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**CONTRASTING LIFE-HISTORY TRAITS AND
POPULATION DYNAMICS IN TWO CO-EXISTING
GASTROINTESTINAL NEMATODES OF SVALBARD
REINDEER**

Thesis submitted for the degree of Doctor of Philosophy

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STATEMENT OF ORIGINALITY

I hereby acknowledge that this dissertation is solely based upon original work carried out by myself and has not been submitted for consideration previously for a higher degree at this or any other university. Any references henceforth used have been appropriately acknowledged.

Collaborative authorship is acknowledged at the beginning of each paper.

Robert Justin C. Irvine

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ABSTRACT

By definition, parasites are expected to have fitness consequences for their hosts by reducing survival and fecundity. If such events are density dependent they may play a regulatory role in their host's dynamics. However, there are few studies in the wild that provide empirical evidence to support these suppositions. To understand the impact of parasites it is necessary to explore the interactions between parasite and host and the mechanisms that regulate nematode populations. The aims of this work are to: 1) identify the species specific patterns of infection; 2) investigate the interactions between and within nematode species and 3) examine the regulatory mechanisms that control nematode fecundity. The distribution of parasites between hosts and the variation between years, seasons, reindeer age and location are also examined. Nematode infections of Svalbard reindeer are dominated by two species: *Ostertagia gruehneri* and *Marshallagia marshalli* and their contrasting life-histories and population dynamics highlights the importance of investigating at the level of individual species. For *O. gruehneri*, there is significant annual variation but no strong seasonal pattern in abundance. Susceptible calves do not acquire infection until their second summer. Egg output is highly seasonal with a peak in July and controlled through density dependent effects on worm development. In contrast *M. marshalli*, shows a strong seasonal cycle which does not vary between years and the peak occurs in late winter suggesting winter transmission. Egg output is low and also confined to the winter months. The quantification of these traits is important in allowing parameterisation of models with data from the study system. In many studies parameters are estimated from studies of domestic host parasite systems and these may be inappropriate in this natural system. The role of immunity and arrested development and the relationship between transmission and environmental heterogeneity are discussed.

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CHAPTER 1.

General Introduction.

THE IMPORTANCE OF PARASITE POPULATION DYNAMICS IN THE CONTEXT OF HOST POPULATION DYNAMICS

Gastrointestinal helminths tend to cause morbidity rather than mortality in their hosts so tend to reduce body condition, even when food is not limiting (Symons, 1985, Arneberg *et al.*, 1996). However, nematodes may have fitness consequences for their hosts through reductions in survival and fecundity (Anderson & May, 1991). Theoretical models that consider parasites as a functional predator suggest that gastrointestinal nematodes, can have a regulatory effect on their vertebrate hosts because of parasite mediated density dependent effects on host fecundity and survival. (Anderson & May, 1978, May & Anderson, 1978). Also it is clear that parasite virulence can have contrasting effects on the dynamics of hosts depending on whether the impact of infection is mostly on increased host mortality (stabilising) or on a reduction in host reproductive rate (de-stabilising) depending on the pattern of parasite distribution in the host (Anderson & May, 1978, May & Anderson, 1978). However, despite the potential significance of parasite-host interactions in explaining the dynamics of natural populations, there are very few studies that provide empirical evidence to support these models, in part, because of the logistical difficulties in collecting the necessary long-term demographic data from wild populations. The best example is the study of *Trichostrongylis tenuis* infections in red grouse (*Lagopus lagopus scoticus*) which has shown that parasites can drive cycles through their time delayed impact on fecundity (Hudson *et al.*, 1985, Hudson, 1986, Dobson & Hudson, 1992 and Hudson, Dobson & Newborn, 1998). Results from other systems suggest that parasites can have an impact on the host survival or reproduction in natural host populations (Boonstra *et al.*, 1980, Haukisalmi *et al.*, 1988, Keith & Cary, 1990, Gulland, 1995).

Whether this is sufficient to regulate these populations has not been demonstrated because of the

complications of interactions with other potentially regulating mechanisms such as predation and competition with other herbivores. Furthermore, the necessary long term

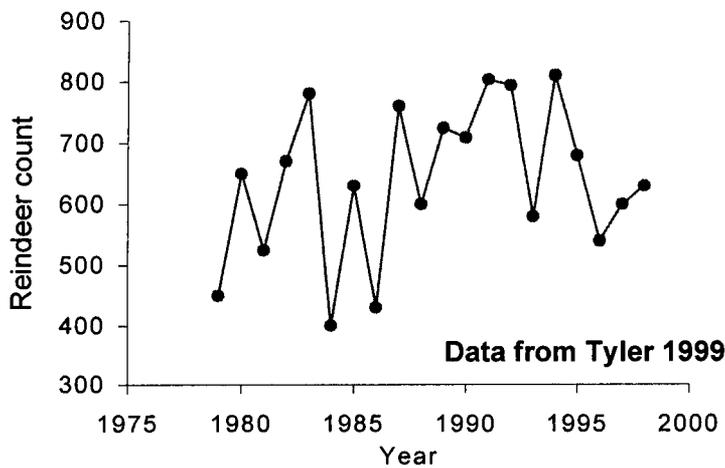


Fig 1.1. Two-fold fluctuations in the annual count (1979-1999) of reindeer in Adventdalen on Nordenskiöldland, Svalbard. (Tyler & Oritsland 1999).

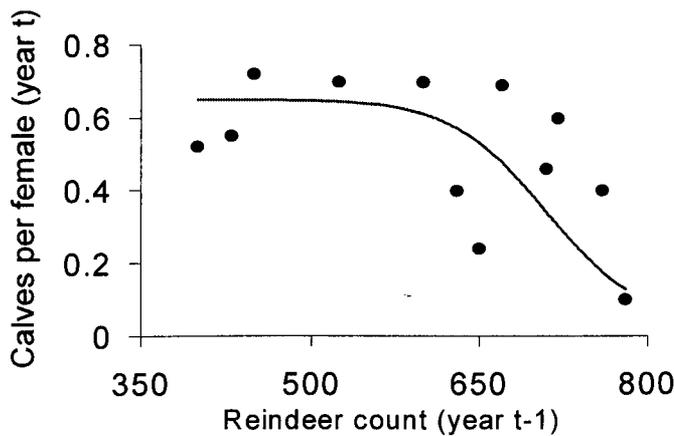


Fig 1.2. The probability of calving depends on the population density of reindeer the previous summer (t-1)

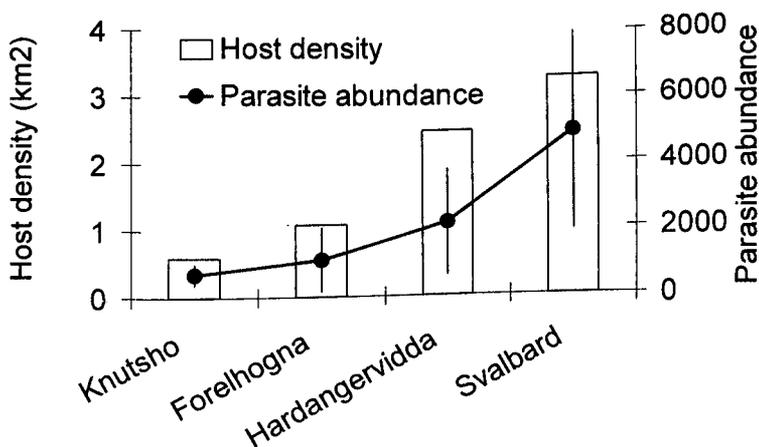


Fig 1.3. The abundance of abomasal nematodes and density of reindeer in 3 locations from mainland Norway and from Svalbard (Bye & Halvorsen, 1983, Halvorsen & Bye 1987)

individually based data is difficult to acquire. Ideally, the best way to determine the impact of parasites is to explore a system where other complicating and competing mechanisms can be either accounted for or are largely absent. With its absence of predators or competitors, the Svalbard reindeer-abomasal nematode system is an ideal model to test theories about parasite mediated regulation of host population dynamics for three reasons. First, the reindeer population fluctuates two-fold (Fig 1.1). Second, this is mainly due to host density dependent calving rate (Fig 1.2) exacerbated by stochastic weather events. Third, the reindeer harbour comparatively high burdens of parasites that vary between years at a population level (Fig 1.3, Bye, 1983) and at an individual level high parasite burdens depress host body condition (Halvorsen & Bye, 1986; Langvatn *et al.*, 1999)

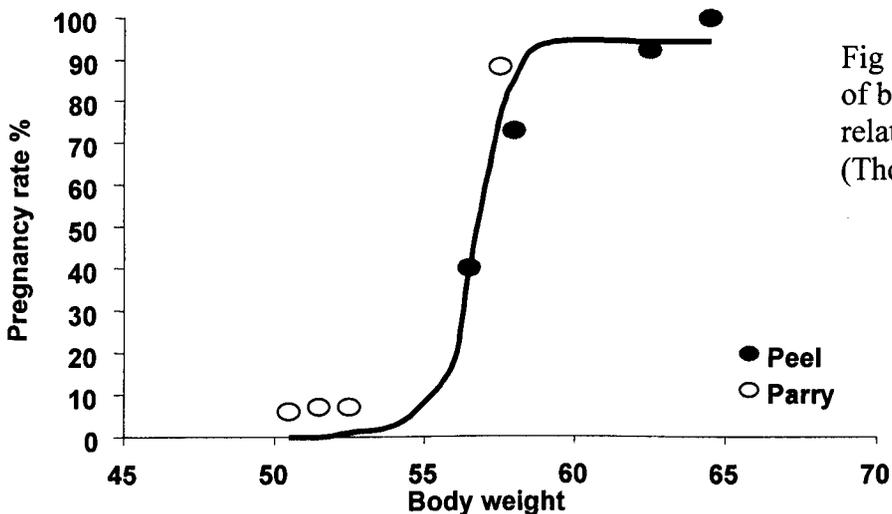


Fig 1.4. The probability of being pregnant is related to body weight (Thomas 1982).

Since in many cervid species, including caribou (Fig 1.4 and Thomas, 1982), the age at first reproduction and the probability of ovulation is closely related to autumn fat levels (Albon *et al.*, 1986), we can expect that parasites may influence the reproductive rate of reindeer on Svalbard (see also Thomas & Killian, 1990) and this, in turn, may be an important regulatory factor (Anderson, 1980). Recent work on the Svalbard reindeer-abomasal nematode system has identified two nematode species that dominate the parasite fauna of reindeer (Irvine *et al.*, 2000: Chapter 5). The first, *Marshallagia marshalli* appears not to be correlated with any fitness trait and seems to focus its transmission and fecundity

in the winter. The second and most abundant, *Ostertagia gruehneri*, is negatively related to body condition (Langvatn, 1999) and through anthelmintic experiments, it has been

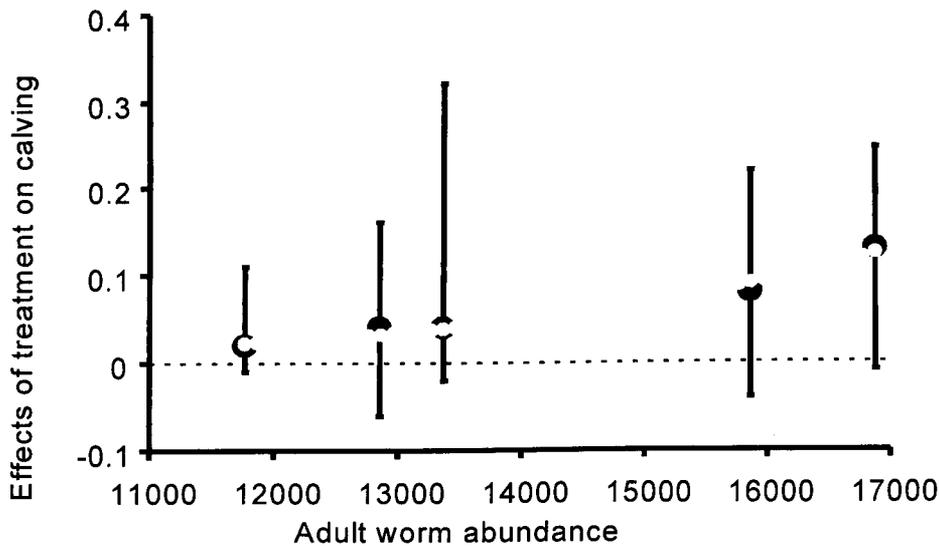


Fig 1.5. The difference in the calving rate (estimated regression line: difference = $1/(1+\exp(8.13-0.00366 \cdot \text{Ostertagia gruehneri abundance}))$) of reindeer treated with anthelmintics the previous April-May and controls, in relation to the estimated *O. gruehneri* abundance in October. Error bars are 95% confidence limit. The magnitude of the treatment effect was significantly positively related to the abundance of adult *O. gruehneri* worms in animals sampled in the previous October ($F_{1,3} = 143.92$, $P = 0.001$ (Albon et al., submitted)).

possible to quantify the effect of parasites on reindeer fitness. Treated animals have a higher probability of reproduction than controls but there is little effect of treatment on survival (Albon et al., submitted). The impact on calving rates varies with the intensity of infection (Fig 1.5) in a density dependent manner. Furthermore, theory suggests (May & Anderson, 1978, Dobson & Hudson, 1992) that in this case parasites should produce a destabilising effect on the host population. In support of the role parasites might play is the positive relationship between the annual variation in abundance of the pathogenic *O. gruehneri* and the annual variation in reindeer density (Fig. 1.6).

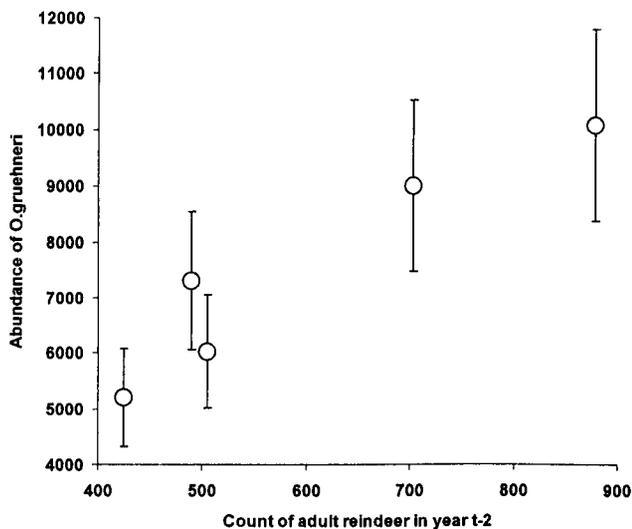


Fig 1.6.
The relationship between mean abundance of *O. gruehneri* and the population density of reindeer (Colesdalen, Nordenskiöldland). Host density is lagged by two years. Mean *O. gruehneri* abundance was estimated accounting for the negative binomial distribution. Error bars are 95% confidence limits. (Albon et al., submitted)

While the overall project on Svalbard reindeer has been geared to examining the impact gastro-intestinal parasites have on their hosts and, in particular, the extent to which they may regulate their numbers, the overall aim of my thesis is to quantify the population dynamics of the individual species in this naturally infected reindeer population.

THE COST OF PARASITES IN DOMESTIC ANIMALS: PRODUCTION LOSSES, LIFE CYCLE AND PHYSIOLOGICAL IMPACT.

Gastrointestinal trichostrongylid nematode infections of domestic ruminants have been the subject of much research because of the financial losses associated with parasite reduced growth rates, wool production, increased length of time to reach market weights and the cost of anthelmintic treatment (Armour, 1980). Evidence from experiments comparing worm-free and infected animals has shown differences in survival and weight gain in both domestic and feral sheep (Armour, 1980, Grenfell, 1988). The most important gastrointestinal parasites of domestic livestock are the abomasal nematodes such as *Teladorsagia circumcincta* and *Haemonchus contortus* in sheep and *Ostertagia ostertagi* in cattle (Soulsby, 1986). These are directly transmitted parasites. Eggs passed in the faeces, develop when temperature and humidity are favourable, through to infective larvae and

migrate onto the pasture and are subsequently ingested by the grazing herbivore. Once inside the host the infective larvae lose their protective second stage larval sheath and migrate into the abomasal mucosa before developing to 4th stage, eventually emerging back into the abomasal lumen and mucous layers as adults. It is the damage done to the mucosa which is responsible for the pathogenic effects including disruption of the acid producing parietal cell and the proteolytic efficacy of pepsinogen. (Holmes, 1987). Associated inflammation of the mucosa can be detected as elevated levels of pepsinogen in the plasma through leakage across the mucosa wall. Anorexia may also occur (reviewed in Kyriazakis *et al.*, 1999) due to the stimulation of circulating gastrin levels (Fox, 1997) and the result is reduced digestive efficiency and food intake. Therefore, body condition and growth rates can be affected. The essential assumption about host-parasite relationships is that the impact of parasites is related to the intensity of infection in an individual host and the impact of parasites on the host population depends on the distribution of the parasites between hosts (Hudson & Dobson, 1995). Therefore it is important to understand the epidemiological factors that are responsible for the intensity of infection within and between hosts.

THE USE OF EPIDEMIOLOGICAL TECHNIQUES IN UNDERSTANDING THE PATTERNS OF INFECTION

The aggregated distribution of parasite numbers among hosts. Parasite infections are typically characterised by large variation in parasite abundance between individuals and as such, it is important to consider the frequency distribution of parasite burdens in the host population (Crofton, 1971, Anderson & May, 1978, 1991). Typically only a few hosts harbour the majority of the parasites and the variance in parasite numbers exceeds the mean. This type of distribution can be described by the negative binomial which is

governed by two parameters; the mean and k which is an inverse measure of the aggregation (Hudson & Dobson, 1995, Wilson, Grenfell & Shaw, 1996, Shaw, Grenfell & Dobson 1998). These distributions come about for a number of reasons. First, hosts are not distributed randomly in the environment so that exposure to infection even with randomly distributed larvae may produce aggregated parasite distributions in the host population (Keymer & Anderson, 1979). However, it is unlikely that the larvae will be distributed evenly if the hosts are non-randomly distributed and eggs are passed in faeces deposited in pulses. Second, the biological processes (nutritional status and immunity) affecting parasite fecundity, mortality and transmission will vary between hosts. Third, genetic predisposition varies so that some animals will repeatedly be more likely to have high infections than others. Fourth, as the sampling and counting techniques may well produce distorted variation in the estimates of parasite abundance. For example, if the sample size is too small then there is a possibility that the observed distribution does not reflect the population because sampling may not have detected the few individuals with high burdens, or vice versa (Gregory & Woolhouse, 1993). Again, as in age-intensity profiles, care must be exercised when combining data from classes of animals that may have different distributions such as sex differences. For example male mammals tend to carry more nematodes than females (Poulin, 1999) because exposure or susceptibility is higher (Grenfell, 1992). One implication of an aggregated parasite distribution is that if intensity of infection is related to virulence then the impact of the parasites will only affect a small number of the hosts because only a few individuals harbour many of the parasites. Conversely the impact of parasite density-dependent host factors such as immunity will affect large number of the parasites. Therefore the effect of increased aggregation will be to stabilise the interaction between the dynamics of the hosts and parasites, unless

aggregation is extremely high in which case the host escapes the regulatory role of the parasite.

Season and Parasite Burden. The population dynamics of these gastrointestinal nematodes have been studied extensively (Smith & Grenfell, 1985; Smith, 1994; Grenfell, 1992) and important aspects of their population dynamics include the seasonality in transmission, development and establishment, and host development of acquired immunity. In temperate climates nematode transmission rates are commonly low in the late autumn/winter period due to reduced survival and development rates of the free-living stages of the nematodes, and the practice of housing domestic animals during cold periods (Smith & Grenfell, 1985). Typically, there is a spring rise in parasite burdens and faecal egg counts that may be associated with relaxation of immunity in peri-parturient animals (Crofton, 1958; Salisbury & Arundel, 1970) but still occurs in non-breeders. In domestic animals worm burdens tend to increase over the summer and decline in autumn and over the winter (Michel, 1969). The decline is due in part to the death rate of the adult nematodes exceeding the ingestion of larvae that will develop into adults because of phenomena such as arrested development of ingested larvae. High numbers of arrested larvae may re-emerge in the end of the harsh climatic period and cause disease in the beginning of the grazing season coinciding with host reproduction (Armour, 1970; Armour & Duncan, 1989). This seasonal pattern is particularly relevant in temperate and polar regions and has been interpreted as an adaptation to withstand periods when development, survival and transmission of free living stages in the external environment is low. Parasites, therefore, adopt a life cycle that varies with season so that transmission is focussed on the summer months and production of new infective stages is timed to coincide with favourable environmental conditions. The seasonal pattern may also be a product of acquired immunity which is known to occur in domestic livestock. Sheep and cattle develop high

worm burdens during the first months of the animals' life. Thereafter acquired immunity is considered to reduce the rate of nematode establishment and cause a reduction in the adult worm burden with increasing host age (Smith, 1994). This is thought to be the main reason for the low levels of infection commonly observed in adult animals compared to calves and lambs (Armour, 1980). Trichostrongyle nematodes show strongly seasonal dynamics in ruminants in temperate environments with transmission and peak burdens occurring in the main summer grazing period (Smith & Grenfell, 1985, Armour, 1980).

Weather and parasite burdens; variation among years. There are a number of processes that may produce variation in the annual abundance of parasites in the host. First, factors affecting the availability of infective larvae to the host. For example, the degree of pasture contamination with nematode egg laden faeces, the environmental conditions affecting egg and larval development on the pasture, the survival of infective larvae and the migration of larvae onto herbage that will be grazed (Armour, 1980; Stromberg, 1997). Second, the presence of susceptible hosts on the infected pasture when infective larvae are present. In the first case, the source of larvae on the pasture are either from over-wintering larvae (Smith & Archibald, 1969) or from larvae developing from eggs deposited in the same season (Young, 1980). This will be dependent on a) the combination of the density of hosts grazing the pasture and the size of the egg population that is deposited in the current summer as these will set the size of the over wintering larval population. And b) The resulting number of infective larvae will also be weather dependent. The development of the free-living stages are strongly correlated with temperature and humidity. For example, high infection rates may be experienced on spring pastures, especially in years with mild and wet weather favourable for the nematode free-living stages (Smith & Grenfell, 1985; Armour, 1970; Armour, 1980). Transmission may occur over six months or more and

provides the opportunity for nematode species with different life-history strategies to occur with successional changes in abundance of species with different developmental rates (Crofton, 1957, 1963, Boag & Thomas 1977). Thus annual variation in parasite burdens and seasonal infection cycles are likely to be closely linked to temperature either directly through its effect on the larval populations on the pasture or indirectly through its effect on host activity such as food (and therefore larval) intake rates and the seasonal availability of susceptible hosts.

Studies of domestic hosts-parasite systems usually take place over a short time span which in some cases is long enough to capture the seasonal dynamics but there are very few studies that have produced data suitable for an analysis of annual variation. There are however some examples from wild host-parasite systems such as the Red grouse-*Trichostrongylis tenuis* work (Hudson & Dobson, 1995; Moss et al., 1993) and the Soay sheep-gastrointestinal nematode system (Gulland & Fox, 1992)

Host age and parasite burden. One of the most instructive and fundamental epidemiological tools available is the analysis of the intensity of infection in relation to host age, which can yield information on parasite transmission and longevity and the basic reproductive rate, R_0 (Halvorsen, et al., 1986, Anderson & May 1991). Typically parasite intensity increases with host age due to ingestion of parasites over the given time period. In fact, the rate of increase and the shape of the curve is a balance between parasite infection rates (immigration) and parasite mortality due to death of parasites and animals containing the parasite. If both rates are constant, the curve will rise to an asymptote, the level of which will be determined by the balance between the two rates (Fig 1.7a); for example, the brain worm *Elaphostrongylis rangiferi* in reindeer (Halvorsen, 1986b).

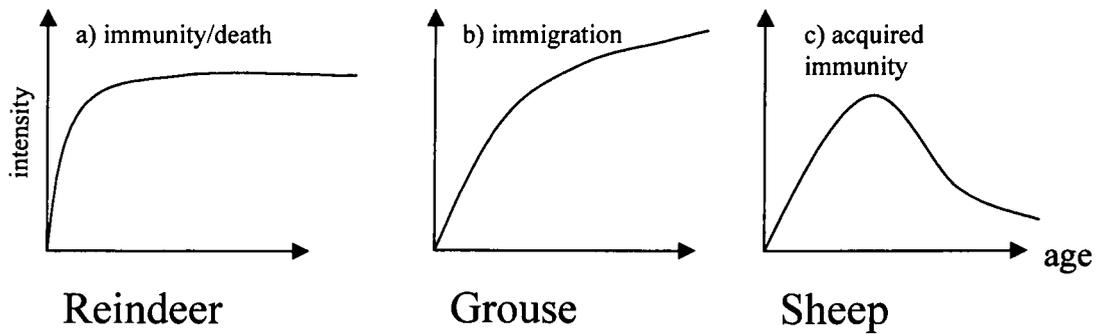


Fig 1.7 a), b) & c). Types of age intensity curves with possible interpretations.

If immigration is greater than the death rate (Fig 1.7b.) as seen in the red grouse-*T. tenuis* system (Hudson & Dobson, 1996), the intensity of infection can carry on increasing with age. If mortality is greater than immigration after initial infection, the relationship can initially rise but then decline in a convex manner (Fig 1.7c). This pattern is observed in systems where acquired immunity is thought to occur such as the domestic sheep-*T. circumcincta* system (Barger *et al.*, 1985). However, other processes could produce this convex functional form such as parasite-induced host mortality, age-dependent (behavioural) changes in infection rate and age-related changes in exposure (Rousset, 1996). The limitations of age intensity curves are firstly that all these factors may be working together and therefore it is not possible to determine which mechanism is responsible without more direct measures or through experimentation (Hudson & Dobson, 1995). And secondly, that variations in sample size can make it appear that convexity occurs while in fact this is a consequence of sampling from an aggregated distribution (Pacala & Dodson, 1988). Ideally age-intensity curves should be constructed from longitudinal data by repeated, serial sampling of the same individuals. However, estimating gastrointestinal parasite burdens is difficult without killing the animal. Therefore, when constructing age-intensity curves using culled animal the effects of age, sex, social status, sample size, parasite aggregation and season must be considered. One should also consider

that the culling policy may affect the future sampling through impacts on the sex and age structure of the host population.

An alternative to age-intensity relationship is age prevalence. These can be determined for gastrointestinal helminths from non destructive measures such as faecal egg counts (Grenfell *et al.*, 1995) or nematode specific levels of circulating plasma antibodies (Lloyd, 1995). However, the relationship between these measures and the intensity of infection is not simple and they are of limited use in an examination of the parasite population dynamics (Hudson & Dobson, 1995).

Density-dependent processes and parasite burden. Long term studies of host-parasite systems feature various dynamics from stability to cycles (Kennedy & Rumpus, 1977, Hudson *et al.*, 1985). Although these systems can display various dynamics, they are often characterised by regular patterns or at least a low degree of aggregation, suggesting that regulation of either the density of the parasites or the hosts occurs. If the host is viewed as presenting a closed population of parasites then it is theoretically possible to determine density dependent effects on the parasite population but because the host usually has to be killed in order to estimate densities then the mechanism governing the process may be difficult to elucidate. Parasite dynamics such as establishment, development, fecundity, survival and arrested development may all be density-dependent. It follows that if the parasite population is concentrated into few hosts with high worm burdens then any of these density dependent processes may be large enough to constrain the parasite population (Keymer, 1982). The impact of density-dependent processes may affect the observed parasite distribution by reducing the degree of aggregation. For example, density-dependent acquired immunity will reduce worm burdens and therefore animals that have been exposed for longer, i.e. older animals may have lower worm burdens and consequently k will be affected (Pacala & Dobson, 1988) However, estimates for

aggregation in the older age classes may be unreliable because sample sizes are typically smaller in this group.

GAPS IN THE KNOWLEDGE

Although coupling knowledge of age-intensity relationships and the factors responsible for parasite distributions should help in understanding parasite dynamics, there are three factors, crucial to parasite population dynamics, for which there is a paucity of empirical data. First, there is a need to understand the mechanisms that govern nematode fecundity. This typically varies with season and species. Combined with development and survival rates on the pasture this governs the availability of new infections to susceptible hosts (Hudson & Dobson, 1995). Second the pattern of arrested development determines the degree of damage due to arresting and de-arresting worms and introduces a time lag in the impact of parasitism and may also vary with species. Third the dynamics of immunity (Tompkins & Hudson, 1999) and the heterogeneity in immune response of individual hosts to parasites (Grenfell et al., 1995).

Parasite Fecundity. This is usually defined as the number of eggs per female worm that are passed out in the faeces per day. In experimentally infected calves *O. ostertagi* egg output rises to a peak 2-3 weeks following initial egg production and then declines exponentially (Michel, 1969). During this latter phase there is little evidence for a correlation between egg output and the population size of adult female worms. This contrasts with *H. contortus* where there is a good relationship between egg output and worm burden (Coyne *et al.*, 1991). This suggests that *O. ostertagi* is strongly regulated but *H. contortus* is not. Domestic livestock systems are characterised by seasonal patterns of egg output (Anderson

et al., 1979). Usually susceptible young animals on pasture will develop an infection in the spring and egg output will rise as the infection develops to reach a seasonal peak. Also, the phenomenon of the peri-parturient rise in lambing ewes produces a spring rise in egg output. This evidence has suggested that egg output is at least partly regulated through an immune response and is likely to be exposure dependent (Quinnel *et al.*, 1990). However, egg output also rises in non-pregnant ewes and males as well indicating other factors are also partly responsible (Gulland, 1992). Further evidence comes from observed high egg output in immuno-suppressed ewes or naïve animals (Gulland, 1992). There is some evidence that fecundity in *T. circumcincta* of sheep is related to the length of the female worms with the interpretation that shorter worms are less well developed and therefore have reduced fecundity. In this latter study the level of circulating antibody IgA was related to worm length (Stear *et al.*, 1995) and this suggests that immunity may indeed play a role in nematode fecundity. However, Michel (1970) has shown that egg output rises with worm burden until the rate of egg output per worm starts to drop with increasing worm burden and an asymptotic relationship is observed. Therefore fecundity may be regulated through density-dependent competition for space and resources. Furthermore, in a natural infection of a wild host population there may be a number of species present and there is the possibility that one species may affect the fecundity of the other through inter-specific competition. However, to date, this has not been determined in natural gastrointestinal infections of wild host populations.

Arrested development. Gastrointestinal parasites show varying degrees of arrested development between species and within species from different host localities (Gibbs, 1987). Arrestment or hypobiosis is defined as a pause in the development of 4th stage larvae when they are in the gastric glands of the mucosa (Michel, 1974). The mechanisms

that stimulate larvae to arrest and then to de-arrest or resume development are not completely resolved (for a review see Gibbs, 1987). However, arrestment seems to be partly stimulated by environmental conditions on the pasture including low temperatures (Armour & Bruce, 1974) or periods of drought (Smith & Grenfell, 1985). This suggests that arrestment is a strategy to survive periods when transmission and survival on the pasture is limited by adverse conditions. Another potential mechanism is within-host responses such as density dependence such that incoming larvae have an increasing probability of arresting as the resident burden of adults increases (Michel 1974). This may be due to immune stimulation triggering arrestment until the immune system is compromised by an event such as parturition in spring when larvae de-arrest (Michel, 1974) or, may be due to density-dependent competition for space and resources (Hong, *et al.*, 1986). However, the latter is also confounded by time so that over the course of a trickle challenge experiment, more larvae arrest and this may be due to the age of the larvae with older larvae ingested towards the end of the experiment arresting more readily than larvae ingested early on.

The mechanisms controlling de-arrestment are even less well resolved. There is some evidence that the spring or peri-parturient rise is due to relaxation of immunity (Lloyd, 1995) but the fact that non-lambing ewes exhibit a seasonal rise at this time of year suggests that other mechanisms may also be responsible such as intrinsic length of the arrestment phase; i.e. larvae will arrest for a more or less fixed period corresponding to the length of the adverse conditions. In Britain, this also corresponds with the period animals are housed over-winter and this may be the selection pressure that the nematodes have responded to in developing this strategy (Halvorsen & Bye, 1999). There is evidence that nematodes of Svalbard reindeer do not have the expected degree of arrestment in the

parasite population and that it is possible for transmission to continue even in the arctic winter, albeit at lower levels (Halvorsen & Bye, 1999). If there are differences within a nematode species in the propensity to arrest (Al Khalid, 1989, El Azazy 1995) then there are likely to be differences between species. In a mixed natural infection one species may be more virulent and through density dependence or immune stimulation may have a direct or indirect impact on the propensity of the other species to arrest. However, whilst taxonomy based on morphology of adult nematodes is possible, determining the species of 4th stage larvae is more difficult and requires molecular techniques. The advent of species specific PCR probes will make this easier and it will then be possible to quantify which species is responsible for the observed levels of arrested development. These data can then be used to take into account the time delays in the impact of a pathogenic species correcting for the different life-history of a coexisting species.

Host immunity. The observed variation between hosts in the size of an infection is also likely to be the result of heterogeneity in the immune response between different animals. There may be variation in the genetic predisposition to infection so that some hosts are more resistant than others. Furthermore there is evidence for a large degree of variation between animals in the major histo-compatibility complex (MHC) which manifests as differences in the potential an animal has to mount an immune response (Paterson, 1998). However, the degree of immune response is also dependent on the nutritional status of the animal since we may expect that an animal has to partition resources to the immune system (Van Houtert & Sykes, 1997). Therefore, there is a body of evidence that animals in poor nutrition, particularly if protein is limited are somewhat immune compromised (Coop & Kyriazakis, 1999). However, measuring immunity directly is difficult in a free ranging ungulate such as the Svalbard reindeer. Experiments that manipulated nutritional status,

parasite burden and measuring antibody titres would be most informative. Preliminary studies indicate that assays developed to measure sheep immunoglobulin isotypes work on reindeer sera and *O. gruehneri* antigen (Irvine and Huntley, unpublished data). However, in this thesis, some insight into the role of immunity in the reindeer-parasite dynamics has been attempted through the inclusion of body condition as a surrogate for immunocompetence in models that explain variation in intensity of infection.

SPECIFIC AIMS OF THE STUDY

This study aims to describe the population dynamics of the abomasal nematodes of Svalbard reindeer (*Rangifer tarandus platyrhynchus*) and, from this, identify the contrasting life histories and population dynamics of the two major abomasal nematodes. The specific objectives were first, to identify the pattern of infection with respect to seasonal abundance, arrested development and acquired immunity. Second, by resolving the adult nematodes to their component species, to determine the contrasting seasonal and annual patterns of abundance and look for evidence of interactions between the species. Third, to examine the potential regulatory mechanisms that control nematode fecundity. Fourth, to determine the contrasting pattern of arrested development between the two species. As such this work will provide, a valuable understanding of the epidemiology, life history and population dynamics of a mixed natural nematode infection of a wild host.

The theoretical models often rely on assumptions about the parasite population which are based on demographic rates derived from single species, experimental nematode infections of domestic livestock (Hudson & Dobson 1995, Kao et al., 2000). However, natural infections usually comprise more than one species. Even in domestic livestock,

such as sheep, the abomasum will tend to harbour multiple species that infect the host in a natural succession of species (Crofton, 1957). This may be due to species differences in over-winter survival of infective larvae or the development of free living stages (Boag & Thomas, 1977). Depending on the species concerned there are a number of studies that find either positive, negative or no effect of one co-existing species on the abundance of the other (Holmstad & Skorpning, 1998; Nilssen et al., 1998). These different effects may be due in part to interactions with the hosts immune response and how specific this is to alternative species. There is some evidence that protective immunity to one species may cross over to affect the establishment of a second species (see Christensen, 1987). Another interaction may be due to physiological effects whereby one species affects the environment such that it either favours or reduces the establishment of a second species. For example, it has been suggested that in the case of *T. circumcincta*, infection disrupt the structure of the abomasum which makes it difficult for *Haemonchus contortus* to establish (Dobson & Barnes, 1995). However, our knowledge of the life histories and population dynamics of mixed infections of gastrointestinal nematodes in wild ruminant populations points to substantial differences from domestic systems (see above and Halvorsen & Bye, 1999) and this thesis aims to provide the detailed epidemiological knowledge on which to base realistic models of host-parasite dynamics.

CHAPTER 2.

Svalbard reindeer and their parasites.

The first step in the process of identifying and measuring the impact of a program is to define the program's objectives and outcomes. This involves a clear understanding of the program's purpose and the specific results it is intended to achieve. Once the objectives are defined, the next step is to identify the key indicators that will be used to measure the program's impact. These indicators should be relevant, measurable, and achievable. The final step in this process is to develop a data collection and analysis plan that will ensure the accuracy and reliability of the data used to measure the program's impact.

CHAPTER 1

The purpose of this chapter is to provide a comprehensive overview of the process of identifying and measuring the impact of a program. This chapter will discuss the importance of defining program objectives and outcomes, the role of key indicators, and the development of a data collection and analysis plan. The chapter will also provide a detailed description of the various methods used to measure program impact, including both quantitative and qualitative approaches.

Identifying and Measuring Program Impact

The process of identifying and measuring the impact of a program is a complex and multi-step process. It begins with a clear understanding of the program's purpose and objectives. This involves a thorough review of the program's mission statement and a clear definition of the specific outcomes it is intended to achieve. Once the objectives are defined, the next step is to identify the key indicators that will be used to measure the program's impact. These indicators should be relevant, measurable, and achievable. The final step in this process is to develop a data collection and analysis plan that will ensure the accuracy and reliability of the data used to measure the program's impact.

CHAPTER 2. Svalbard reindeer and their parasites:

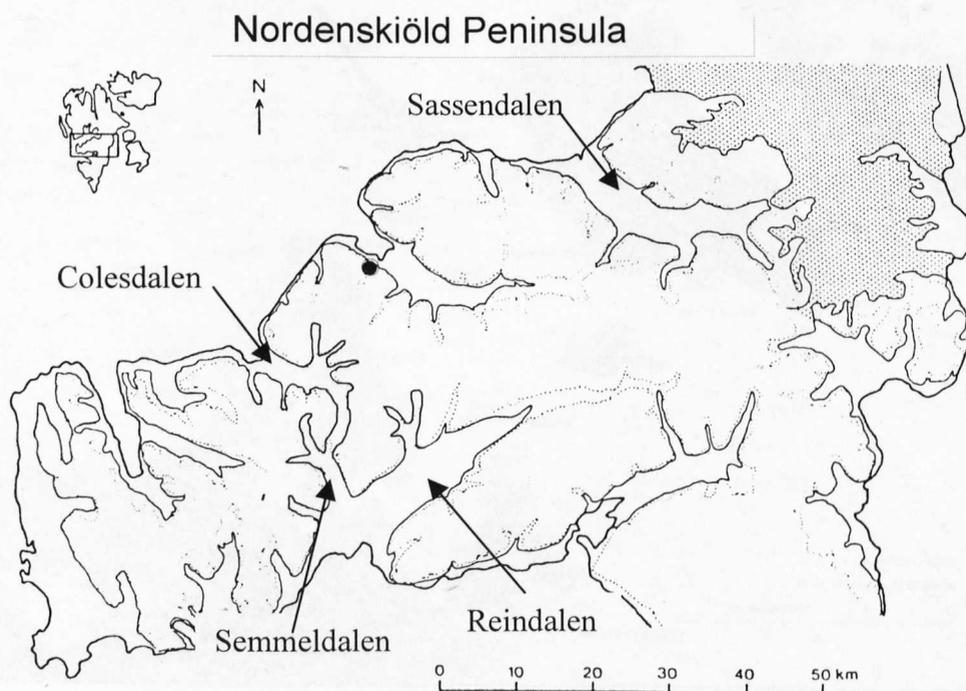
A REVIEW OF THE STUDY SYSTEM

Arctic Region



THE STUDY AREA ON SVALBARD.

Svalbard is a high arctic archipelago situated between 76° and 81° North roughly half way between the North Cape of Norway and the North Pole. It is a highly seasonal environment with periods of 24 hour darkness (Polar night) from 28th October until 14th February and 24 hour daylight from 21st April to 21st August. The ground is frozen and largely snow covered from October until June, therefore plant growth is restricted to a short period of about 8 weeks from mid June onwards. The mean annual precipitation (1975-1985) is around 195 mm and mean annual temperature is -6.5°C (Førland Hanssen-Bauer & Nordli, 1997). In summer the permafrost may thaw to a depth of 2-3 m in some places. The snow free period lasts for only three months and mean summer temperature (June to August) is 3.6°C and rainfall averages 53 mm (Førland, et al., 1997). However, because of oceanic influences, temperatures are highly variable and can rise above zero even in mid-winter. Within Svalbard much of the reindeer ecology has been studied on the Nordenskiöld peninsula. Relatively, this is one of the more vegetated areas on Svalbard



Coastal areas and valleys are vegetated up to about 200m above sea level and little grows above this altitude except on some higher plateaux. Higher up glaciers dominate and mountains rise to about 1000m above sea level (Brattbakk, 1985). Yearly primary production has been estimated at between 20 and 80g dry matter/m² (van der Wal, *pers. comm.*) The main valleys are wide and U-shaped and dominated by braided river beds. There are only 4 resident mammals on Svalbard; the polar bear (*Ursus maritimus*), the arctic fox, (*Alopex lagopus*), the Svalbard reindeer (*Rangifer tarandus platyrhynchus*) and voles (*Microtus arvalis*). The latter are restricted solely to bird cliff areas. The reindeer represent the major herbivore but there are localised populations of Pink Foot (*Anser brachyrhynchus*) and Barnacle (*Branta leucopsis*) geese that use the range for part of the year. Ptarmigan (*Lagopus mutus hypoboreus*) represent the only resident bird species. Some domestic livestock have been kept at mining settlements and an introduced muskox (*Ovibos moschatus*) herd numbering between 10 and 60 survived between 1929 and 1984 (see Halvorsen & Bye 1999).

SVALBARD REINDEER

Svalbard reindeer are a distinct subspecies and, genetically, its closest relative is the Peary Caribou (*R. t. pearyi*) of the Canadian arctic islands. (Roed & Witten, 1986). Svalbard reindeer differ from their nearest neighbour, the Eurasian tundra reindeer (*R. t. tarandus*). Like the Peary caribou, they are comparatively small and stocky with short legs ears and face and the extremely thick winter coat emphasises these features. However, inconsistent with other reindeer/caribou species, Svalbard reindeer do not migrate (Tyler & Øritsland, 1989) but may emigrate in years when there is an acute shortage of food in winter (Aanes *et al.*, 2000). The lack of migration is thought to be due to the lack of competitors,

predators and/or harassing insects. Possibly because of this reindeer on Svalbard exist at local densities of up to 8km^{-2} which is 2-3 times higher than that found on the Norwegian mainland (Fig 1.3 and Reimers, 1977).

