by grid, with neonates of each female nested within grid. Data were log-transformed before analyses.

	Mean Litter Weight		
	Treatment 1	Treatment 2	Control
Litter 1[a]	42.5 ± 4.9 (20)	57.9 ± 9.6 (10)	45.6 ± 4.9 (23)
Litter 2[b]	49.1 ± 10.8 (28)	52.0 ± 8.0 (16)	50.3 ± 11.6 (46)

a Control significantly higher then Treatment1 $F_{(1, 38)} = 13.345$, $P = 0.001$
Control significantly lower then Treatment2 $F_{(1,38)} = 75.511$, $P = 0.000$

b No difference between grids $F_{(2, 68)} = 1.345$, $P = 0.265$

apparently not in response to treatment. Litter 1 neonates from the control grid were significantly heavier then neonates from the Treatment 1 grid, but significantly lighter than neonates from Treatment 2 grid. Neonate body weights at parturition of litter two did not differ between the three grids (Table 4.10).

Discussion

Collection of both treated and untreated hares from the two treated grids (manipulated grids) showed that ivermectin was successful at reducing the numbers of those nematodes, *Protostrongylus boughtoni* and *Nematodirus triangularis*, most likely to negatively affect this population of hares. Treatment significantly reduced both prevalence and mean intensities of both parasites. Furthermore, treated hares had higher indices of bone-marrow than did the untreated hares, suggesting that reductions in worm numbers improved their condition. The manipulation was therefore effective in reducing nematode numbers in treated hares on the manipulated grids.

Is there evidence that parasites were responsible for regulating the hare population? This question can be answered by looking at two different types of measures. First, there are measures of the population, such as density recruitment or survival, which measure the dynamics on the grid as a whole. Second, there are measures applicable to individuals, such as reproduction or weight and body condition, which measure the attributes of individuals on each grid. I will first address measures of the population.

Parasites were not the most important factor affecting changes in hare numbers during the initial period of hare decline. This is suggested by a decline in hare density that began at the same time on all of the study grids.

Furthermore, hare densi'y during the summer of 1991 did not recover from its overwinter loss on any of the study grids. It is important to point out that population turnover on all of the study grids was high (Fig 4.2). At peak hare densities all suitable hare habitat is occupied and it is difficult for recruits to establish home ranges (Keith 1966), so it is not surprising that movements of hare populations on the grids were then high. Also, at this point in the cycle survival rates are low and populations of individual hares on any one area are changing rapidly. As a result, less than 20% of the hare population on the manipulated grids received continued treatments of ivermectin. Over 50% of the hares were captured once, and received only one treatment of ivermectin before disappearing from the study area.

My study was designed to reduce the numbers of parasites in all hares on the grid, not just in the treated hares. Ivermectin itself would reduce numbers of worms in treated hares and the treatment should have reduced transmission of parasites because of lower numbers of adult parasites on the manipulated grids. However, my earlier experiments showed that hares must be regularly treated for ivermectin to be effective at reducing numbers of hare nematodes (see Chapter 3, Discussion). Ineffective treatment due to the high rate of population turnover eliminated the potential to effect transmission and limited the effects of treatment to the treated resident hares.

Changes in hare density were calculated from all animals trapped on each grid and high population turnover led to low proportions of hares receiving many treatments. Because of this the ivermectin manipulation did not have any clear effects on hare density, survival, or recruitment. It was impossible to then establish whether or not parasites influence hare population cycles.

Under these circumstances, measurements applied to individuals would better reveal the effects that parasites have on the hare population. Indices measuring individual attributes like changes in weight, condition or fecundity are examples of measurements that can be calculated on identifiable resident hares that have received repeated treatments with ivermectin. Indeed, condition of resident treated hares, as calculated by percent-bone-marrow, was improved.

The change in weight occurring overwinter in resident male hares was examined to determine if reductions in worm numbers affected an individual's ability to maintain body weight overwinter. The males from the manipulated grids used to calculate weight change and body condition indices were all residents that had received 3.3($\pm$0.9) treatments of ivermectin over an eight month period, still a relatively low rate of treatment. Overwinter changes in weight did not differ among the study grids, nor was there a difference in the index of body condition.

This suggests that parasites had no affect on a hare's ability to maintain body weight during the winter period when food resources were limited and risk to predators was high. I did examine only surviving males and differences in this subpopulation my initially be small making it difficult to effect that treatments with ivermectin might have. Alternatively, the number of parasites infecting a given host might have been too low to affect hare body weight. However, this seems unlikely as parasites are at or near their peak mean intensities just after the peak in hare density is reached (Erickson 1944, Keith *et al.* 1985). Also, infection with *Nematodirus triangularis* in this population of hares was higher than reported in other surveys of snowshoe hares. Finally, at this point in the cycle, winter food resources are limited and reductions in worm numbers may not lead to increases in body weight simply

because food is too scarce, making it impossible for any hare, regardless of treatment, to maintain body weight.