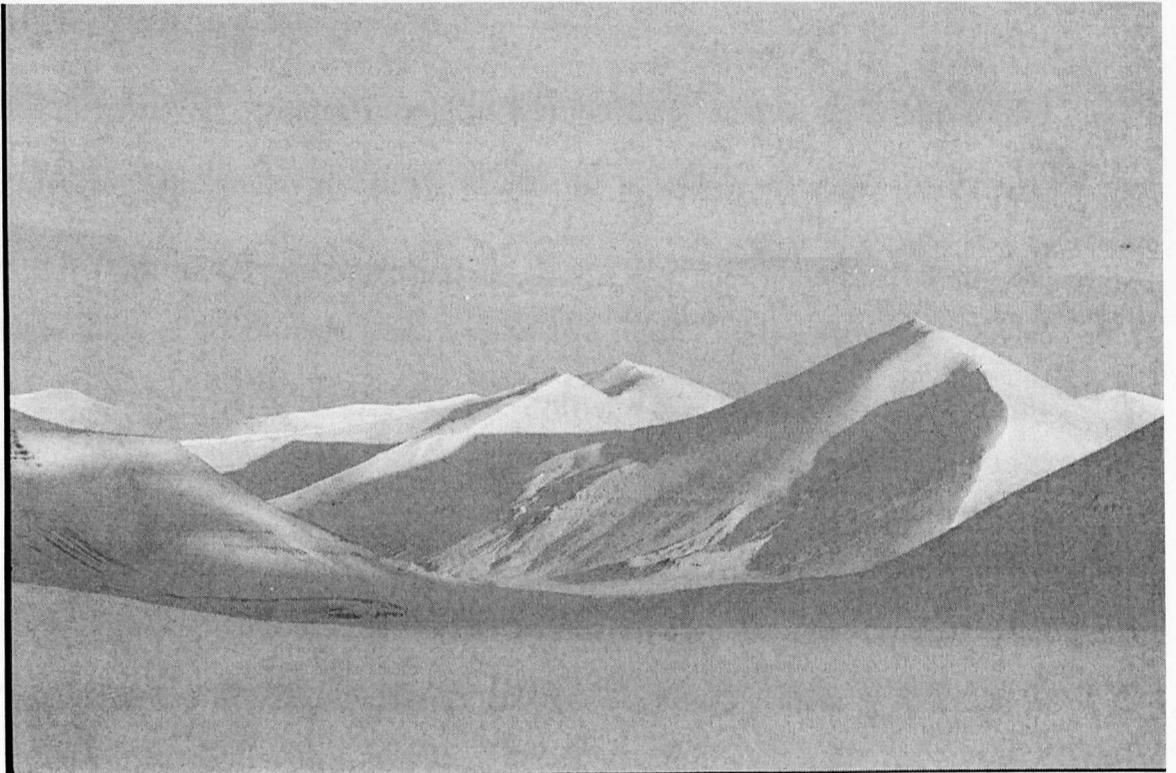
Rates of reproduction are low in young animals reaching up to 75% when animal reach the age of 3. (Langvatn, 1999). 90% of calving occurs between 2nd and 9th June (Tyler, 1987). Males and females grow antlers up to August when the velvet is cleaned and antlers are hardened off. The breeding season occurs in mid October and ovulation rates are invariably high at around 85-100% (Langvatn, 1999). All animals lay down fat through summer and by October back fat depth on the rump is around 20mm in an adult female. Mothers with a calf at foot may still be suckling calves by October but some will have been weaned by this time. Body weight for females in October is 67.3kg (+6.0) for a non-lactating female and 61.7kg (+2.8) for a lactating female (Tyler, 1987) but this varies between years. It is well established in reindeer that pregnancy rates is positively related to body weight (Fig 1.4 and Thomas, 1982). By April, towards the end of winter, adult females will weigh round 50kg and calf weight will have dropped from around 30kg to about 22kg. Most mortality is due to starvation and occurs in the late winter when ice prevents access to forage. Calves and old animals show the highest rates of mortality. Females will live to around 12 years of age and males to around 6. Two aspects of the reindeer population dynamics are notable. First, annual calving rates are very variable with calving commonly up around 70 % but can be as low as 10% and that there is a non-linear relationship between this and population density such that calving rates are disproportionately lower at high reindeer densities (Fig 1.3 and Tyler & Øritsland, 1999). Second the reindeer population exhibits a two-fold fluctuation which is largely accounted for by changes in recruitment to the population (Fig 1.2 and Tyler & Øritsland, 1999). The years with low calving rates are often associated with icing events during the winter which reduces access to winter forage.

PARASITES OF SVALBARD REINDEER

Svalbard reindeer harbour relatively few species of parasites (reviewed in Halvorsen & Bye, 1999). There are reports of *Sarcocystis* species in various muscles (Bye, 1985 - in Halvorsen & Bye, 1999). The tapeworm *Monezia benedini* has been found occasionally in calves and cysticerci of *Taenia ovis krabbei* have been found in adult reindeer (Bye, 1985b, 1985c - in Halvorsen & Bye 1999).

Research has focused more on the gastrointestinal nematodes. Bye & Halvorsen (1983) described six nematode species or species complexes in the Svalbard reindeer abomasa. These have subsequently been reclassified and are dominated by *Ostertagia gruehneri* (and its morph *O. g. arctica*, Dallas *et al.*, 2000a, Chapter 3.4) and *M. marshalli* (and its morph *M. m. occidentalis*, Dallas *et al.*, 2001, Chapter 3.5) (Irvine *et al.*, 2000). *T. circumcincta* occurs at less than 5% of total nematode abundance. Halvorsen & Bye (1986) also found no nematodes in the rest of the gastrointestinal tract of 24 reindeer although *Nematodirus* spp. eggs have also been identified in faecal egg counts. *O. gruehneri* is mainly a reindeer parasite but the others have all been reported in reindeer (reviewed in Bye & Halvorsen, 1983). *M. marshalli* has been mainly reported in domestic livestock but has been reported in muskox which were present on Svalbard between 1929 and 1984 (see Bye *et al.*, 1987). Both *O. gruehneri* and *M. marshalli* have a direct life cycle and infection is principally transmitted with food. The classic trichostrongyle life cycle is assumed and has the following pattern: Eggs are passed out in the faeces, develop to 1st and then 2nd stage larvae in the faeces and then the 3rd stage larvae inside the 2nd stage sheath. It is thought that these larvae migrate onto the vegetation in the moisture film on the leaf surface and then ingested with the vegetation, ex-sheath in the rumen pass into the abomasum where they

migrate into the mucosa and either develop into 4th stage larvae and return to the abomasum lumen to develop into adults or remain in the mucosa as arrested larvae.



CHAPTER 3.

Methods.

CHAPTER 3. 1

DATA COLLECTION AND DEVELOPMENT OF METHODS.

HOST SAMPLING.

Culled animals. Culling was carried out by experienced hunters under licence from the Governor of Svalbard. Animals were approached on foot in the field and shot in the neck or thorax. The stratification of the cull animals by sex, age and location was designed to fulfil the objectives of the greater Svalbard reindeer project with particular emphasis on sampling adult females and calves. Sampling mainly took place towards the end of winter (April) and the end of summer (October) in two valleys; Colesdalen and Sassendalen (see Chapter 2 Fig 2.1) (although some animals were culled in mid-summer, July, and Autumn, September). In 1998 and 1999 some individuals were caught and treated with an anthelmintic (see below). A sub-set of these were culled either 14 days, 12 weeks or 24 weeks post treatment (Irvine, 2000, Chapter 2.2) and analyses of parasite population dynamics took this experiment into account. Body condition measures included total body weight, subcutaneous back fat depth, kidney fat index (percentage that peri-nephric fat represents of kidney plus fat weight), hind leg length (skeletal size) and dressed carcass weight. Reproductive status was based on ovulation or pregnancy status, and lactational status was recorded as milk or yield. Ovaries were stored for analysis of retrospective reproductive status. Animals were aged by extracting an incisor and counting the annuli in the cementum of microtome histological sections (Reimers & Nordby, 1968). Abomasum samples for gastrointestinal parasitology were extracted as outlined below.

Live animal sampling. This study utilised a marked population of known age animals to provide faecal egg counts from known individuals from April to September by locating animals with telescopes and binoculars and collecting freshly excreted faeces. The marked population was captured and marked individually using a net trawled between two snow-mobiles. Each reindeer was fitted with ear tags and a modified plastic cattle collar (Dalton Ltd, Nettlebed, UK). Blood and skin samples were collected, body weight was recorded and pregnancy diagnosis and back fat depth was measured using an ultrasound machine. An anthelmintic experiment was carried out by either injecting individuals with 4mg/kg live weight moxidectin or administering an ivermectin slow release bolus. Analyses of egg output from treated animals was corrected for treatment group. The efficacy of the moxidectin treatment was assessed from monitoring faecal egg output in control versus treated individually marked females. Furthermore the efficacy against adult and larval stages was determined from a selective cull of treated and control animals 2, 12 and 24 weeks post-treatment (Irvine 2000, Chapter 2.2).

PARASITOLOGY.

Parasitology techniques are adapted from MAFF/ADAS (1986) and laboratory manuals of the Parasitology Division at the Moredun Research Institute (Frank Jackson pers. comm.) and are described below:

Faecal egg counts. Faeces were collected from each the rectum or immediately after excretion from each reindeer and an egg count was carried out within 48 hours on the fresh material. No frozen faeces were used for egg counts. 3g of faeces were ground through a nylon mesh tea sieve suspended in 42 ml of saturated salt (NaCl) solution using a pestle. One third of the resulting suspension was put into a centrifuge tube using a syringe fitted

with 5cm of silicon tubing. The 15ml tube was filled to the top and a cover-slip was placed on the positive meniscus. The tubes were spun at 1000 rpm for 3 minutes. The cover slip was removed using a decided positive upward motion and placed on a microscope slide. The eggs adhering to the underside of the cover slip were assumed to represent the number of eggs per gramme and the whole of the cover-slip was examined at 100x magnification.

Extraction and enumeration of adult and larval nematodes from the abomasal lumen.

Immediately after animals were culled the gastrointestinal tract was removed from the abdomen and the abomasum was ligated at the omasum-abomasum junction and at the duodenum close to the pyloric sphincter. The abomasum was frozen within 2 hours of removal. On thawing, the abomasum was weighed and then opened along the distal curvature. The content was tipped into a 5 litre beaker and the pH was taken using a standard meter and freshly prepared standards were prepared every few days for calibration. The mucosa surface was rinsed under cold running water and care was taken to ensure all debris was removed by washing under all the mucosa folds. The washed mucosa was weighed, labelled and refrozen. The content in the beaker was diluted to 4 litres and under constant agitation sub-sampled using a tap mounted pump to draw of 6 by 200ml (5%) aliquots in conical flasks. The content of each flask was examined under a stereo microscope with sub-stage lighting using a modified perspex pollen counting tray 6 channels wide each channel within one field of view wide at 16x magnification (objective) and 16cm long. 100 males and 100 females were collected in 70% ethanol. If less than 100 were available in the first 5% then another 5% was counted up to a maximum of 20% of the abomasum content. Juvenile worms (lumen larvae) were also counted and collected up to a maximum of 100 but if 100 of each of the adults was achieved in the 1st 5% but not

100 lumen larvae then the number of larvae reached was the final number collected. A back up sample of 20% of the content was stored in formalin.

Extraction of larvae in the abomasum mucosa larvae. The mucosa larvae which may represent the arrested 4th stage larvae were extracted from the empty abomasum using the standard acid-pepsin digest fluid. Briefly, the abomasum was chopped up into 2cm squares and put into the digest fluid 500ml per 250g of mucosa. The fluid was prepared daily and made from 8g of pepsinogen, 8.5g salt (NaCl) and 10ml of hydrochloric acid (HCl) per litre of water. The chopped abomasum and fluid was incubated for 12 hours and then poured through a 38 μ m sieve. The residue was washed with hand-hot water to reduce the amount of fat. The resulting washings were diluted to 1 litre and four 100ml sub-samples were extracted using a tap mounted filter pump. These were examined as above and a minimum of 100 4th stage larvae (mucosa larvae) were picked out and stored in 70% ethanol. The technique was subsequently adapted to improve the quality of larval DNA so that species-specific PCR probes could be used on each larvae (see Irvine & Dallas, submitted, Chapter 2.3). Essentially, the larvae released after 2 hours were recovered for PCR analysis and the abomasum plus fluid was returned to the incubator for a further 10 hours to maximise the numerical return.

Taxonomy: morphology and PCR probes. The adult nematode population was classified to species using the morphological characteristics of the males and the taxonomy key of Dródz (1965) and Lichtenfells *et al.*, (1993). A PCR probe was developed to identify males, females and larvae and the accuracy of this was tested on a number of adult males and females (50 females identified on morphology by Lynda Gibbons, Royal Veterinary College). The PCR probes identified that *O. gruehneri* and its morph *O. g. arctica* were

genetically indistinguishable when using the ITS-II region and this was the same for *M. marshalli* and its morph *M. m. occidentalis*. The probes was then able to tell whether an individual worm was either *O. gruehneri*, *M. marshalli* or *T. circumcincta* (Dallas *et al.*, 2000a (Chapter 2.4), Dallas *et al.*, 2000b (Chapter 2.5) & Dallas *et al.*, 2001 (Chapter 2.6)) This probe then allowed relatively quick identification of the species of individual lumen larvae and mucosa larvae and this allowed analysis of the contrasting species specific patterns of arrested development.

CHAPTER 3.2

Use of moxidectin treatment in the investigation of abomasal nematodiasis in wild reindeer (*Rangifer tarandus platyrhynchus*).

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2000

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USE OF MOXIDECTIN TREATMENT IN THE INVESTIGATION OF ABOMASAL NEMATODIASIS IN WILD REINDEER (*RANGIFER TARANDUS PLATYRHYNCHUS*).

R. J. Irvine

ABSTRACT

Gastro-intestinal parasitism is endemic in many wild ruminant populations. An experiment was conducted to evaluate the role of anthelmintics as a tool for understanding the impact of parasitism using wild Svalbard reindeer (*Rangifer tarandus platyrhynchus*) and moxidectin. Adult females were injected sub-cutaneously with moxidectin at a dose rate of 0.4mg/kg body weight. Groups of animals were culled within the expected period of efficacy (around 14 days post-treatment) or around 12 weeks or 24 weeks post-treatment. Moxidectin was effective in eliminating the abomasal worm burdens. Although reindeer became re-infected, worm burdens were significantly lower in treated animals up to 24 weeks after treatment compared to untreated controls. Nematode egg output did not reappear until 5 weeks, a similar period to that claimed by the manufacturer for sheep and cattle. Animals culled 12 and 24 weeks post-treatment had been re-infected and harboured a wide range in abomasal worm burdens which contributed to the understanding of the seasonal variation in the faecal egg count – worm burden relationship and underlines the value of experimentation in wild life diseases.

INTRODUCTION.

It is well documented that parasitic gastro-enteritis in livestock has a substantial financial and welfare impact. However, much of our understanding of gastrointestinal nematodes in wild ruminants is derived from domestic livestock. Parasitism has traditionally been associated with mortality and morbidity with the study of it in wild animals limited to the pathology of carcasses, a somewhat extreme case (see Gulland, 1995 for a review). The lack of studies on wild life is partly due the difficulties of collecting data in the field. Observational data frequently shows negative effects of parasites on host fitness but this does not discount the possibility that a third unknown factor is causing these observed patterns. Unfortunately, evidence from controlled experiments that indicate the impact of parasitism on wildlife is even more scarce because manipulating the parasite burden in a wild population is often constrained by logistical problems such as limited opportunities for handling. Alternatively faecal egg counts can be collected from wild animals but these may be unreliable because the relationship with actual worm burden is not clear.

Svalbard Reindeer (*Rangifer tarandus platyrhynchus*) are a wild reindeer population on the Svalbard Archipelago, Norway (76-81°N) in which it has proved possible to study the impact of parasites on growth, reproduction and survival. They have no predators, do not migrate and have no competitors. These reindeer occur at relatively high population densities (Tyler, 1987, Skogland, 1984) and harbour high parasite burdens (Halvorsen & Bye, 1999) dominated by *Ostertagia gruehneri* and *Marshallagia marshalli* (Irvine and others, 2000). There is evidence that body condition is negatively related to the abundance of *O. gruehneri* (Langvatn and others, 1999). Mortality is low except in winters when early rainfall followed by sub-zero temperatures causes extensive icing over of the vegetation and severely limits feeding access. Calving rates are high, approximately 75%, but can

drop to around 10% (Tyler & Øritsland, 1999). The snow free period lasts from mid June to mid September and this provides the only opportunity for females to regain on condition after calving in June for lactation, ovulation and subsequent pregnancy.

This study investigates the efficacy of moxidectin in reducing egg counts and removal of adult, immature and arrested stages of abomasal nematodes. Furthermore, the degree of re-infection with abomasal nematodes in moxidectin treated Svalbard reindeer within the same summer was measured. Moxidectin is a member of the milbemycin group, with a similar mode of action to ivermectin (Shoop and others, 1995). Research in ovine and bovine trichostrongylosis, suggests that its persistence of activity is at least as great as ivermectin against some abomasal nematode species such as *Teladorsagia circumcincta* (Grimshaw and Hong, 1997) and *Ostertagi ostertagi* (Deroover and others, 1997). The value of using the new generation macrocyclic anthelmintics in wild animal populations is discussed.

MATERIALS AND METHODS

Animal population. 298 female reindeer were caught by netting from snow-scooters in late winter (April/May, 1997, 1998 and 1999), aged using categories of calf, yearling and adult. In 1997 and 1998, every second animal within each age group was treated. In 1999 to ensure sufficient treated animals were re-sighted for culling a batch of 35 adult females were treated in addition to the usual treatment protocol. All animals were marked with individually numbered plastic ear tags and a modified cattle collar.

At the relevant sampling intervals animals were culled and sampled according to the protocols outlined in Halvorsen and others (1999) and Irvine and others (2000). Lactational status was recorded as lactating or not lactating.

Anthelmintic treatment. The treated groups (42 in both 1997 and 1998, 76 in 1999) were injected sub-cutaneously at the shoulder with 0.4mg moxidectin (Cydectin; Fort Dodge) per kg body weight to an accuracy of 2.5kg (Salter 100 x 0.5 kg clock face spring balance) between 14th April and 9th May. The dose rate was twice that recommended for sheep and cattle but within levels used in other methods of drug delivery and tested on red deer, another cervid species (Mackintosh and others, 1993). This dose rate was chosen to maximise efficacy of the drug on the nematodes when the pharmacokinetics of moxidectin in reindeer losing weight may have a negative impact on the drugs performance (Oksanen & Nieminen, 1998).

In 1998, five adult animals treated on 17th or 18th April were located and culled between 12 and 14 days post-treatment. Five adult controls were also culled at the same time. During 1999, 8 treated adult animals were culled together with eight adult controls in late July (12 weeks post-treatment) and again in late October (24 weeks post-treatment). This allowed assessment firstly of the efficacy of the drug shortly after treatment and within the effective period and secondly to examine the extent of re-infection in treated animals compared to control groups.

Parasite population. Abomasal worm burdens and faecal strongyle egg counts were estimated using standard methods (MAFF/ADAS 1986) with modifications as outlined in Halvorsen and others (1999). No *Nematodirus* eggs were detected in the study animals. In Svalbard reindeer, gastrointestinal nematodiasis is limited to the abomasum (Halvorsen & Bye, 1986) so it was assumed that the strongyle egg output was derived from the abomasal nematodes. Strongyle egg counts did not include tapeworm or fluke eggs. Data were not available for the abundance of mucosa larvae in July and October 1999. Individual

strongyle faecal egg counts were recorded for all marked animals in the study area during all three summers.

Statistical Analysis. Abomasal worm and larvae burdens are presented as geometric means with 95% confidence limits. Egg counts are presented using natural log transformation (count+1) to allow use of zero counts in the analysis. Solid lines to guide the eye were fitted using the spline function in Genstat (Lawes Agricultural Trust). Prevalence was estimated as the proportion of animals having detectable strongyle egg output and standard errors about this proportion were calculated accordingly. Egg count and worm burden data was analysed using generalised linear regression analysis (SAS version 6.12, 1996, SAS Institute, USA). Parasite data was assumed to be poisson distributed. Statistical significance was taken at the 5% probability level and calculated using the F test.

RESULTS.

Abomasal worm burdens during treatment. All five animals culled between 12 and 14 days post treatment in 1998 (Fig 3.2.1), had zero adult worm burdens compared with a mean of 12750 for the control group. The mean abundance of lumen larvae (the larval fraction found in the abomasal contents) in the treated group was 44 compared to 2572 in controls (Fig 3.2.1c, $F_{1,8}=35.82$, $P<0.0001$). The abundance of mucosal larvae (the larval fraction found in the abomasal mucosa (L4s)) was 115 in the treated group compared to a mean of 6500 in the controls ($F_{1,8}=65.92$, $P<0.0001$).

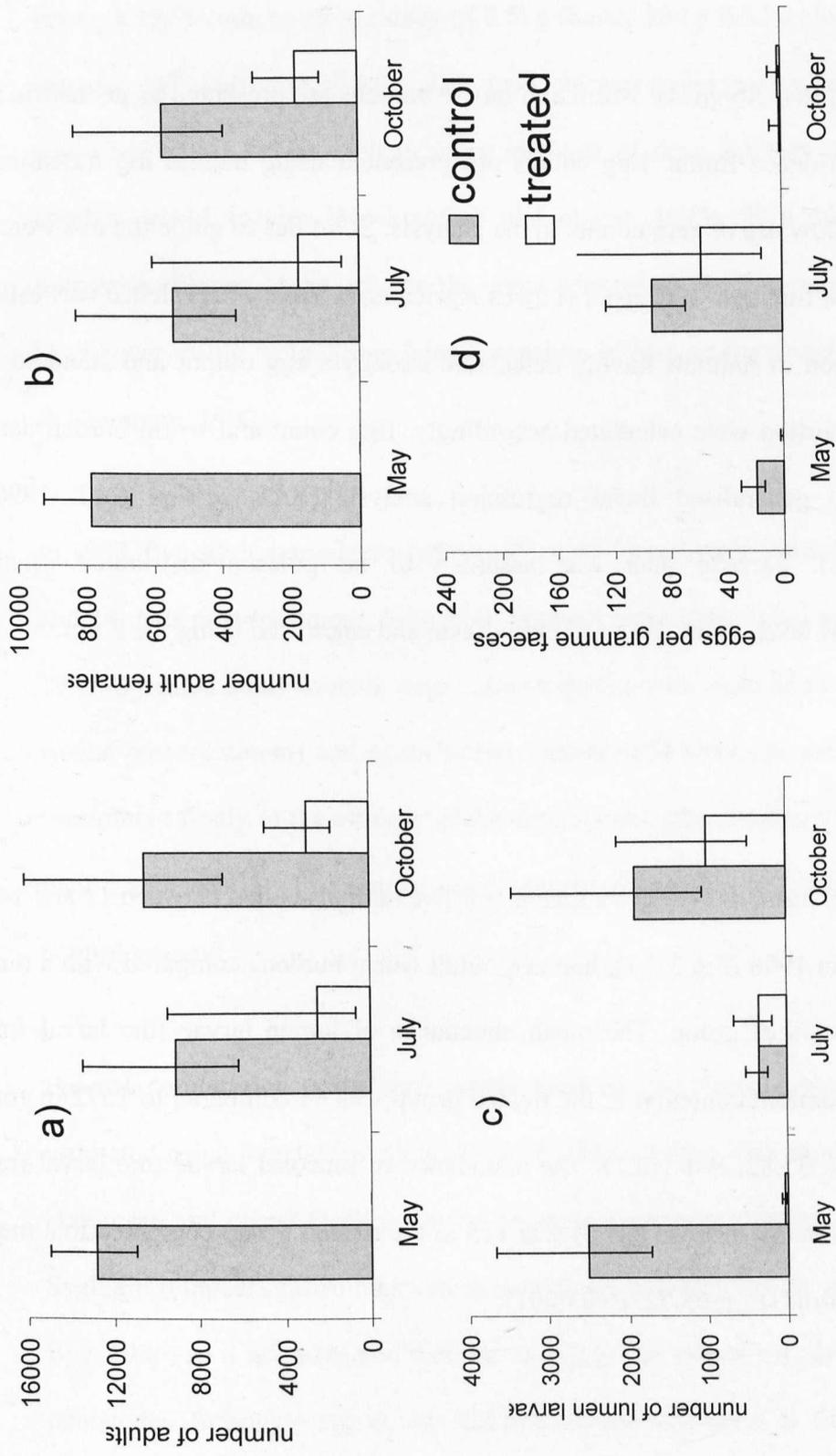
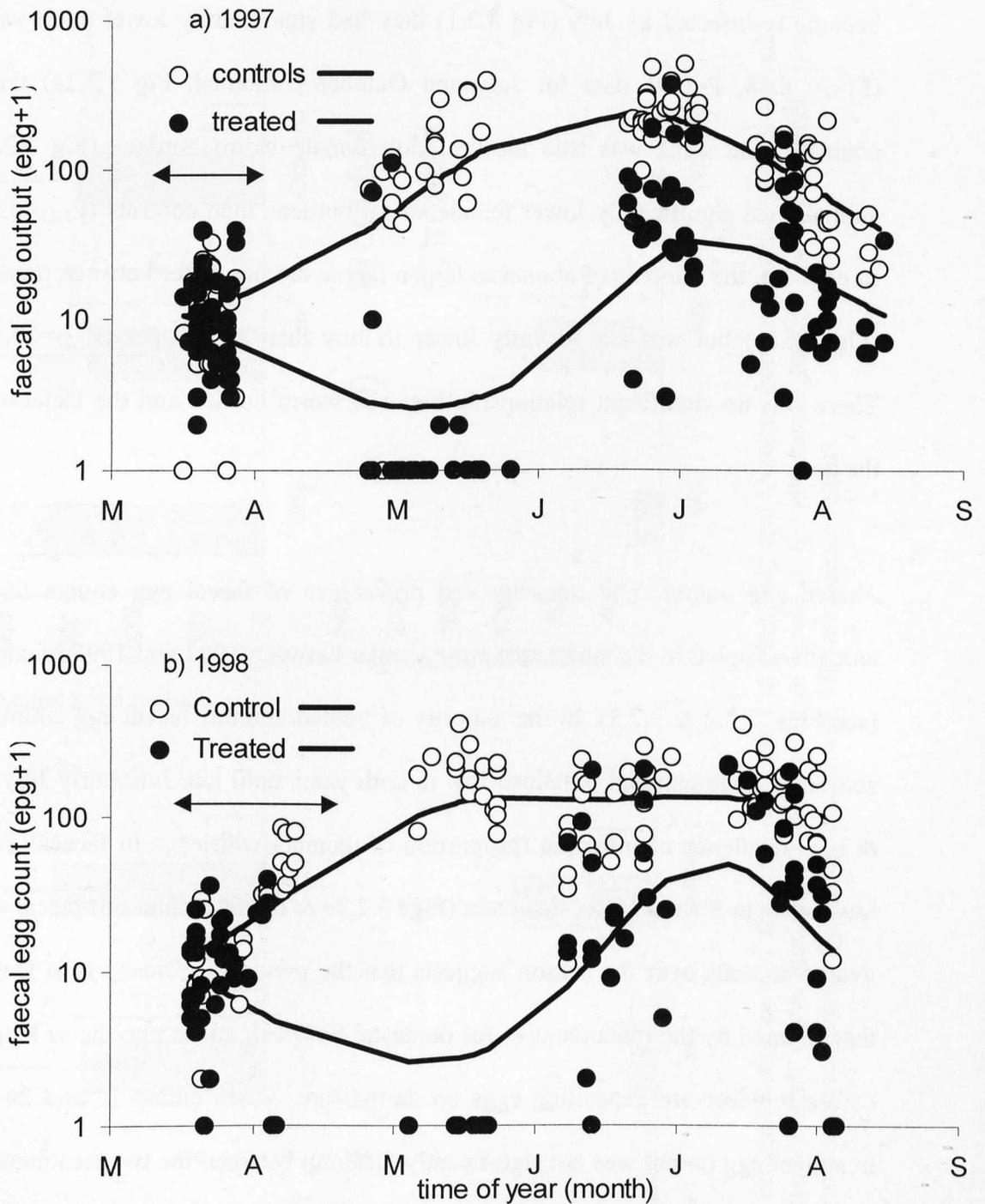


FIG 3.2.1: The abundance of different categories of abomasal nematodes in May, July and October from treated and control animals: a) total adult worm burden, b) total female adult worm burden, c) total number of larvae in the abomasum lumen. The fourth panel; d) shows the associated faecal egg output from the same animals. (geometric means with 95% confidence limits).

Abomasal worm burdens 12 and 24 weeks post-treatment. Even though treated animals had become re-infected by July (Fig 3.2.1) they had significantly lower adult worm burdens ($F_{1,27}= 8.38$, $P<0.01$ data for July and October combined, Fig 3.2.1a) than untreated controls. The same was true for the adult female worm burdens (Fig 3.2.1b); treated animals had significantly lower female worm burdens than controls ($F_{1,27}=6.35$, $P<0.02$). In contrast, the burdens of abomasal lumen larvae did not differ between treatment groups (Fig 3.2.1c) but was significantly lower in July than in October ($F_{1,27}=19.17$, $P<0.01$). There was no significant relationship between worm burden and the lactational status of the host.

Faecal egg output. The intensity and prevalence of faecal egg counts for all marked animals sampled in the study area were similar between 1997 and 1998 in control animals (see Figs. 3.2.2 & 3.2.3). In the majority of treated animals faecal egg counts dropped to zero after treatment and remained low in both years until late June/early July (Figs 3.2.2a & b). Prevalence of infection (proportion of animals with eggs in faeces) also remained low for up to 8 weeks after treatment (Figs 3.2.3a & b). The pattern of faecal egg counts in treated animals over the season suggests that the period of efficacy is at least as long as that claimed by the manufactures for domestic livestock and it may be as long as 8 weeks before reindeer are depositing eggs on the pasture. When culled 12 and 24 weeks post-treatment egg output was not significantly different between the two treatment groups (Fig 3.2.1d).

FIG 3.2.2: The intensity of strongyle faecal egg counts (eggs per gramme +1, on the Log_e



scale) in adult female reindeer between April and September a) 1997 and b) 1998. Solid circles indicate animals treated with moxidectin, open circles are controls. Time of capture and range of treatment administration dates is indicated by the double-ended arrow. Egg counts in this period are taken prior to anthelmintic administration. Solid lines fitted to guide the eye and are predicted from the spline function in Genstat.

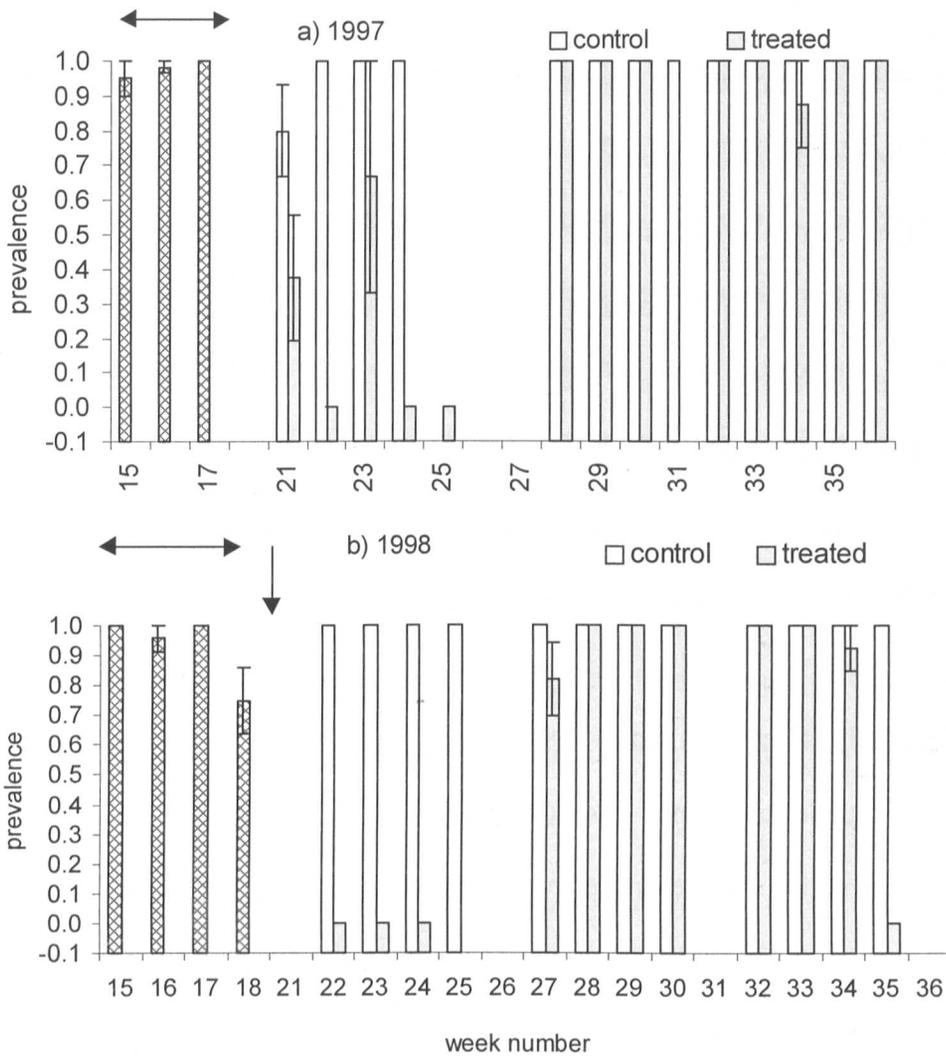


FIG 3.2.3: The prevalence of infection (proportion of adult female reindeer producing eggs in faeces) in treated animals (grey bars) and controls (open bars) for a) 1997 and b) 1998. Data are shown for weekly samples from April to September. Weeks after the vertical arrow indicate prevalence after treatment. Time of capture and range of treatment administration dates indicated by the double-ended horizontal arrow. Prevalence within the capture period (hatched bars) indicate the presence or absence of eggs in the faeces at time capture before the effects of the anthelmintic. Standard errors are not plotted for prevalence of zero and one.

Relationship between egg output and worm burden. In May 1998 when 10 reindeer were culled 12-14 days post-treatment, there was no relationship between egg output and worm burden in the five control animals ($F_{1,3}=0.47, P>0.49$) and the treated animals had zero eggs per gramme faeces.

In July/August 1999, 12 weeks post treatment, there was a significant positive relationship between faecal egg count and worm burden when both groups were combined ($r=0.554$; $F_{1,14}=6.71$, $P=0.021$). There was a tendency for a significantly positive relationship in

treated animals and for no significant relationship in controls (interaction: $F_{1,12}=3.41$, $P=0.090$). This relationship was unaffected when total adult burden was substituted by using only the adult female component of the population in the analysis. Within the adult worm population, the numbers of adult females were also related to egg output ($F_{1,14} 6.29$, $P=0.025$). In October there was no relationship between worm burden and faecal egg output because faecal egg output was close to zero (Fig 3.2.1d)

DISCUSSION.

Moxidectin is effective in removing abomasal nematodes from reindeer and the use of this or other anthelmintics may provide a useful tool in the study of parasitism in wild populations rather than resorting to the opportunistic analysis of carcasses. Other studies on this drug and the related drug ivermectin have shown them to be effective and safe in semi-domestic reindeer (Dietrich & Craigmill, 1990, Oksanen and others, 1992) and in red deer (Mackintosh and others, 1993). The period of efficacy for moxidectin appears to vary between species and may be longer in reindeer than cattle and sheep (Dietrich & Craigmill, 1990). Also, the efficacy of moxidectin may be more persistent than for ivermectin (Deroover and others, 1997, Taylor, 1993, Barth and others, 1997). Although the efficacy injectable moxidectin is much shorter than a long acting bolus (e.g. Ivomec Maximizer, MSD Agvet), administration of the drug is much more certain, particularly when animals have to be released into the wild immediately.

Although animals were re-infected in July and October, the worm burdens in the treated group were still significantly lower than in the controls. This longer-term impact of using anthelmintics such as moxidectin needs further investigation for two reasons. First, this

may have implications for the impact of reinfection and the associated pathogenesis and cost of parasitism on the host. Second, experiments such as this can give valuable data on re-infection, survival and development of abomasal parasitism in wild animals. The observation that treated animals had become re-infected 12 weeks post-treatment is not surprising. However, the range in the observed burdens needs some explanation. It is possible that this illustrates individual differences in susceptibility to infection or local variation in infection risk because of heterogeneity in pasture contamination. However, it may also reflect differences in the length of the effective period of the drug between individuals. The drug is known to be lipophilic and reindeer carry large amounts of back fat. For instance, an animal that is pregnant and/or has a calf may have a more dynamic fat reserve due to lactation than a yield animal and this may lead to faster metabolism of the drug and a shorter effective period. However, the activity of the drug may be reduced in animals with high back-fat reserves because the release rate may be sub-lethal to the nematodes. Whether body condition interferes with the pharmacokinetics and alters the effective dosing rate is unknown but may have implications for the route of drug administration in animals such as Svalbard reindeer that accumulate large amounts of sub-cutaneous back fat.

The absence of other nematodes in the small and large intestine in these reindeer (Halvorsen & Bye, 1986) makes this system an ideal candidate to study the relationship between abomasal worm burdens and faecal strongyle egg output. The egg count/worm burden relationship in treated adult animals in July was significant and positive. Egg output increased in a linear fashion with worm burden until worm burden reaches a point where egg output per female worm is reduced so that although worm burden increases, faecal egg output remains stable. This pattern has been described before and is briefly reviewed in Coyne and Smith (1991). The experiment allowed re-infection over the summer and

generated a wide variation in worm burdens with some low burdens that also had low faecal egg counts. Therefore, the effect of treatment could be viewed as artificially creating the situation found in young animals in that treated animals had low worm burdens and egg counts. This suggests that egg counts in the summer may have potential for predicting the degree of infection in young Svalbard reindeer up to 3 years of age because in this host species, unlike sheep and cattle, worm burdens carry on increasing up to this age. However, in older animals, there is no evidence of immunity; worm burdens remain high and egg counts are highly variable (Halvorsen and others, 1999). Being able to monitor worm burdens via egg output in young animals may be helpful in assessing the role of parasites in determining the age at first reproduction and hence have implications for the population dynamics of the reindeer. The lack of relationship in winter is to be expected and highlights the importance of timing in determining when to sample and monitor gastro-intestinal parasitism in wild and domestic ruminants.

Environmental considerations may also be important in deciding which drug to use against nematodes in wild host populations. Oksanen & Nieminen (1998) found that moxidectin and ivermectin were equally effective against nematodes in an experimental reindeer herd but ivermectin had higher insecticidal efficacy. However, it is claimed that moxidectin residues in the dung may be less harmful to dung dwelling insects than ivermectin. Timing of anthelmintic may also have implications for the insect fauna. Treatment in winter results in high ivermectin residues in the concentrated, pelleted faeces but at this time of year faeces from untreated animals tends not to be populated with these detritivores (Nilssen and others, 1999). Furthermore, faecal output may be lower at this time of year than in summer so that a relatively small proportion of the annual faecal production will be contaminated with ivermectin residues (Nilssen and others, 1999). The persistency and simplicity of an

anthelmintic treatment in wild animals and the impact on the wider ecosystem are important considerations for management decisions and requires further investigation.

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CHAPTER 3.3

RESEARCH NOTE: TRADE-OFFS BETWEEN RECOVERY AND POLYMERASE CHAIN REACTION (PCR) SUCCESS IN ARRESTED LARVAE OF GASTROINTESTINAL NEMATODES OF NATURALLY INFECTED SHEEP AND REINDEER.

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RESEARCH NOTE

OPTIMISING THE RECOVERY AND SUCCESS OF POLYMERASE CHAIN REACTION (PCR) ANALYSIS OF ARRESTED ABOMASAL NEMATODE LARVAE OF NATURALLY INFECTED SHEEP AND REINDEER..

R.J. Irvine, J.F. Dallas

ABSTRACT

The relationship between recovery and success rates of polymerase chain reaction (PCR) analysis was studied in arrested L4 larvae of gastrointestinal nematodes extracted from abomasa of four naturally infected lambs and two reindeer. Recovery and PCR success were unaffected by whether material was fresh or freeze-thawed. Recovery increased and PCR success remained constant (60-80%) with duration of neutral N-acetyl cysteine digestion. Recovery increased and PCR success declined to zero with duration of acid-pepsin digestion. Studies requiring both optimised PCR analysis and data on intensity of infection of mucosa larvae should combine 2-4 hour and 16 hour digests.

A common feature of the life cycles of gastrointestinal nematodes of ruminants is the presence of arrested larval stages within the mucosa of the intestinal tract (Eysker, 1997). Theoretical (Smith & Grenfell, 1985) and empirical (Coyne & Smith 1994) studies involving communities of gastrointestinal nematodes suggest that species differences in the propensity to undergo arrest can influence species-specific pathogenic effects and transmission rates. Few studies, however, have succeeded in detailed parameterisation or testing of theoretical models in wild hosts. This is largely because estimating species composition in arrested larvae is presently hindered by the difficulty of species identification using morphological traits in adult worms (Drodz, 1965; Suarez & Cabaret, 1991). Live larvae extracted into saline solution from the mucosa can be allowed to develop into identifiable adult stages [Jackson, F. pers. comm.]. Such development is, however, time-consuming and inapplicable to field-based studies in which the only feasible method for storage of mucosa is freezing.

Species-diagnostic tests using the polymerase chain reaction (PCR), which provide an alternative means of species identification, have been developed for a wide range of gastrointestinal nematodes (Roos & Grant, 1993; Schneider et al., 1999; Gasser et al., 1999; Hung et al., 1999, Dallas et al., 2000a). The majority of such tests involve detection of the second internal transcribed spacer (ITS2) of nuclear ribosomal genes. The success of such tests in arrested larvae obtained by digestion of frozen mucosa has, however, yet to be demonstrated. The most commonly used digestion fluid contains 0.25 M HCl, which cleaves DNA by depurination (Wahl et al., 1979). Extended digestion of mucosa in this fluid could therefore lead to degradation of larval genomic DNA, especially when freeze-thawing has killed the larvae. In addition, few studies have attempted to estimate the

influence of extraction methods on numbers of larvae recovered (but see Hung et al., 1999), which are essential data for obtaining estimates of infection intensity.

The present study investigates the effects of (a) fresh versus freeze-thawed mucosa, (b) digestion in acidic versus neutral fluid, and (c) duration of digestion period on numbers of larval nematodes recovered and success rates of PCR detection of the ITS2 of individual nematodes. Experimental material consisted of abomasa from naturally-infected sheep *Ovis aries* and Svalbard reindeer *Rangifer tarandus*.

The first experiment involved nematode larvae from abomasa of four freshly-killed sheep reared in an agricultural environment in northeast Scotland. Sheep were chosen because their natural infections of abomasal nematodes are well described (Soulsby, 1982). The pyloric half of the mucosa was cut into 3cm squares and each piece was randomly allocated to one of the following treatments. Material was either digested fresh or following one or two freeze-thaw cycles. Digest fluid was either using acid-pepsin (MAFF/ADAS, 1986) or neutral 60mM N-acetyl cystiene (Darwin-Murrell *et al.*, 1997). Digest periods were 1hr, 4hr or 16 hr. Not all possible combinations of treatments were carried out. Recovery of larvae was estimated by washing the resulting digest fluid through a 38 μ mesh sieve, then diluting the material collected to one litre and counting the larvae from 10% aliquots in perspex counting chambers until at least 100 had been extracted for PCR purposes. Recovered larvae were stored in 70% ethanol.

Total cellular DNA was isolated from individual larvae by incubation in 10 mM Tris.HCl, 0.1 mM EDTA, pH 8.0, containing 20 μ g/ml proteinase K. Incubation was carried out in microtitre plate wells containing 20 μ l of this fluid overlaid with 30 μ l mineral oil (Sigma)

for 6 h at 55°C, followed by heating at 95°C for 10 min. DNA samples were stored at 4°C. The ITS2 was detected by PCR using the conserved primers NC1 and NC2, which detect ITS2 in all species of nematodes known to infect sheep in agricultural environments (Gasser *et al.*, 1993). PCR conditions were as described (Dallas *et al.*, 2000a), except that reaction volume was 10 µl, containing 5 µl of undiluted larval DNA, and the PCR program was continued for 40 cycles. PCR products were visualised by electrophoresis for 2 hours at 5 V/cm in 2% (w/v) agarose, 0.5 x Tris-borate EDTA gels containing 0.25 µg/ml ethidium bromide. The presence of a single fragment of the expected size (ca. 320 bp) indicated successful reaction of ITS2.

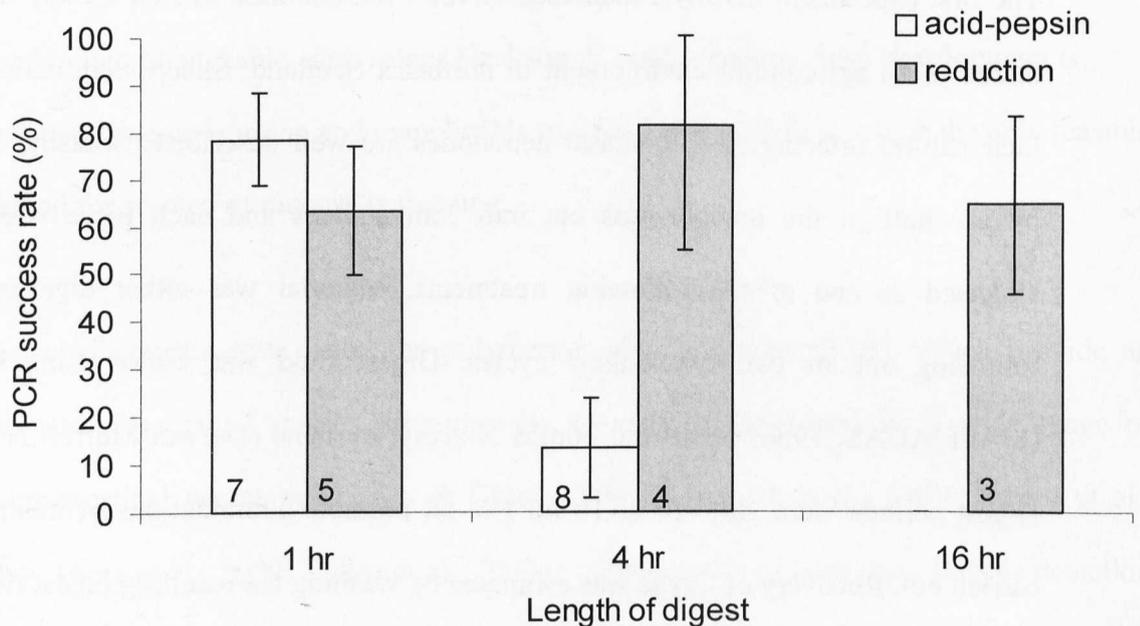


Fig 3.3.1. Success rates of PCR detection of the 320 bp ITS2 fragment in larvae extracted from lamb abomasa digested with either acid-pepsin or N-acetyl cysteine. Mucosa was digested for 1 hour, 4 hours or 16 hours. The numbers at the foot of the columns indicate sample sizes.

Using logistic regression or generalised linear models, neither PCR success rates nor numbers of larvae recovered differed significantly between fresh and freeze-thaw treatments ($p > 0.2$). Data were therefore pooled across pre-treatments. Further analysis detected a significant interaction between type of digestion fluid and duration of digest on

PCR success rate ($F_{4,24}=5.84$, $p<0.01$). PCR success rates for the acid-pepsin fluid declined sharply from around 80% after one hour to zero after 16 hours digestion (Fig. 3.3.1). In contrast, PCR success rates for the reduction fluid were 65-85% independent of digest period. Numbers of larvae recovered were similar between the two fluids ($F_{1,40}=0.04$, $p>0.5$), and over the four digest periods ($F_{2,39}=0.39$, $p>0.5$). The latter result is probably due to the small mucosa samples used being rapidly digested.

We also investigated the relationship between recovery and PCR success rate for acid-pepsin digestion of abomasa of Svalbard reindeer. This choice was motivated by the need to ensure continuity with protocols previously used to estimate intensities of arrested larvae (MAFF/ADAS, 1986). Briefly, two male reindeer were culled on Svalbard in October 1998 and two females in October 1999. Abomasa were ligated, removed by dissection, transported frozen to Britain and stored at -20°C . In May 2000, abomasa were thawed to allow complete removal of adult nematodes destined for other studies. Empty abomasa were refrozen, re-thawed two days later and digested in acid-pepsin for 16 hours. Larvae were removed from digests after 1, 2, 4, and 16 hours. For each period, the digest fluid was poured through a $150\mu\text{m}$ sieve placed over a $38\mu\text{m}$ sieve. After each extraction, the material retained on the lower sieve was diluted to 1 litre and at least 100 larvae were collected. The remaining abomasal material retained on the upper sieve was returned to the incubator with fresh fluid to continue digestion. The mucosa larvae from the four digest periods were identified as either *Marshallagia marshalli* or *Ostertagia gruehneri* by PCR as described (Dallas *et al.*, 2000). However, larvae from the 16 hour digest of the October 1998 animals were not analysed by PCR owing to the low or zero PCR success rates observed in 4 and 16 hour acid-pepsin digests of larvae from lamb mucosa (this study) and

the zero PCR success rates observed in earlier analyses of larvae from 16 hr acid-pepsin digests of five reindeer abomasa (Dallas, unpublished observations).

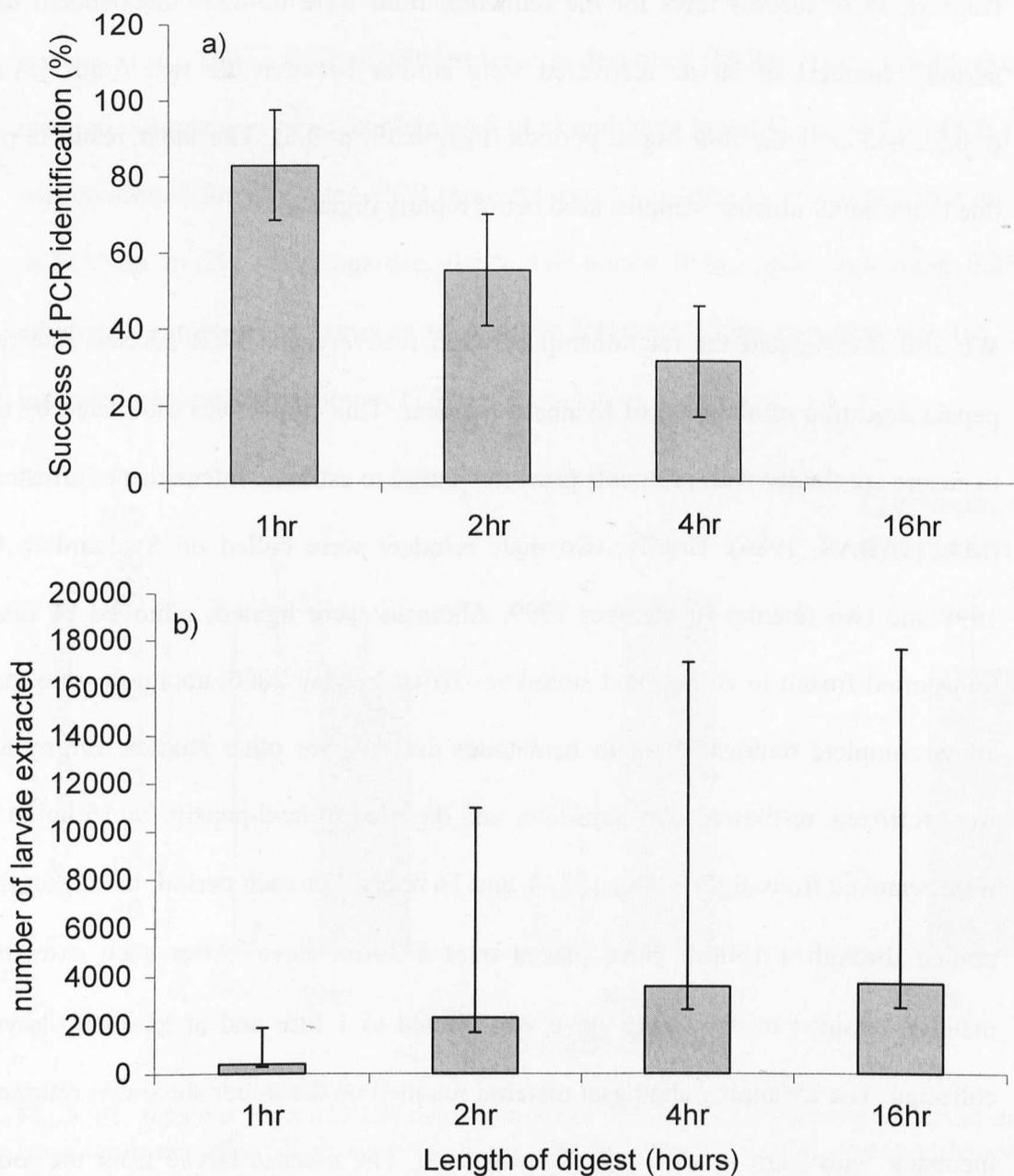


Fig 3.3.2

- a) Mean success rates (and standard error bars) for PCR identification of larvae extracted from four adult reindeer abomasa digested using acid-pepsin. The material was digested for 1 hour, 2 hours, 4 hours and 16 hours (analysed using Logistic regression).
- b) The mean cumulative number of larvae recovered (and confidence limits) of four adult reindeer abomasa digested using acid-pepsin. The digest periods were 1 hour, 2 hours, 4 hours and 16 hours (analysed using generalised linear models).

A trade-off between recovery and PCR success rate was evident in this experiment. PCR success rates were initially similar to those of the one hour digest of the lamb abomasa and

declined to zero with increasing digest period. (Fig 3.3.2.a&b). Significantly higher PCR success was achieved from one hour digests over 4 hour ($t=2.51$, $p=0.03$) and 16 hour ($t=3.28$, $p=0.008$). However, the success of PCR after one hour, although greater, was not significantly different from 2 hour digests ($p>0.2$, logistic regression). Numbers of larvae recovered increased from one to two hours digest although the trend was not significant ($\chi^2=3.4$, $df=1$, $p=0.065$). However, significantly more larvae were recovered after 4 hours ($\chi^2=5.29$, $df=1$, $p=0.02$). Although there were more larvae recovered after 16 hours this was not significantly greater than the number recovered after 4 hours ($p>0.4$, Fig. 3.3.2.b). Therefore, one ideal combination would be to digest for two hours for in order to maximise PCR success rate and continue the digest for longer, (up to 16hours) to maximise numerical recovery of larvae.

The present study found evidence for a trade-off between recovery and PCR success rate in larvae extracted using acid-pepsin digestion from fresh and freeze-thawed abomasum from sheep and reindeer. Although the larvae from the sheep were not identified, their external appearance is accepted in the literature as indicating the presence of nematode larvae and data obtained by counting such larvae are commonly used to estimate infection rates (Coyne & Smith, 1994). We have merely added a PCR stage to this type of analysis. The increase in recovery with digestion period might be influenced by the size of the pieces into which the mucosa were cut up prior to digestion. The decline in PCR success rate with digestion period might be even steeper than found here for target DNA sequences of lower copy number than the nuclear ribosomal genes (200-500 copies in most eukaryotes). In conclusion, for studies which require both PCR analysis of arrested larvae and data on their intensity of infection, we suggest that the sample is subjected to a maximum of two hour acid-pepsin digest in the first instance. Resulting larvae will be suitable for PCR.

Subsequently, the remaining material can be digested for up to 16 hours to maximise recovery. The reduction method also appears suitable as an alternative but comparing results from samples subject to different digest methods may be flawed because of the interaction between length of digest and digest method in yielding larvae.

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CHAPTER 3.4

Research Note: Identification by polymerase chain reaction (PCR) of *Marshallagia marshalli* and *Ostertagia gruehneri* from Svalbard reindeer

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RESEARCH NOTE: IDENTIFICATION BY PCR OF *Marshallagia marshalli* AND *Ostertagia gruehneri* FROM SVALBARD REINDEER*

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ABSTRACT

A PCR assay to identify two common abomasal nematodes *Marshallagia marshalli* and *Ostertagia gruehneri* of Svalbard reindeer was developed. Species-specific PCR primers were designed from ITS-2 sequences of rDNA and validated using morphologically identified adult male and female nematodes. Using the species-specific primers, a 110 bp fragment was amplified from *M. marshalli* and its minor morph *M. occidentalis* and a 149 bp fragment was amplified from *O. gruehneri* and its minor morph *O. arctica*. No PCR products were amplified from the third rare species, *Teladorsagia circumcincta*, or DNA from the reindeer host. The assay provides a useful tool to estimate species composition for both sexes in this nematode community.

** Note: Nucleotide sequence data reported in this paper are available in the EMBL and Genbank™ databases (accession numbers AJ250655, AJ250656, AJ400715 and AJ400716).

The abomasal nematode community of Svalbard reindeer, *Rangifer tarandus platyrhynchus*, consists of three polymorphic species of the order *Strongylida*. Over 99% of the community consists of *Marshallagia marshalli* (major morph)/*M. occidentalis* (minor morph) and *Ostertagia gruehneri* (major morph)/*O. arctica* (minor morph), and less than 1% consists of *Teladorsagia circumcincta* (major morph)/*T. trifurcata* (minor morph)/*T. davtiani* (minor morph) (Halvorsen & Bye, 1999). The minor morphs exist only in males. A recent study suggested that the first two species have different life histories, as shown by the annual and seasonal abundance of adult males and seasonal egg output (Irvine *et al.*, 2000a). Such contrasts, however, assume that the species compositions of the adult male and female fractions of the community are equal. If this assumption is unwarranted, the life histories of *M. marshalli* and *O. gruehneri* may be more similar than previously recognised. Unfortunately, species composition of adult females cannot easily be estimated because they cannot readily be identified to species using morphological traits (Lichtenfels *et al.*, 1997). To overcome this obstacle, we have developed a PCR assay to identify *M. marshalli*/*M. occidentalis* and *O. gruehneri*/*O. arctica* from Svalbard reindeer. The primers were designed from sequences of the second internal transcribed spacer (ITS-2) of rDNA because this region shows high levels of intraspecific homogeneity and interspecific divergence in sequence for several species of strongylid nematodes (Hoste *et al.*, 1995; Heise *et al.*, 1999). The primers were validated for detection of species-specific DNA fragments using morphologically identified nematodes.

Nematodes were obtained during 1997 from wild reindeer from three sites in Nordenskiöldland, Svalbard: Colesdalen (78°05'N, 15°20'E), Reindalen (77°57'N, 15°40'E) and Sassendalen (78°15'N, 17°30'E). Abomasa were removed from culled female

reindeer and stored frozen at -20°C for 3-25 weeks. Abomasa were thawed and nematodes collected immediately by washing the abomasal lumen with water. Adult male nematodes were identified as *M. marshalli*, *M. occidentalis*, *O. gruehneri*, *O. arctica* or *T. circumcincta* according to the key of Drózd (Drózd, 1965). Adult female nematodes were identified as *M. marshalli* or *O. gruehneri* according to the key of Lichtenfels and Hoberg (Lichtenfels & Hoberg, 1993). Nematodes were stored in 70% ethanol at 4°C. Total cellular DNA was isolated from individual nematodes by incubation in 100 µl of 10 mM Tris.HCl, 0.1 mM EDTA, pH 8.0, containing 20 µg/ml proteinase K for 18 h at 55°C followed by heating at 95°C for 10 min. DNA samples were stored at -20°C.

ITS-2 sequences were determined for 16 adult males each of *M. marshalli* and *O. gruehneri* from six reindeer from Colesdalen, six from Sassendalen, and one from Reindalen. The ITS-2 of individual nematodes was amplified by PCR using the primers NC1 and NC2 (Gasser *et al.*, 1993). PCR volume was 50 µl, containing 1 x NH₄ buffer, 0.5 u *Taq* DNA polymerase (BioLine), 2.5 mM MgCl₂, 200 µM dATP, dCTP, dGTP, dTTP, 0.5 µM of each primer, and 5 µl of DNA solution. The latter consisted either of 100x diluted stock DNA when PCR yields were high or of undiluted stock DNA when PCR yields were low. The PCR program was 92°C / 2 min. 15 s, [90°C / 15 s, 55°C / 15 s] x 35 cycles, 72°C / 1 min. Single fragments of the expected size (ca. 320 bp) were purified using Qiaquick spin columns (Qiagen), and both strands sequenced on an ABI 377 automated DNA sequencer using NC1 and NC2. The sequences obtained were aligned using Clustal X (Thompson *et al.*, 1997). All differences between sequences and all variable bases were checked against the raw data from the sequencer.

Two sequences containing the complete ITS-2 and part of the 28S gene were detected in *M. marshalli* (Fig. 3.4.1). These differed at one position (28), at which five individuals

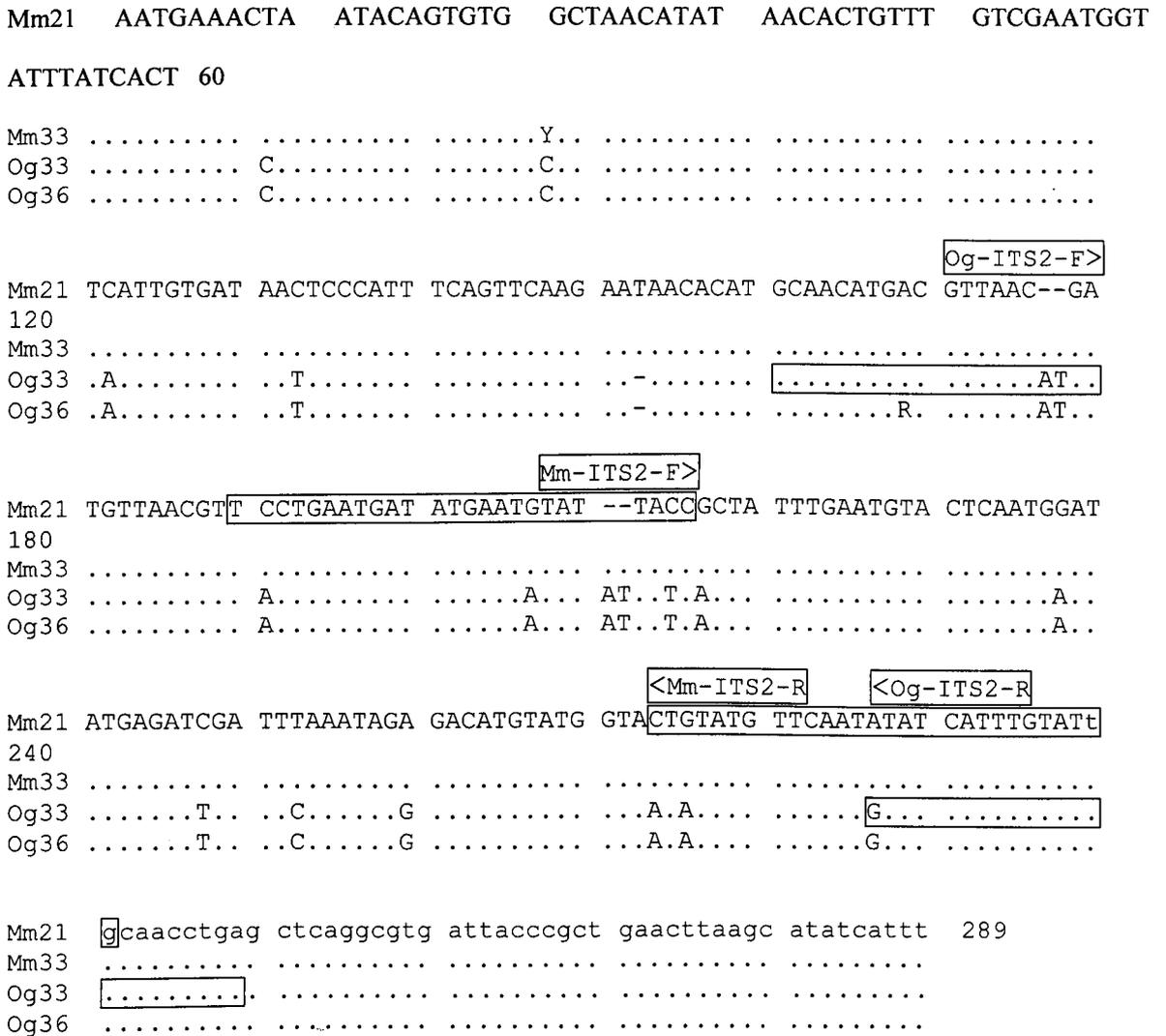


Fig. 3.4.1. Alignment of four sequences containing the complete ITS-2 and part of the 28S rRNA gene detected in 16 adult males each of *Marshallagia marshalli* (Mm) and *Ostertagia gruehneri* (Og) from 13 Svalbard reindeer. The numbered sequences refer to individual reindeer. Bases identical to those of Mm21 are indicated by a dot. Positions 28 and 109, at which polymorphic bases were detected, are indicated by IUPAC codes. The four oligonucleotide primers designed for species identification by PCR are shown as boxed sequences. Positions 1 and 239 correspond to the 5' and 3' ends, respectively, of ITS-2, and positions 240 to 289 (lower case) correspond to the end of the 26S gene of *Caenorhabditis elegans* (GenBank accession number X03680).

possessed T and 11 individuals possessed C/T. Two sequences were detected in *O. gruehneri*. These differed at one position (109) at which five individuals possessed A and

11 individuals possessed A/G. The numbering of the positions differed slightly from that given in a parallel study (Dallas *et al.*, 2000b) owing to the gaps in the present alignment. Both of the variable bases detected within species occurred in all three study sites on Svalbard. The differences between the ITS-2 sequences of *M. marshalli* and *O. gruehneri* consisted of 14 substitutions (3% divergence), three indels, and the two variable bases described. These ITS-2 sequences thus showed intra- and interspecific differences similar to those of several other strongylid nematodes (Hoste *et al.*, 1995; Heise *et al.*, 1999).

Species-specific PCR primers were designed to detect one ITS-2 fragment in *M. marshalli* (110 bp) and another in *O. gruehneri* (149 bp, Fig.3.4.1) using OLIGO 4.0 (National Biosciences, Inc.). The primers were: Mm-ITS2-F 5' TCCTGAATGATATGAATGTATTACC 3', Mm-ITS2-R 5' CAATACAAATGATATATTGAACATACAG 3', Og-ITS2-F 5' GCAACATGACGTTAACATGA 3' and Og-ITS2-R 5' TCAGGTTGCAATACAAATGATAC 3'. The ability of these primers to detect the expected fragments was tested using morphologically identified adult males of all the taxa present in the abomasal community of Svalbard reindeer, except the two minor morphs of *T. circumcineta*, *T. trifurcata* and *T. davtiani*, and adult females of *M. marshalli* and *O. gruehneri*. PCR conditions were as above, except that the annealing temperature was 50°C, reactions contained four primers (Mm-ITS2-F/R at 1.0 µM and Og-ITS2-F/R at 0.5 µM), and 2 µl of DNA was used in a total reaction volume of 10 µl. PCR products were separated by electrophoresis for 1.5 hours at 200 volts in 2% (w/v) agarose, 0.5 x Tris-borate EDTA gels containing 0.25 µg/ml ethidium bromide. A single fragment of ~ 110 bp was amplified from 32 males and 16 females of *M. marshalli*, and from 8 males of its minor morph *M. occidentalis* from Svalbard. A single fragment of ~ 149 bp was amplified from 32 males and 16 females of *O. gruehneri*, and from 16 males of its minor morph *O. arctica* from Svalbard and Norway. No fragments

were amplified from 16 males of *T. circumcincta* or from Svalbard reindeer DNA. Examples of the two species-specific fragments are shown in Fig. 3.4.2. In a parallel study containing details of the Norwegian study sites, we showed that *O. gruehneri* and its minor morph *O. arctica* have virtually identical ITS-1 and ITS-2 sequences (Dallas *et al.*, 2000b). The species-specificity of both fragments was verified by direct DNA sequencing using Mm-ITS2-F/R and Og-ITS2-F/R as sequencing primers. The 57 bp sequences determined for four adult male *M. marshalli* and the 106 bp sequences determined for four adult male *O. gruehneri* from Colesdalen and Sassendalen were identical to those shown between the primers Mm -ITS2-F/R and Og-ITS2-F/R, respectively (Fig. 3.4.1).

The PCR assay described here correctly identified adult males and females of *M. marshalli*, and adult males of its minor morph, *M. occidentalis*, from Svalbard. The assay also correctly identified adult males and females of *O. gruehneri*, and adult males of its minor morph, *O. arctica*, from Svalbard and Norway. No products were amplified from *T. circumcincta* and the reindeer host. The presence of *T. circumcincta* can be distinguished from a negative PCR result by using primers NC1 and NC2 (see Stevenson *et al.*, 1996) (J. Dallas, unpublished data). This assay therefore provides a means of positively identifying the two species comprising over 99% of the abomasal nematode community of Svalbard reindeer, and is almost certainly applicable to all life cycle stages (Gasser *et al.*, 1993)

M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32

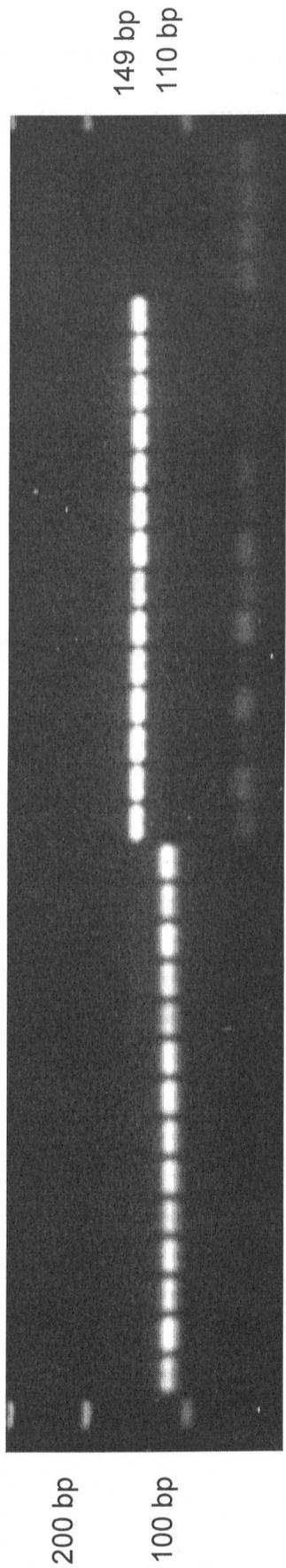


Fig. 3.4.2. PCR products amplified from *Marshallagia marshalli* and *Ostertagia gruehneri* using the primers Mm-ITS2-F/R, Og-ITS2-F/R. Lanes: M, 100 bp size standard; 1-7, adult male *M. marshalli* from Colesdalen; 8-14, adult male *M. marshalli* from Sassendalen; 15-21, adult male *O. gruehneri* from Colesdalen; 22-28, adult male *O. gruehneri* from Sassendalen; 29-32, DNA from Svalbard reindeer (host controls).

This study expands the range of PCR assays available to identify gastrointestinal nematodes of animals (Roos & Grant, 1993; Schneider *et al.*, 1999; Gasser *et al.*, 1999; Hung *et al.*, 1999). The ITS-2 sequences on which the PCR primers are based were virtually identical for *M. marshalli* from Svalbard (this study) and for *O. gruehneri* and *O. arctica* from Norway and Svalbard (Dallas *et al.*, 2000b) implying that this assay will be useful in both geographical locations. Nevertheless, both *M. marshalli* and *O. gruehneri* have much wider distributions. *M. marshalli* is widespread, having 142 occurrences recorded for 13 groups of wild and domesticated ungulates. *O. gruehneri* is more restricted, having 21 occurrences recorded for six groups of wild ungulates (Suarez & Cabaret, 1991). The analysis of isolates representing broader geographical scales and host ranges of both *M. marshalli* and *O. gruehneri* will be required to assess the global applicability of the present PCR assay.

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CHAPTER 3.5

RESEARCH NOTE: DNA EVIDENCE THAT *Ostertagia gruehneri* AND *Ostertagia arctica* (Nematoda: Ostertagiinae) IN REINDEER FROM NORWAY AND SVALBARD ARE CONSPECIFIC*

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RESEARCH NOTE: DNA EVIDENCE THAT *Ostertagia gruehneri* AND *Ostertagia arctica* (Nematoda: Ostertagiinae) IN REINDEER FROM NORWAY AND SVALBARD ARE CONSPECIFIC*

J.F. Dallas, R.J. Irvine, O. Halvorsen

ABSTRACT

DNA sequences of ITS-1 and ITS-2 of rDNA were determined for 16 individual adult males each of *Ostertagia gruehneri* and *Ostertagia arctica* from Svalbard reindeer (*Rangifer tarandus platyrhynchus*) and Eurasian tundra reindeer (*R. t. tarandus*). Each ITS was virtually identical in *O. gruehneri* and *O. arctica* and the three mixed bases detected were shared by both species. Our results strongly suggest that *O. gruehneri* and *O. arctica* are dimorphic males of the same species.

* Note; Nucleotide sequence data reported in this article are available in the EMBL, GenBankTM and DDJB data bases under the accession numbers AJ250656 - AJ250659.

Ostertagia gruehneri Skrjabin, 1929 and *Ostertagia arctica* Mitskevich, 1929 (Nematoda: Ostertagiinae) are intestinal parasites of reindeer and caribou, *Rangifer tarandus*, and their distribution extends throughout the range of the host species (Lichtenfels *et al.*, 1990). These nematodes are suspected to represent polymorphic males of the same species on the basis of their co-occurrence and synopse (cuticular ridges) structure (Lichtenfels *et al.*, 1990; Drózd, 1995; Suarez & Cabaret, 1991). However, there have been no studies of crosses or genetic similarity between *O. gruehneri* and *O. arctica* to our knowledge. As a test of the polymorphism hypothesis (Daskalov, 1974) and to clarify their taxonomic status (Lichtenfels *et al.*, 1997) we compared DNA sequences obtained by PCR between isolates of *O. gruehneri* and *O. arctica* from four locations: two on the Svalbard archipelago and two in Norway. We also used these data to compare the relative levels of DNA sequence divergence in ITS-1 and ITS-2 and to assess the monophyly of *Ostertagia*. The host on Svalbard is the endemic Svalbard reindeer (*Rangifer tarandus platyrhynchus*) and the host in Norway is the Eurasian tundra reindeer (*R. t. tarandus*). The study animals from Svalbard and south Norway were wild reindeer and those from north Norway were from semi-domesticated herds. We have recently described several aspects of the biology and ecology of the abomasal nematode community of reindeer on Svalbard (Halvorsen *et al.*, 1999; Halvorsen & Bye, 1999; Irvine *et al.*, 2000a).

Abomasa were removed from culled reindeer from four locations, Reindalen (77°57'N, 15°40'E) and Sassendalen (78°15'N, 17°30'E) on Svalbard, Hardangarvidda in southern Norway (60°15'N, 7°30'E), and Målselv in northern Norway (69°45'N, 19°30'E). The Målselv samples were collected during 1986, and the Hardangarvidda and Svalbard samples were collected during 1997. *O. gruehneri* comprised up to 94 per cent of the abomasal nematodes in adult Svalbard reindeer, 99 per cent in adult reindeer at

Hardangarvidda, and was the dominant species in reindeer in Målselv. *O. arctica* comprised from one to five per cent at Svalbard and less than one per cent at Hardangarvidda (Halvorsen & Bye, 1999; Bye *et al.*, 1987; Bye, 1987). Abomasa were stored frozen at -20°C before processing. Abomasa were thawed and nematodes were collected immediately by washing the abomasal lumen with water. Adult male nematodes were classified as *O. gruehneri* or *O. arctica* according to the key of Drózd (Drózd, 1965) and stored in 70% ethanol. One pair of individual adult male *O. gruehneri* and *O. arctica* was chosen from 16 reindeer: one from Reindalen, six from Sassendalen, five from Hardangarvidda and four from Målselv. Total cellular DNA was isolated from individual worms by incubation in 100 µl of 10 mM Tris.HCl, 0.1 mM EDTA, pH 8.0, containing 20 µg/ml proteinase K, for 18 h at 55°C in microtitre plate wells overlaid with paraffin oil. After incubation, the digests were heated at 95°C for 10 min then stored at -20°C.

The ITS-1 of individual nematodes was amplified by PCR using the primers NC5 (5' GTAGGTGAACCTGCGGAAGGATCATT 3') and NC1R (5' AACAACCCTGAACCAGACGT 3'), and the ITS-2 was amplified using NC1 (5' ACGTCTGGTTCAGGGTTGTT 3') and NC2 (5' TTAGTTTCTTTTCCTCCGCT 3'). These primers had been designed from conserved sequences of the 5.8S 18S and 26S genes of *Caenorhabditis elegans* (Gasser *et al.*, 1993, Gasser & Hoste, 1995). The PCR reaction volume was 50 µl, containing 1x NH₄ buffer, 0.5 units *Taq* DNA polymerase (BioLine), 2.5 mM MgCl₂, 200 µM dATP, dCTP, dGTP, dTTP, 0.5 µM of each primer, and 10 µl of DNA solution. The latter consisted either of 100x diluted stock DNA for samples giving high PCR yields or of undiluted stock DNA for samples giving low PCR yields. The PCR program used was: 90°C / 2 min 15 s, [90°C / 15 s, 55°C / 15 s] x 35 or 40 cycles, 72°C / 1 min. The PCR products detected were single fragments of the expected lengths (ca. 570 bp for ITS-1 and ca. 320 bp for ITS-2). PCR

products were purified using Qiaquick spin columns (Qiagen), then sequenced on an ABI 377 automated DNA sequencer using the PCR primers. Both strands of each fragment were sequenced. Sequences were aligned using Clustal X (Thompson *et al.*, 1997). All differences between sequences and all mixed bases detected by alignment were checked against the ABI electropherograms. Published ITS-1 sequences of two pairs of polymorphic species *O. ostertagi* (AF044933) / *O. lyrata* (AF044930), and *O. mossi* (AF044932) / *O. dikmansi* (AF044928), and of four other trichostrongylid species, *O. leptospicularis* (AF044931), *Teladorsagia circumcincta* (AF044934), *Haemonchus contortus* (AF044927) and *H. placei* (AF044929), and published ITS-2 sequences of *O. leptospicularis* (X86025) and *O. ostertagi* (X86027), were obtained from the Genbank™ database. Levels of divergence in ITS-1 and ITS-2 among three species, *O. gruehneri*, *O. leptospicularis* and *O. ostertagi* were quantified as the number of observed pairwise differences expressed as a percentage of the total aligned length of ITS-1 (404 bp) and ITS-2 (241 bp). Numbers of pairwise base substitutions were obtained using MEGA [S. Kumar, K. Tamura and M. Nei 1993, The Pennsylvania State University, University Park, PA 16802], and numbers of pairwise indels were counted by eye. A bootstrapped neighbour-joining tree of ITS-1 sequences was obtained using Clustal X. Alignment gaps were not counted, no correction was made for multiple mutations at the same position and 1000 bootstrap replicates were performed.

In *O. gruehneri* and *O. arctica*, all 32 ITS-1 sequences were 400 bp and all 32 ITS-2 sequences were 238 bp in length. The four ITS-1 variants detected were virtually identical, differing only by the presence of C/T mixed bases at two positions, 46 and 89. The two ITS-2 variants detected were also virtually identical, differing only by the presence of A/G mixed bases at position 108. Thus, *O. gruehneri* and *O. arctica* from four geographically

diverse locations possessed virtually identical ITS-1 and ITS-2 sequences. Furthermore, all the mixed bases detected were shared between *O. gruehneri* and *O. arctica*. The C/T at position 46 of ITS-1 was shared by both species at Hardangarvidda and Sassendalen, and the A/G at position 108 of ITS-2 was shared by both species in all four sites. The C/T at position 89 of ITS-1 was shared by both species at both locations on Svalbard and appeared to be absent from both Norwegian locations.

These results strongly suggest that *O. gruehneri* and *O. arctica* are dimorphic males of the same species. The virtual identity of ITS-1 and ITS-2 sequences in species previously suspected to be morphs has also been shown in *Teladorsagia*, *Cooperia* and *Ostertagia* (Stevenson *et al.*, 1996; Newton *et al.*, 1998; Zarlenga *et al.*, 1998). Both ITS regions are known to evolve rapidly among closely related species of nematodes that include several members of the Trichostrongylidae (Hoste *et al.*, 1999, Hoste *et al.*, 1995). Therefore, it is unlikely that these regions are so conserved as not to detect a genuine species difference between *O. gruehneri* and *O. arctica*. The biological significance of male dimorphism in the Ostertagiinae remains unresolved, but it may relate to longevity of infective larvae and variable susceptibility in the host (Suarez *et al.*, 1995).

Table 3.5.1. Levels of sequence divergence of ITS-1 and ITS-2 among three *Ostertagia* species

	<i>O. gruehneri</i>	<i>O. leptospicularis</i>	<i>O. ostertagi</i>
<i>O. gruehneri</i>	-	3.7 – 4.0	7.7 – 10.1
<i>O. leptospicularis</i>	2.5 – 2.9	-	7.7 – 10.1
<i>O. ostertagi</i>	7.1 – 8.7	8.3 – 10.0	-

Values for ITS-1 and ITS-2 are shown above and below the diagonal, respectively. Values are the number of observed pairwise differences expressed as a percentage of the total aligned length of ITS-1 (404 bp) and ITS-2 (241 bp). The first value in each comparison is for base substitutions only and the second value is for base substitutions and indels combined.

The relative levels of sequence divergence in ITS-1 versus ITS-2 in *Ostertagia* were compared using sequences obtained for *O. gruehneri* in this study and those of *O. leptospicularis* and *O. ostertagi* referred to above. Both ITS regions show similar levels of divergence among the three species, and base substitutions contribute more than indels to divergence (Table 3.5.1). These results imply that both regions would be equally useful for species discrimination using PCR (Gasser & Monti, 1997).

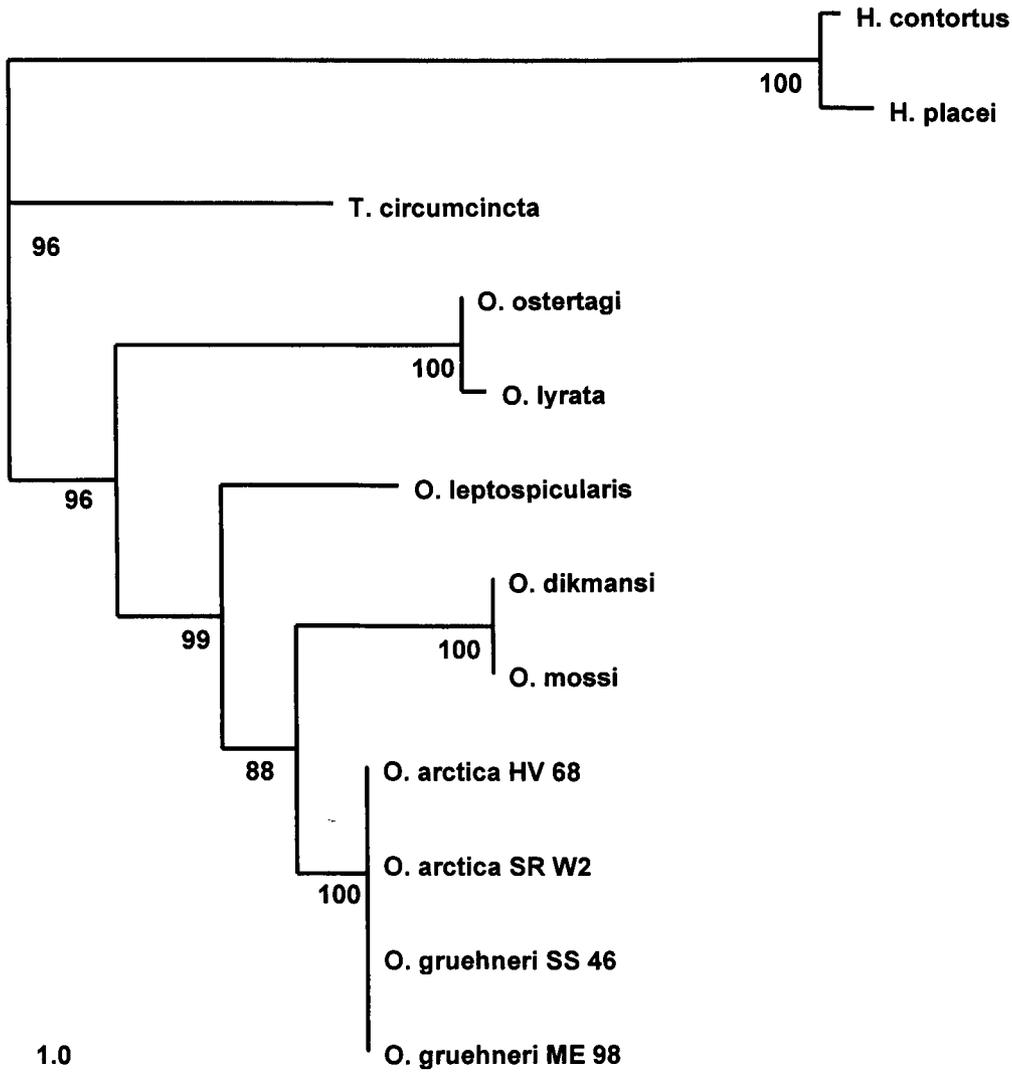


Fig. 3.5.1. Neighbour-joining tree of ITS-1 sequences of *Ostertagia gruehneri* and *O. arctica* together with those of other trichostrongylid species obtained from Genbank™. The latter include the two polymorphic species, *O. ostertagi* / *O. lyrata* and *O. mossii* / *O. dikmansii*. The two *Haemonchus* species were defined as outgroups. The designations following the *O. gruehneri* and *O. arctica* sequences refer to the four collection sites (HV, Hardangarvidda; SR, Reindalen; SS, Sassendalen; ME, Målselv) and to codes for individual reindeer. The numbers on the nodes of the tree are the percentage bootstrap values out of 1000 replicates. The scale indicates one-percent sequence divergence along the horizontal branches of the tree.

Fig 3.5.1 shows a neighbour-joining tree of the relationship of the four ITS-1 sequences detected in *O. gruehneri* and *O. arctica* with those of other trichostrongylid species previously subjected to phylogenetic analysis of the ITS-1 sequences by Zarlenga and co-authors (Zarlenga *et al.*, 1998). The single root of the ITS-1 tree of the seven nominal *Ostertagia* species shown here strengthens these authors proposals that *Ostertagia* is a monophylletic group.