Fecundity, and ultimately neonate survival, are two additional individual level measures that can be used to determine the effect that parasites have on hare populations. The treated females used in the study of reproduction were all residents on the manipulated grids; those hares used during litter one had received 4.6($\pm$2.3) treatments of ivermectin and those used during litter 2 had received 5.4($\pm$2.7) treatments, over an eight or nine month period, respectively. The results of examinations of treated hares at the end of the experiment suggests that worm numbers were reduced in these female hares. There was a general decline in fecundity of female hares between 1989-1990 and 1991 (see Results). It was evident that during the 1991 reproductive season all female hares were struggling to reproduce. Of the 22 female hares held captive on the study areas during gestation of the third litter, only one treated female was pregnant. Reproducing females, from the untreated population, were unable to maintain their body weight, and body weights after parturition of second litter were much lower than were weights previous to pregnancy. In addition, increases in body weight during gestation of the second litter were much lower in untreated hares in 1991 than they were in 1990. It seems that for untreated females, all energy reserves and nutrition acquired during gestation were channeled to their developing embryos.

Reproduction in 1991 was not as taxing for the treated females. Their increase in body weight during gestation of 1991's second litter was similar to the increases observed in 1990, and treated females lost significantly less weight after parturition of the second litter than did the treated females. Each

treated female also produced approximately two more young than untreated females, but fewer than produced per female in 1989/1990 (Fig. 4.5)

The ability of treated females to maintain body weight during reproduction suggests that they may have been in better condition, and it is also possible that they were more able to meet the lactation demands of their offspring. If this were true there should be some evidence to support this idea from data collected on the radio-collared neonates. Indeed, offspring born to untreated females succumbed to exposure/starvation at a much higher rate than did offspring born to treated females (Fig. 4.8).

Untreated females may have abandoned their litters after parturition, but this is unlikely (Boutin *et al.* 1988). Reproduction for females is costly; it is unreasonable to assume that a female in good condition would produce a litter and then abandon it after parturition. I can think of at least two explanations, other than litter abandonment, that could explain the higher proportion of exposure/starvation deaths in the untreated neonate population. It could be that untreated females were caring for their offspring, but were incapable of meeting their offspring's lactational demands. Alternatively, for these resource stressed females infected with high numbers of worms, reproduction and/or parturition may have been so traumatic that they either died or were predated shortly after giving birth. In fact, one untreated female died in her cage after giving birth to a viable litter. It could be argued that handling these females and keeping them captive introduced additional stress. However, handling of treated and untreated females was identical, and the consequences, in the absence of any effect of treatment, should have been identical among the groups. That it was not indicates that parasitism does affect female reproduction at the initial stages of decline in the Kluane hare population.

Females from treated grids produced more offspring per female, were able to maintain body weight during reproduction, and appeared more capable of meeting the nutritional demands of their offspring. It follows then, that neonate survival on the manipulated grids should be higher. However, increasing the condition of the mother by removing parasites does not necessarily preclude her offspring from risk to predation. In comparison to the control group, a high proportion of offspring of treated females were taken by predators (Fig. 4.7). Some of these "predation" deaths may actually be deaths caused by exposure or starvation with the carcasses then being scavenged. However, I located every radio-collared neonate each day, so only high one-day scavenging rates would lead to a bias in the deaths classified as predation. A study on scavenging rates of dead juvenile hares indicated that one-day scavenging rates were low (O'Donaghue 1992). Therefore, although some of the deaths attributed to predation may actually have been scavenged, most were probably correctly classified.

Figure 4.7 appears to suggest that predation of offspring of treated females was lower in 1991 than in 1989/90 and that increases in exposure/starvation deaths in both the treated and untreated population was the major factor leading to low rates of neonate survival in 1991. However, it is highly likely that a majority of the deaths classified as "radio signal lost" were actually predator kills. It is unlikely that the lost signals could be attributed to dispersing juveniles. Radio-collared neonates whose signals were lost disappeared within the first 3.67 days ($\pm$0.13) of their life. In a previous study of hare reproduction conducted at Kluane no leverets were known to disperse before the fifth week of their life (O'Donaghue 1992). Also, the areas where the lost neonates had last been located at was extensively checked by radio telemetry. In one instance an entire litter of four young that was last located

together disappeared, and this litter was classified as "radio signal lost". On the second day of this litter's disappearance I returned to the litter's last known location. Through an extensive search using radio telemetry I located the faint signals of both radio transmitters, less the antennas, and the eartag from one of the uncollared young, 1km away in a great horned owl pellet. I suspect that most neonates that disappeared were similarly removed by predators; however, I can not discount that some disappearances may simply represent failed radios. All radios were working at the time the young were released and radio failure is not a common problem within the first week of transmission, so it is likely that only a few, if any, of the radios actually failed. If those hares that disappeared and were classified as "radio signal lost" are added to the predation group the proportion of predated hares in 1991 is approximately 10% higher than it was in 1989/90. In essence both increases in exposure/starvation deaths and predation deaths were responsible for the lack of increased neonate survival in the treated group in 1991.