The conspecificity we have inferred from our results implies that *O. gruehneri* and *O. arctica* are synonyms. The consequences of this for nomenclature will be dealt with separately.

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CHAPTER 3.6

Research Note: DNA evidence that *Marshallagia marshalli* Ransom 1907 and *M. occidentalis* Ransom 1907 (Nematoda: Ostertagiinae) from Svalbard reindeer are conspecific

J.F. Dallas^a, R.J. Irvine^{bc}, O. Halvorsen^d. 2001

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Systematic Parasitology 50, 101-103

RESEARCH NOTE: DNA EVIDENCE THAT *Marshallagia marshalli* Ransom 1907 AND *M. occidentalis* Ransom 1907 (Nematoda: Ostertagiinae) FROM SVALBARD REINDEER ARE CONSPECIFIC*

John F. Dallas, R. Justin Irvine & Odd Halvorsen

ABSTRACT

The gastrointestinal parasitic nematodes of ruminants *Marshallagia marshalli* and *Marshallagia occidentalis* are morphs of a single species according to indirect evidence. In this study, their taxonomic status and molecular identification were assessed in isolates from the abomasal nematode community of Svalbard reindeer (*Rangifer tarandus platyrhynchus*) more directly using genetic data. DNA sequences of the first and second internal transcribed spacers of nuclear ribosomal RNA genes were obtained from individual nematodes by the polymerase chain reaction (PCR). Both taxa contained virtually identical sequences of each ITS and shared most of the polymorphisms detected. A PCR assay based on ITS-2 sequences previously developed to identify *M. marshalli* and *Ostertagia gruehneri*, the second common species in this community, gave indistinguishable results for *M. marshalli* and *M. occidentalis*. Genetic data thus confirmed that *M. marshalli* and *M. occidentalis* are conspecific.

* Note; Nucleotide sequence data reported in this article are available in the EMBL, GenBankTM and DDJB data bases under accession numbers AJ250655, AJ400715, and AY013242–4.

The proposal that many species of the trichostrongylid subfamily Ostertagiinae Lopez-Neyra, 1947 have polymorphic adult males (Daskalov, 1974) is presently accepted (Drózd, 1995; Lichtenfels et al., 1997). Nonetheless, supporting evidence is generally indirect, consisting of the occurrence of morphs in males only, the consistency of the relative abundance of morphs within hosts, and the co-occurrence of pairs of morphs in the same areas and under similar climatic conditions (Suarez & Cabaret, 1991). DNA sequence data can yield direct evidence for assessing taxonomic status. Furthermore, the detection of species-specific DNA sequences by the polymerase chain reaction (PCR) is increasingly being used to identify nematode species (McKeand, 1998). One such assay is intended for quantifying species abundance in mixed infections containing suspected morphs (Dallas et al., 2000a). It is therefore important to confirm the taxonomic status of suspected morphs using the DNA sequences on which the assay is based, and to compare assay results in the morphs present.

In this study, the taxonomic status of the suspected morphs *Marshallagia marshalli* Ransom, 1907 and *Marshallagia occidentalis* Ransom, 1907 was assessed using DNA sequence data in samples from the abomasal nematode community of Svalbard reindeer *Rangifer tarandus platyrhynchus*. *M. marshalli* is one of two common species in this community (Halvorsen & Bye, 1999) and, according to ecological and morphological criteria (Drózd, 1995), *M. occidentalis* is its rare morph. Sequences of the first and second internal transcribed spacers of nuclear ribosomal RNA genes (ITS-1 and ITS-2) of *M. marshalli* and *M. occidentalis* were obtained by PCR and compared. The other common species in this community is *Ostertagia gruehneri*, whose rare morph is *O. arctica* according to ecological, morphological (Drózd, 1995) and ITS-1 and ITS-2 sequence (Dallas et al., 2000b) criteria. In addition, the performance of a PCR assay based on ITS-2

sequences developed to identify *M. marshalli* and *O. gruehneri* in isolates from Svalbard reindeer (Dallas et al., 2000a) was tested in *M. occidentalis*. Most of the ITS-2 sequences of *M. marshalli* were reported in the latter study.

Samples of abomasal nematodes were obtained from adult reindeer culled during 1997 at Colesdalen (78°05'N, 15°20'E), Nordenskiöldland, Svalbard. Adult male nematodes were identified according to external morphology as *M. marshalli* or *M. occidentalis* as previously described (Irvine et al., 2000). The mean dominance of *M. marshalli* in individual hosts was 23–46% and that of *M. occidentalis* was up to 0.2%. Seven adult male *M. marshalli* and eight adult male *M. occidentalis* from the same seven reindeer were analysed. Total cellular DNA was isolated, and DNA fragments containing ITS-1 and ITS-2 were amplified by PCR from individual nematodes, sequenced and aligned as previously described (Dallas et al., 2000b).

The 15 ITS-1 sequences determined were all 384 bp and the 15 ITS-2 sequences were all 235 bp in length. Both spacer sequences were virtually identical in *M. marshalli* and *M. occidentalis*, and sequences differed only by a total of 12 polymorphic sites. Five of the eight substitution polymorphisms found in ITS-1 were shared between *M. marshalli* and *M. occidentalis*: A,C or M at site 21, A or W at site 102, A or R at site 124, K or T at site 211 and C,T or Y at site 344. Letters other than A, C, G, or T indicate IUPAC codes for polymorphic bases. The remaining three substitution polymorphisms were detected once in either *M. marshalli* or *M. occidentalis*. In addition, four *M. marshalli* and three *M. occidentalis* individuals showed within-individual polymorphism for the same 1 bp indel at site 101. Two of the three substitution polymorphisms found in ITS-2 were shared between *M. marshalli* and *M. occidentalis*: T or Y at site 28 and C or T at site 53. The remaining

substitution polymorphism was detected once in *M. marshalli*. In addition, one *M. marshalli* and one *M. occidentalis* individual showed within-individual polymorphism for the same 1 bp indel at site 107, and four *M. marshalli* and four *M. occidentalis* individuals showed within-individual polymorphism for the same 2 bp indel at sites 112/113. No individual possessed both indel polymorphisms. Thus, the samples of *M. marshalli* and *M. occidentalis* analysed possessed virtually identical ITS-1 and ITS-2 sequences, and they shared all sequence polymorphisms detected in two or more individuals.

A PCR assay based on diagnostic ITS-2 primers developed to identify *M. marshalli* and *O. gruehneri* in isolates from Svalbard reindeer was tested using 16 *M. marshalli* and eight *M. occidentalis* individuals. PCR and electrophoresis conditions were as previously described (Dallas et al., 2000a). The 110 bp DNA fragment previously shown to be diagnostic for *M. marshalli* was detected in all 16 *M. marshalli* and all eight *M. occidentalis* individuals.

The sequences of ITS-1 and ITS-2 of *O. gruehneri* and *O. arctica*, which were obtained in parallel using the same PCR primers (Dallas et al., 2000b), differed from those determined here at several positions. This finding and the detection of sequence polymorphisms make it most unlikely that the virtual identity of *M. marshalli* and *M. occidentalis* ITS sequences found here is an artefact of PCR carry-over contamination.

The present study confirmed that *M. marshalli* and *M. occidentalis* are conspecific using DNA sequence data, and that a PCR assay to identify *M. marshalli* gave identical results for both morphs. Both ITS-1 and ITS-2 have evolved rapidly among closely related trichostrongylid nematodes (Hoste et al., 1995; 1998), making it unlikely that a genuine species difference between *M. marshalli* and *M. occidentalis* would have gone undetected.

Genetic data likewise confirm the existence of morphs in the genera *Teladorsagia* (Stevenson et al., 1996) and *Ostertagia* (Gasnier et al., 1993; Zarlenga et al., 1998; Dallas et al., 2000b) in the trichostrongylid subfamily Ostertagiinae, and in the genus *Cooperia* in the trichostrongylid subfamily Cooperiinae (Newton et al., 1998). The present study completed an ITS sequence dataset that confirms the conspecific status of all seven suspected morphs in the abomasal nematode community in Svalbard reindeer. According to both morphological and ITS sequence data, this community consists of three species: *M. marshalli*/*M. occidentalis* (this study), *O. gruehneri*/*O. arctica* (Dallas et al., 2000b) and *Teladorsagia circumcincta*/*T. trifurcata*/*T. davtiani* (Stevenson et al., 1995).

Our results, based on samples from one host species from one site on Svalbard, implied that *M. marshalli* and *M. occidentalis* are synonyms. Nonetheless, these taxa are among the most widespread of gastrointestinal parasitic nematodes, having 142 and 100 records, respectively, in many domesticated and wild ruminants (Suarez & Cabaret, 1991). Analysis of ITS sequences representing global geographical scales and host ranges should therefore be carried out before nomenclature is changed.

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CHAPTER 4

Evidence for continued transmission of parasitic nematodes in reindeer during the arctic winter.

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EVIDENCE FOR CONTINUED TRANSMISSION OF PARASITIC NEMATODES IN REINDEER DURING THE ARCTIC WINTER.

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ABSTRACT

Living in the high Arctic the Svalbard reindeer (*Rangifer tarandus platyrhynchus*) and its trichostrongyle nematodes experience a long cold winter from October to late May / early June. Over this period, transmission would be expected to be low. However, in culled reindeer the abundance of infection increased from autumn to late winter providing evidence for continued transmission within this period. To our knowledge this is the first time this has been demonstrated in a climate with temperatures consistently below 0° C. In one winter (1996-97) the average fraction of nematodes found as larvae in the abomasum mucosa increased from around 10 % to 50% between October and March. This suggests that arrested development took place throughout the winter. We found no evidence for an efficient acquired immune response towards the nematodes. The abundance of infection did not tend to decrease with increasing host age after an earlier peak but levelled off instead, as predicted by a simple immigration – death model. In the late winter when the nutritional plane is low both adult reindeer and calves had high worm burdens at intensities that may affect their condition and fitness.

INTRODUCTION

Nematodes of the family Trichostrongylidae cause significant losses in domestic livestock through negative effects on host survival and growth (Soulsby et al, 1982). The population dynamics of these gastrointestinal nematodes have been studied extensively (Smith & Grenfell, 1985; Smith, 1994; Grenfell, 1992) and important aspects of their population dynamics include the seasonality in transmission, development and establishment, and host development of acquired immunity. In temperate climates nematode transmission rates are commonly low in the late autumn - winter period due to reduced survival and development rates of the free-living stages of the nematodes, and the practice of housing domestic animals during cold periods (Smith & Grenfell, 1985). High infection rates are experienced on spring - summer pasture and may cause disease in late summer - autumn, especially in years with mild and wet weather favorable for the nematode free-living stages (Smith & Grenfell, 1985; Armour, 1970; Armour, 1980). In late summer and autumn after transmission to the host, the larvae enter a state of arrested development and high numbers may accumulate in the host abomasum and intestinal mucosa. Arrested larvae have high survival rates (Smith & Grenfell, 1985) and the arrestment is thought to be an adaptation by the parasite to survive periods of unfavourable climate for the development, survival and transmission of free-living stages (Armour & Duncan, 1989). High numbers of arrested larvae may re-emerge in the end of the harsh climatic period and cause disease in the beginning of the grazing season coinciding with host reproduction (Armour, 1970; Armour & Duncan, 1989). Sheep and cattle develop high worm burdens during the first months of the animals' life. Thereafter acquired immunity is considered to reduce the rate of nematode establishment and cause a reduction in the adult worm burden with increasing host age (Smith, 1994). This is thought to be the main reason for the low levels of

infection commonly observed in adult animals compared to calves and lambs (Armour, 1980).

The Svalbard reindeer (*Rangifer tarandus platyrhynchus*) inhabits the archipelago of Svalbard in the high arctic (76 - 81°N, 9 - 33°E). It is the only free-living large herbivore on the islands and the total population size estimated to be approximately 8500 animals (Hindrum et al, 1995), consists of a set of relatively isolated sub-populations separated by sea, large areas of glaciers and sparsely vegetated mountains. Bye, Halvorsen and Nilssen 1987 reported seven species of trichostrongyle nematodes from the abomasum of the Svalbard reindeer (*Ostertagia gruehneri*, *Marshallagia marshalli*, *Grosspiculagia occidentalis*, *Skrajabinagia arctica*, *Teladorsagia circumcincta*, *Teladorsagia davtiani* and *Teladorsagia trifurcata*) and found that worm burdens were relatively high compared to those found in wild reindeer on the Norwegian mainland (Bye, 1986). Today, four of the species reported by Bye, Halvorsen and Nilssen [9] are recognised as morphs of a total of three species (Gasnier et al, 1993; Drozdz, 1995).

At Svalbard the monthly average air temperature is normally above 0° C only between June and September, and the maximum temperature is seldom above 1°C through the winter (Fig. 4.1).

The upper meter of ground may thaw in the summer, but typically this part is frozen and covered by snow from October to the end of May/early June. The low temperatures together with snow covered ground are thought to be an unfavourable environment for the free-living stages of trichostrongyle nematodes. Since the reindeers' food intake is also lower in winter than in summer (Staaland, 1986) we would expect parasite transmission rates to be very low through the winter. This should promote selection for larval arrestment and lead to a drop in the abundance of infection, especially the abundance of adult nematodes, due to nematode mortality over the winter. Larval arrestment in autumn

and winter should cause an increase in the proportion of arrested larvae over the same period as adults die. However, Halvorsen, 1986 found that mature worms and larvae were abundant all year round in Svalbard reindeer. He interpreted this to suggest that transmission occurred throughout the winter and that arrested development was not an important strategy in this harsh environment.

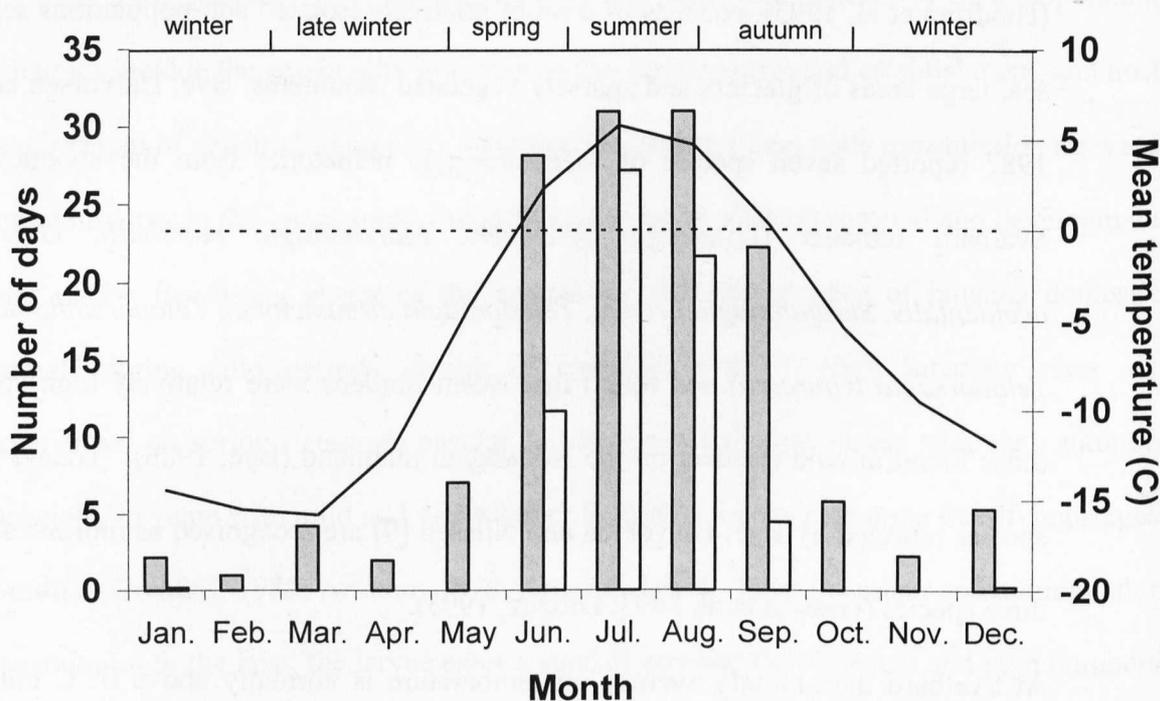


Fig. 4.1. Monthly mean number of days with maximum temperature above 1 °C (shaded bars), maximum temperature above 6 °C (open bars) and monthly mean temperature (line) at Svalbard Airport. The bars are based on data from 1994-1998 and the line on data from the years 1912-1996.

This paper has three objectives answered by performing quantitative analyses of worm abundance in the abomasums of Svalbard reindeer sampled in autumn and winter months. First, to test the hypothesis that transmission of trichostrongyle nematodes occurs through the arctic winter. Second, to investigate the seasonal occurrence of arrested larvae in abomasum mucosa. Third, to look for evidence of reindeer developing acquired immunity to their trichostrongyle parasites. In the abomasum we distinguished between mucosa (harbouring larval stages) and lumen (containing both larval and adult stages). The larvae in the lumen are individuals that have recently entered the host and individuals that have

emerged from the mucosa (arrested from previous infection), ready to develop into adults. One would predict strong selection in favour of arrested development due to freezing temperatures in autumn and concomitantly reduced transmission rates. As a result one would expect a decrease in the abundance of lumen nematodes, accompanied by an increase in mucosal larvae until the late winter, when these trends reverse due to mucosal larvae beginning to emerge and develop. If transmission rates are actually reduced, a decrease in total worm abundance may also occur during the winter period. The reindeer are exposed to the infectious stages of the nematodes throughout their lives and protective immunity may be acquired as the reindeer increase in age. We look for evidence of acquired immunity by exploring the relationship between intensity of infection and host age.

MATERIALS AND METHODS

Reindeer and sample sites. Abomasums were collected from adult female reindeer and calves of both sexes culled in Sassendalen, Colesdalen and the lower part of Reindalen valleys in Nordenskjoldland, Spitsbergen (77° 50'-78° 20'N, 15° 00'-17° 30'E). The reindeer were sexed from a distance and then shot with a rifle by experienced hunters. Female reindeer were selected for the cull to enable us to investigate the relationship between parasite burden and reindeer fecundity, results that will be published elsewhere. Colesdalen and Reindalen are connected by a 15 km valley system and reindeer are known to move between these two valleys (pers. obs.). Sassendalen is approximately 40 km East-North-East of the area where animals were culled in Colesdalen and Reindalen, and movements of reindeer are believed to be rare between these valleys and Sassendalen (Øritsland et al., 1986; Tyler & Øritsland, 1987). Reindeer were culled in August,

September, October, February, March and April in the years 1994-1997, but not from all these months each year (Table 4.1). The ages of reindeer were determined from the number of annuli in sections of the incisor teeth (Reimers & Norby, 1968).

Table 4.1. Sample sizes from the different months and years in the two areas Sassendalen and Colesdalen-Reindalen.

Year	Month	Sassendalen		Colesdalen-Reindalen	
		nadult females	ncalves	nadult females	ncalves
1994	August	2	1	2	4
1994	October	21	10	7	2
1995	April	3	6	-	-
1995	October	16	4	10	5
1996	March	7	2	-	-
1996	April	-	-	4	2
1996	September	11	5	5	1
1996	October	15	1	5	2
1997	February	-	-	5	-
1997	March	10	-	5	-
1997	April	6	-	3	-
1997	October	15	3	-	-
Total		106	32	46	16

nadult females = the number of females reindeer 1 year and older sampled
 ncalves = the number of calves sampled

Nematode samples. Abomasums were removed and frozen within 3 h of culling. After thawing for examination the abomasum was opened along the greater curvature and the lumen content was washed out in 4 l of water. Six 5% sub-samples were extracted using a vacuum pump during thorough agitation of the suspension. Each 5% aliquot was put through a 150 µm sieve and the retained part examined for nematodes. Subsequent 5% sub-samples were investigated until at least 100 adult female and 100 adult male nematodes were found, or until 20% of the abomasum content had been examined. Nematodes in the abomasum lumen were classified as adults if they had clearly defined

genital structures, otherwise they were classified as larvae. In the sample from August 1994 larvae in the abomasum lumen were not counted. The empty, washed abomasum was digested for 12 h at room temperature in 1 l of acid/pepsin digestion fluid (20 ml conc HCl, 8 g pepsin and 8.5 g NaCl per l water) per 500 g of abomasum. The subsequent digest was poured through a tier of two sieves: a 150 μm on top and a 38 μm at the bottom. The content held in the 38 μm sieve was washed into a beaker and diluted to 1 l. As described previously 5% sub-samples were extracted and examined until at least 100 larvae were counted or 20% of the digest fluid had been examined. The intensity of infection due to the different parasite stages was estimated from the number of nematodes found in the fraction of mucosa or lumen content examined. Parasites were not identified to species, so indices such as prevalence, intensity and abundance refer to overall trichostrongyle infection.

Meteorological data. Data on temperatures and summer precipitation were obtained from the Norwegian Institute of Meteorology, Oslo. The measurements were taken between Sassendalen and Colesdalen at Longyearbyen Airport, Spitsbergen (78° 15'N, 15° 28'E).

Statistical analyses. The terms prevalence, intensity and abundance of infection are used following the definition of Margolis *et al.*, 1982. The prefix total is used for the sum of adult and larval nematodes. The parasite population in an individual host is called infrapopulation (Margolis *et al.*, 1982).

Both the total abundance of nematodes and the abundance of adult nematodes in adult reindeer tended to approach an asymptote with increasing host age. The main determinant of this pattern is thought to be the immigration-death process fundamental in governing the

growth of nematode infrapopulations. Assuming a constant net immigration rate (Λ) and per capita death rate (μ) of nematodes in the hosts the change in parasite abundance with time or host age can be described by the simple expression

$$\frac{dA(t)}{dt} = \Lambda - \mu A(t) \quad (1)$$

where $A(t)$ is the abundance of infection at time t [19]. If the host is uninfected at $t = 0$ the solution to this differential equation is

$$A(t) = A^* (1 - \exp(-\mu t)) \quad (2)$$

where $A^* = \frac{\Lambda}{\mu}$ and gives the asymptotic equilibrium abundance of infection as $t \rightarrow \infty$. We

modelled the relationship between host age (Age) and expected abundance of infection statistically using this model ($A(\text{Age}) = E[i(\text{Age})] = A^* (1 - \exp(-\mu \text{Age}))$), where the expected value of the individual intensities of infection at each host age ($E[i(\text{Age})]$) is the abundance, $A(\text{Age})$, as before, and we now consider changes in parasite abundance with respect to age (i.e. parasite populations would be in equilibrium with respect to time). Parameters A^* and μ were estimated from the data. The reciprocal of the mortality rate, $1/\mu$, can be interpreted as a crude estimate of the nematodes average life expectancy and $A^* \mu$ as a crude estimate of the average net transmission rate of parasites. To test the hypothesis that the worm populations were in equilibrium with respect to time and space, month, year, and locality of sampling were fitted as additive effects on the asymptotic abundance of infection giving $A^* = \alpha + \beta X$ where α is the intercept, β a vector of regression coefficients and X the matrix of predictor variables. The data did not support models with μ varying with month, year and locality ($P > 0.5$). When analysing the data on the abundance of larvae in abomasum lumen and mucosa we used ordinary linear models with no age effect and identity link function. For all these analyses the error was assumed to

follow a negative binomial distribution (Wilson et al, 1996). The models were fitted using maximum likelihood. The likelihood ratio statistic was used to test for significant effects of the predictor variables and likelihood ratio based confidence intervals (C.I.) as measure of the precision of parameter estimates (McCullagh & Nelder, 1989). The proportion (p) of larvae in the abomasum mucosa to the total worm burden was modelled using the logit link function and a quasi-likelihood approach assuming the variance being proportional to $\pi(1 - \pi)$, where π is the expected value of p . Tests of significant effects of month, year and locality of sampling were done using analysis of deviance (McCullagh, Nelder, 1989).

RESULTS

Age - intensity relationships. All calves and adult females examined were infected with abomasal nematodes (prevalence=100%). The abundance of total infection increased rapidly during the first two years of life, after which the increase decelerated and appeared to approach an asymptotic level at about 5 years of age (Fig. 4.2 A).

This pattern was also evident in the abundance of adult nematodes (Fig. 4.2 B). In contrast, the abundance of larvae in abomasum lumen and mucosa were low during the reindeers' first year and showed no evidence of a continued increase thereafter (Fig. 4.2C and D). Residual plots showed that a model of the form $A(\text{Age}) = A^*(1 - \exp(-\mu \text{Age}))$, with A^* and μ being parameters for the relationship between host age (Age) and abundance of infection ($A(\text{Age})$) adequately fitted the data on total abundance of infection and abundance of adult nematodes in adult reindeer. There was no evidence that these measures of abundance of infection decreased with increasing host age after an earlier peak (Fig. 4.2A and B).

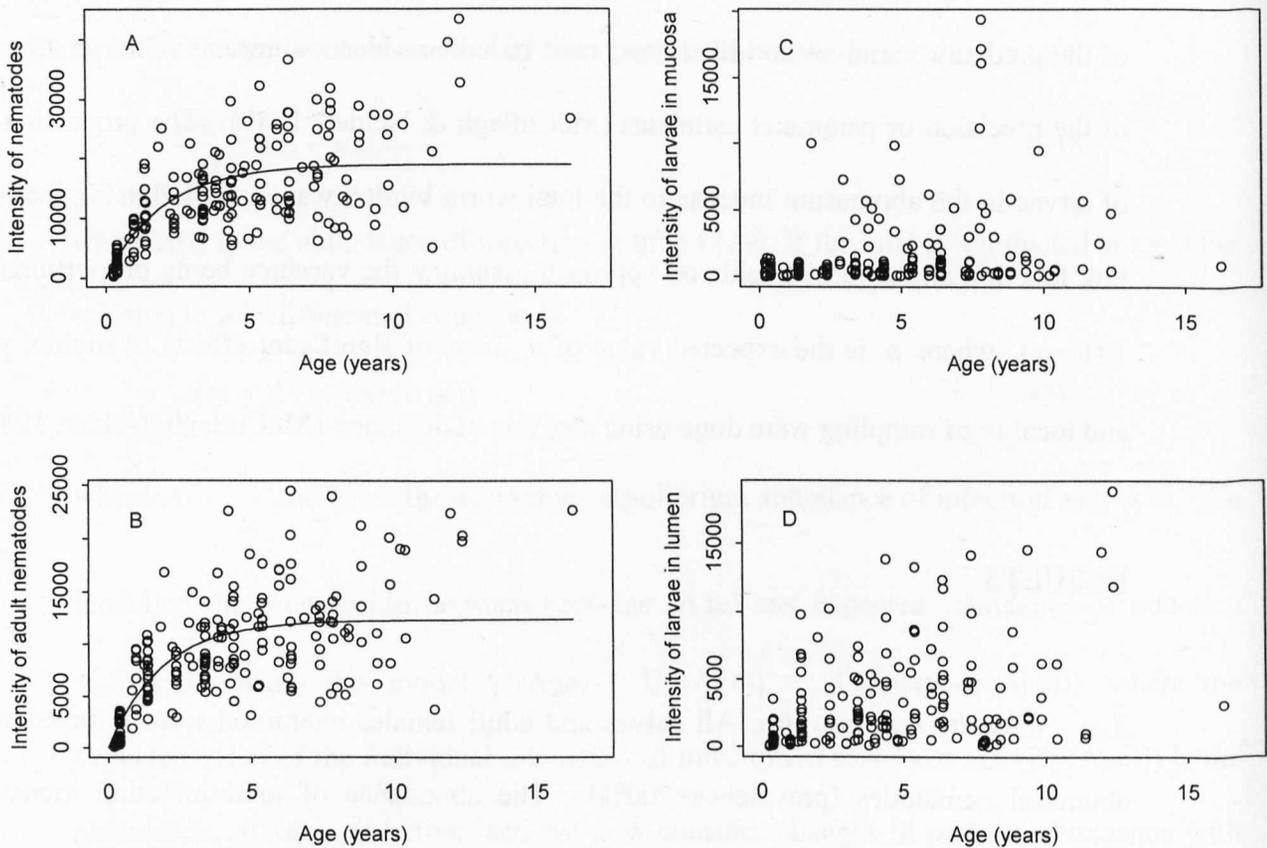


FIG. 4.2. The intensity of different stages of trichostrongyle nematodes plotted against the age of their Svalbard reindeer host. Age is measured on a continuous scale assuming all hosts born on 1st June. A) The total abundance of infection in abomasum lumen and mucosa. The regression line is the best fit line of the form $A(\text{Age}) = A^*(1 - \exp(-\mu \text{Age}))$ with parameter estimates $A^* = 18867$ worms/host (95% C.I. = [16659, 22045]), $\mu = 0.50$ per yr (95% C.I. = [0.34, 0.77]), $k = 5.60$ (95% C.I. = [4.3, 7.1]). B) The abundance of adult nematodes in abomasum. The regression model is the same as above with parameter estimates $A^* = 12356$ worms/host (95% C.I. = [11026, 14193]), $\mu = 0.53$ per yr (95% C.I. = [0.37, 0.79]), $k = 6.19$ (95% C.I. = [4.8, 7.3]). C) The abundance of larval nematodes in abomasum mucosa. D) The abundance of larval nematodes in abomasum lumen.

The parameter estimates from the model for total abundance of infection suggested a crude measure of the parasites life expectancy to be $1/\mu = 2$ years and a crude estimate of the average transmission rate to be $A^* \mu / 365 = 26$ larvae per reindeer per day (Fig 4.2A). The estimate for the adult life expectancy was 1.9 years (Fig. 4.2B). The model did not capture well the increase in total and adult abundance of infection in the youngest calves, which

tended to have lower intensities of infection than predicted. Due to these generally low levels of infection in calves we have analysed their worm burden separately.

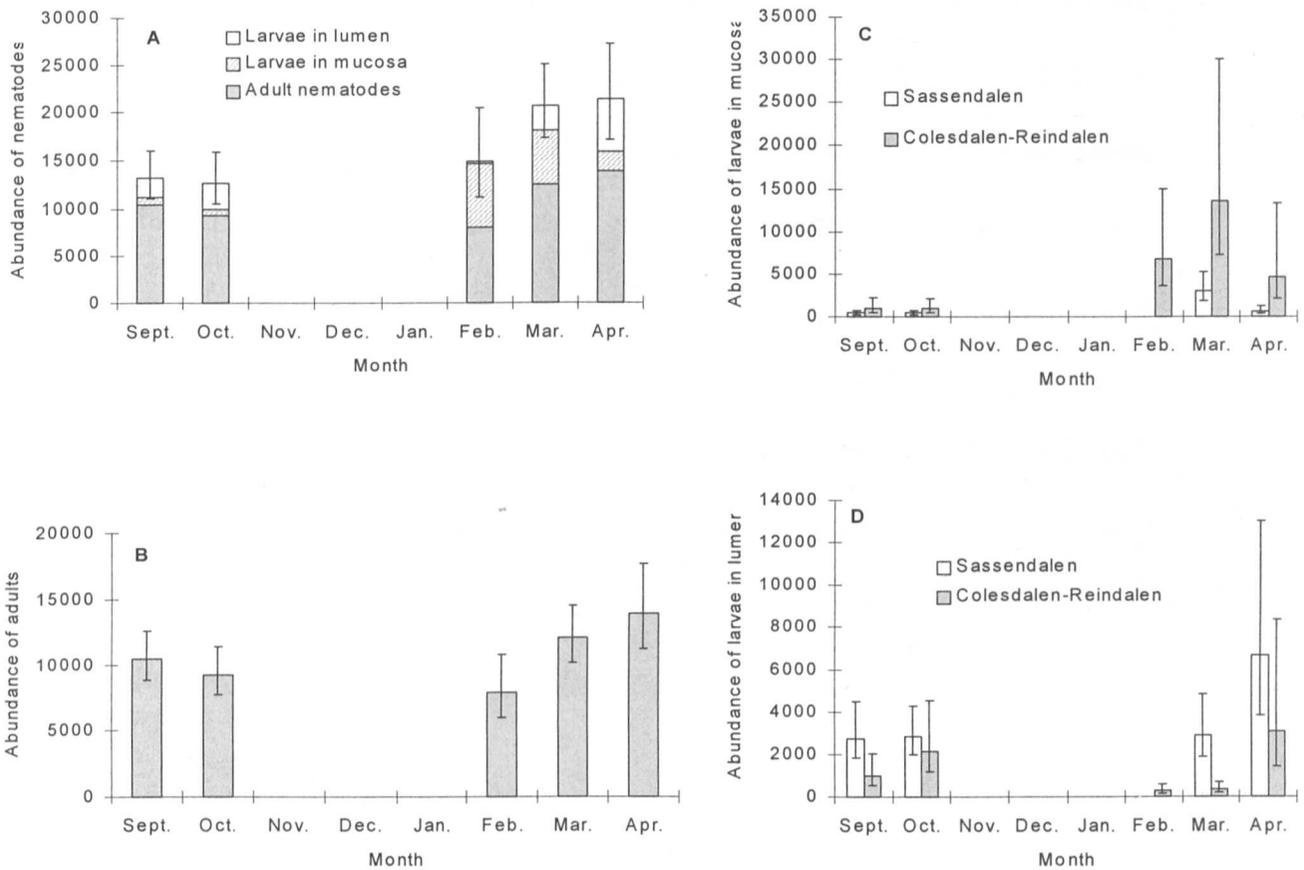


FIG. 4.3. Estimates of the abundance of different stages of abomasum nematodes in adult female Svalbard reindeer with 95% confidence limits, for samples from different months from September 1996 to April 1997. A) The asymptotic abundance of nematodes in the abomasum mucosa and lumen (Other parameter estimates: $\mu=1.05$ per yr, 95% C.I. = [0.53, ∞), $k=8.52$, 95% C.I. = [6.0, 11.7]). The bars are split up according to the contribution from the different parasite stages. B) The asymptotic abundance of adult nematodes in abomasum lumen (Other parameter estimates: $\mu=0.95$ per yr, 95% C.I. = [0.53, ∞), $k=9.0$, 95% C.I. = [6.3, 12.4]). C) The abundance of larvae in abomasum mucosa for each of the two sample areas ($k=1.6$, 95% C.I. = [1.1, 2.2]). D) The abundance of larvae in abomasum lumen for each of the two sample areas ($k=1.8$, 95% C.I. = [1.3, 2.4]). The asymptotic estimates of worm burden in A) and B) are estimated as the parameter A^* in $A(\text{Age})=A^*(1-\exp(-\mu \text{Age}))$, where $A(\text{Age})$ is the abundance of nematodes, Age is the age of the sampled Svalbard reindeer and μ a parameter that was estimated from data.

Adult reindeer - total worm burden. The estimated asymptotic total abundance of nematodes in October differed significantly between years ($\chi^2 = 23.71$, d.f.=3, $P < 0.0001$) and localities ($\chi^2 = 11.09$, d.f.=1, $P = 0.0009$), but there was no significant interaction ($P = 0.99$). The total abundance of infection was higher in Colesdalen-Reindalen than in Sassendalen (mean difference = 5419, 95% C.I. = [2101, 9560]). When correcting for additive year and locality effects the asymptotic abundance of infection differed significantly between months in the autumn - winter period ($\chi^2 = 27.05$, d.f.=4, $P < 0.0001$). Sampling in this period was performed most regularly from September 1996 to April 1997 (Fig. 4.3A) during which there was a significant increase in the abundance of total nematodes from September-October to February-April (Fig. 4.4, $\chi^2 = 18.94$, d.f.=1, $P < 0.0001$), but no significant difference between the localities ($\chi^2 = 0.43$, d.f.=1, $P = 0.51$), any additional month effect ($\chi^2 = 3.88$, d.f.=3, $P = 0.27$), nor any locality-month interaction ($\chi^2 = 8.25$, d.f.=6, $P = 0.22$).

There was also a significant increase in the abundance of nematodes from October 1995 to March - April 1996 (Fig. 4.4, $\chi^2 = 20.57$, d.f.=1, $P < 0.0001$). However, during the previous winter there was a non - significant drop from the October 1994 sample to the three samples collected in April 1995 (Fig. 4.4, $\chi^2 = 3.20$, d.f.=1, $P = 0.07$). Within years in Sassendalen, the asymptotic abundances of infection were similar in March-April and September-October in 1995 and 1997, while it decreased from March-April to September-October in 1996 (Fig. 4.4). Also the asymptotic abundance of infection tended to decline from April to September-October 1996 in Colesdalen-Reindalen (difference = 5886, 95% C.I. = [-1495, 14888]).

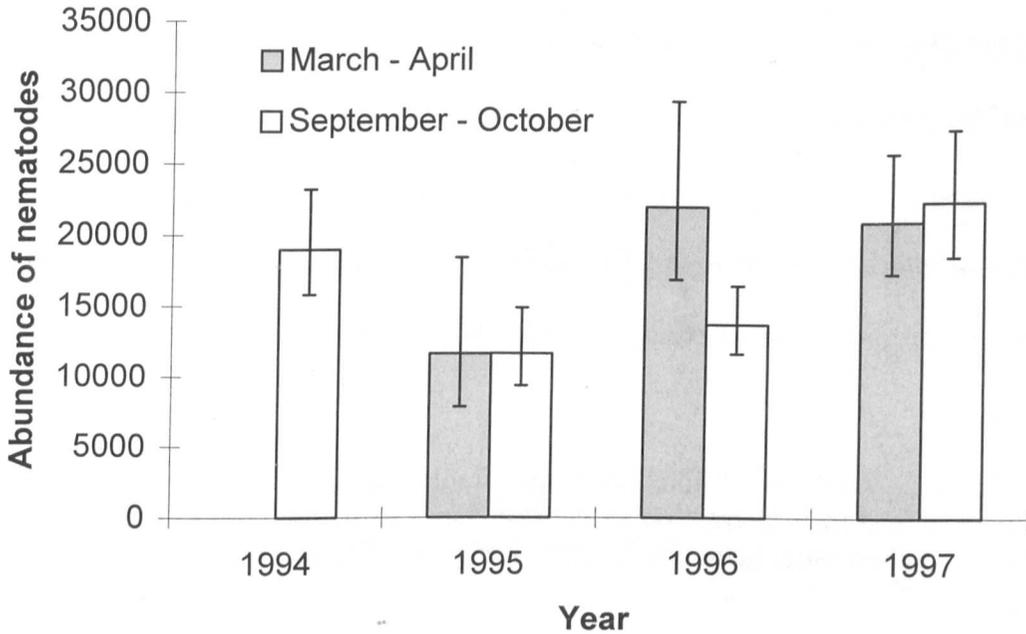


FIG. 4.4. The estimated asymptotic abundance of nematodes (A^*) with 95% confidence limits for reindeer culled in Sassendalen in March-April and September-October in different years. Parameter A^* is estimated from fitting the model described in Fig. 4.2 and 4.3 with additional estimated parameters: $\mu=0.57$ per yr (95% C.I. = [0.38, 0.96]), $k=7.5$ (95% C.I. = [5.7, 9.7]).

Adult reindeer - adult nematodes. The total abundance of infection increased over the winter period partly because of changes in the abundance of adult nematodes. The asymptotic abundance of adult nematodes differed between months from September 1996 to April 1997 (Fig. 4.3B, $\chi^2 = 13.48$, d.f.=4, $P=0.009$), with no significant effects of locality ($\chi^2 = 0.44$, d.f.= 1, $P= 0.50$) nor any month - locality interaction ($\chi^2 = 0.87$, d.f.=3, $P=0.83$). As for the total abundance of infection, the abundance of adults was higher in March and April than in September and October (Fig. 4.3B). The estimate for February was lower than the estimate for March-April ($\chi^2 = 5.81$, d.f.=1, $P=0.02$), but not significantly different from the September-October estimate ($\chi^2 = 2.02$, d.f.=1, $P=0.15$). Also in Sassendalen from October 1995 to March 1996 the asymptotic abundance of adult nematodes increased significantly (difference=9089, 95% C.I. = [4791, 14623]). Over the same winter there was a non-significant increase in adult nematode abundance in

Colesdalen-Reindalen (difference=3584, 95% C.I. = [-813, 8872]). While in the 1994-95 winter there was no evidence of an increase (October-April difference = -774, 95% C.I. = [-5114, 5702]).

Adult reindeer - larval stages. The abundance of larvae in both the abomasum mucosa and lumen not only differed between months but also localities in the 1996-97 winter (Table 4.2).

Table 4.2. Analysis of likelihood ratio table for the effect of month and locality of sampling and their interaction on the abundance of A) larvae in abomasum mucosa and B) larvae in abomasum lumen in Svalbard reindeer culled in the autumn-winter 1996-97.

A)

Model	-2Loglik	Residual d.f.	χ^2	P
Intercept	1137.07	63		
Month	1108.76	59	28.3	<0.0001
Locality	1067.07	58	41.7	<0.0001
Month:locality	1050.73	55	16.3	0.001

B)

Model	-2Loglik	Residual d.f.	χ^2	P
Intercept	1153.22	63		
Month	1129.18	59	24.0	<0.0001
Locality	1108.79	58	24.4	<0.0001
Month:locality	1105.36	55	3.43	0.33

-2Loglik = the -2 log likelihood value for the model. The data were assumed to be distributed following the negative binomial distribution where the index of dispersion (k) was estimated from the data.

χ^2 = the difference in -2 log likelihood between the model with the factor and the simpler model on the row above follow approximately a chi-square distribution with d.f. equal to the difference between those of the models under comparison.

In addition there was a significant interaction between these two predictors of the abundance of larvae in mucosa (Table 4.2A). In both Colesdalen-Reindalen and Sassendalen the abundance of larvae in abomasum mucosa showed a peak in March (Fig. 4.3C) while the abundance of larvae in the abomasum lumen showed a peak in April (Fig.

3D). Thus it appeared that, as the abundance of larvae in the mucosa dropped, there was an increase in the lumen (Fig. 4.3C and D). The high abundance of larvae in abomasum mucosa in February and March accounted for a high proportion of the total worm burden (Fig. 4.5), and caused a significant between month variation in the mean proportion mucosa larvae when the locality effect was taken into account ($F = 17.42$, $d.f.=4, 59$, $P<0.0001$).

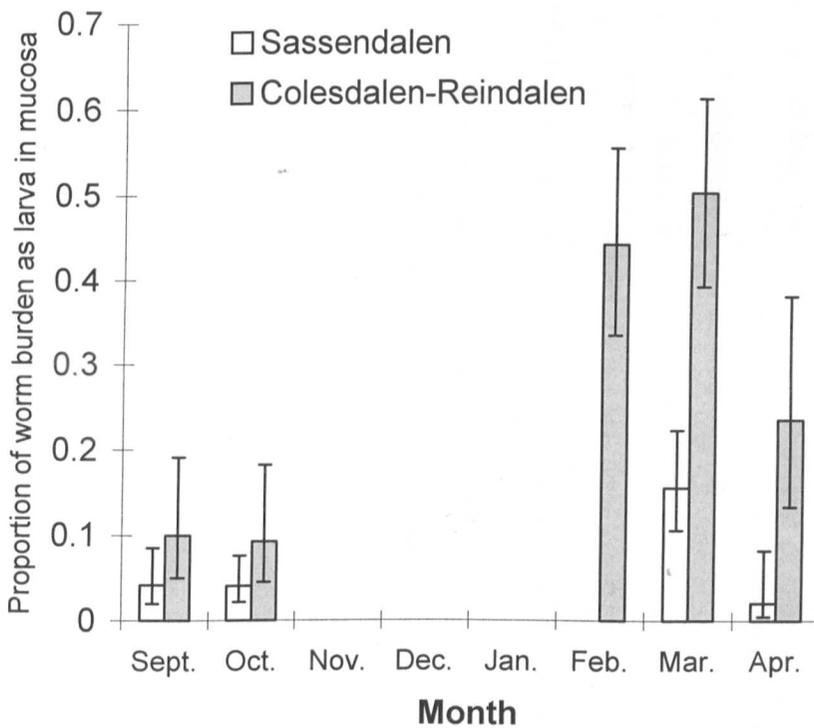


FIG. 4.5. The mean proportion of mucosa larvae to total intensity of infection in adult female Svalbard reindeer with 95% confidence limits, for samples from different months and locations from September 1996 to April 1997.

These patterns in the abundance of the larval stages were not as clear in the patchy samples from the other years. The abundance of larvae in mucosa was higher and the abundance of lumen larvae lower in Colesdalen-Reindalen in April 1996 than in the previous October (Table 4.3). The net result was an increase in the mean proportion of mucosal larvae in the infrapopulation from October 1995 to April 1996 (Table 4.3). However, in Sassendalen the

Table 4.3. Estimated mean differences between samples from different localities, and month/year of sampling in: the abundance of larvae in abomasum mucosa, the mean proportion of larvae in mucosa to total intensity of infection, and the abundance of larvae in abomasum lumen in Svalbard reindeer. 95 % confidence limits for the estimates are given in brackets.

Locality	Mo-yr	Proportion of larvae in mucosa		
		Larvae in mucosa	to total intensity of infection	Larvae in lumen
Sassendalen	Oct.-94 vs Apr.-95	-590 (-1017, -129)	-0.031 (-0.05, 0.006)	-5071 (-2872, -7681)
Sassendalen	Oct.-95 vs Mar.-96	52 (-239, 518)	-0.019 (-0.04, 0.001)	1810 (245, 4347)
Colesdalen-Reindalen	Oct.-95 vs Apr.-96	3775 (127, 16800)	0.15 (0.02, 0.30)	-1698 (-2744, -1081)

mean proportion of mucosal larvae tended to decrease from October 1995 to March 1996 and from October 1994 to April 1995 (Table 4.3).

Calves. In both valleys the total worm abundance in calves differed significantly between months (Fig. 4.6A, $\chi^2 = 48.6$, d.f.=4, $P < 0.0001$) and is clearly an over winter increase (from August-September to March-April).

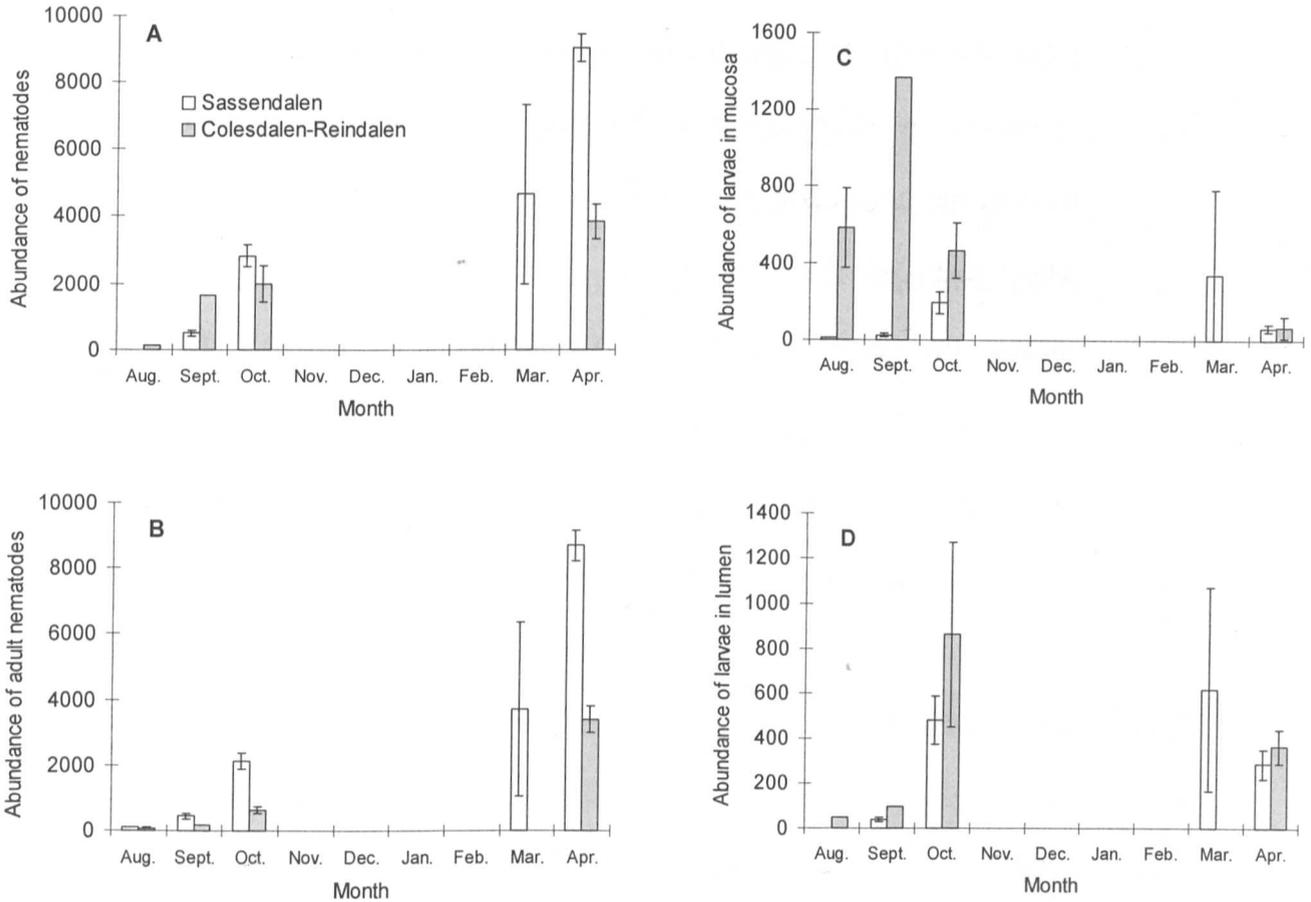


Fig. 4.6. Estimates of the abundance of different stages of abomasum nematodes in Svalbard reindeer calves with S.E.M. bars, for samples from different months in the years 1994-97 in each of the two sample areas Sassendalen and Colesdalen-Reindalen. Columns with no error bars represent samples of only one individual. A) The total abundance of nematodes in the abomasum. B) The abundance of adult nematodes in abomasum. C) The abundance of larvae in abomasum mucosa. D) The abundance of larvae in abomasum lumen.

Despite the fact that adults in Colesdalen had significantly higher adult worm burdens than in Sassendalen, calf worm burdens increased more slowly in Colesdalen-Reindalen than in Sassendalen (Fig. 4.6A). Although the data was partly

confounded in time and space with few observations from each year-month-locality combination, the total worm burden tended to increase from October to March-April within all years and localities. The pooled sample was dominated by data from Sassendalen from October 1994 ($n=10$) and the following April ($n=6$), and the same pattern as found in the pooled samples was evident in these with a marked increase in the total abundance of infection from October to April (difference=5979, 95% C.I. = [3208, 10199]). In calves the increase in worm burden over the winter reflected an increase in the abundance of adult nematodes (Fig. 4.6B). The abundance of larvae in both the lumen and mucosa did not show a tendency towards higher levels in March and April (Fig. 4.6C and D). Rather, the abundance of larvae in abomasum mucosa decreased from October to April ($\chi^2 = 10.71$, d.f.=1, $P=0.001$) and the abundance of larvae in abomasum lumen showed a tendency to decline over the same period ($\chi^2 = 3.27$, d.f.=1, $P=0.07$). It was 21 female and 27 male calves in the sample, but no strong effect of sex on any of our measures of worm burden ($P>0.15$).

DISCUSSION

The increase in total abundance of infection over the winter was surprising. This result implies that the reindeer continue to ingest infective larvae from snow-covered pasture during the long arctic winter as suggested previously by Halvorsen, 1986. In temperate areas research on the free-living stages of *Ostertagia ostertagi* suggests that this species experiences low development rates at temperatures approaching freezing (Smith et al, 1986). However, in New Zealand, considerable development of free-living eggs and larvae may occur, and sheep are found to pick up high numbers of trichostrongyle nematodes from pasture at close to freezing temperatures

(Familton & McAnulty, 1994). This suggests that the temperature-dependent development of trichostrongyle nematodes may be a highly adaptable trait permitting transmission through cold winters if hosts are available. Larval development at temperatures consistently below zero is still unlikely, but unfortunately, little work has been done on trichostrongyle nematode transmission under such conditions because, livestock generally will be housed and therefore do not pick up infection from pasture. However, the infective stages of trichostrongyle nematodes can have a relatively high survival rate at sub-zero temperatures (Crofton, 1971; Tharaldsen, 1976; Slocombe, 1974; Grenfell et al, 1986) so the reindeer possibly pick up larvae through the winter which have already developed to the infective stage during the summer and autumn.

The total abundance of infection did not decline with increasing host age after peaking when juvenile as in the case of many temperate ruminants. Instead, worm abundance increased at a decelerating rate until it levelled off at 5 years old and onwards. This confirms the earlier findings by Halvorsen and Bye, 1986 of high gastro-intestinal worm burdens in adult reindeer and *Elaphostrongylus rangiferi* in the brain/lungs of semi-domesticated reindeer (Halvorsen, 1986). The high intensity of infection often found in adult reindeer suggests that they do not develop as an efficient immune response towards their nematode infections as found in sheep and cattle. However, nutrition is known to be important in both the ability of ruminants to withstand the effects of gastrointestinal infections and their ability to resist infection (van Houtert & Sykes, 1996). Our samples were collected immediately before and at the end of the arctic winter, a period of starvation over which female reindeer lose on average 30 % of their body weight (Tyler, 1987). This stress may

reduce their ability to mount an efficient immune response to their worm burden over the winter. Interestingly, the abundance of nematodes did not increase from March-April to September-October, which includes the spring, a period believed to be most important for parasite transmission. Future studies should focus on the summer period and the possibility that immune responses may be detected at that time.

The crude estimates of parasite mortality rates were relatively low, giving an estimated parasite life expectancy of 2 years. Published estimates of trichostrongyle life expectancies from domestic livestock systems are much lower, with maximum values of 0.14 years for *Ostertagia ostertagi* and *Haemonchus contortus* (Smith & Grenfell, 1985; Smith, 1988) and 0.4 years for *Trichostrongylus colubriformis* (Dobson et al, 1990). However, a longer life expectancy (1 yr [33]) has been estimated for *Trichostrongylus tenuis* in red grouse. This suggests that trichostrongyle life expectancies may be higher in natural host populations than in livestock with the shorter life expectancies possibly reflecting adaptations to the use of anthelmintics in livestock host populations (pers. com. Peter J. Hudson). With such a high life expectancy the reindeer have to ingest, on average, only 26 infective larvae per day for the high abundance of infection observed in adult reindeer to develop and persist. It should be acknowledged that our estimates are crude. The effect of host mortality, density dependent processes, seasonal, annual and parasite species specific transmission and survival are not specified when estimating parasite mortality and transmission rates. More information on the parasite population dynamics is needed, or preferably experiments have to be conducted, to get better estimates of these components. One interesting indication from our estimates is that the reindeer do not necessarily have to ingest a high number of larvae per day to

obtain observed levels of infection, and that pasture contamination therefore may be very low. Indeed preliminary work suggests this is the case (pers. obs.).

In calves, the total abundance of infection was low in the autumn and peaked in the late winter. This differs from the seasonal pattern observed in domestic livestock in temperate areas where newborn get infected by high numbers of nematodes through their first summer and autumn but then have declining intensities of infection over winter (e.g. Crofton, 1955; Anderson et al, 1969; Waller et al, 1981). The small increase in total abundance of infection over their first summer again suggests the nematode transmission rate to be relatively low compared with that experienced by livestock on densely populated pastures. This is probably due to low densities of infective larvae on the pasture and reduced intake of larvae due to suckling in the first of the three available summer-autumn months (Skogland, 1990). The intensities of infection at the end of winter were higher than those observed in an experimental study of semi-domesticated reindeer calves, in which parasite burdens were found to have a significant negative effect on host food intake and weight gain (Arneberg et al, 1996). The observed levels of infection may therefore have a significant effect on calf nutrition and growth through their first year and thereby affect their survival and future reproductive success.

In adult reindeer the abundance of larvae in abomasal mucosa tended to be higher in February-April than in the previous October, causing a marked peak in the mean proportion of mucosa larvae to total infection in 1997. This suggests that arrested development may take place, but may be affected by factors that vary between years. For example, the climatic conditions in autumn and winter (Armour & Bruce, 1974)

or perhaps between-year variability in host immunocompetence (Michel et al, 1979). In contrast, the abundance of larvae in the abomasum lumen did not show any clear trend over the winter. This may be because this group of nematodes consists of both emerged larvae from abomasum mucosa developing into the adult stage and new larvae from recent infections. Therefore, both the timing of larval emergence from abomasal mucosa and transmission from pasture affect their abundance. Between-year and locality variation in both or one of these processes may therefore remove a general seasonal trend. The abundance of adult nematodes also increased from September-October to March-April, so development to the adult stage appears to take place through the winter. This suggests that arrested development is not such an important strategy as is the case of some trichostrongyle nematodes of livestock, and may support Halvorsen's (Halvorsen, 1986) hypothesis that housing is a stronger selective force for arrested development in livestock systems in cold climates than the winter temperature *per se*. The peak abundance of larvae in the abomasum lumen in April 1997 may anyway have been due to a relatively synchronised emergence of larvae from abomasum mucosa. If so, disease may be induced in the late winter when the reindeer are also stressed by limited food availability. In calves we found no evidence of larval arrestment but more data is needed to clarify this point.

Bye *et al.* 1987 found differences in parasite load and taxonomic composition between samples collected from different areas at Spitzbergen. We found that worm burden also differed between localities within one of their sample areas. This supports the idea that the reindeer at Nordenskjöldland are structured as relatively isolated sub-populations, and also suggests that important physical and biological

factors for trichostrongyle nematodes vary significantly within the spatial scale of these studies.

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CHAPTER 5

Life-history strategies and population dynamics of abomasal nematodes in Svalbard reindeer (*Rangifer tarandus platyrhynchus*).

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LIFE-HISTORY STRATEGIES AND POPULATION DYNAMICS OF ABOMASAL NEMATODES IN SVALBARD REINDEER (*Rangifer tarandus platyrhynchus*).

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SUMMARY

The observation that the total abundance of adult nematodes in the abomasum of Svalbard reindeer increases between October and April suggests adaptation to cope with the Arctic winter. Here we investigate the extent to which selection has led to similar life history strategies in the three most numerous trichostrongyle species. The life histories are found to differ markedly. This is done using flexible statistical models for the abundance and dispersion of parasites in the host population. One of the taxa, *Marshallagia marshalli*, was most abundant and had its highest egg output in the winter. In contrast, the abundance of the most common taxon, *O. gruehneri*, m. *gruehneri* was stable or declined from autumn to late winter, and the closely related taxa, *O. gruehneri*, m. *arcticus*, showed a similar over-winter drop. The faecal egg output of these two taxa was highest in summer, as found in temperate trichostrongyle species. Despite the apparent contamination of summer pastures with *O. gruehneri*, calves showed negligible burdens until their second summer and the abundance of infection reached an asymptote within their third year. In contrast, the abundance of *M. marshalli* in calves showed a rapid increase over the first summer and by late winter was similar to peak levels found in adults (8000 worms). This increase could not be accounted for by the developing abomasum larvae population and is therefore evidence for transmission over the winter for this taxa. While *M.*

marshalli showed little between year variation, *O. gruehneri* showed two-fold fluctuation in the abundance of infection. *O. gruehneri* may therefore play a role in the fluctuating population dynamics of the host. Since there was no apparent decline in abundance with host age in any of the three species there was no evidence of reindeer mounting an immune response.

INTRODUCTION

Trichostrongyle nematodes show strongly seasonal dynamics in ruminants in temperate environments with transmission and peak burdens occurring in the main summer grazing period (Smith & Grenfell, 1985, Armour, 1980). Both primary production and development of the free-living stages of the nematode life cycle are strongly correlated with temperature and humidity. Transmission may occur over six months or more and provides the opportunity for nematode species with different life-history strategies to occur with successional changes in abundance of species with different developmental rates (Crofton, 1957, 1963, Boag & Thomas 1977). In contrast, during the winter period low temperature limits egg and larval development and as a result reduces transmission (Familton & McAnulty, 1995, Smeal, Fraser & Robinson, 1981). In order to survive this unfavourable period trichostrongyle infections have developed two main strategies: some species survive in the host (e.g. *Teladorsagia circumcincta*), while others survive on the pasture (e.g. *Nematodirus battus*) (Soulsby, 1982). Over wintering in the host provides the opportunity to resume development and start reproduction as soon as the ambient conditions are favourable for the development of free-living stages and may be less risky than being on pasture.

The extreme environmental conditions prevailing in the arctic would be expected to focus nematode transmission and reproduction in the short arctic summer. In the Svalbard archipelago the snow-free period lasts for only three months and mean summer temperature (June to August) is 3.60 C and rainfall averages 53 mm (Førland, Hanssen-Bauer & Nordli, 1997). Compared to mainland Norwegian reindeer herds, Svalbard reindeer (*Rangifer tarandus platyrhynchus*) are non-migratory (Tyler & Øritsland, 1987), occur at locally high population densities (Alendal & Byrkjedal, 1976, Reimers, 1977) and experience high levels of parasitic infection (Bye & Halvorsen, 1983), sufficient to depress fecundity (unpublished data). Previous studies of the abomasal nematodes of Svalbard reindeer (Halvorsen 1986, Halvorsen & Bye 1999, Halvorsen et al. 1999) have revealed dynamics in the infections indicative of life-history traits at variance with those found in related species studied in sheep and cattle. These include continued transmission of nematodes in the arctic winter, reduced role of arrested development and extended life expectancy of the parasitic phase. Earlier work has dealt mainly with the infection as infra-communities without separating the different taxa. Seven nominal taxa of abomasal nematodes representing two dimorphic and one trimorphic species have been found in the Svalbard reindeer (Halvorsen & Bye 1999). Here we report the abundance of these taxa in two reindeer populations in Svalbard. Two taxa dominate: the major morph of *Ostertagia gruehneri* and the major morph *Marshallagia marshalli*, which together account for more than 95% of the adult worm burden. The minor morph of *O. gruehneri*, i.e. morph *arcticus*, accounts for about 4% of the total worm population. We compare and contrast the life-cycle strategies and population dynamics of these taxa.

The variation in adult abundance of the nematode taxa are analysed in relation to host age, season, year and reindeer population and variation in egg output in relation to season, to investigate whether taxa have evolved different life histories. The relationship between the abundance and distribution of infection and host age can also provide evidence for density dependent processes in the parasite population dynamics such as parasite induced host mortality or immunity (Anderson & Gordon 1982, Pacala & Dobson 1988, Grenfell, Dietz & Roberts 1995a, Rousset et al. 1996, but see Fulford et al. 1992) and heterogeneities in host responses to infection (e.g. host survival and immunity, Grenfell et al. 1995b). With no density dependence or heterogeneities in host response to infection the aggregation of parasites in the host population, measured by the dispersion parameter (k) of the negative binomial distribution, is expected to track the abundance of infection (Grenfell, et al. 1995). However, density dependent processes combined with heterogeneities in host exposure may cause k to increase and the abundance of infection to decrease at increasing host age (Pacala & Dobson 1988, Grenfell et al. 1995a), but heterogeneities in host immune responses may cause k to decrease at increasing host age (Grenfell et al. 1995b). To evaluate this aspect we incorporate an analysis of how k varies with both abundance of infection and host age.

MATERIALS AND METHODS

Reindeer hosts. Animals were culled every year in the autumn, October (1994-1997) and again in late winter, either February (1997 - 1998), March (1996 - 1998) or April (1995-1998). An additional 25 in late August 1994 and 23 in early September 1996 provided material in late summer. The focus of the culling was on female reindeer, because the relationship between fecundity and worm burden was of primary interest for the overall research project, but also males and calves were culled, especially in

1994-1995. Accurate ages of culled animals were derived from annuli in the cementum of extracted (I₁) incisors (Reimers and Nordby, 1968).

Culling took place in two different valley systems in Nordenskiöldland, Spitsbergen (77° 50' - 78° 20'N and 15° 00' - 17° 30'E). Approximately two thirds of the animals (120 adult females, 17 adult males and 33 calves) were culled in Sassendalen about 40km East of Longyearbyen and the rest (64 adult females, 9 adult males and 18 calves) in Colesdalen 20 km South West of Longyearbyen. Movement of reindeer between these valleys is thought to be unlikely (Øritsland and Arendal, 1996, Tyler and Øritsland, 1987) and we refer to these two locations as separate populations. On Svalbard, reindeer grazing density is estimated at 3.2/km² (Tyler, 1987) and is higher than what commonly found at the Norwegian mainland (range 0.6-2.5 reindeer/km², Skogland, 1984).

Parasitology – abomasum worm burdens. The species composition and abundance of trichostrongyle nematodes in the abomasa of 261 culled reindeer were analysed (Table 4.1). The abomasum was ligated and extracted from the shot animal and frozen within 3 hours of death. After thawing the abomasum was opened along the greater curvature and the contents washed out into 4 litres of water. Six 5% subsamples were extracted using a vacuum pump during thorough agitation of the suspension. Each 5% was washed through a 150-micron sieve and the retained proportion examined for adult nematodes and larvae. Nematodes were counted until at least 100 of both male and female worms were extracted or until 20% of the content had been examined. Any worm with clearly defined genital structures was regarded as an adult. Species profiles of the individual host worm burdens were based on the taxonomy of the extracted adult male worms (Dròdz, 1965) and the proportions were assumed to be the same in the adult female fraction. References to

Marshallagia marshalli in the text will refer to the major morph *marshalli* unless otherwise stated and *Ostertagia gruehneri* will refer to major morph *gruehneri* unless morph *arcticus* is appended. *Grosspicularis occidentalis* was reclassified as *Marshallagia marshalli*, morph *occidentalis* (Dròzd, 1995).

Parasitology – faecal egg counts. Faecal egg count data was obtained from individually marked reindeer from April to October in 1994 to 1998 in the Colesdalen reindeer population. Faeces from individually marked animals were collected immediately after excretion, stored at around 4°C and processed within 48 hours to avoid complications of larval development. Three grammes of faeces were suspended in 42ml of saturated salt solution and one third of this was put in a glass centrifuge tube with a ground rim. The tube was filled until there was a positive meniscus and a cover slip was placed on the top. Tubes were centrifuged at 1000 rpm for two minutes. The cover slip was removed and placed on a microscope slide. The whole area was counted at 100x magnification and the number of eggs was recorded as eggs per gramme of faeces. Eggs of the *M. marshalli* taxa are larger (100x70 µm) than other strongyle eggs and have more developed morulla than nematodirus eggs, and can therefore be readily distinguished from other trichostrongyle eggs (MAFF/ADAS, 1986). Given that the nematode fauna is dominated by *O. gruehneri*, m. *gruehneri* and *M. marshalli*, it seems reasonable to assume that the other trichostrongylid fraction is largely from the *O. gruehneri* m. *gruehneri* and *O. gruehneri* m. *arcticus* taxa and we will call this *Ostertagia* eggs.

Anthelmintic treatment. The hypothesis that these abomasal parasites influence the hosts' fecundity is the subject of ongoing work. However treated animals were

utilised here to determine if animals became re-infected. Reindeer caught in April/May 1998 were randomly allocated to anthelmintic treatment groups. The treated group was injected sub-cutaneously with 0.2mg Moxidectin per kg live weight. The control group received nothing. Faecal egg output was estimated as above.

Statistical analysis. Because of the few males available in the data set we have focused our analysis of worm burdens on female reindeer, but included male calves. Adult worm burdens were analysed assuming the nematode counts came from a negative binomial distribution (Wilson, Grenfell & Shaw 1996, Wilson & Grenfell 1997) and the models were fitted by maximum likelihood method. Neglecting constant terms in the log likelihood function for the negative binomial distribution this is equivalent to minimising $\log l$ given by

$$\log l = \ln(\Gamma(y + k)) - \ln(\Gamma(y + 1)) - \ln(\Gamma(k)) + y \ln(\hat{y}/(\hat{y} + k)) - k \ln(1 + \hat{y}/k), \quad (1)$$

where y is the observed nematode intensity, \hat{y} is the predicted abundance of nematodes, k is the negative binomial dispersion parameter and Γ the gamma function. Recent studies (Grenfell et al. 1995a, and Shaw, Grenfell & Dobson, 1998) have suggested that the dispersion parameter k is likely to be positively related to the abundance of infection. Figure 4 in Shaw et al., (1998) suggests that linear or log-linear models are candidates for this relationship. To investigate this we tried three simple models for k : 1) k constant ($k = \text{constant}$), 2) k a linear function of \hat{y} ($k = k_1 + k_2 \hat{y}^{10^{-3}}$ with constraints $k_1 > 0$ and $k_2 > 0$ to ensure k to be positive for all \hat{y}), and 3) k an exponential function of \hat{y} ($k = \exp(k_1 + k_2 \hat{y}^{10^{-3}})$). When fitting these models for k we used an over-parameterised model for \hat{y} , with the parameter α in the Gompertz function described below varying with reindeer population and γ

varying with population, month and year of sampling and the interactions between these predictor variables. Additional patterns of variation in k at increasing host age were investigated by 1) estimating k for each age class of reindeer and 2) using an extended version of the above models for k , while modelling the abundance of infection using the best-fit model from the analysis with no age effect on k . In the second approach, we constrained our model of the effect of age on k to the data for animals older than the age at which the abundance of infection had reached its early maximum (c) using the models:

$$k = \begin{cases} \exp(k_1 + k_2 * \hat{y} * 10^{-3}) & \text{for age} \leq c \\ \exp(k_1 + k_2 * \hat{y} * 10^{-3} + k_3 * \text{age}) & \text{for age} > c \end{cases}$$

for the species with a log linear relationship between k and the abundance of infection, and

$$k = \begin{cases} k_1 + k_2 * \hat{y} * 10^{-3} & \text{for age} \leq c \\ k_1 + k_2 * \hat{y} * 10^{-3} + k_3 * \text{age} & \text{for age} > c \end{cases}$$

for species with a linear relationship between k and the abundance of infection. This was to investigate the possibility of a change in k with increasing host age after the initial age related increase in \hat{y} , but independent of the effects on k of seasonal and between year variation in \hat{y} .

For the systematic component of the models (i.e. modelling \hat{y}), we used both linear and non-linear models. The age-intensity relationship were assumed to follow a Gompertz function given by

$$\hat{y} = \alpha + (\gamma - \alpha)\exp(-\exp(-\kappa(\text{Age} - \phi))), \quad (2)$$

where \hat{y} is as above, α is the lower asymptotic abundance of infection for Age $\rightarrow -\infty$, γ is the upper asymptotic abundance of infection for Age $\rightarrow \infty$, Age = ϕ is the inflection point and κ determines the rate at which the curve approaches the asymptote. Seasonal variation, locality and year effects on worm burden were modelled as additive effects on α , γ , κ and ϕ through a log link function, giving for example $\alpha = \exp(\mathbf{X}\beta)$, where β is a vector of regression coefficients and \mathbf{X} a matrix of predictor variables. To investigate the structure of seasonal effects we also fitted a sine function for γ :

$$\gamma(\text{season}) = a + A(\sin(2\pi(\text{season} - \delta))) \quad (3)$$

where season is a continuous measure of time of year between 0 and 1, a is the abundance of infection at the inflection point, A the amplitude of the wave, and δ determines the time of year the curve passes the seasonal inflection point. Also in this model the parameters a and A were allowed to vary with predictor variables through a log link function. Models were compared using likelihood ratio tests (McCullagh & Nelder, 1989) where the difference in $-2 \log$ likelihood of two nested models were assumed to be distributed as χ^2 with degrees of freedom equal to the difference in residual degrees of freedom between the two models. Likelihood ratio based confidence intervals (C.I.) were used as measure of the precision of parameter estimates (McCullagh & Nelder, 1989). Nematode egg count data from faeces were $\log_e (X+1)$ transformed and the seasonal pattern in mean egg counts was described using a smoothing spline (Hastie & Tibshirani, 1990).

RESULTS

Species composition, prevalence and profiles. Taxonomic analysis of Svalbard reindeer abomasum helminths confirmed the existence of six taxa found in an earlier study (Bye & Halvorsen, 1983). *O. gruehneri* and *M. marshalli* accounted for more than 95% of the abomasum parasite population found in adult reindeer of both sexes and all adult reindeer were infected with both (Table 4.1). However, the relative proportions of these two species appeared to differ between late summer/autumn (August/September/October) and late winter (February/March/April). The proportion of *M. marshalli* was higher, relative to *O. gruehneri* in the late winter because of an apparent increase in the abundance of *M. marshalli* (Table 4.1). Male and female reindeer had similar species profiles and abundances in late summer/autumn but in late winter the small sample of males appeared to have a disproportionately high proportion of *M. marshalli* relative to *O. gruehneri*.

O. gruehneri, *m. arcticus*, the third most abundant abomasum nematode, accounted for no more than 5 % of the total worm burden but infected more than 90% of the adult reindeer (females=93%, males 100%). In late summer the abundance in adult female and male reindeer were similar at 385 (SD=319) and 438 (SD=307) worms per host. In calves prevalence was around 5% in late summer but abundance was on average less than one worm per abomasum. In late winter abundance tended to be higher (41 nematodes per host) but prevalence was still low (9%). The other three species occurred erratically at low prevalence and abundance (Table 5.1).

Table 5.1. Prevalence (%), abundance of infection and species profiles for nematode taxa in the abomasum of Svalbard reindeer. Estimates are given separately for adult females, males and calves and for samples collected in the late summer period (August-October) (upper table) and late winter period (February-May) (lower table). Data are combined for both reindeer populations.

Species	Females			Males			Calves		
	Prevalence	Abundance (SD)	Species profile %	Prevalence	Abundance (SD)	Species profile %	Prevalence	Abundance (SD)	Species profile %
<i>O. morph gruehneri</i>	100	7124 (3999)	72	100	7039 (5391)	68.6	44	15 (26)	1.3
<i>M. morph marshalli</i>	100	2290 (1761)	23	100	2621 (1622)	25.5	97	1192 (1140)	98.6
<i>O. morph arcticus</i>	93	385 (319)	3.9	100	438 (307)	4.3	5.2	0.7 (3.0)	0.06
<i>T. circumcincta</i>	26	49 (154)	0.5	60	142 (192)	1.4	0	0	0
<i>T. trifurcata</i>	1.8	1.4 (11)	0.01	10	18 (54)	0.17	0	0	0
<i>M. morph occidentalis</i>	0.9	2.0 (21)	0.02	5	4.0 (18)	0.04	2.6	0.3 (2.1)	0.03
Total		9852 (4562)			10262 (5940)			1209 (1133)	

Species	Females			Males			Calves		
	Prevalence	Abundance (SD)	Species profile %	Prevalence	Abundance (SD)	Species profile %	Prevalence	Abundance (SD)	Species profile %
<i>O. morph gruehneri</i>	100	6712 (3774)	52	100	2788 (1480)	25	58	201 (531)	3.1
<i>M. morph marshalli</i>	100	5951 (3587)	46	100	8275 (3772)	74	100	6422 (3000)	96.1
<i>O. morph arcticus</i>	79	221 (286)	1.7	50	126 (178)	0.9	17	42 (128)	0.6
<i>T. circumcincta</i>	25	46 (108)	0.36	33	20 (31)	0.1	8	3.8 (13)	0.06
<i>T. trifurcata</i>	2.8	2.3 (14)	0.02	0	0	0	0	0	0
<i>M. morph occidentalis</i>	11	11 (32)	0.08	17	19 (46)	0.2	17	5.2 (13)	0.09
Total		12943 (5016)			11227 (4012)			6675 (3210)	

Modelling k. For both *M. marshalli* and *O. gruehneri* models with k varying with \hat{y} were found to fit the data significantly better than models with a constant k (Table 4.2). For *M. marshalli* the log-linear model gave a marginally lower $-2 \log$ likelihood value than the linear model for k . The linear model for k had a much lower $-2 \log$ likelihood value for *O. gruehneri* than the log-linear model, while the opposite was true for *O. gruehneri*, m. arcticus. We used the best-fit dispersion models in the subsequent formal analyses of worm burdens of *M. marshalli*, *O. gruehneri* and *O. gruehneri*, m. arcticus, respectively. Analyses of residuals showed that these gave a good descriptions of the variance structure in the data.

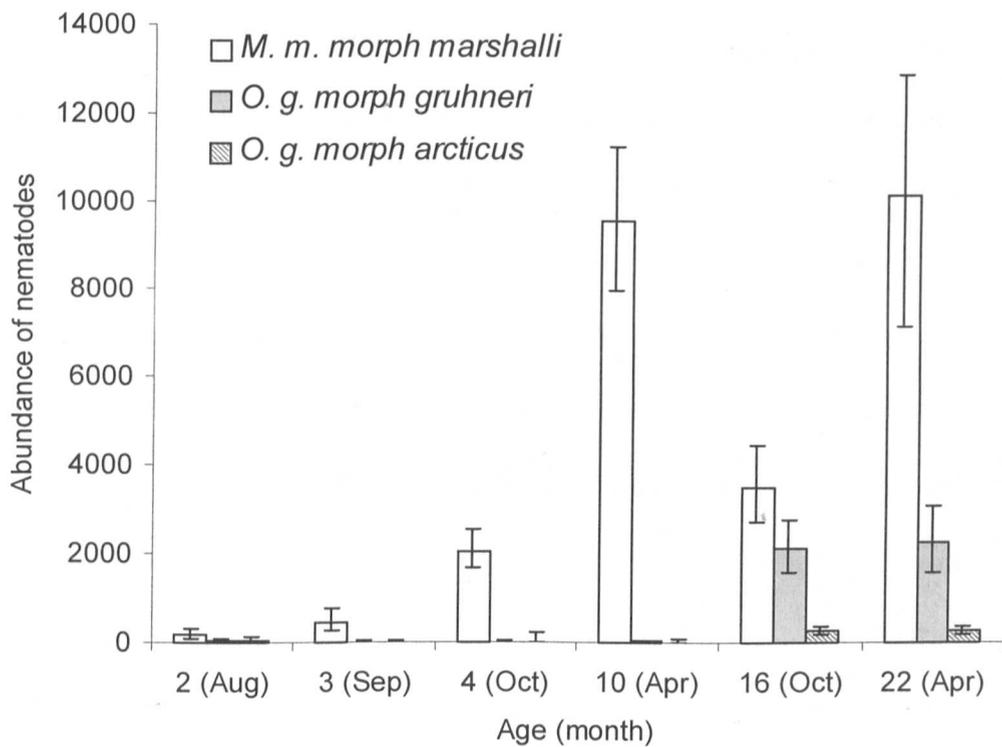
Table 2. Analysis of likelihood ratio table for the fit of different functions for the negative binomial dispersion parameter k to the data on the intensity of *M. marshalli*, *O. gruehneri* and *O. gruehneri*, m. arcticus in calves and adult female Svalbard reindeer. $-2 \log l$ gives the $-2 \log$ likelihood of the model, np the number of parameters fitted, and P, the P-value, for the test for a significantly better fit using the linear and log-linear functions when compared to the model with a constant k . The predicted abundance of infection (\hat{y}) was estimated using an over-parameterised model including a non-linear age - intensity relationship, and asymptotic values with age depending on year, month and reindeer population sampled (see main text).

Function	<i>M. marshalli</i> , <i>M. marshalli</i>			<i>O. gruehneri</i> , m. gruehneri			<i>O. gruehneri</i> , m. arcticus		
	-2 log l	np	P	-2 log l	np	P	-2 log l	np	P
$k = \text{constant}$	3938.08	30		3884.81	30		2459.35	30	
$k = k_1 + k_2 \hat{y} 10^{-3}$	3932.00	31	0.01	3684.27	31	<0.0001	2398.84	31	<0.0001
$k = \exp(k_1 + k_2 \hat{y} 10^{-3})$	3930.77	31	0.00	3703.24	31	<0.0001	2387.98	31	<0.0001

Age intensity relationships. At the end of their first summer (October), calves were infected with on average 2100 adult *M. marshalli* and 22 *O. gruehneri* in Sassendalen (Fig. 5.1). By April of their first winter the abundance of *M. marshalli* had increased to around 9000 (Fig. 5.1), similar to that found in yearlings of 22 months (Fig. 5.1) and not significantly different from adults in late winter ($\chi^2=0.22$,

df=1, P=0.63, see Fig 5.2a). Thus after the first winter there was no evidence of the abundance of *M. marshalli* infection changing with age (Fig 5.2a & b). The abundance of *M. marshalli* in Sassendalen was about twice as high as in Colesdalen and this was significant for both calves and yearlings ($\chi^2=58.76$, df=1, P<0.001) and adult reindeer ($\chi^2=25.77$, df=1, P<0.001, see Fig. 5.2a & b).

Fig 5.1. The estimated abundance (with 95 % confidence intervals) of adult *M. marshalli*, *O. gruehneri* and *O. gruehneri*, m. arcticus in reindeer from Sassendalen in calves and yearling females. The abundances were estimated using a linear model with log link function and assuming a negative binomial error distribution with k



fitted as a linear function of the estimated abundance of infection for *O. gruehneri* and a log linear function for *M. marshalli* and *O. gruehneri*, m. arcticus. For Colesdalen the estimates are a multiplicative factor α of the plotted ones, with estimates: $\alpha_{M. marshalli} = 0.30$, $\alpha_{O. gruehneri} = 3$, $\alpha_{O. gruehneri, m. arcticus} = 0.77$. Estimates of parameters in the model for the negative binomial parameter k : $k_1 M. marshalli = 0.91$, $k_2 M. marshalli = 0.25$, $k_1 O. gruehneri = 0.016$, $k_2 O. gruehneri = 2.59$, $k_1 O. gruehneri, m. arcticus = -4.66$, $k_2 O. gruehneri, m. arcticus = 23.97$.

In contrast to *M. marshalli*, the abundance of *O. gruehneri* did not increase significantly from October to late winter in the reindeer's first year (Fig. 5.1, $\chi^2=1.81$, df=1, P=0.18). However, during the second summer of life the abundance

increased by a factor of 40 (from less than 50 to more than 2000, Fig. 5.1). The pattern of the age intensity relationship differed between the two populations ($\chi^2=12.72$, $df=1$, $P=0.0001$, Table 4.3: Model 9 versus 7). In Colesdalen the increase in abundance was relatively fast, reaching an asymptote of about 11000 in their second year, whereas in Sassendalen, the rate of increase was slower reaching the asymptote in their third year (Fig 5.2c & d). The asymptotic levels of infection differed significantly between our two study populations ($\chi^2=4.49$, $df=1$, $P<0.05$). Interestingly the population differences in *O. gruehneri*, m. *gruehneri* abundance were reversed compared to *M. marshalli* with *O. gruehneri* abundance in adult reindeer lower in Sassendalen and on average 0.73 of that in Colesdalen (Figure 5.2c & d).

Age intensity patterns in *O. gruehneri* m. *arcticus* were similar to those observed for *O. gruehneri*. The abundance of *O. gruehneri*, m. *arcticus* was very low through the reindeers' first year but by the end of their second summer burdens had increased to the asymptotic value at around 400 (Fig. 5.2e & f). Although relatively low, significantly higher abundances were found in Sassendalen ($\chi^2=3.89$, $df=1$, $P<0.05$).

There was no evidence for acquired immunity since there was no decrease in the abundance of infection in any of the species with increasing age (Fig. 5.2). The dispersion parameter *k* also increased from calves to older animals, but showed no evidence for a continuing increase at higher ages (Fig. 5.2). The estimates for *k* in adult reindeer were in the range of 1.1-5.9 for *M. marshalli*, 2.7-16.9 for *O. gruehneri*, m. *gruehneri* and 0.1-2.6 for *O. gruehneri*, m. *arcticus* and showed little evidence for a change with age except for a decrease for *O. gruehneri*, m. *arcticus* in the samples from Sassendalen (Fig. 5.2f)

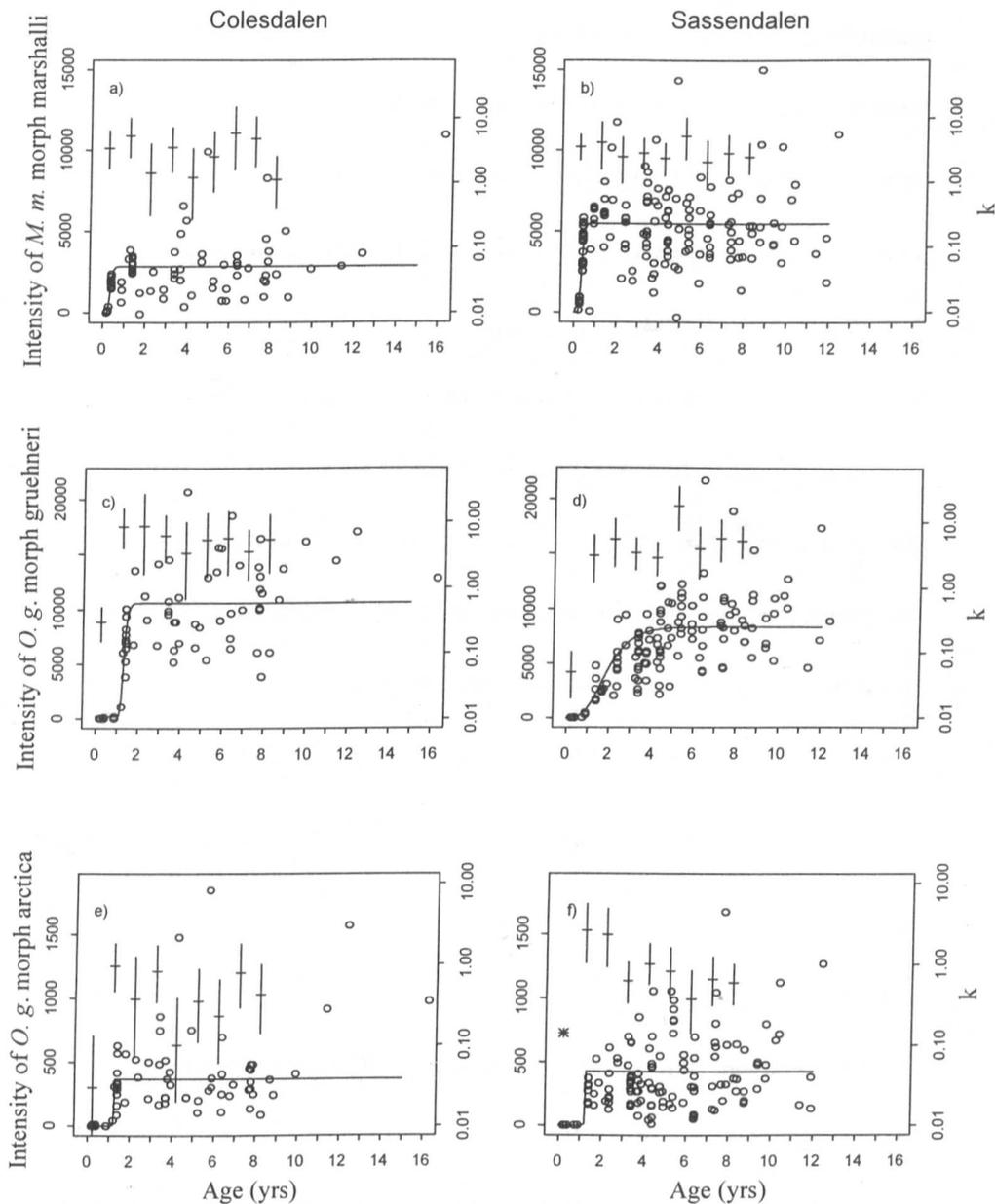


Fig 5.2. Gompertz age-intensity curves and estimates of k (crosses) with 95% confidence intervals for adult *M. marshalli* (a, b), *O. gruehneri* (c, d) and *O. gruehneri*, m. arcticus (e, f) sampled from the calves and adult female reindeer in Colesdalen (a, c, e) and the Sassendalen (b, d, f). Observed values for the nematode taxa corrected for seasonal and between year variation in nematode abundances are plotted around the curves. The seasonal components of the model are shown in Fig. 5.3. For *M. marshalli* there were no significant between year variation in the asymptotic abundance of infection (Table 3). The fitted curves and adjusted residuals for *O. gruehneri* and *O. gruehneri*, m. arcticus are standardised for the 1997-98 winter. Estimates of k are given for each age class of reindeer with animals older than 8 years combined. The 95% confidence interval for *O. gruehneri*, m. arcticus in calves in Sassendalen was $[0, \infty)$ and only the parameter estimate is plotted (*). (Estimates of the negative binomial parameter k in the overall model: $k_1 M. marshalli = 0.67$, $k_2 M. marshalli = 0.12$, $k_1 O. gruehneri = 0.043$, $k_2 O. gruehneri = 0.69$, $k_1 O. gruehneri$, m. arcticus = -3.18, $k_2 O. gruehneri$, m. arcticus = 8.55).