Conclusions

I was unable to examine the interaction between predation and parasitism. It was evident from examining hares taken by predators that predators first consume lungs and intestines, those organs within which the parasites reside. As a result, comparison of numbers of worms in predated hares versus hares collected from the general population was impossible. Understanding how parasites affect predation during the peak and decline phases of a hare cycle would explain much about the regulatory potential of parasites. Measures of the population suggest that parasites do not regulate this population of hares. However, conclusions drawn from these measures

must be viewed with caution as high population turnover on the manipulated grids reduced the ability of the manipulation to have an affect on measurements of density, recruitment and survival. High rates of population turnover made the individual level measures much better indicators of how parasites affect this population of hares; these measures can be applied to identifiable individuals whose treatment histories are known. Parasites did affect condition of untreated hares and did regulate reproduction in untreated females, lowering their reproductive output and causing a high number of their progeny to die of exposure and starvation. However, in this population of hares, parasitism was not responsible for regulating neonate survival. Removing parasites from the female population did not increase the survival of their progeny to recruitment. Ultimately predation overrode the effects of the manipulation and those offspring born to treated females, offspring that did not succumb to exposure or starvation, were taken by predators. The increase in exposure and starvation deaths of neonates caused by parasitism of their mothers was compensatory to that resulting from predation. It appears that parasitism interacts with predation and food resources to affect hare reproduction and neonate survival at the initial stage of decline in this population of hares.

Care should be taken when conducting field studies designed to measure the influence that parasites have on wild host populations. A study like Hudson *et al.*'s (1992) where necropsy data can be used to define the interactions between parasites and predators is desirable, and a host species with relatively low population turnover should be selected, so that a population of repeatedly treated hosts can be maintained. In this way the effects of treatment can be maximized. It would also be useful to know more

about how resource stressed females cope with the duties of parental care in populations of uninfected hosts and hosts infected with many worms.

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Chapter 5: Concluding Discussion

Factors affecting the size of snowshoe hare populations at the decline phase of hare cycles suggest that parasites might regulate that decline. Predation is one important factor leading to reductions in the size of hare populations at the initiation of the decline (Keith *et al.* 1984, Boutin *et al.* 1986, Krebs *et al.* 1992). Parasites can affect the asbility of red grouse avoid predation (Hudson *et al.* 1992a), and this should be general in situatons where prey are difficult for the predator to kill (i. e., the number of kills relative to predation attempts is low) (Temple 1987). Snowshoe hares are difficult to kill for lynx (*Lynx canadensis*) and coyotes (*Canis latrans*). Both are major predators of hares at Kluane (Murray 1991). Under such circumstances, one would expect that selective predation would lead to reductions in the number of hares infected with many worms. The effect that this would have on the hare (or parasite) populations depends upon the dynamics of infection. If enough parasites are present to significantly increase rates of predation, then parasite induced predation may help regulate the hare population. This might happen if selective predation enhanced parasite transmission (as is true of taeniid larvae infecting hares), or if high parasite numbers are due to reduced resistance of nutritionally stressed hares. However, if selective predation eliminates enough adult worms so that parasite transmission is reduced, then this might regulate the parasite populations at numbers too low to induce regulatory effects on the host population. Understanding how parasites affect predation during the peak and decline phases of a hare cycle would explain much about their regulatory role.

In my study, it was impossible to examine the interaction between predation and parasitism. In examining hares taken by predators it was

evident that those predators preferentially consume the organs within which the parasites reside, making it impossible to compare numbers of worms in predated hares to numbers in hares collected from the general population. Those data could have been obtained through estimation of parasite numbers by fecal analyses, and by then comparing estimates among predated and random samples of hares. Unfortunately, constraints of time and personnel precluded collection of the large samples required for such an analysis.

Two additional factors affecting hare population size at the decline phase of the cycle are reductions in recruitment and increases in the proportion of "starvation" deaths (Keith and Windberg 1978, Boutin *et al.* 1986). Hudson and his coworkers (Hudson 1986, Hudson and Dobson 1989, Hudson *et al.* 1992b) demonstrated that similar reductions in recruitment and non-predatory deaths in red grouse, *Lagopus lagopus scoticus*, could be ascribed to infections with *Trichostrongylus tenuis*. Models, manipulative field studies and other empirical evidence suggested that *T. tenuis* was responsible for the naturally occurring population cycles of red grouse (Dobson and Hudson 1992). The similarities in observed patterns suggest that similar parasites infecting snowshoe hares might be responsible for the naturally occurring population cycles of snowshoe hares.