When modelling the change in k as a continuous function of increasing age while controlling for the effect of the abundance of infection on k , the cut off points (c) were chosen as $c=1$ year for *M. marshalli*, $c =2$ years for *O. gruehneri*, m. arcticus and $c =3$ years for *O. gruehneri* on the basis of the age intensity profiles (Fig. 5.2). The analysis suggested that k decreased with increasing age for *M. marshalli* ($\chi^2=8.40$, $df=1$, $P=0.004$, $k_3 M. marshalli = -0.075$) even though there was no such pattern in k when estimated for each age class separately (Fig. 5.2a,b). The difference in outcome of these two analyses is likely to be due to the confounding effect on k of the strong seasonal variation in abundance of *M. marshalli* which was not controlled for when the age specific estimates of k were calculated. The slopes for k against age were also negative for *O. gruehneri*, m. gruehneri and *O. gruehneri*, m. arcticus but not significantly different from zero (respectively: $\chi^2=0.63$, $df=1$, $P=0.43$, $k_3 O. gruehneri, m. gruehneri = -0.14$, and $\chi^2=2.87$, $df=1$, $P=0.09$, $k_3 O. gruehneri, m. arcticus = -0.072$).

Seasonality of infection

Worm burdens. The abundance of adult *M. marshalli* showed a significant seasonal cycle captured by fitting the annual sine curve (Table 5.3 and Fig 5.3a). In all four years there were consistently higher abundances at the end of winter than in late summer. The two populations differed significantly in the mean and amplitude of the cycle in abundance of adult *M. marshalli* (Table 4.3, model 5 versus 4 & 4 versus 3) However there was no significant variation in this pattern between years (Table 4.3, models 6,7,8 versus 5, $P>0.4$). Female reindeer in Sassendalen had a seasonal mean abundance of 5473 and amplitude of 2983, compared to a mean abundance of 2826 and amplitude of 1725 in Colesdalen. Although the amplitude of the fluctuation was

Table 3. Comparison of models for the abundance of adult *M. marshalli*, *O. gruehneri* and *O. gruehneri*, *m. arcticus* in calves and adult female Svalbard reindeer. ai denotes the Gompertz age – intensity model given by $\hat{y} = \alpha + (\gamma - \alpha)\exp(-\exp(-\kappa(\text{Age}-\phi)))$ where α , γ , κ and ϕ are parameters estimated from the data. $\mathcal{N}(S)$ denotes fitting γ in the Gompertz function as a sine function $\gamma = a + A(\sin(2\pi(\text{season} - \delta)))$ where a , A and δ are parameters estimated from data. Parameters were allowed to vary with the predictor variables reindeer population (P) and year (Y) of sampling through a log link function. For example, model 5 is a model with a sine function for γ , and with α and a varying with reindeer population. PxY denote an interaction between the predictor variables, reindeer population (P) and year (Y). Adding a term letting κ and ϕ vary between the reindeer populations in the most complex model above with significant terms for α and γ is denoted + $\kappa(P) + \phi(P)$, and dropping a term giving a constant amplitude in the seasonal fluctuations in both populations is denoted - $A(P)$. np gives the number of parameters fitted in the model, -2 logl the -2 loglikelihood value of the model and P the significance level when comparing the model with the last significant model above. The best fitting models are highlighted with bold numbers. For all models the error distribution were assumed to be negative binomial with dispersion parameter $k = \exp(k_1 + k_2 \hat{y} 10^{-3})$ for *M. marshalli* and *O. gruehneri*, *m. arcticus*, and $k = k_1 + k_2 \hat{y} 10^{-3}$ for *O. gruehneri*.

Model	Terms	<i>M. marshalli</i> , <i>M. marshalli</i>				<i>O. gruehneri</i> , <i>m. gruehneri</i>				<i>O. gruehneri</i> , <i>m. arcticus</i>			
		Np	-2 logl	P	-2 logl	P	-2 logl	P	-2 logl	P	-2 logl	P	
1	Intercept	2	4204.74		4231.58		2680.12						
2	Ai	6	4109.98	<0.0001	3764.20	<0.0001	2461.64	<0.0001					
3	ai($\alpha(P)$)	7	4096.13	0.0002	3753.73	0.001	2457.70	0.05					
4	ai($\alpha(P)$, $\gamma(P)$)	8	4081.83	0.0001	3738.58	0.0001	2451.03	0.01					
5	ai($\alpha(P)$, $\mathcal{N}(S(\alpha(P)$, $A(P))))$)	11	3950.96	<0.0001	3726.66	0.008	2424.97	<0.0001					
6	ai($\alpha(P)$, $\mathcal{N}(S(\alpha(P+Y)$, $A(P))))$)	14	3950.43	0.91	3721.09	0.13	2410.88	0.003					
7	ai($\alpha(P)$, $\mathcal{N}(S(\alpha(P+Y)$, $A(P+Y))))$)	17	3945.15	0.44	3699.25	0.0001	2404.53	0.10					
8	ai($\alpha(P)$, $\mathcal{N}(S(\alpha(PxY)$, $A(PxY))))$)	23	3943.60	0.83	3694.96	0.64	2397.86	0.16					
9	+ $\kappa(P) + \phi(P)$	+1	3947.89	0.21	3686.53	0.002	2410.88	0.99					
10	- $A(P)$	-1	4006.43	<0.0001	3687.53	0.21	2413.26	0.12					

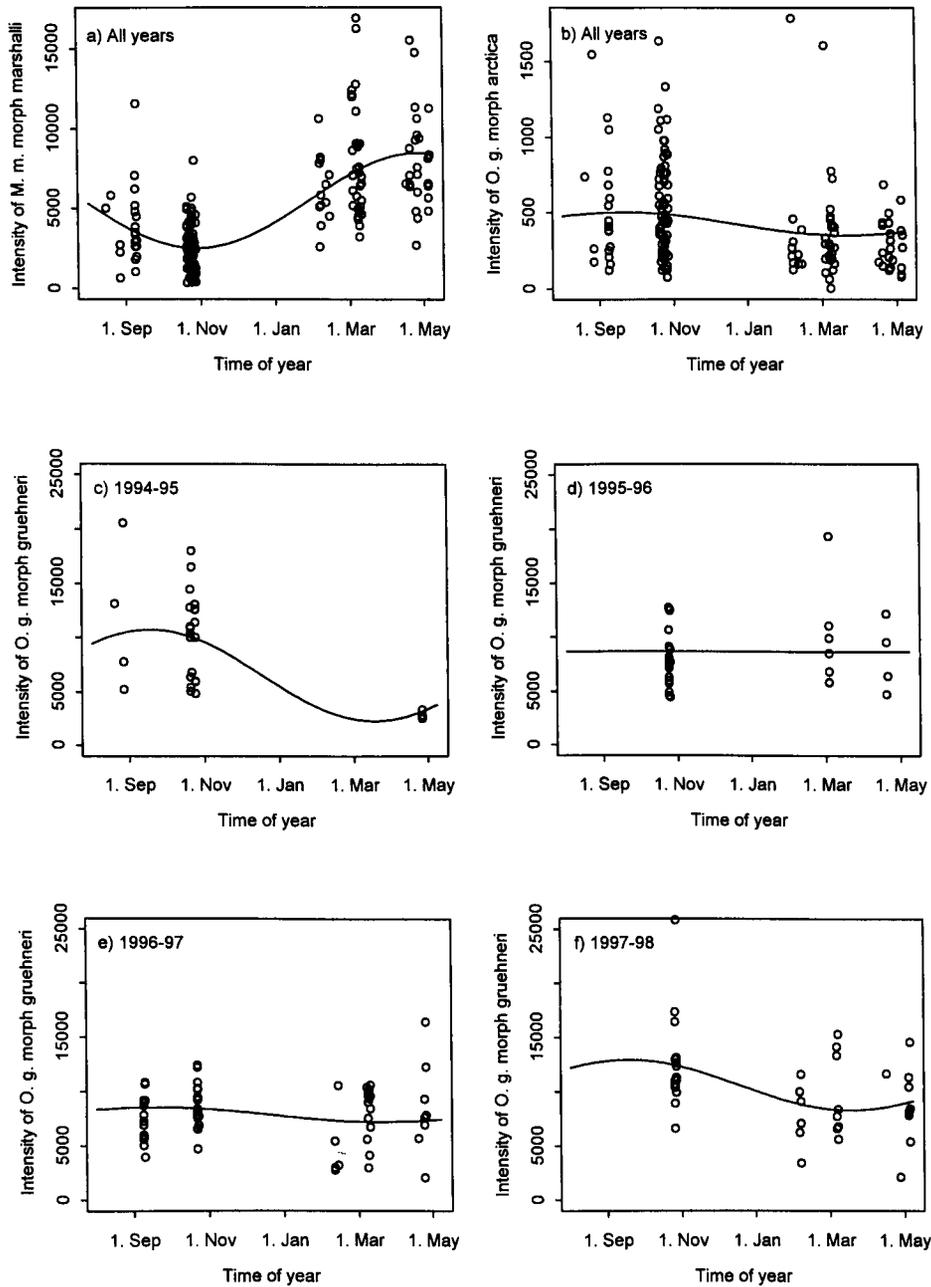


Fig 5.3. Sine curves describing the seasonal component of the variation in the abundance of adult abomasal nematodes from calves and adult female Svalbard reindeer. a) *M. marshalli* (all years), b) *O. gruehneri*, m. arctica (all years) and c-f) *O. gruehneri*. Observed values in nematode abundances are plotted around the curves for the nematode taxa correcting for reindeer age and between year variation from the best-fit models. In Fig 5.3 a) and b) the curve for *M. marshalli* and *O. gruehneri*, m. arctica is standardised to the Sassendalen reindeer population and for *O. gruehneri*, m. arctica the asymptotic abundance with reindeer age is for the 1997-98 winter. The curves for *O. gruehneri* are given for samples from the Colesdalen reindeer population and the winters c) 1994-95, d) 1995-96, e) 1996-97 and f) 1997-98.

higher in Sassendalen than in Colesdalen it was a similar proportion of the mean in each population (0.55 and 0.61 respectively). The seasonal fluctuations were significant in both populations ($P < 0.0001$) and did not differ significantly in phase (governed by δ , $\chi^2 = 0.45$, $df = 1$, $P = 0.50$).

There was also a significant seasonal component in the abundance *O. gruehneri* (Table 4.3), but in contrast to *M. marshalli* infections, this seasonality was not consistent between years and giving a significant interaction with year ($\chi^2 = 27.41$, 6df, $P < 0.0001$, Table 4.3, Model 7 vs 5). In two out of four years, 1995-96 and 1996-97, the abundance of *O. gruehneri* did not change significantly from the late summer to the late winter period ($P > 0.1$, Fig 5.3b, e). However, in 1994-95 and 1997-98, there was a drop in abundance from late summer to late winter ($P < 0.005$, Fig 5.3c, f). Also for *O. gruehneri*, *m. arcticus* there was a seasonal drop in abundance over the winter period as indicated in some years for *O. gruehneri* ($\chi^2 = 49.14$, 1df, $P < 0.0001$, Figure 5.3b).

Faecal egg output. Unfortunately to date we have no abomasum nematode abundance data from animals between May and mid-August because no reindeer were culled in this period. However, we do have substantial faecal egg count data collected from live animals in one of the study populations (Colesdalen/Reindalen) between April and September. In April output of both the *M. marshalli* taxa eggs and *Ostertagia* eggs was low and very similar at about 5 (SD=3.9) and 8 (SD=6.2) eggs per gramme (Fig 5.4a), but prevalence was high at 91% and 98%, respectively. As expected, as snow melt accelerated in June, the output of the *Ostertagia* eggs increased dramatically and continued to rise to a peak in July with a mean of 235 (SD=124) eggs per gramme of faeces. This peak level did not differ significantly between summers ($F_{4,675} = 0.688$, $P = 0.601$). From August onwards, *Ostertagia* egg

output declined. although *Ostertagia* eggs were still 100% prevalent in September. By October the *Ostertagia* egg prevalence had fallen to 65% and mean egg output had dropped to 2.5 (SD=2.8) eggs per gramme faeces. In contrast, in June the *M. marshalli* taxa egg output declined so that by July eggs were found in very few (1.3%) of the faecal samples (Fig. 5.4a).

In the autumn *M. marshalli* taxa prevalence increased to 41% but the mean eggs per gramme in the faeces was still less than one (0.82, SD=1.01).

Adult reindeer treated with anthelmintics in late April/early May were infected with *Ostertagia* eggs by mid-July (Figure 5.5b), and although egg output was lower in treated than in untreated animals, the decline from July to September paralleled that found in the untreated adults. Despite low abundance of adult *O. gruehneri* in naïve calves the prevalence of *Ostertagia* eggs in July was 25% (n=20). By August this had increased to 55% (n=51, mean=14) and by September prevalence had declined again to 17% (n=24, SD=46.3). The decline in calves of *Ostertagia* egg output was parallel but lower than that of the treated adult reindeer group (Fig 5.4b). By this time prevalence of the *M. marshalli* taxa eggs in calves was 46% although mean egg output was less than one in both groups.

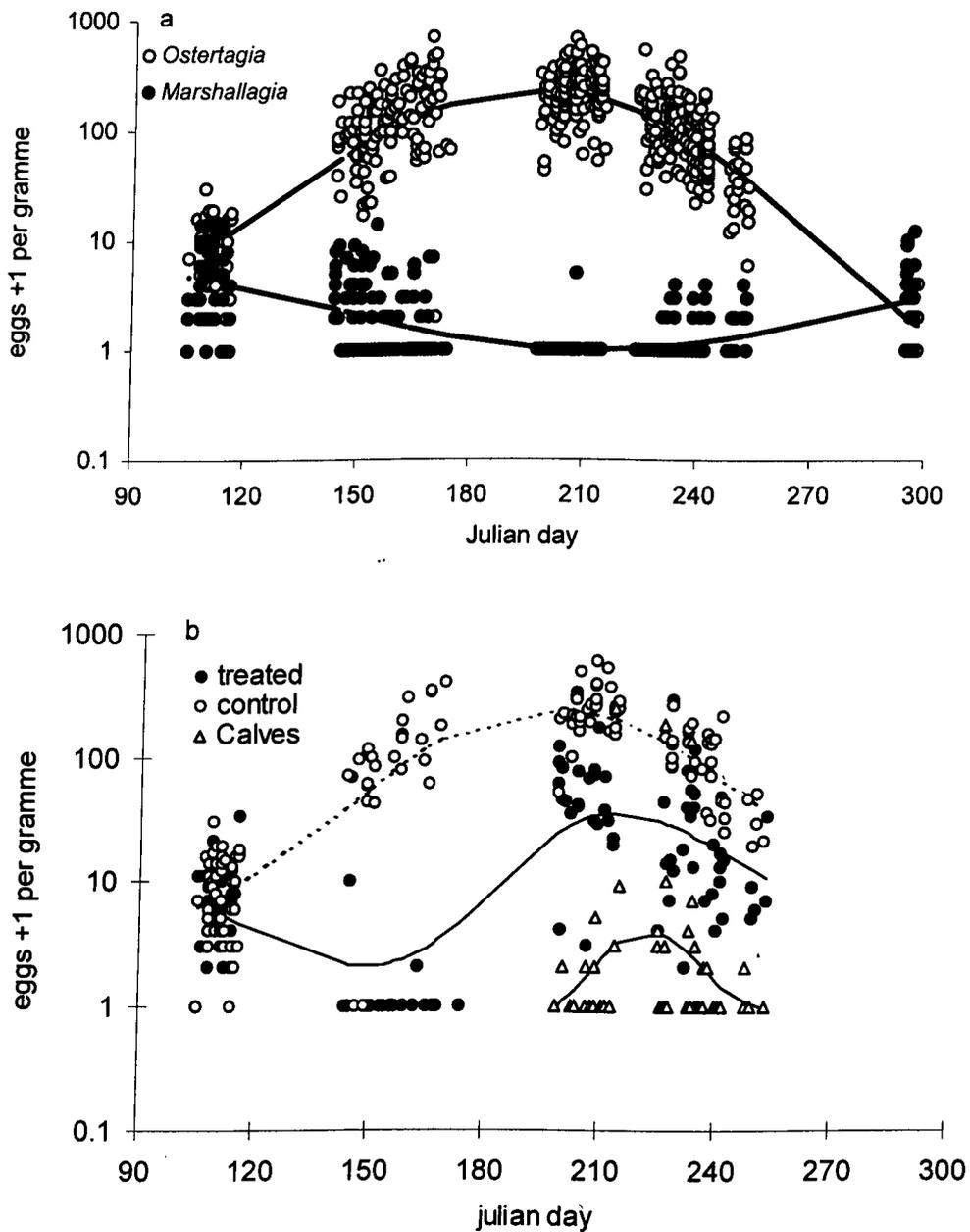


Fig 5.4 a) Faecal egg count data collected from adult females between April and September 1997 plotted against Julian day (days after 1st January) for trichostrongyle eggs. Filled circles: *M. marshalli* taxa, open circles: *Ostertagia* eggs (largely *O. gruehneri*, *m. gruehneri* and *O. gruehneri*, *m. arcticus*). A smoothing spline was fitted to both data sets to guide the eye.

b) Seasonal pattern in *Ostertagia* egg output for adult reindeer either treated with an anthelmintic (filled circles) or untreated (open circles) and untreated calves (open triangles) with fitted smoothing splines. This figure demonstrates that treated adult animals produce patent infections after the treatment wears off and that the autumn decline is similar in all cases.

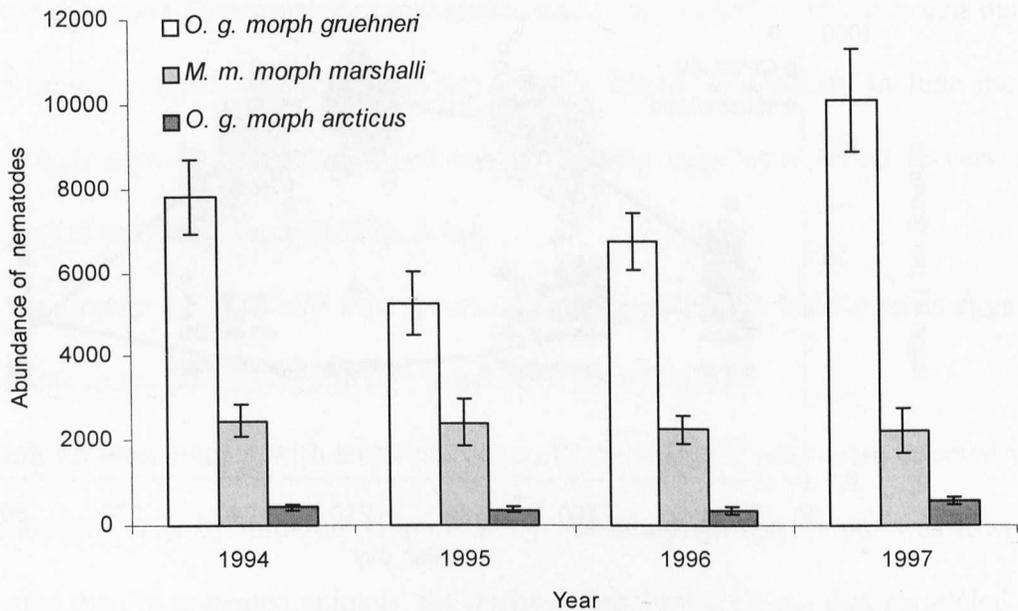


Fig 5.5. The abundance with 95% confidence intervals of *M. marshalli*, *O. gruehneri* and *O. gruehneri*, m. arcticus in October in adult female Svalbard reindeer from Sassendalen (1994-1998).

Annual variation in abundance. The abundance of adult *M. marshalli* did not differ significantly between the four years from 1994 to 1997 (Table 4.3, models 6,7,8 versus 5). In contrast, the abundance of both *O. gruehneri* and *O. gruehneri*, m. arcticus varied significantly between years (Table 4.3, Model 7 versus 5). In October, the abundance of *O. gruehneri* varied significantly between years from around 5000 in 1995 to 11000 worms per host in 1997 (Fig 5.5). The between year pattern in *O. gruehneri*, m. arcticus was similar to that found for *O. gruehneri* in October, with highest abundance in 1997.

Correlations in the intensity of nematode species. In adult reindeer, there was no correlation between *O. gruehneri* and *M. marshalli* in either late winter ($r=-0.045$, $P=0.71$, $n=96$) or in late summer ($r=-0.042$, $P=0.69$, $n=93$) and within each season there was no relationship within each reindeer population ($P>0.2$). As could be expected, there was a strong positive relationship between the closely related taxa *O.*

gruehneri and *O. gruehneri*, m. arcticus ($r=0.588$, $P=0.001$, $n=261$), This was true for adult reindeer from both reindeer populations ($r=0.496$, $P<0.001$, $n=129$ and $r=0.528$, $P<0.001$, $n=55$ for Sassendalen and Colesdalen respectively). There was no relationship between *O. gruehneri*, m. arcticus taxa and *M. marshalli* ($r=-0.058$, $P=0.407$, $n=207$).

DISCUSSION

Our study of gastrointestinal nematodes in Svalbard reindeer hosts provides compelling evidence of differences in the life histories and population dynamics of the two most abundant nematode species.

Aetiology of infection. Although worm burdens remain elevated throughout the adult age range, there are species differences in the rate of infection of hosts. Infection with *M. marshalli* increased rapidly in the host's first year of life, whereas very low levels of infection with *O. gruehneri* and *O. gruehneri*, m. arcticus were found in animals during their first summer and winter and increased mainly over their second summer. This is late compared with sheep and cattle where peak infection is reached during the first summer grazing (Anderson *et al.*, 1979, Armour, 1989). From the anthelmintic experiment we know that treated adult reindeer become re-infected within the same summer as treatment, therefore it is possible for transmission and development of a patent infection to occur within the host within one summer. We therefore suspect the low intensities of *O. gruehneri* in calves to be due to either 1) transfer of protective antibodies from the mother to the calf. 2) The gut morphology of immature ruminants differs from adults (McDonald Edwards & Greenhalgh, 1984) it may be unsuitable for *O. gruehneri* in calves. Or, 3) A difference between

nematode taxa in their distribution across vegetation communities the calf grazing strategy and feeding preferences may cause low contact rates with the infective larvae of *O. gruehneri* while they still pick up *M. marshalli*.

Contrasting seasonal patterns in the dominant taxa. *M. marshalli* had significantly higher adult worm abundance in late winter than in late autumn. Our previous observation of an increase in total abundance of nematodes over winter (Halvorsen *et al.*, 1999) was mainly due to the change in the abundance of adult *M. marshalli*. In calves, *M. marshalli* increased from around 2000 in October to around 9500 in April in Sassendalen. This increase could not be accounted for solely by the development of larvae present in the animals at the end of summer because the abundance of larvae was only 895 (range=186 to 4800, Halvorsen *et al.*, 1999). Therefore a large proportion of the increase in calves must be due to transmission from the pasture during the winter, and is also likely to cause the increase in *M. marshalli* in adult reindeer. Faecal egg output in this species also occurred mainly in the winter months.

The evidence for over winter transmission and egg production by *M. marshalli* suggests that this species has adapted to long winters. Still, preliminary work indicates that eggs deposited in the winter have high survival but no development at sub-zero temperatures (unpublished observations). Our working hypothesis is that the transmission strategy adopted by *M. marshalli* is one of remaining as eggs in the faeces through the winter and developing to the infective stage in the summer. If infective larvae stays in faeces, there is the possibility that coprophagy is of major importance for transmission in the winter when the availability of grazing for the reindeer is very sparse. *M. marshalli* has a wide

geographic distribution and host range (Urquhart *et al.*, 1996), but comparable data are lacking from other climatic zones. Whether the observed life-history pattern is an adaptation to High Arctic conditions or a general trait of *M. marshalli* is therefore unclear.

In contrast to *M. marshalli*, the output of *Ostertagia* eggs showed a seasonal pattern similar to that found in temperate trichostrongylosis with very low levels in the winter but a peak in mid summer (Anderson *et al.*, 1979). In addition the abundance of *O. gruehneri* in adult reindeer did not increase from late autumn to late winter. In fact, in two years, *O. gruehneri* declined over the winter months suggesting a rate of transmission and larval development that do not compensate for adult worm mortality. *O. gruehneri*, m. arcticus behaved in a similar way to *O. gruehneri*.

In models of the population dynamics of trichostrongyle nematodes, the seasonality in egg output is generally assumed to be due to changes in adult nematode population size sometimes modified by density dependent effects on nematode fecundity (e.g. Smith & Grenfell 1985, Leathwick Barlow & Vlassoff 1992). We found that in the late summer, *Ostertagia* egg output showed a similar decline between groups of animals with different worm burdens (calves, treated and untreated adults), suggesting that the drop in egg output in late summer is not purely due to density dependent effects on nematode fecundity. Also, egg output approaches zero in animals that have on average 10000 adult abomasal nematodes. This suggests that external environmental factors may have a strong influence on the seasonality in *Ostertagia* egg production in this system. Data on worm burdens from animals culled during the summer period will allow a more detailed analysis of this phenomenon.

Contrasting annual patterns between the dominant taxa. The pattern of increase in *M. marshalli* over the winter did not vary between years. This suggests that *M. marshalli* population dynamics is not strongly influenced by external environmental factors such as climate or host density. For example, *M. marshalli* burdens in Sassendalen did not vary with a two-fold change in reindeer population size (range = 400 - 1000 in the period 1994-98. Syssemannen unpublished helicopter counts). This pattern suggests that the nematode population dynamics may be governed by intrinsic density dependent mechanisms within the host. Compared to *M. marshalli*, the between year variation in abundance of *O. gruehneri* suggests that its population dynamics are sensitive to external factors and that it may play a role in the fluctuating population dynamics of its host.

Population differences in species profile. At the reindeer population level the abundance of *M. marshalli* and *O. gruehneri* were inversely related. *M. marshalli* burdens were higher in Sassendalen, the population with the lower *O. gruehneri* levels. This could suggest the existence of competitive interactions between species as has been found for other trichostrongyle systems (Diez-Baños, Cabaret & Diez-Baños, 1992). However, this interpretation was not supported at the level of individual reindeer since we found no evidence for a negative correlation between the intensity of *O. gruehneri* and *M. marshalli*. The lack of any significant correlation between these two species is consistent though with the idea that they have different dynamics in time and space. This is likely since grazing in winter, when *M. marshalli* appears to concentrate its transmission effort, is confined to relatively small wind blown ridges and areas where snow cover is thin. In contrast, in

the summer, when *O. gruehneri* appears to have its transmission window, reindeer forage over wide areas in the valley floors.

Immunity. The age intensity profiles of *M. marshalli*, *O. gruehneri* and *O. gruehneri*, *m. arcticus* do not provide evidence for any strongly acquired immune response affecting the abundance of these nematode taxa. While in domestic livestock, the abundance of abomasal nematodes is high in calves and lambs and lower in adults due to acquired immunity, in Svalbard reindeer, once the asymptote of infection is reached it remains high with increasing age.

It has been suggested that the dispersion parameter, k , may increase with age in the host population due to the density dependent effect of acquired immunity (Pacala & Dobson, 1988, Grenfell *et. al.*, 1995a,b). In this study we find no evidence for an increase in k with host age. After correcting for the effect of the abundance of infection on k , k was found to decrease with increasing age for *M. marshalli*. The crude age specific estimates of k also indicated a drop in k with age for *O. gruehneri*, *m. arcticus* in Sassendalen. This pattern has previously been found for trichostrongyle nematodes in sheep (Barnes & Dobson 1990). A drop in k with age may be the outcome of acquired immunity in combination with heterogeneities in the hosts' immune response with some hosts responding more efficiently than others (Grenfell *et. al.* 1995b). However, the effect of different possible population processes on the distribution of parasites has not been fully investigated (Fulford *et al.* 1992, Smith *et al.* 1995). Evidence for acquired immunity based on patterns in k will therefore be weak. We therefore find it unreasonable to interpret the inconsistent patterns in the k estimates as evidence for acquired immunity when this conflicts with the evidence from the age intensity relationship.

The probability of reindeer being pregnant in late winter is negatively related to the abundance of *O. gruehneri* (unpublished observations). One would expect this pathogenic effect to cause selection for immunity. Since the nutritional status or general condition of hosts may influence their ability to mount an immune response (Lloyd, 1995), the absence of evidence for acquired immunity in Svalbard reindeer may indicate the cost associated with an immune response is relatively high for these animals. Alternatively, direct measures of immune competence may be necessary to uncover its importance in this host–parasite interaction.

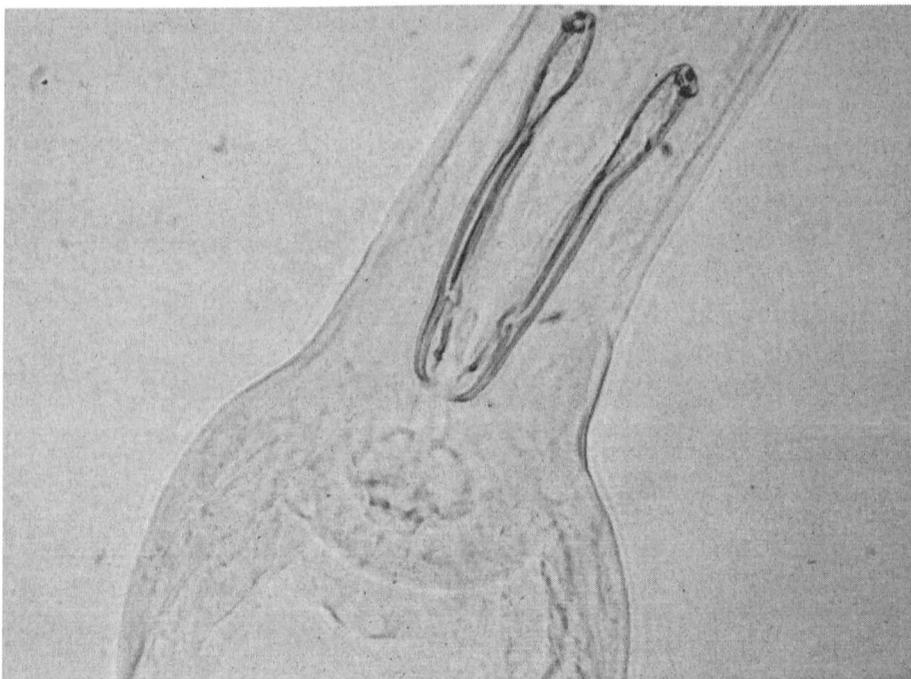
Statistical modelling of variation in k. Our analysis supported the suggestion that the dispersion parameter k of the negative binomial distribution varies with abundance (Grenfell *et al.*, 1995a,b). The best fit functional relationship between k and mean worm burden varied between the nematode taxa. We therefore suspect that if a common functional form for the relationship between k and the abundance of infection exists, it will be a more complex function than those we adopted. Still, analyses of residuals suggested that our models for k gave a good description of the variance structure in the data. It was also our experience that modelling these data using a constant k affected our conclusions significantly. The assumption of a constant k should therefore be checked when analysing complex data sets. Shaw *et al.* (1998) have recently suggested methods for analyses of grouped data on macroparasites with separate estimation of k 's within groups. Our suggestion of modelling k as a simple continuous function of the mean may be an alternative to their approach when analysing data with respect to continuous predictor variables or when sample sizes in different groups are small. Even though our simple models for k may not capture the true relationship between k and the mean, the method is likely to be robust to relatively substantial errors in the functional form of this relationship

as found for models for overdispersion in log-linear models (McCullagh & Nelder 1989). Simulated studies are needed to evaluate this claim.

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Ostertagia gruehneri.



CHAPTER 6

Contrasting regulation of fecundity in two abomasal nematodes of Svalbard reindeer (*Rangifer tarandus platyrhynchus*).

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**CONTRASTING REGULATION OF FECUNDITY IN TWO
ABOMASAL NEMATODES OF SVALBARD REINDEER
(*Rangifer tarandus platyrhynchus*).**

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SUMMARY

Stability of trichostrongylid populations indicates that some form of density dependent regulation occurs which could act through fecundity. We present evidence for intra-specific density dependent effects in one of two, dominant, abomasal nematode species (*Ostertagia gruehneri*) of Svalbard reindeer (*Rangifer tarandus platyrhynchus*). We found evidence in *O. gruehneri*, for density dependent regulation of female worm length in April, July and October 1999. However, it is only in July that female worm length explains the variation in the number of eggs *in utero* which is also related to egg production per female worm only in this month and not at other times of the year. The seasonal pattern in faecal egg output in this species focuses egg production in the summer months when conditions are favourable to transmission. In contrast, we found no evidence in the other common species (*M. marshalli*) for density dependent regulation of female worm length during or the number of eggs *in utero*. Faecal egg output in *M. marshalli* was positively related to worm burden but not to the mean number of eggs *in utero*. Neither inter-specific interactions nor host body condition appeared to influence worm fecundity. The contrasting pattern of density dependent regulation of fecundity provides further evidence for divergent life-histories in this nematode community.

INTRODUCTION

The long-term stability or cyclic behaviour found in many gastrointestinal parasite populations suggests that they are regulated by density dependent processes (Hudson & Dobson, 1995). One possible candidate is female worm fecundity which is often strongly affected by nematode abundance (Barger, 1985; Scott & Lewis, 1987) and is therefore an important parameter in the understanding of the dynamics of nematode populations (Anderson & Michel, 1977, Anderson & May 1991).

There are at least three possible density dependent mechanisms that may affect nematode fecundity and all involve retarded worm development. First, trickle challenge experiments with gastrointestinal nematode infections of sheep and cattle have shown that the faecal egg output is related to the size of the adult worm population in a density dependent manner (Michel, 1969; Boag & Thomas, 1977). This suggests direct intra-specific competition for space and/or resources that may reduce worm development and maturation (Tompkins & Hudson, 1999). Second, increasing burdens of nematodes are likely to stimulate a greater immune response that may contribute to the density dependent reduction in fecundity (Quinnell, Medley & Keymer, 1990). Specifically, the impact of the immune response in sheep has been found to reduce female worm development (Stear *et al.*, 1995) giving shorter, less well developed, worms that have fewer eggs *in utero*. However a complicating factor is that the size of the immune response is likely to be determined by the nutritional status of the host (reviewed in Van Houtert and Sykes 1996) so that animals in poor condition may allow larger worm burdens to establish. Third, inter-specific competition may also limit the fecundity and ultimately the population size of a cohabiting species (Dobson, 1985; Adamson & Noble, 1993). There is, however,

no evidence for inter-specific density dependent fecundity in gastrointestinal nematodes of ruminants. These three mechanisms may combine to generate the characteristic seasonal pattern of fecundity in gastrointestinal nematodes in which faecal egg output increases in spring in parallel with rising nematode burdens due to high transmission then declines again because density dependence reduces *per capita* egg production (Michel 1974). Worm burden and egg output may be reduced through a density dependent immune response (Quinnell *et al.*, 1990) but the drop in egg output in the autumn may also be due to senescence rather than changes in worm density (Shaw & Moss, 1989) and this may generate the illusion of density dependence. Nevertheless, previous studies have not been able to determine which of these mechanisms is the more important in the regulation of nematode fecundity. It is only recently that analysis of the inter-specific density dependent effects has been possible through the use of PCR techniques to aid in the often difficult identification of adult female worms (Dallas, 2000).

The aim of this study was to determine which of these mechanisms could explain the contrasting patterns of fecundity observed in the two, dominant, abomasal nematode species (*Ostertagia gruehneri* and *Marshallagia marshalli*) of Svalbard reindeer (*Rangifer tarandus platyrhynchus*). Both species show strong seasonality in faecal egg output which in *O. gruehneri*, is confined to the summer but in *M. marshalli* appears to be in winter (Irvine *et al.* 2000). Here we investigate whether: 1) Nematode fecundity is regulated through density dependent effects on female worm length, number eggs *in utero* or faecal egg output. 2) Seasonality in faecal egg output is due to changes in the abundance of infection, nematode fitness measured as worm length, or density dependent effects on reproduction. 3) Nematode fecundity is affected by seasonality in host condition suggesting the

possibility of an immune response that, combined with contrasting seasonality in the observed dynamics, may affect the two species differently. 4) Female fecundity is limited by inter-specific competition.

MATERIAL AND METHODS.

Reindeer hosts. Fifty adult female reindeer were culled during 1999 (10 in late April, 16 at the end of July and 24 in late October). Total body weight was recorded using a clock face spring balance (100 x 0.5kg). Body condition was assessed using a measure of back fat depth over the rump (10 cm in and at 45° from the base of the tail). Abomasa and faecal samples were recovered for the parasitology protocol outlined below. For the female worm measures we randomly selected 10 reindeer from April and July and 11 from October. Faecal egg counts and worm burdens from a larger data set of 128 adult female reindeer culled during the same months in 1995 to 1999 were also obtained.

Parasite populations. Adult abomasal worm burdens and faecal egg counts were collected and counted as described by Halvorsen *et al.* (1999) using methods modified from MAFF/ADAS (1986). Adult male worms were identified to species based on morphology as described in Irvine *et al.* (2000). For each of the 31 reindeer selected for the female worm measurements, 25 adult female worms were chosen at random and examined microscopically. In two hosts, fewer than 25 female worms were available. The number of eggs *in utero* in each female was counted using a compound microscope. (10x ocular and 40x objective). The image of each worm was captured using a camera mounted on a stereoscope at 6.25x magnification. The

length of each worm was measured using a segmented line drawn on screen and calibrated for the magnification used. Each female worm was then identified to species using a species-diagnostic DNA assay based on a polymerase chain reaction (PCR; Dallas *et al.* 2000).

Table 6.1. The proportion of each abomasal nematode species expressed as a percentage of the total number of adult female nematodes that were identified from 31 reindeer culled in April (10), July (10) and October (11). Numbers in brackets indicate the number of nematodes that were identified to species. (Note the low numbers of *M. marshalli* found in July and that these were distributed in only 5 reindeer hosts.)

Species	April	July	October
<i>O. gruehneri</i>	59% (n=147)	94.6% (n=209)	53.7% (n=144)
<i>M. marshalli</i>	41% (n=102)	5.4% (n=12)	42.3% (n=124)

Measures of adult female worm fecundity. Mean number of eggs *in utero* and mean female worm length was calculated for each nematode species in each reindeer host and, when analysed, weighted for the number of worms on which the mean was based. These means were assumed to be normally distributed. Few *M. marshalli* females were found in July (Table 6.1) and when less than three worms of a species were present per host, the data point was excluded from the analysis. The analysis tested whether a non-linear power function ($y=\alpha(x)^\beta$) provided a better fit than a linear relationship ($y=\alpha+\beta x^1 + \beta x^2 + \dots$) using linear and non-linear regression. Season was added as month (April, July or October) and both the slope and the intercept were allowed to vary. The abundance of the cohabiting species was fitted additively to determine the inter-specific density dependent effects. Total body mass or back fat thickness was added to the best-fit models to determine the effect of body condition as an indirect method of detecting the effects of an immune response. The analysis consisted of three stages. First, factors responsible for the variation in

female worm length were determined, second the number of eggs *in utero* was analysed. Third, the relationship between faecal egg output (a measure of fecundity) and the number of eggs *in utero* (standing crop) was then established. The number of eggs *in utero* can vary between individuals and is only an instantaneous measure but can give individual based information in support of analyses of faecal egg output. Individual reindeer faecal egg count data by species was divided by the number of females of that species in each host to give a measure of eggs per gramme faeces per female worm (epgf) for *O. gruehneri* and, because of the low egg output, eggs per kilogramme faeces per female (epkgf) for *M. marshalli*.

Analysis of faecal egg counts. Faecal egg counts and adult worm burden estimates from 128 adult and yearling female reindeer including the 31 deer used in the analysis of female worms were collected. In all samples the two species were identified and recorded separately (Irvine *et al.*, 2000). The relationship between faecal egg counts and worm burdens was analysed using generalised non-linear models with a negative binomial error distribution (Wilson & Grenfell, 1997). Curves were fitted by the maximum likelihood method. The negative binomial factor k was allowed to vary linearly with the mean or was held constant (Irvine *et al.*, 2000). Following other studies (Croll *et al.*, 1982, Anderson & Schad, 1985 and Michael & Bundy 1989) a power function was fitted to the faecal egg count data and in the absence of compelling biological rationale this was chosen in preference to the more complex Ricker or Gompertz functions (Lebreton, 1989). Preliminary examination of the data indicated that the gamma-type function (Bishop & Stear, 2000) was not appropriate for this data because no decline in egg output was observed at higher worm burdens. Intra-specific density dependence of *O. gruehneri* faecal egg output with the number of adult *O. gruehneri* was determined. Inter-

specific competition was analysed by additively fitting the abundance of adult *M. marshalli* to the model and we allowed the impact of *M. marshalli* to differ from that of *O. gruehneri* by fitting a separate parameter for each species. The *M. marshalli* faecal egg output was analysed the same way. The above models were re-run using the smaller data set (n=31) to confirm that these animals were representative and that the best-fit models were the same in both the larger and the sub-data set.

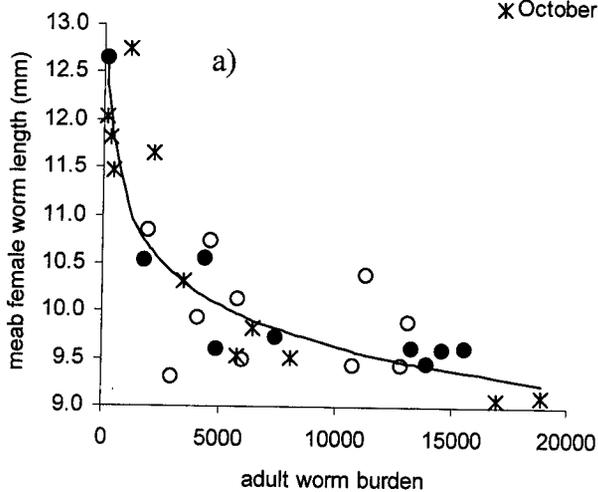
RESULTS

Factors affecting female worm length. Female worm length and worm burden in *O. gruehneri* showed a clear, negative density dependent relationship (Fig 6.1a) which did not vary with season. This was best described by a power function which explained more of the total variance (68%) than a linear function (62%) and used the same number of degrees of freedom (Table 6.2). There was no significant improvement in the model by allowing either the elevation term (α) or the power term (β) to vary with month. Variation in *O. gruehneri* worm length was unrelated to either *M. marshalli* burden ($P>0.3$) or host body condition (back fat depth or dressed carcass weight, $P>0.4$). The rest of the female worm fecundity analyses follows the structure given in Table 1, but only the statistics from the best fit models are quoted in the text. All other relevant models can be assumed to be non-significant.

In contrast to *O. gruehneri*, there was no linear or non-linear relationship between *M. marshalli* worm length and worm burden in any of the three months sampled (Fig 1b). In practice the analysis was based on 19 hosts from April and October because insufficient data were available from July for analysis. The two data points plotted for July refer to hosts where more than two *M. marshalli* females were measured.

O. gruehneri

○ April
● July
✱ October



M. marshalli

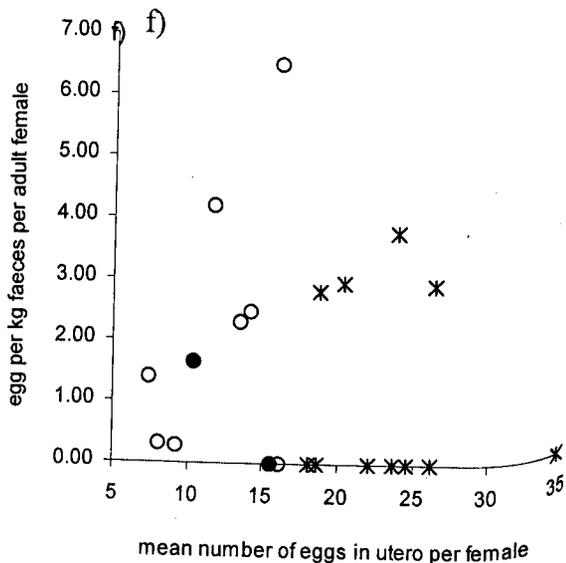
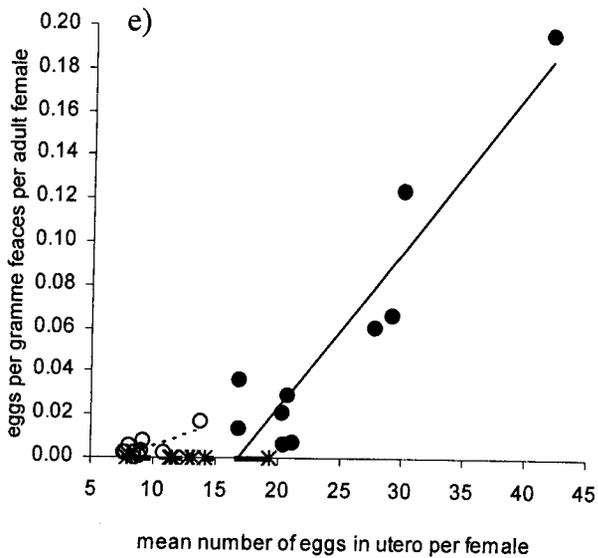
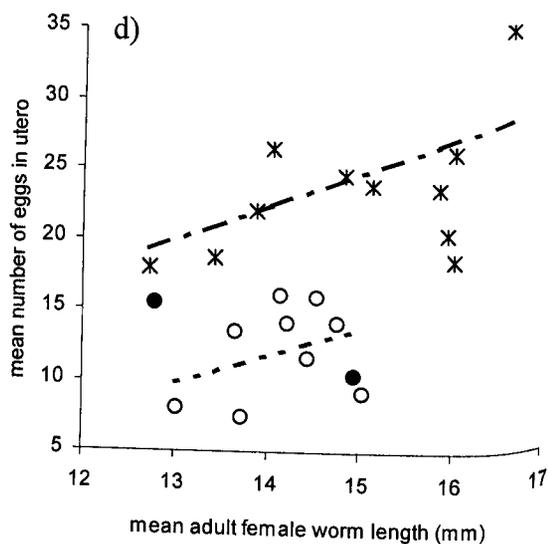
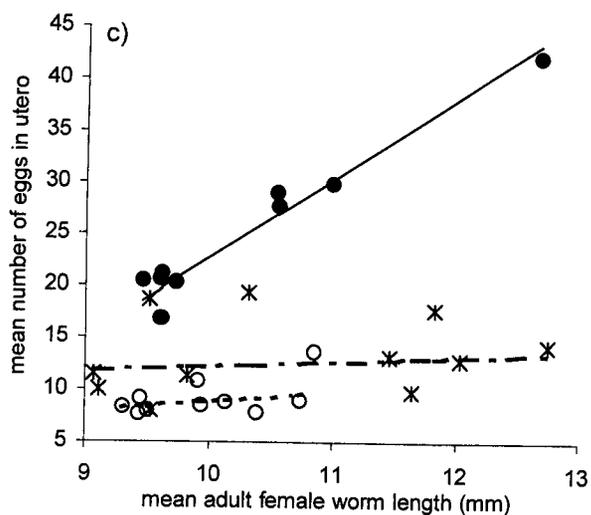
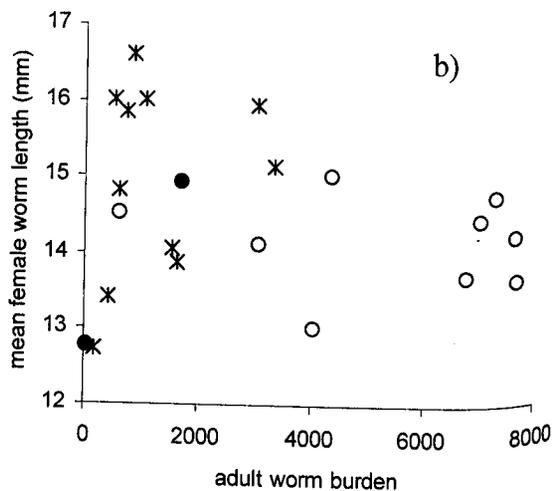


Fig 6.1. Data and predicted values for the best-fit models from the analysis of the relationships between the fecundity variables. plots a), c) & e) refer to *O. gruehneri* and plots b), d) & f) refer to *M. marshalli*. Data for April is represented by open circles, for July by filled circles and October by crosses.

- a) The relationship between *O. gruehneri* female worm length (Oglength) and the number of adults (Og). The fitted line represents the best-fit model where worm length is predicted by a power function $Oglength = \alpha(Og)^\beta$. (Parameter estimates for the fitted line are: $\alpha=17.3$, 95% CI = [14.91 to 16.98] and $\beta=-0.064$, 95% CI of -0.08 to -0.05. ($F_{1,29}=60.57$, $P<0.01$).
- b) *M. marshalli* female worm length (Mmlength) plotted on the abundance of *M. marshalli* in the same host. There was no significant relationship between the two variables.
- c) The relationship between mean number of *O. gruehneri* eggs *in utero* in females (eiu) and mean female Oglength (mm). The fitted lines represent the best fit from a model where eiu is predicted by month, Oglength and the interaction between month and Oglength. There is a positive relationship in July and no significant relationship in April and October. (Parameter estimates (\pm standard errors) for July are: $Ogeggs = -54.17_{(\pm 9.23)} + 7.68_{(\pm 0.92)} Oglength$)
- d) The relationship between mean number of *M. marshalli* eggs *in utero* (eiu) in females and the mean Mmlength (mm) in April and October. The fitted lines represent the best fit from a model where eiu is predicted by month and Mmlength. In July no line was fitted because the abundance and prevalence of *M. marshalli* was very low. (Parameter estimates (\pm standard errors) for the fitted lines are: *M. marshalli* $eiu_{April} = -20.81_{(\pm 13.68)} + 2.32_{(\pm 0.96)} Mm\ length_{April}$ and *M. marshalli* $eiu_{October} = -10.09_{(\pm 14.57)} + 2.32_{(\pm 0.96)} Mm\ length_{October}$)
- e) The relationship between *O. gruehneri* eggs per gramme faeces per female worm (epgf) and the mean number of eggs *in utero* (eiu) per adult female *O. gruehneri*. The fitted lines indicate the best-fit model where epgf was predicted by month with a month by eiu interaction. There was a significant positive relationship in July but not in April and October. (Parameter estimates (\pm standard errors) for the fitted line in July are *O. gruehneri* $epgf = -0.11_{(\pm 0.017)} + 0.007_{(\pm 0.0007)} Og\ eiu$)
- f) *M. marshalli* eggs per kilogramme faeces per female worm (epkgf) plotted on the mean number of eggs *in utero* per adult female *M. marshalli* for April, July and October. No significant relationship was detected even if the highest April epkgf is excluded.

Factors affecting the number of eggs in utero. The number of eggs *in utero* was significantly related to female worm length in *O. gruehneri* (Fig 6.1c). The model including the interaction of worm length and month explained 90% of the variation ($F_{5,25}=49.81$, $P<0.001$). The relationship was only significantly positive in July (Fig 6.1c). In April and October the numbers of eggs *in utero* were similar and low independent of worm length.

In contrast, the number of eggs *in utero* was significantly related to female worm length in *M. marshalli* in both April and October (Fig 6.1d). The relationships had similar slopes but different elevations because the number of *M. marshalli* eggs *in utero* was significantly higher in October than in April ($F_{1,17}=53.05$, $P<0.01$). The model explained 77% of the variation ($F_{2,17}=29.46$, $P<0.01$, Fig 6.1d).

Table 6.2 Comparison of models for the mean female length of adult female *O. gruehneri* (Oglength). Table shows analysis of sums of squares (Res SS) for the model of Oglength predicted by month, and worm burdens of *O. gruehneri* (Og) and *M. marshalli* (Mm) separately or in combination. A power function of the form $\alpha(\text{Og})^\beta$ (model 3) provided a better fit than a linear relationship (model 2). The mean female worm length for each reindeer host was weighted for the number of females of that species used in calculating that mean. MS=mean squares, df = extra degrees of freedom used in that model. The best fit model, 3, is in bold

Model	Resid df	Res SS	MS	Comparison	F	P
1= α (intercept)	30	384.41	12.81			
2= $\alpha+b(\text{Og})$	29	219.12	7.55	2v1	19.48	<0.01
3=$\alpha(\text{Og})^\beta$	29	121.52	4.34	3v2 (better)		
4= $\alpha(\text{month})(\text{Og})^\beta$	27	117.83	4.53	4v3	0.41	Ns
5= $\alpha(\text{Og})^{\beta(\text{month})}$	27	118.91	4.57	5v3	0.29	Ns
6= $\alpha(\text{month})(\text{Og})^{\beta(\text{month})}$	25	99.31	4.14	6v3	1.34	Ns
7= $\alpha(\text{Og}+\text{Mm})^\beta$	28	194.80	6.98	7v3		Ns

Eggs per gramme faeces per female and the number of eggs in utero relationships.

A strong positive relationship between eggs per gramme faeces per female and the mean number of eggs *in utero* was found for *O. gruehneri* in July but not in April or October. The best fit model included eggs *in utero*, month and the eggs *in utero* by month interaction ($F_{5,21}=26.27$, $P<0.01$, Fig 6.1e). The values of egg output per gramme faeces per female were very low and similar in April and October (Fig 6.1e).

In contrast variation in *M. marshalli* eggs per kilogramme faeces per female was not related to any of the variables tested (Fig 6.1f) even if the outliers are excluded.

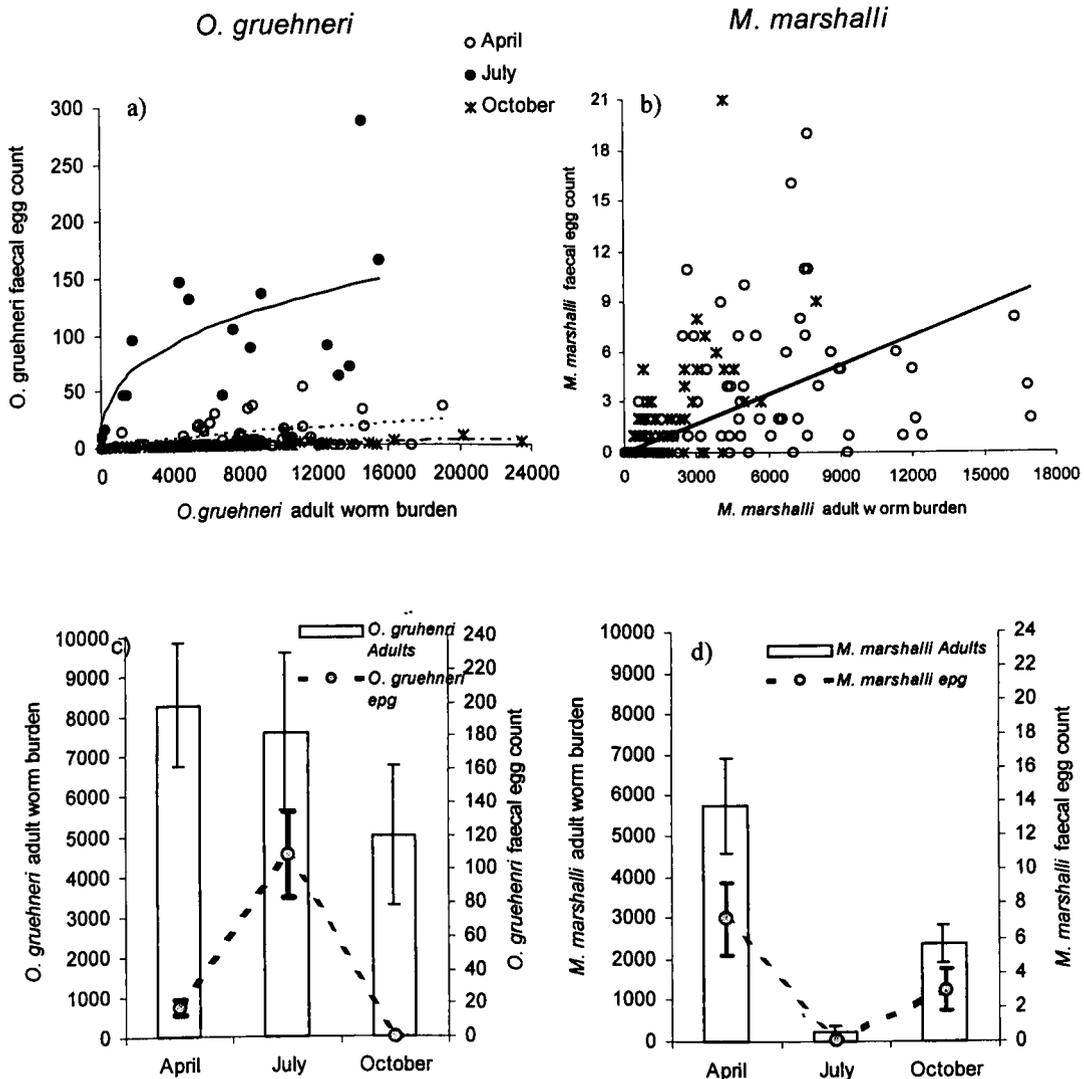


Fig 6.2. The relationship between faecal egg counts and adult worm burdens. Plots a) & c) refer to *O. gruehneri*, b) & d) refer to *M. marshalli*. The larger data set is displayed in a) & b) and the sub-set used in the analysis of individual female worms is shown in c) & d)..

- The relationship between faecal egg output (epg) and adult worm burden (Og) in April, July, and October for 128 reindeer sampled between 1995 and 1999. The fitted lines for each month are derived from a model where epg was predicted from adult worm burden using a power function ($\text{epg} = \alpha * \text{Og} * \text{Og}^\beta$). (Parameter estimates for fitted lines: $\alpha_{\text{April}} = 0.034$, 95% CI = [0.00023, 7.59], $\beta_{\text{April}} = -0.34$, 95% CI = [-0.95, 0.28]; $\alpha_{\text{July}} = 5.02$, 95% CI = [0.22, 63.57], $\beta_{\text{July}} = -0.65$, 95% CI = [-0.94, -0.30]; $\alpha_{\text{October}} = 0.0000121$, 95% CI = [0.000000007, 0.0093], $\beta_{\text{October}} = -0.65$, 95% CI = [-0.94, -0.30])
- The relationship between faecal egg output (epg) and Mm worm burden in April, July, and October for 122 reindeer sampled between 1995 and 1999. The best-fit model provides a linear fit that does not vary with month ($R^2 = 0.48$, $P < 0.01$).
- The mean and standard error for *O. gruehneri* adult worm burden (columns) and *O. gruehneri* faecal egg counts (line) for April July and October 1999 (n=31).
- The mean and standard error for *M. marshalli* adult worm burden (columns) and *M. marshalli* faecal egg counts (line) for April July and October 1999 (n=31).

Faecal egg counts and worm burden relationships. In the larger data set representing 128 adult female animals for which we had data on faecal egg output and worm burdens, *O. gruehneri* faecal egg output was higher in July than in April and October consistent with the seasonal peak for this species (Irvine *et al.*, 2000). A power function that allowed eggs per gramme faeces to vary with month provided a significantly better fit for the relationship between faecal egg output and worm burden than the equivalent linear model ($\chi^2=9.9$, $df=1$, $P<0.001$). Both the slope (α : $\chi^2=147.3$, $df=2$, $P<0.01$) and the power parameter (β : $\chi^2=7.3$, $df=2$, $P<0.01$) varied significantly with month (Fig 6.2a). β was negative and less than unity in July suggesting egg output during this month was density dependent (Fig 6.2a). In contrast, β was not significantly different from zero in April or October (Fig 6.2a). The pattern in *O. gruehneri* abundance and faecal egg output was the same in the smaller subset ($n=31$) of animals sampled in 1999 and the analysis confirmed that the same model provided the best fit. As found in an earlier study (Irvine *et al.*, 2000), the abundance of *O. gruehneri* for the sub-set of data remains high in all seasons yet the faecal egg output varies considerably (Fig 6.2c).

There was a simple linear relationship between faecal egg output and worm burden in *M. marshalli*. ($R^2=0.48$, $P<0.01$, $n=123$, Fig 6.2b) for the larger sample of animals. The percentage of the total variance explained was similar for both linear (8.2%) and power functions (8.8%). There were no significant differences between months or interactions between worm burden and month. The pattern on *M. marshalli* abundance and faecal egg output was the same in the smaller sub-set of animals sampled in 1999 and the analysis confirmed that the linear model also provided the best fit in this case. The abundance of *M. marshalli* exhibits a strong seasonal pattern with peak intensities in April and almost negligible levels in July

and faecal egg output mirrors this pattern confirming that worm burden is the main predictor of faecal egg counts in this species (Fig 6.2d).

In both these species, no additional variation in egg output could be explained by adding the rival cohabiting species and fitting a parameter that allows the impact of one worm species to differ from that of the other ($P > 0.1$).

DISCUSSION

This is the first study to demonstrate contrasting patterns of density dependence in fecundity of two cohabiting gastrointestinal nematode species in the wild. We found evidence in *O. gruehneri* for density dependent effects on worm length irrespective of season. Nonetheless, the effect of worm length on the number of eggs *in utero* was significant only in July, when peak egg production occurs. Furthermore, it appears that the observed seasonal changes in the number of eggs *in utero* in female *O. gruehneri* is matched by the seasonal pattern of *O. gruehneri* faecal egg output. In contrast, we found no evidence to support the hypothesis that *M. marshalli* worm length is related to the density of adult *M. marshalli*. However, there was evidence of a positive relationship between *M. marshalli* worm length and the number of eggs *in utero* but the latter was not strongly related to faecal egg output. Evidence for contrasting life histories in these two species has been described before (Irvine *et al.*, 2000) and it is notable that the pathogenic *O. gruehneri* (Langvatn *et al.*, 1999) is where we find strong evidence for density dependent fecundity.

Analysis of nematode fecundity is prone to Type 1 errors (Keymer & Slater, 1987) because of the highly skewed distributions commonly found for both the number of nematodes in the hosts and the estimates of their fecundity. In our analysis

of worm length and eggs *in utero* this problem was avoided since the worm burdens in the host sample set were relatively evenly distributed across the worm burden range (Fig 6.1a &b), and since the sample size (number of females), on which the estimates of worm length and eggs *in utero* were based, showed no increase with increasing worm burdens. The statistical problems associated with analysing faecal egg output is more difficult to solve, but can be partly overcome by using an appropriate error structure in the statistical analysis (negative binomial). The results from our analysis of faecal egg output are also corroborated by the worm length and eggs *in utero* analysis, and this is in agreement with the observed density dependent relationship suggesting the result may not be a statistical artefact.

For *O. gruehneri*, the intra specific density dependent relationship between adult worm burden and female length is notable in that there were no significant differences between months in either the elevation (α) or the degree of density dependence measured by the negative exponent (β) of the power function (Fig 6.1a). This is consistent with studies of *Teladorsagia circumcincta* in blackface sheep (Stear *et al* 1995) and *Heterakis gallinarum* in ring necked pheasants (Tompkins & Hudson, 1999). In both these studies, fecundity was strongly related to female worm length and in the case of *H. gallinarum* female worm length alone explained the variation in the number of eggs *in utero*. Nonetheless, our study is the first to show seasonal variation in the number of eggs *in utero*, which cannot be fully explained by density dependent regulation in female worm length. Worm length was only a good predictor of the number of eggs *in utero* in July. In both April and October when the mean temperature is below zero and the ground is snow covered, the number of eggs *in utero* was low. The seasonal pattern in eggs *in utero* is reflected in the low faecal egg output in winter (April and October) and high output in summer (July) and it is

this intrinsic seasonal pattern in eggs *in utero* that may be responsible for the observed seasonality in faecal egg output while density dependence is only important in the summer. However, because the egg output is markedly high in the summer, a large proportion of the annual egg output is probably under density dependent regulation. We suggest that these results provide empirical evidence that the seasonal pattern of faecal egg output may be an adaptive strategy to focus transmission efforts in the summer, months when the ground is snow free and the temperature is above zero and therefore infective stages of these nematode parasites can develop and successfully transmit to naïve hosts (Halvorsen *et al.*, 1999).

Although this study is the first to demonstrate density dependence in nematode fecundity in the wild, and confirms similar findings in sheep and pheasants (Gulland, 1992; Stear, Park & Bishop 1996; Tomkins & Hudson 1999), other studies have failed to detect this form of regulation (Shaw & Moss, 1989; Coyne & Smith, 1991; Hudson & Dobson 1997) and have implicated worm senescence or density dependent survival as the regulatory mechanisms. For example, in the red grouse/*Trichostrongylus tenuis* system where there is also no evidence for immunity (Hudson & Dobson 1997), it has been suggested that, rather than density dependent regulation of fecundity, egg output decreases due to the decrease in fecundity in older worms (Shaw & Moss, 1989). The seasonal fecundity in the present study might be explained by worm age. If worms were young and immature in April, of prime age and fecund in July and, in October, senescent, we might predict the observed pattern. However, we do not have any data on demography that may indicate worm age, other than worm length and there are no differences in length, possibly because worm burdens are consistently high across seasons and length is strongly governed by density dependence. Although it lies outside the scope of this

study, the role of worm demography in nematode population dynamics may be worth exploring to fully understand the pattern of fecundity in naturally infected wild and domestic ruminant systems.

Density dependent effects of immunity on fecundity have been described (Smith & Grenfell, 1985; Quinzel *et al.*, 1990). In the blackface sheep study (Stear *et al.*, 1995) there was evidence that worm length was governed by a density dependent immune response in the form of plasma immunoglobulin (IgA) which appeared to retard worm development and, consequently, reduce the numbers of eggs *in utero*. Without discounting the potential effects of immunity on fecundity, there are three lines of evidence that point to its limited involvement in the present study. First, there is no observed decline in total egg output at high worm burdens (Fig 6.2a). Second, analysis of the relationship between host age and worm intensity provides no strong evidence for acquired immunity (Irvine *et al.*, 2000). Third, using body condition as surrogate for nutritional status and assuming poor nutrition compromises the ability to mount an immune response (Van Houtert & Sykes, 1996) then the lack of a relationship between body condition and female worm length and the number of eggs *in utero* may also suggest that immunity is not important.

In the analysis of the *M. marshalli* population, we found no evidence for density dependent female worm length. However, as found in *O. gruehneri*, worm length was the best predictor of the number of eggs *in utero* and although worms had more eggs *in utero* in October than April, the slope of the relationship was similar in both months (Fig 6.1d). There was no relationship between faecal egg output per female worm and eggs *in utero* and this may be due to the very low level of faecal egg output observed in this species and raises questions about the value of the linear

relationship between faecal egg output and the density of adult *M. marshalli* (Fig 6.2c) when only 8% of the variation is explained. Regulation of fecundity in *M. marshalli* is difficult to explain using the density dependent hypothesis but the relationship between worm length and the number of eggs *in utero* may also reflect changes in nematode demography such as worm senescence. It is interesting, to note that when worm burden is low in October, mean eggs *in utero* is highest while the converse is true in April (Fig 6.2d). We also know that in July hosts have very few adult *M. marshalli* and that by October they have a resident *M. marshalli* population with high numbers of eggs *in utero*. The drop in the number of eggs *in utero* by April may be due to the age of the worms. However, relevant worm age data is difficult to obtain for a natural infection but would provide a valuable addition to our understanding of natural systems.

Earlier work has again not provided any indirect evidence for immunity to this species in reindeer (Halvorsen *et al.*, 1999; Irvine *et al.*, 2000) and, we found no relationship between body condition (surrogate for immunity) and either nematode worm length, or eggs *in utero*. *M. marshalli* shows very stable within year cycles in the adult population that do not vary between years or host age classes (Irvine *et al.*, 2000) indicating that regulation of this population occurs. It is unlikely that factors such as host grazing patterns coupled with the fluctuating host density (Langvatn *et al.*, 1999) are important because of this observed stability. The cycles may suggest a short lived adult population and the potential role of immunity can only be elucidated through developing direct measures of immune response such as ELISAs to circulating nematode specific antibodies.

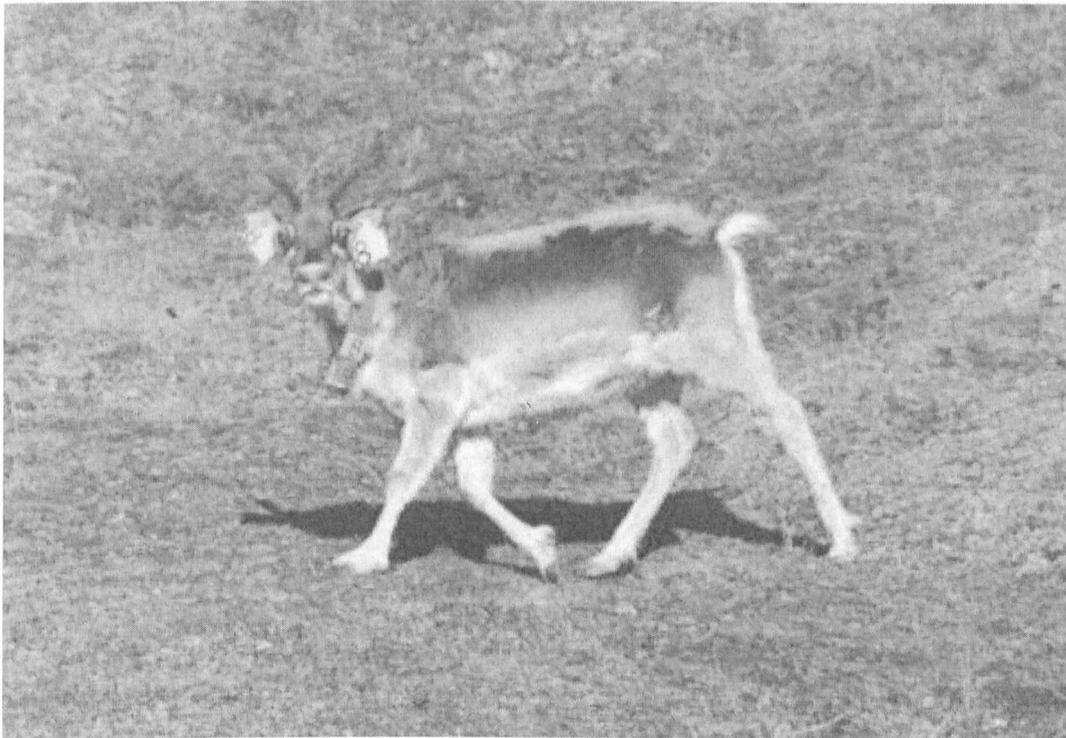
The lack of evidence for inter-specific competition is consistent with a previous study (Adamson & Noble, 1993). These two species may also avoid competition by adopting seasonally polarised periods of reproduction. Certainly, the lack of adult *M. marshalli* in July eliminates any possibility of inter-specific interactions but we also found no effects in winter when *O. gruehneri* abundance remains high and *M. marshalli* burden is increasing (Irvine *et al.*, 2000). This conclusion potentially simplify studies of fecundity traits in communities of parasitic nematodes. However, in the case of *M. marshalli*, sample sizes for the number of female worms measured are small, therefore, it might be profitable to investigate the density dependent effects of *O. gruehneri* on *M. marshalli* development and standing crop of eggs in a larger data set.

This study provides clear evidence that one mechanism governing fecundity in *O. gruehneri* is density dependent affects of the adult *O. gruehneri* population on female worm development. The role of immunity in the regulation of nematode fecundity is not clear and the development of direct measures of immune response are needed to explore this candidate mechanism. However, we also highlight the importance of seasonality in the pattern of fecundity and caution against studies that fail to take into account this possibility when analysing nematode dynamics. Whether the degree of density dependent fecundity found in *O. gruehneri* is sufficient to regulate the nematode population and thereby affect the host-parasite dynamics (Anderson & May, 1978) has not been investigated but there may be other density dependent mechanisms acting on other stages of the life cycle that are important. However, it is clear that the fecundity of nematode species in mixed natural infections can be

strikingly different and therefore resolution of data to the species level will be important in any analysis of natural populations.

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CHAPTER 7.

General Discussion and Future Directions

GENERAL DISCUSSION.

Traditionally ecological research has focussed on understanding the processes that lead to population regulation with many studies investigating mammalian herbivores and game birds (Grenfell et al., 1998, Hudson & Dobson 1998). Much research has focussed on candidate mechanisms that include direct density dependence (for resources), competition (intra- and inter-specific) (Van der Wal et al., 1998) and the role of predators (Rettie & Messier, 2000). The impact of parasites has also been suggested as a potential regulatory mechanism (Anderson & May 1978, May & Anderson, 1978). However, although parasites can undoubtedly have an impact on an individual animal (Gulland, 1995) it has been much harder to demonstrate that these impacts are responsible for the patterns of host dynamics at the population level. Parasites are intimately associated with their host species and the degree to which they interact, which depends on the species involved, has implications for the host population dynamics. Because of the diversity in parasite life-history strategies, investigations into the potential mechanisms that may regulate a natural host population need to determine the parasite population dynamics at the species level. This will increase the resolution of the data over studies that aggregate all the component species as one.

There is a very large literature dealing with parasites such as gastrointestinal nematodes in domestic animals. However, there are very few studies that have included parasites in an analysis of wild host population dynamics because data for parameterising the necessary models is often not available and theory generally advances faster than the availability of supporting empirical evidence. The only options may be to parameterise models by extrapolating from domestic literature

(e.g. nematode fecundity in Smith & Grenfell, 1985; Grenfell et al., 1995). Otherwise, aspects of the host parasite relationship have been treated as a phenomenon because the mechanisms have not been determined (e.g. the role of arrested development). This thesis therefore represents an attempt to understand the dynamics of a mixed natural parasite infection using reindeer as a model system and thereby support models (not presented here) of the host-parasite dynamics with the relevant parameter estimates. Ultimately, the best test of the effect of parasites is through experimental manipulation of parasite burdens. This has been successfully carried out on the Svalbard reindeer through the use of anthelmintics (Irvine, 2000, Chapter 3.2) that are routinely used in domestic livestock husbandry. The use of the molecular tools (Dallas et al., 2000a,b, & 2001; Chapter 3.3, 3.4 & 3.5), the ability to manipulate the parasite burdens, and advances in statistical analyses (in Irvine et al., 2000, Chapter 5) have facilitated an understanding of the parasite dynamics that has not been reached in any other natural system with the possible exception of the red grouse-*Trichostrongylus tenuis* system (Hudson & Dobson, 1995) and this approach may be of use in other studies.

Epidemiology of nematode infection in Svalbard reindeer. Earlier work from the 1970s and 1980s revealed patterns in parasite abundance observed in the Svalbard reindeer that differed from that found in sheep and cattle systems (Halvorsen & Bye 1999). Early analysis of the abomasal worm population data in this thesis revealed two main findings. First, there was no evidence for acquired immunity (Halvorsen et al., 1999, Chapter 4, Fig. 4.2.) based on an analysis of the relationship between the abundance of infection and the age of the host. In fact, adult animals harboured high burdens of parasites throughout the age range. Interpreting the role of immunity from

age-intensity curves is not straightforward and immunity is likely to reduce variation. There was a large amount of between individual variation that could be due to host factors such as genetic predisposition or to the interaction between nutritional status and grazing pattern; for example animals with high food intake requirements may graze for longer in graminoid areas that have higher infective larval populations (Van der Wal *et al.*, 1999 & see “Future Directions” section below). The analysis also suggests that these nematodes are long lived (up to two years). Second, there was evidence that the intensity of infection increased over the winter even taking into account the developing larvae (Halvorsen *et al.*, 1999, Chapter 4, Fig. 4.3).

Species comparisons within the nematode population. Because it was known that the abomasal nematode populations were made up of a number of species the natural next question was to determine whether these species had similar or contrasting seasonal and annual patterns of abundance. Traditional morphologically based taxonomy enabled identification of the adult male nematodes (Irvine *et al.*, 2000, Chapter 5) and molecular techniques involving the PCR confirmed that the rarer types were morphs of the two main species (Dallas *et al.*, 2000a & 2001, Chapter 3.5 & 3.6). Furthermore, using the same techniques, it is possible to determine the species composition of the female and larval stages including the arrested component (Chapter 3.3). Therefore, the dynamics of each species can be fully investigated and the effects these stages have on the host can be quantified. We took advantage of these methodological advances to analyse the seasonal and annual dynamics of these two species, allowing for the aggregated distribution in a dynamic way by allowing k , the measure of aggregation to vary with the mean (Irvine *et al.*, 2000, Chapter 5).

There were three main findings from this. First, *M. marshalli* is responsible for the increase over winter and shows a strong seasonal pattern with a low level in summer but, no annual variation (Chapter 5, Fig 5.3). *M. marshalli* fecundity is largely limited to the winter months. Second, *O. gruehneri* shows little consistent seasonality but varies significantly between years (Chapter 5, Fig 5.5). *O. gruehneri* egg output is mainly focussed on the summer months (Chapter 5 Fig 5.4a.). Third, calves don't pick up *O. gruehneri* in their first summer despite the summer egg output by this species yet, they do pick up significant levels of *M. marshalli* (Chapter 5, Fig 5.1). Evidence for the two species competing is weak. Despite the population level differences in abundance of the two species, there was no relationship between the two species within and individual reindeer. The rate of infection with *O. gruehneri* in calves may be related to the abundance of the primary *M. marshalli* infection, but the data is not strong enough for this analysis. Furthermore it is known that *O. gruehneri* is virulent because body condition in animals culled in October declines with increasing intensity of *O. gruehneri* (Langvatn, 1999). These findings highlight the benefits of analysing the parasites at the individual species level and show that *O. gruehneri* rather than *M. marshalli* is a good candidate to investigate in the impact of parasitism on the host because both the parasite and the reindeer host densities vary between years (Fig 1.6). The hypothesis that the reindeer population is regulated through density dependent relationship with *O. gruehneri* is explored in Albon et al., (submitted) and the main findings are presented in Fig 7.3.

Transmission dynamics and nematode fecundity. Another important unknown in the host parasite relationship is the regulation of transmission. Measuring transmission directly in the wild is difficult as is measuring the size of the challenge on the pasture. However, on-going work has shown that development rates of eggs and

larvae on the pasture are quite quick and that infective larvae can survive for long periods (up to 100 days, unpublished data). Combining these data with defecation rates supplied in the literature and the seasonal pattern of faecal egg output (Chapter 5; Fig 5.4a; Irvine *et al.*, 2000) we can parameterise a model to simulate the seasonal risk of infection from the pasture (Fig 7.1).

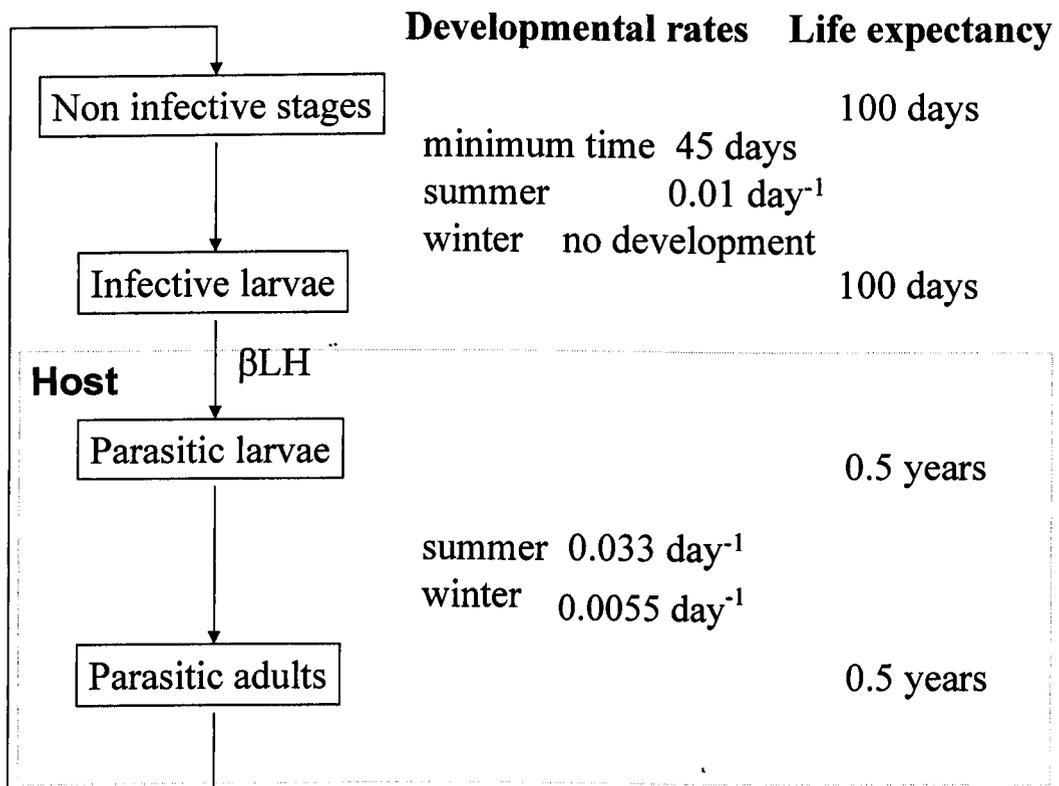


Fig 7.1. Model and parameter estimate for transmission dynamics based on data is from the Svalbard reindeer-*O. gruehneri* system.

The regulation of nematode fecundity and understanding what generates the seasonal pattern of egg output is an important component in this process. This will affect transmission and may help explain annual variation in abundance of parasites in hosts and is essential in modelling the host-parasite dynamics. A direct analysis of the number of eggs in-utero and female worm length for both main species across three seasons indicates that there is a strong seasonal egg production in *O. gruehneri* and that this is negatively related to the length of the worms which is in turn reduced as *O. gruehneri* intensity increases (Fig 6.1, Chapter 6; Irvine *et al.*, 2001). Whereas

M. marshalli fecundity is very low and seems to be more directly related to the number of worms present and not related at all to female worm length (Fig 6.1, Chapter 6; Irvine *et al.*, 2001). Therefore, whilst *O. gruehneri* behaves in a similar way to nematodes of domestic livestock (see Smith & Grenfell, 1985, Armour 1980),

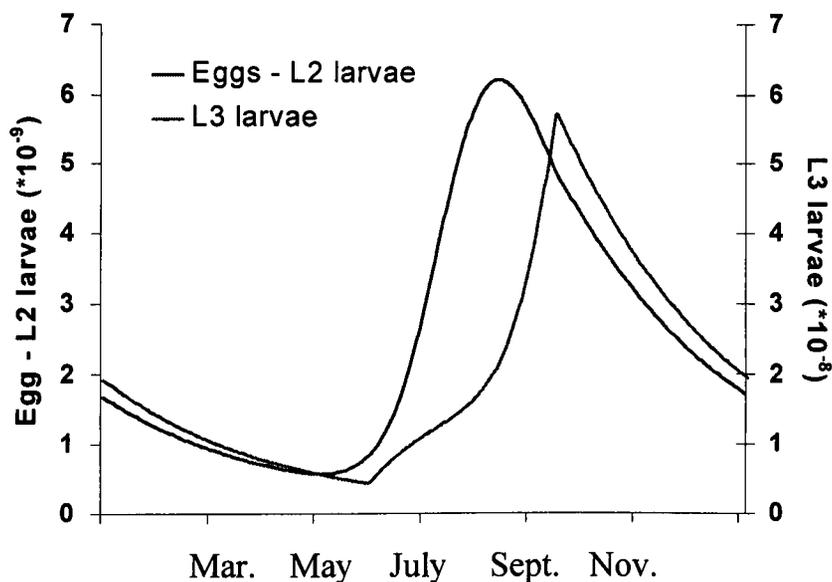


Fig 7.2. Simulated abundance of eggs and larvae on the pasture. The equation system for the model for the number of eggs and non-infective larvae on pasture (EL2), infective larvae on pasture (L3), parasitic larvae in the host (PL) and mature parasites in the host (M):

$$\frac{dEL2}{dt} = \lambda(t, M, H) - \mu_{EL2}EL2 - \sigma_{EL2}EL2(t - \tau_{EL2})e^{\mu_{EL2}\tau_{EL2}}$$

$$\frac{dL3}{dt} = \sigma_{EL2}EL2(t - \tau_{EL2})e^{\mu_{EL2}\tau_{EL2}} - (\mu_{L3} + \beta H)L3$$

$$\frac{dPL}{dt} = \beta HL3 - (\mu_{PL} + \sigma_{PL} + \mu_H)PL$$

$$\frac{dM}{dt} = \sigma_{PL}PL - (\mu_M + \mu_H)M$$

where μ_i is the stage specific mortality rate, σ_i is the development rate, τ_i is the minimum developmental time, β is the transmission coefficient, H is the number of hosts. The function $\lambda(t, M, H)$ describes the egg output rate on pasture and is a nonlinear function that describes the density dependence and the strong seasonality observed in the nematode egg production.

the relationship it has with the reindeer host is quite different, particularly in respect of the slow accumulation of nematodes in young animals and the maintenance of high burdens in adults. Whereas *M. marshalli*, primarily a nematode of sheep (see

Bye et al., 1987), and usually occurring in arid areas, has adopted an unexpected strategy of focussing transmission over winter.