The hares at Kluane were infected with seven species of helminths. One of these species (*Mosgovoyia pectinata*) is non-pathogenic and probably does not have an impact on this population of hares. Another four species, *Trichuris leporis*, *Passalurus* sp., *Taenia macrocystis* and *T. pisiformis* can be pathogenic and can affect host survival and reproduction. However, these four species were few in number and in too few hares to havef much influence on hare numbers at Kluane. The other two species, *Protostrongylus boughtoni* and *Nematodirus triangularis*, occurred both in high numbers and

in a high proportion of hares. Both are likely to be pathogenic in hares (see discussion in Chapter 2), and both showed moderate amounts of clumping. Theoretical models of host parasite systems have shown that moderate amounts of parasite clumping are important for regulation of host population size by parasites. In addition, the rapid reinfections with *P. boughtoni* and *N. triangularis* observed in the experiments with ivermectin (Chapter 3) suggest that their rates of transmission and net reproductive output are high. The theoretical models show that this is also important for host population regulation.

As usual, the initial decline in snowshoe hare numbers began in winter at a time when: 1) infections of *N. triangularis* were at peak mean intensities (significantly higher than the previous winter), 2) food resources are limited, 3) predation is a significant cause of hare mortality. Hare densities on my study grids in the spring following the winter of decline were the same as a year previous, but they can be as much as 50% lower (Keith *et al.* 1984, Krebs *et al.* 1986). This decline was followed in the summer by reductions in survival of young hares, at a time when infections of *P. boughtoni* are at high mean intensities an lactating females are caring for the needs of their growing offspring.

These patterns suggest that parasites, particularly *P. boughtoni* and *N. triangularis*, could affect hare numbers during the peak and initial decline phases of the Kluane hare cycle. However, there was no direct evidence for parasite-induced mortality or reductions in neonate survival in this system. A manipulative field study could show if parasites were having such effects; and because parasite numbers are high at the peak, this manipulation would require reducing numbers of *P. boughtoni* and *N. triangularis* in selected populations of hares at Kluane.

My experiments on the chemotherapeutic effects of ivermectin demonstrated that treatment significantly reduces numbers of adult *P. boughtoni* and *N. triangularis*, but does not affect fourth stage larvae within lung parenchyme (*P. boughtoni*) or within the intestinal mucosa (*N. triangularis*) nor is a single injection able to prevent reinfection in hares after 4 weeks post-treatment. As a result, repeated treatment of individual hares would be required. I planned a manipulation that would accomplish this by trapping and treating hares on selected treatment grids every 4 weeks in summer and twice over the winter period. However approximately 70% of the hares on these treatment grids were transients, moving through the study grids, and only 30% of the hares on the treatment grids were given the necessary repeated treatments with ivermectin. The high turnover of the hare population and consequent inability to treat a large portion of the population made the manipulation ineffective. The inability of the manipulation to demonstrate any effect on measures of the population such as density, recruitment, and survival are therefore uninformative.

The measures applied to individuals were much better indicators of how parasites affect this population of hares; these measures can be applied to identifiable individuals whose treatment histories are known. In fact, treated hares collected at the end of the experiment that had received repeated treatments of ivermectin had significantly fewer worms and were in significantly better condition than untreated hares collected at the same time and from the same study grids. Parasites did regulate reproduction in untreated females, lowering their reproductive output and causing a high number of their progeny to die of exposure and starvation. However, in this population of hares, parasitism was not responsible for regulating neonate survival. Removing parasites from the female population did not increase the

survival of their progeny to recruitment. Ultimately, predation overrode the effects of the manipulation and those offspring born to treated females, offspring that did not succumb to exposure or starvation, were ultimately taken by predators.

It appears that parasitism interacted with nutritional stress to affect hare fecundity at the initial stage of decline in this population of hares. High mean intensities of helminths and reduced nutritional condition ultimately compromised host energy budgets. However, predation of young hares in the first 14 days of life was a major factor limiting the recruitment of young into the adult hare population at this time, and the effects of parasites appear to have been compensatory to the effects of predation. For these reasons, I believe that parasites contributed to, but were not the most important factor, regulating hare demise at the initial stage of the decline in this hare population. Ultimately, the inability to measure how parasitism affects predation of snowshoe hares and the inability of the manipulation to affect overwinter survival on the manipulated grids, because of high rates of population turnover, make it difficult to state how parasites contribute to snowshoe hare cycles. However, data that I was able to collect support the contention that if the mortality or suppression of reproduction caused by a parasite proves to be only compensatory, then parasites will be unimportant as agents of population regulation (Holmes 1982, Spratt 1990).

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