Understanding the relationship between worm burden and nematode fecundity in this system and how this varies seasonally and annually is important in parameterising a model to determine the transmission dynamics of this system (Fig 7.1). In combination with experiments determining the development and survival rates of the free living stages, this may lead to an understanding of the potential challenge on the pasture resulting from the annual variation in the abundance of infection with *O. gruehneri* (Fig 7.2).

Fine scale resolution at the individual species level has clearly demonstrated the contrasting life-history traits for the two main gastrointestinal nematode parasites and highlights the importance of working at the individual species scale.

FUTURE DIRECTIONS

Arrested development and the time-lag in the host parasite relationship.

One major aspect of the life-history of gastrointestinal nematodes that is potentially important but I have not yet studied in detail is the propensity to undergo arrested development. This can introduce a time delay into the parasite dynamics so that the abundance of adult nematodes in the hosts is related to the transmission some time previously. Arrested development is thought to be an adaption to survive adverse conditions and may be triggered by environmental conditioning, the intensity of the current infection or through the effect of an immune response. The propensity to arrest can vary between species and it is thought that the degree of synchrony of arrestment and de-arrestment may be one of the more pathogenic effects of these

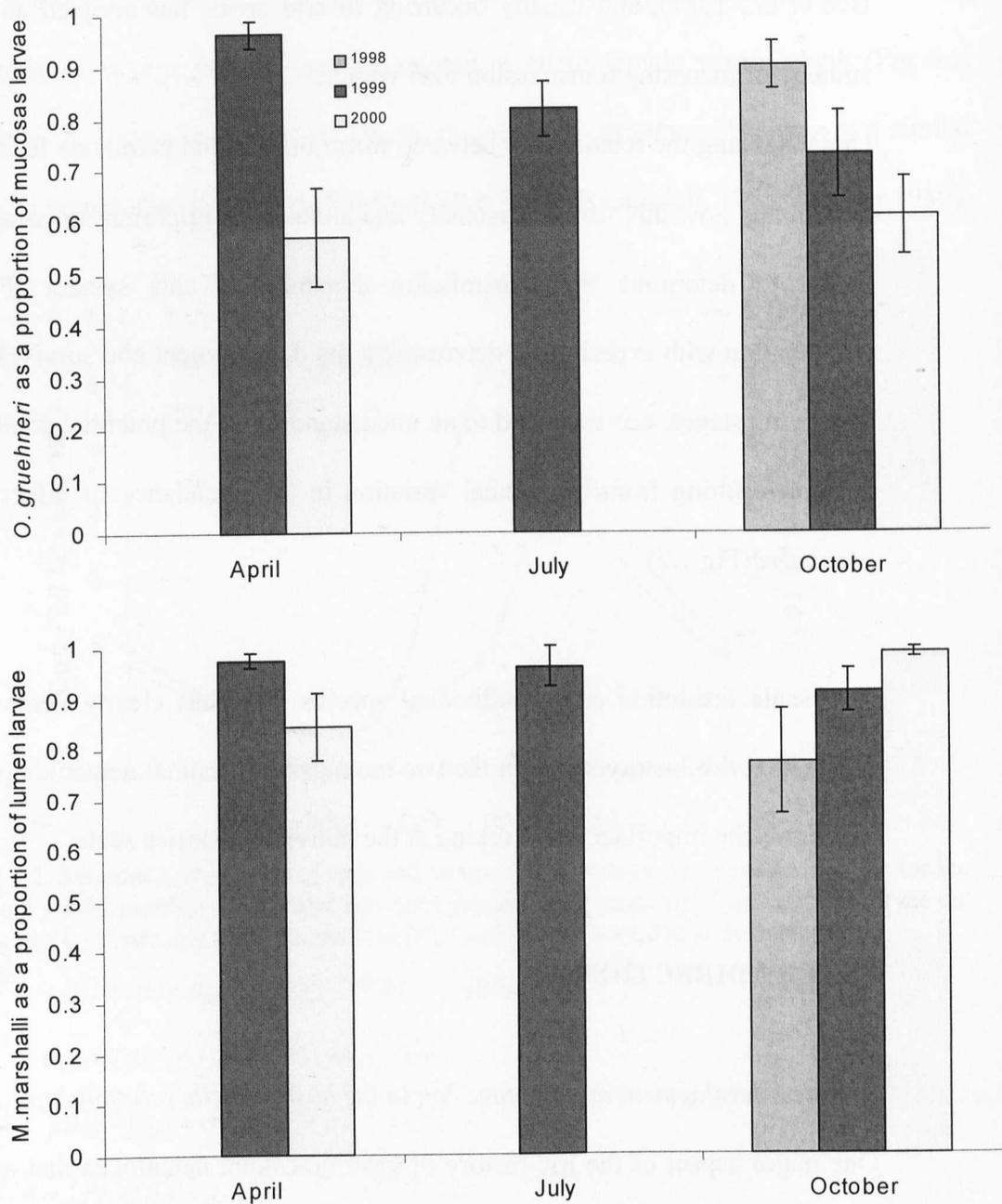


Fig 7.3. Mean larval proportions based on PCR analysis of individual larvae from hosts culled between October 1998 and October 2000. Empty columns indicate no samples. a) The estimated proportion (and standard error bars) of mucosa larvae that is *O. gruehneri*. b) The estimated proportion (and standard error bars) of lumen larvae that is *M. marshalli*.

parasites as they burrow in and out of the abomasal mucosa and therefore may affect measures of host fitness such as body condition, reproduction and as such, host fitness. Techniques to extract the mucosa (arrested) larvae so that they can be identified using PCR methods have been developed (Chapter 3.2). Using these

techniques we have resolved the abundances of the larval stages to the species level. On going work has indicated that the two main species have contrasting patterns of arrestment. Nearly all the larvae found in the mucosa wall are *O. gruehneri* whereas nearly all the larvae found in the abomasal lumen are *M. marshalli* (Fig 7.3a & b). Further work will use population growth models using the abundances of the different stages of each species to investigate how the contrasting seasonal and annual variation between these two species can be accounted for. Interestingly, it appears that the pathogenic *O. gruehneri* does arrest and therefore introduces a time lag into the system.

This could be considered as a partial explanation for the two year time lag we observe between the abundance of parasites and the reindeer counts (Fig 1.6). Further explanation of this time lag may lie in the fact that calves don't tend to pick up infection in their first summer (Fig 5.1, Chapter 5; Irvine *et al.*, 2000). This suggests that the main window for transmission may be early in the season before calves have a significant herbage intake.

Further evidence for this comes from anthelmintic experiments. Treated animals, clear of infection until June become re-infected with high numbers of nematodes by late July (although the abundances were lower than in controls (Irvine, 2000). Analysis of the species present in both controls and treated animals show that *M. marshalli* is virtually absent indicating that the main challenge to the animals in June and July is from *O. gruehneri* (Stein, unpublished manuscript). Indeed the effect of treatment on subsequent pregnancy is significant and because of this seasonal low in *M. marshalli*, the treatment effect must only have operated on *O. gruehneri*. This rationale has been the basis for the development of a simulation model. It is now possible to model the challenge on the pasture through our understanding of

nematode fecundity regulation and survival of free living stages (Fig 7.2). Now, combining our data on the host population with the experimental data on parasite manipulation, a simulation model was parameterised using calf survival estimates from the literature of 0.75 (Solberg *et al.*, *in press*) and 0.82 (Tyler & Øritsland 1999) and a mean adult survival of 0.88 (Albon *et al.*, submitted) and reindeer densities of 1.0-2.5 reindeer/km². Without the parasite effect on calving rate in the model there is no population regulation even though random walk close to the extinction boundary may cause the population to persist at low numbers for extended

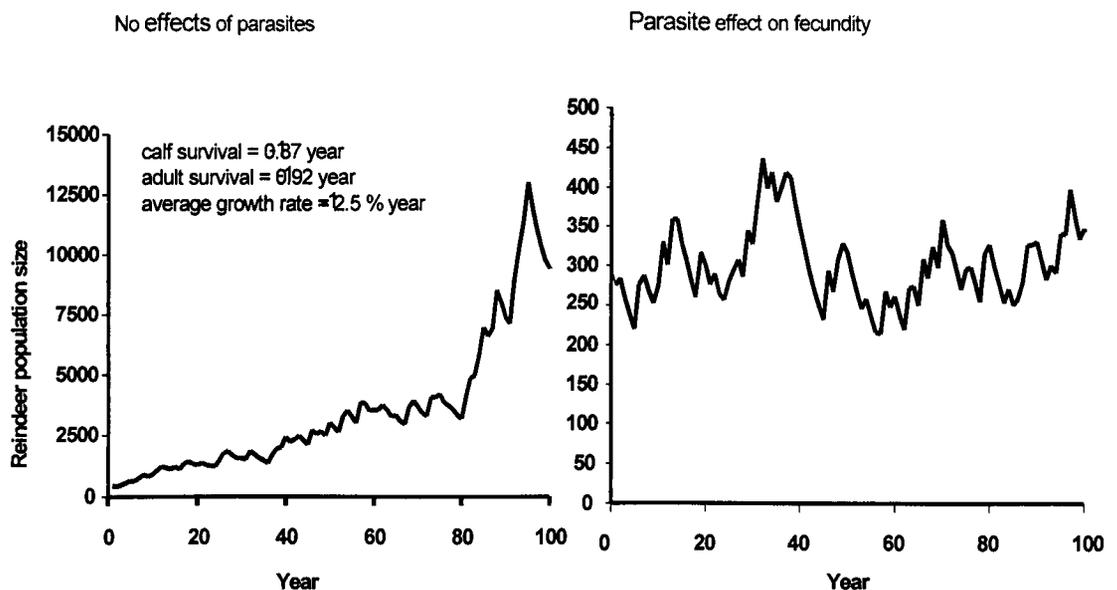


Fig 7.3. a) Simulated population growth trajectory for a model including the stochastic effects of weather. b) population trajectory for the same population but including the density dependent effects of *O. gruehneri* on female reindeer fecundity (compare with Fig 1.1). Using survival parameter values of 0.82 and 0.88 for calves and adults, respectively and host populations densities of 1.0-2.5 reindeer/km², the model predicted a population growth rate of 1.4 % year⁻¹ in the absence of the parasite, but with a parasite-mediated reduction in calving rate greater than 0.065 (Albon *et al.*, submitted), the population growth rate would become negative. Simulations with constant winter precipitation also show that the effect of the parasite is stabilising with no evidence for cyclic dynamics in the regulated range of host population dynamics.

periods of time. But with a parasite-mediated reduction in calving rate greater than 0.065 (Albon *et al.*, submitted), the population growth rate would become negative. Simulations with constant winter precipitation also show that the effect of the parasite is stabilising with no evidence for cyclic dynamics in the regulated range of

host population dynamics. Also the model predictions appeared to be insensitive to the scale of the time delay in parasite abundance in response to host densities, demonstrating that without nematodes in the system the population is unstable. However, the inclusion of the effect of nematode parasites on host fecundity is sufficient to stabilise the population trajectory so that it fluctuates about 2-fold and is within the range that we observe in the field (Fig 7.3. a,b & Albon et al., submitted to PRSB).

The role of host heterogeneity in the observed parasite distribution.

This thesis has determined that the two main nematode species have contrasting life-histories and population dynamics and that any pathogenic effect is likely to be caused by *O. gruehneri*. However, the causes of the between host variation in *O. gruehneri* abundance have not been fully identified. Abundance of parasites in hosts is not normally distributed as outlined in the general introduction. Understanding the mechanisms that generate this heterogeneity in the abundance between hosts is fundamental to understanding the impact parasites have on hosts.

Immunity.

One area of host heterogeneity that has received some attention is host immune response. This can be either due to genetic variability (Paterson, 1996) or the interaction of the immune response with host nutrition (Van Houtert & Sykes, (1997; Coop & Kyriazakis, 1999). Whilst it is difficult to experimentally investigate the immune function in the wild, it is possible to measure nematode specific immunoglobulin levels in Svalbard reindeer. Preliminary work looking at the relationship between circulating antibodies (IgA) and the abundance of mucosa larvae suggests that there are elevated IgA levels in response to higher abundances of

mucosa larvae ($\chi^2=6.77$, $P<0.01$) after accounting for variation in abundance of mucosa larvae due to year month and host valley location. (unpublished data). Although there is no evidence of immunity from the age-intensity analysis, this does not preclude any immune system involvement. Individuals may harbour high worm burdens but the fecundity of these nematodes may be still be regulated by immunity. Although an immune response would tend to reduce worm burdens, differences between hosts in the immune response might also increase heterogeneities in observed parasite burdens.

Grazing.

A second area of host heterogeneity is in the pattern of grazing. In farmed systems grazing animals can be assumed to graze in a homogenous environment. Whereas in a natural system such as on Svalbard, the grazing range is made up of a mosaic of habitats which, because of differing plant phenological dynamics, are used in a sequential or non random manner by the reindeer herbivore (Fig 7.4).

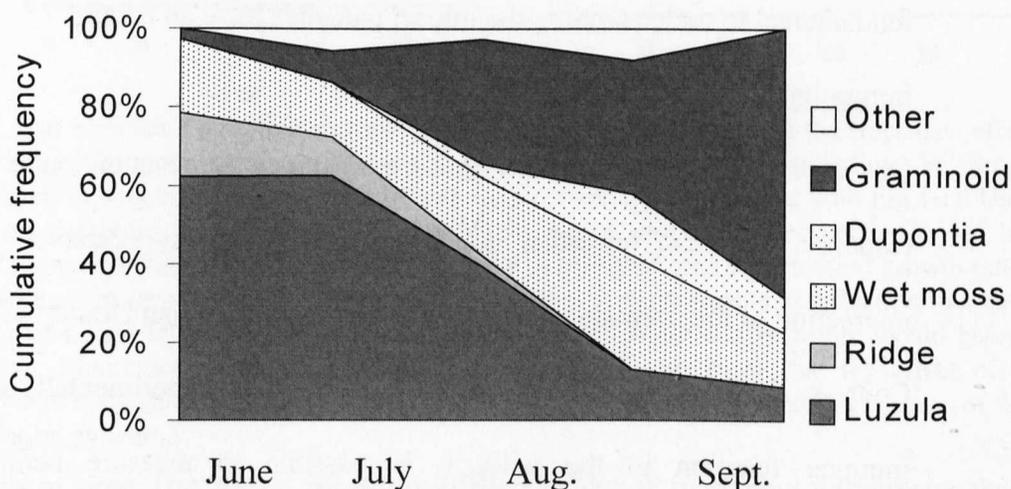


Fig 7.6. The seasonal grazing pattern (use of different habitat types) of Svalbard reindeer of habitat (Van der Wal, unpublished data).

This has two implications for transmission, First, free-living stages will have different development and survival rates on different habitats Second, hosts will utilise different habitats to a lesser or greater extent depending on the season. Therefore different habitats pose a different risk of infection (Van der Wal *et al.*, 1999) which will be modified by heterogeneities in the host immune response. Quantifying this will lead to a better understanding of the host parasite dynamics and allow the production of models based on mechanisms. A study of the effect of environmental heterogeneity in habitats and grazing on parasite dynamics lend itself to close co-operation with ideas from the optimal foraging theory where, besides decisions based on plant quality and quantity, and plant defences, parasites can be included not only from the perspective of decision based on the risk of infection (Hutchings *et al.*, 1998a) but also the decisions that parasitised animals may make in being more selective (Hutchings *et al.* 1998b) and even actively selecting chemically well defended plants for their medicinal qualities.

Wider implications.

The results from this thesis, the anthelmintic experiment and from ongoing work on the arrested larvae, free-living nematode stages combined with data about the host fecundity and survival form the basis for a simulation model that demonstrates for the first time that parasites are sufficient to regulate their host population (Albon *et al.*, submitted to PRSB). Fig 7.3a shows the simulated population trajectory using density dependent calving data and including the stochastic effect of weather on mortality. Fig 7.3b shows the trajectory for the same model but including the effect of parasites on reindeer fecundity (parameter estimates based on data from experimental manipulation of the parasite burdens). Interestingly, not only is the

population in this simulation regulated but it fluctuates about two-fold at levels very similar to the reindeer population in Fig 1.1, Chapter 1).

This study is the first to deal with a mixed natural infection of nematode parasites by treating the two species as independent populations in the sense that they have different dynamics. One option that has been touched upon in chapters 5 & 6 is inter-specific competition. Although the data so far do not point to any evidence for this in the adult stages, the observed dynamics may yet prove to be partly caused by competition at the larval stages. Whilst there are similarities between the life-histories of these nematodes and those of domestic livestock, this study also highlights the need for caution in extrapolating from the domestic literature. Because Svalbard reindeer harbour relatively high burdens across the age range, the temptation is to interpret this as demonstrating a benign host-parasite relationship. However, the value of experimentation in this case has demonstrated that there are subtle costs to nematode parasitism in terms of reduced reproductive success and that this appears mainly due to *O. gruehneri*. The analysis at the species level has therefore permitted a species-specific model of parasite transmission to be developed which might not have been possible if the abomasal parasite community had been treated as one. Therefore, there is now a justification for increasing the effort to understand individual parasites species dynamics when investigating wild host population dynamics.

REFERENCES

REFERENCES

- AANES, R., SAETHER, B. E. & ORITSLAND, N. A. (2000). Fluctuations of an introduced population of Svalbard reindeer: the effects of density dependence and climatic variation. *Ecography* **23**; 437-443
- ADAMSON M. L. & NOBLE, S. J. (1993). Interspecific and intraspecific competition among pinworms in the hindgut of *Periplaneta americana*. *Journal of Parasitology* **79**, 50-56.
- ALBON, S. D., MITCHELL, B., HUBY, B. J. & BROWN, D. (1986). Fertility in female red deer (*Cervus elaphus*): the effects of body composition age and reproductive status. *J. Zool.* **209**, 447-460.
- AL KHALID, N. W., ARSALAN, S. H., MUSLIH, N. J. & AL-SAAD, K. M. (1989). Pathogenicity of *Marshallagia marshalli* (Ransom) in sheep. *Indian J. Anim. Sci.* **59**, 917-919.
- ALENDAL, E. & BYRKLEDAL, I. (1976). Population size and reproduction of the reindeer (*Rangifer tarandus platyrhynchus*) on Nordenskiöld Land, Svalbard. *Norsk Polarinstitut Årbok 1974*, 138-152.
- ANDERSON N, ARMOUR J, JENNINGS FW, RITCHIE JSD, URQUHART GM. (1969). The sequential development of naturally occurring ostertagiasis in calves. *Res Vet Sci.* **10**, 18-28.
- ANDERSON, R. M. (1980). Depression of host population abundance by direct lifecycle macroparasites. *J. Theor. Biol.* **82**, 283-311.
- ANDERSON, RM & MAY, RM (1991). Infectious diseases of humans: dynamics and control. Oxford University Press, Oxford, UK.
- ANDERSON, RM & MAY, RM (1978). Regulation and stability of host-parasite population interactions. I. Regulatory processes. *Journal of Animal Ecology* **47**, 219-248.
- ANDERSON, RM & SCHAD, G. A. (1985). Hookworm burdens and faecal egg counts: an analysis of the biological basis of variation. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **79**, 812-825.
- ANDERSON, R. M. & MICHEL, J. F. (1977). Density dependent survival in populations of *Ostertagia ostertagi*. *International Journal for Parasitology* **7**, 321-329.
- ANDERSON, N., DASH, K. M., DONALD, A. D., SOUTHCOFF, W. H. & WALLER, P. J. (1979). Epidemiology and control of nematode infections. In The epidemiology and control of gastrointestinal parasites of sheep in Australia. (ed. Donald, A. D., Southcott, W. H. & Dineen, J. K.) CSIRO, Australia.
- ANDERSON R. M. & GORDON D. M. (1982). Processes influencing the distribution of parasite numbers within host populations with special emphasis on parasite-induced host mortalities. *Parasitology* **85**, 373-398

- ANDERSON, R. M. & MAY, R. M. (1978). Regulation and stability of host-parasite population interactions. I. Regulatory processes. *Journal of Animal Ecology* **47**, 219-
- ARMOUR, J. (1970). Bovine ostertagiasis: A review. *Veterinary Record* **86**, 184-190.
- ARMOUR, J. (1980). The epidemiology of helminth disease in farm animals. *Veterinary Parasitology* **6**, 7-46.
- ARMOUR, J. (1989). The influence of host immunity on the epidemiology of trichostrongyle infections in cattle. *Veterinary Parasitology* **32**, 5-19.
- ARMOUR, J. & BRUCE, R. G. (1974). Inhibited development of *Ostertagia ostertagi* infections – a diapause phenomenon in a nematode. *Parasitology* **69**; 161-174.
- ARMOUR, J. & DUNCAN, M. (1989) Arrested larval development in cattle nematodes. *Parasitol Today* **3**, 171-76
- ARNEBERG, P., FOLSTAD, I. & KARTER, A. J. (1996). Gastrointestinal nematodes depress food intake in naturally infected reindeer. *Parasitology* **112**; 213-219.
- BARGER, I. A. (1985). The statistical distribution of trichostrongylid nematodes in grazing lambs. *International Journal for Parasitology* **15**, 645-649.
- BARGER, I. A. (1985). The statistical distribution of trichostrongylid nematodes in grazing lambs. *International Journal for Parasitology* **15**, 645-649.
- BARGER, I. A., LE JAMBRE, L. F., GEORGI, J. R. & DAVIES, H. I. (1985). Regulation of *Haemonchus contortus* populations in sheep exposed to continuous infection. *International Journal for Parasitology* **15**, 529-533.
- BARNES, E. H. & DOBSON, R. J. (1990). Population-dynamics of *Trichostrongylus colubriformis* in sheep: - computer-model to simulate grazing systems and the evolution of anthelmintic resistance. *International Journal for Parasitology* **20**, 823-831
- BARTH, D. ERICSSON, G. F., KUNKLE, B. N., REHBEIN, S., RYAN, W. G. & WALLACE, D. H. (1997) Evaluation of the persistence of the effect of ivermectin and abamectin against gastrointestinal and pulmonary nematodes in cattle. *Veterinary Record* **140**, 278-279
- BISHOP, S. C. & STEAR, M. J. (2000). The use of a gamma-type function to assess the relationship between the number of adult *Teladorsagia circumcincta* and total egg output. *Parasitology* **121**, 435-440
- BOAG, B. & THOMAS, R. J. (1977). Epidemiological studies on gastro-intestinal nematode parasites of sheep: the seasonal number of generations and succession of species. *Research in Veterinary Science* **22**, 62-77.
- BOONSTRA, R., KREBS, C. J. & BEACHAM, T. D. (1980). Impact of botfly parasitism on *Microtus townsendii* populations. *Can. J. Zool.* **58**, 1683-1692.
- BRATBAKK, I. (1985). Flora og vegetasjon. In Svalbardreinen og dens livsgrunnlag, (ed. Nils A. Øritsland). Universitetsforlaget, Norway. p 10-34.

- BYE, K. (1987). Abomasal nematodes from three Norwegian wild reindeer populations. *Canadian Journal of Zoology* **65**, 677-680.
- BYE, K. & HALVORSEN, O. (1983). Abomasal Nematodes of the Svalbard Reindeer (*Rangifer tarandus platyrhynchus* Vrolik). *Journal of Wildlife Diseases* **19**, 101-103.
- BYE K., HALVORSEN, O. & NILSSEN, K. (1987) Immigration and regional distribution of abomasal nematodes of Svalbard reindeer. *J Biogeogr* **14**, 451-58.
- CHRISTENSEN, N. Ø., NANSEN, P., FAGBEMI, B. O. & MONRAD, J. (1987). Heterologous antagonistic and synergistic interactions between helminths and protozoans in concurrent experimental infection of mammalian hosts. *Parasitol. Res.* **73**, 387-410.
- COOP, R. L. & KYRIAZAKIS, I. (1999). Nutrition-parasite interaction. *Vet. Parasitol* **84**, 187-204.
- COYNE M. J., & SMITH, G. (1994). Trichostrongylid parasites of domestic ruminants (In Parasitic and infectious diseases: epidemiology and ecology, eds Scott, ME and Smith G). Academic Press Ltd, London, UK.
- COYNE, M. J. & SMITH, G. (1991). Fecundity of gastrointestinal trichostrongylid nematodes of sheep in the field. *American Journal of Veterinary Research* **52**, 1182-1188.
- CROFTON H. D. (1955). Nematode parasite populations in sheep on lowland farms. II. Worm egg counts in lambs. *Parasitology* **45**, 99-115.
- CROFTON, H. D. (1957). Nematode parasite populations in sheep on lowland farms. III. The seasonal incidence of species. *Parasitology* **47**, 304-318
- CROFTON H. D. (1958). Nematode parasite populations in sheep on lowland farms. V: Further observations of the post parturient rise and a discussion of its significance. *Parasitology* **48**, 243-250.
- CROFTON, H. D. (1963). Nematode parasite populations in sheep and on pasture. Technical Communication No. 35 of the Commonwealth Bureau of Helminthology. St. Albans, U. K.
- CROFTON, H. D., (1971). A quantitative approach to parasitism. *Parasitology* **63**, 343-364.
- CROFTON HD. (1971). Nematode parasite population in sheep and on pasture. Technical Communication no 35 of the Commonwealth Bureau of Helminthology. Farnham Royal: Commonwealth Agricultural Bureaux.
- CROLL, N. A., ANDERSON, R. M. ,GYORKOS, T. W. & GHADIRIAN, E. (1982). The population biology and control of *Ascaris lumbricoides* in a rural community in Iran. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **76**, 187-197.
- DALLAS JF, IRVINE RJ, HALVORSEN O. & ALBON, SD (2000a). Identification by polymerase chain reaction (PCR) of *Marshallagia marshalli* and *Ostertagia gruehneri* from Svalbard reindeer. *International Journal for Parasitology* **30**, 863-866.

- DALLAS, J. F., IRVINE, R. J. & HALVORSEN, O. (2000b). DNA evidence that *Ostertagia gruehneri* and *Ostertagia arctica* (Nematoda: Ostertagiinae) are conspecific. *International Journal for Parasitology* **30**, 655-658.
- DALLAS, J. F., IRVINE, R. J. & HALVORSEN O. (2001). DNA evidence that *Marshallagia marshalli* and *Marshallagia occidentalis* (Nematoda: Ostertagiinae) from Svalbard reindeer are conspecific. *Systematic Parasitology* in press.
- DARWIN-MURRELL, K., SLOTVED, H. C., ERIKSEN, L., BJERREGAARD, J., NANSEN, P. & ROEPSTORFF, A. (1997). Improved method for the recovery of *Ascaris suum* larvae from pig intestinal mucosa. *J Parasitol.* **83**, 321-324.
- DASKALOV, P. (1974). On the reproductive relationships between *Ostertagia circumcincta*, *Teladorsagia davtiani* and *O. trifurcata* (Nematoda, Trichostrongylidae). *Izvestiya na Tsentralnata Khelminthologichna Laboratoriya* **17**, 59-72. (*Helminthological Abstracts* **44**, 494).
- DIETRICH, R. A. & CRAIGMILL, A. L. (1990). Safety, efficacy and tissue residues of ivermectin in reindeer. *Rangifer* **2**, 53-56
- DIEZ-BAÑOS, N., CABARET, J. & DIEZ-BAÑOS, P. (1992). Interspecific interactions in naturally acquired nematode communities from sheep abomasum in relation to age of host and season in four areas of León (Spain). *International Journal for Parasitology* **22**, 327-334.
- DEROOVER, E., COBB, R., ROCK, D. W. GUERINO, F. (1997) Persistent efficacy: importance and impact of trial design. *Veterinary Parasitology* **73**, 365-371
- DOBSON, R.J., WALLER, P.J. & DONALD, A.D. (1990). Population dynamics of *Trichostrongylus colubriformis* in sheep: the effect of infection rate on loss of adult parasites. *International Journal for Parasitology* **20**, 359-363.
- DOBSON, A. P. (1985). The population dynamics of competition between species. *Parasitology* **91**, 317-347.
- DOBSON, A. P. & HUDSON, P. J. (1992). Regulation and stability of a free-living host-parasite system: *Trichostrongylus tenuis* in red grouse. II. Population models. *J Anim Ecol.* **61**, 487-498.
- DOBSON, R. J. & BARNES, E. H. (1995). Interaction between *Ostertagia circumcincta* and *Haemonchus contortus* infection in young lambs. *International Journal for Parasitology* **25**, 495-501.
- DOBSON, R. J., BARNES, E. H., & WINDON, R. G. (1992). Population dynamics of *Trichostrongylus colubriformis* and *Ostertagia circumcincta* in single and concurrent infections. *International Journal for Parasitology* **22**, 997-1004.
- DONALD, A. D. (1968). Ecology of the free-living stages of nematode parasites of sheep. *Australian Veterinary Journal* **44**, 219-233.

DRÓZDZ, J. (1965). Studies on helminth and helminthiases in Cervidae. I. Revision of the subfamily Ostertagiinae Sarwar, 1956 and an attempt to explain the phylogenesis of its representatives. *Acta Parasit. Polon.* **13**, 445-81.

DRÓZDZ, J. (1995). Polymorphism in the Ostertagiinae Lopez-Neyra, 1947 and comments on the systematics of these nematodes. *Systematic Parasitology* **32**, 91-99.

EL AZAZY, O. M. E. (1995). Seasonal changes and inhibited development of the abomasal nematodes of sheep and goats in Saudi Arabia. *Vet. Parasitol.* **58**, 91-98.

EYSKER, M. (1997). Some aspects of inhibited development of trichostrongylids in ruminants. *Vet Parasitol.* **72**, 265-83.

FAMILTON AS, MCANULTY RW. (1994). Sheep nematode survival: The epidemiological consequences of findings from recent studies. Proceedings of the 24th seminar of the sheep and beef cattle society of the New Zealand Veterinary Association; 186-95.

FØRLAND, E. J., HANSSEN-BAUER, I. & NORDLI, P. Ø. (1997). Climate statistics and long-term series of temperature and precipitation at Svalbard and Jan Mayen. Report No. 21/97, DNMI-Rapport, Norwegian Meteorological Institute, Box 43 Blindern, N-0313 Oslo.

FOX, M. T. (1997). Pathophysiology of infection with gastrointestinal nematodes in domestic ruminants: recent developments. *Vet. Parasitol.* **72**, 285-308.

FULFORD, A. J. C., BUTTERWORTH, A. E., STURROCK R. F. & OUMA J. H. (1992). On the use of age-intensity data to detect immunity to parasitic infections with special reference to *Schistosoma mansoni* in Kenya. *Parasitology* **105**, 219-227

GASSER, R. B., ROSSI, L., & ZHU, X. Q. (1999). Identification of *Nematodirus* species (Nematoda: Molineidae) from wild ruminants in Italy using ribosomal DNA markers. *International Journal for Parasitology* **29**, 1809-1817.

GASSER, R. B., CHILTON, N. B., HOSTE, H. & BEVERIDGE, I. (1993). Rapid sequencing of rDNA from single worms and eggs of parasitic helminths. *Nucleic Acids Res* **21**, 2525-2526.

GASSER, R. B. & HOSTE, H. (1995). Genetic markers for closely related parasitic nematodes. *Mol Cell Probe* **9**, 315-319.

GASSER, R. B. & MONTI, J. R. (1997). Identification of parasitic nematodes by PCR-SSCP of ITS-2 rDNA. *Mol Cell Probe* **11**,:201-9.

GASNIER, N., CABARET, J & SUAREZ, V. (1993). Species and morphs in the Ostertagiinae - an allozyme study of 7 species. *Int J Parasit.* **23**, 765-70.

GIBBS, H. C. (1987). Hypobiosis in parasitic nematodes – an update. *Adv. Parasitol.* **25**, 129-174.

GREGORY R. D. & WOOLHOUSE M. E. J. (1993). quantification of parasite aggregation - a simulation study. *Acta. Trop.* **54**, 131-139.

GRENFELL, B. T. (1988). Gastrointestinal nematode parasites and the stability and productivity of intensive ruminant grazing systems. *Phil. Trans. R. Soc. B.* **321**, 541-563.

GRENFELL B. T. (1992). Parasitism and the dynamics of ungulate grazing systems. *Am. Nat.* **139**, 907-929.

GRENFELL BT, SMITH G, ANDERSON RM. (1986) Maximum likelihood estimates of the mortality and migration rates of the infective larvae of *Ostertagia ostertagi* and *Cooperia oncophora*. *Parasitology* **92**, 643-52.

GRENFELL, B. T., DIETZ K. & ROBERTS M. G. (1995a). Modelling the immunology of macroparasites in naturally fluctuating host populations. In *Ecology of Infectious Diseases in Natural Populations* (ed. Grenfell, B. T. & Dobson, A. P.), pp. 362-383. Cambridge University Press, Cambridge.

GRENFELL, B. T., WILSON, K., ISHAM, V. S., BOYD, H. E. G. & DIETZ, K. (1995b). Modelling patterns of parasite aggregation in natural populations: Trichostrongylid nematode-ruminant interactions as a case study. *Parasitology* **111 (Suppl.)**, S135-S151

GRIMSHAW W. T. R., HONG, C. & HUNT, K. R. (1997) Persistent activity of injectable ivermectin against important gastrointestinal nematodes of sheep. *Veterinary Record* **141**, 422-423.

GULLAND, F. M. D. (1992). The role of nematode parasites in Soay sheep (*Ovis aries*) mortality during a population crash. *Parasitology* **105**, 493-503.

GULLAND, F. M. D. (1995). Impact of infectious diseases on wild animals populations. In *Ecology of Infectious Diseases in Natural Populations*. Ed. Grenfell, B. T. & Dobson, A. P., Cambridge University Press, Cambridge. pp. 20-52.

GULLAND, F. M. D., & FOX, M. (1992). Epidemiology of nematode infections of Soay sheep (*Ovis-aries* l) on St. Kilda. *Parasitology* **105**, 481-492.

HALVORSEN, O. (1986). Epidemiology of reindeer parasites. *Parasitol Today* **2**, 334-39.

HALVORSEN, O. & BYE, K. (1986). Parasitter i Svalbardrein 1. Rundmark I løpen. In *Svalbardreinen og dens livsgrunnlag*, (Ed. Nils A. Øritsland). Universitetsforlaget, Norway. p 169-192

HALVORSEN, O. & BYE, K. (1999). Parasites, biodiversity and population dynamics in an ecosystem in the High Arctic. *Veterinary Parasitology* **84**, 205-227.

HALVORSEN, O., STIEN, A. IRVINE, J., LANGVATN, R. & ALBON S. (1999). Evidence for continued transmission of parasitic nematodes in reindeer during the Arctic winter. *Int. J. Parasitol.* **29**; 567-579.

HALVORSEN, O. & BYE, K. (1986). Parasitter i svalbardrein 1. Rundmark i løpen (in Norwegian). In: Øritsland NA, editor. *Svalbardreinen og dens livsgrunnlag*. Oslo: Universitetsforlaget. 120-133.

- HALVORSEN, O., STIEN, A. IRVINE, J., LANGVATN, R. & ALBON S. (1999). Evidence for continued transmission of parasitic nematodes in reindeer during the Arctic winter. *International Journal for Parasitology* **29**, 567-579.
- HASTIE, T. J & TIBSHIRANI, R. J. (1990). Generalised additive models. Chapman and Hall, London
- HAUKISALMI, V., HENTTONEN, H. & TENORA, F. (1988) Population-dynamics of common and rare helminths in cyclic vole populations. *J. Anim. Ecol.* **57**, 807-825.
- HEISE, M., EPE, C. & SCHNIEDER, T. (1999). Differences in the second internal transcribed spacer (ITS-2) of eight species of gastrointestinal nematodes of ruminants. *J Parasitol* **85**, 431-435.
- HINDRUM, R., JORDHØY, P., STRAND, O. & TYLER N. (1995). Svaldbardrein et nøysomt liv på tundraen (in Norwegian). *Villreinen*, 105-112.
- HOLMES, P. H. (1987). Pathophysiology of nematode infections. *International Journal for Parasitology*. **55**, 85-92.
- HOLMSTAD, P. R. & SKORPING A. (1998). Covariation of parasite intensities in willow ptarmigan, *Lagopus lagopus* L. *Can. J. Zool.* **76**, 1581-1588.
- HONG, C., MICHEL, J. F. & LANCASTER, M. B. (1986). Populations of *Ostertagia circumcincta* in lambs following a single infection. *International Journal for Parasitology* **16**, 63-67.
- HONG, C., MICHEL, J. F. & LANCASTER, M. B. (1989). Development of protection and of self-cure in lambs infected daily with *Ostertagia circumcincta*. *Veterinary Parasitology* **31**, 125-131.
- HONG, C., MICHEL, J. F. & LANCASTER, M. B. (1989). Development of protection and of self-cure in lambs infected daily with *Ostertagia circumcincta*. *Veterinary Parasitology* **31**, 125-131.
- HOSTE H, CHILTON NB, GASSER RB, BEVERIDGE I. (1995). Differences in the second internal transcribed spacer (ribosomal DNA) between five species of *Trichostrongylus* (Nematoda: Trichostrongylidae). *International Journal for Parasitology* **25**, 75-80.
- HOSTE, H., CHILTON, N. B., BEVERIDGE, I. & GASSER, R. B. (1998) A comparison of the first internal transcribed spacer of ribosomal DNA in seven species of *Trichostrongylus* (Nematoda: Trichostrongylidae). *International Journal for Parasitology* **28**, 1251-1260.
- HUDSON, P. J., & DOBSON, A. P. (1995). Macroparasites: Observed Patterns. In *Ecology of Infectious Diseases in Natural Populations* (ed. Grenfell, B. T. & Dobson, A. P.), pp. 144-176. Cambridge University Press, Cambridge.
- HUDSON, P. J., & DOBSON, A. P. (1997). Transmission dynamics and host parasite interactions of *Trichostrongylus tenuis* in red grouse (*Lagopus lagopus scoticus*). *Journal of Parasitology* **83**, 194-202

- HUDSON, P. J., & DOBSON, A. P. (1995). Macroparasites: Observed Patterns. In *Ecology of Infectious Diseases in Natural Populations* (ed. Grenfell, B. T. & Dobson, A. P.), pp. 144-176. Cambridge University Press, Cambridge.
- HUDSON, P. J. (1986). The effect of parasitic nematodes on the breeding production of red grouse. *J. Anim. Ecol.* **55**, 85-92.
- HUDSON, P. J., DOBSON, A. P. & NEWBORN, D. (1985). Cyclic and non-cyclic populations of red grouse: a role for parasitism? In *Ecology and Genetics of Host Parasite Interactions* (eds. D. Rollinson & R. M. Anderson), pp 77-89. Academic Press, London.
- HUDSON, P. J., NEWBORN, D. & DOBSON, A. P. (1992). Regulation and stability of a free-living host-parasite system: *Trichostrongylus tenuis* in red grouse. I. Monitoring and parasite reduction experiments. *J Anim Ecol* **61**, 477-486.
- HUDSON, P.J., & DOBSON, A.P. (1998). Prevention of population cycles by parasite removal. *Science* **282**, 2256-2258.
- HUTCHINGS, M.R., KYRIAZAKIS, I., ANDERSON, D.H., et al. (1998) Behavioural strategies used by parasitized and non-parasitized sheep to avoid ingestion of gastrointestinal nematodes associated with faeces. *Anim. Sci.* **67**, 97-106.
- HUTCHINGS, M. R., KYRIAZAKIS, I., GORDON, I. J., et al. (1999) Trade-offs between nutrient intake and faecal avoidance in herbivore foraging decisions: the effect of animal parasitic status, level of feeding motivation and sward nitrogen content. *J. Anim. Ecol.* **68**, 310-323.
- HUNG, G. C., GASSER, R. B., BEVERIDGE, I. & CHILTON, N. B. (1999). Species-specific amplification by PCR of ribosomal DNA from some equine strongyles. *Parasitology* **119**, 69-80.
- IRVINE, R. J., STIEN, A., HALVORSEN, O., LANGVATN, R. & ALBON S. D. (2000). Life-history strategies and population dynamics of abomasal parasites of Svalbard reindeer (*Rangifer tarandus platyrhynchus*). *Parasitology* **120**, 297-311.
- IRVINE, R. J. (2000) Use of moxidectin treatment in the investigation of abomasal nematodiasis in wild reindeer (*Rangifer tarandus platyrhynchus*). *Verterinary Record* **147**; 570-573.
- IRVINE, R. J., STIEN, A., HALVORSEN, O., LANGVATN, R. & ALBON S. D. (2000). Life-history strategies and population dynamics of abomasal parasites of Svalbard reindeer (*Rangifer tarandus platyrhynchus*). *Parasitology* **120**, 297-311.
- KAO, R. R., LEATHWICK, D. M., ROBERTS M. G. & Sutherland I. A. (2000). Nematode parasites of sheep: a survey of epidemiological parameters and their application in a simple model. *Parasitology* **121**, 85-103.
- KENNEDY, C. R. & RUMPUS, A. (1977). Long-term changes in the size of the *Pomphorynchus laevis* (Acanthocephala) population in the River Avon. *J. Fish Biology* **10**; 35-42.

KEITH, L. B. & CARY, J. R. (1990). Interactions of the tick (*Haemophysalis leporispalustris*) with a cyclic snowshoe hare (*Lepus americanus*) population. *J. Wild. Dis.* **26**, 427-434.

KEYMER, A. E. (1982). Density dependent mechanisms in the regulation of intestinal helminth populations. *Parasitology* **84**, 573-587.

KEYMER, A. E. & SLATER, A. F. G. (1987). Helminth fecundity: Density dependence or statistical illusion? *Parasitology Today* **3**, 56-58.

KEYMER, A. E. & ANDERSON, R. M. (1981). The dynamics of infection of *Tribolium confusum* by *Hymenolepsis diminuta*: the influence of infective stage, density and spatial distribution. *Parasitology* **79**, 195-207.

KYRIAZAKIS, I., TOLKAMP, B. J., HUTCHINGS, M. R. (1998). Towards a functional explanation for the occurrence of anorexia during parasitic infections. *Anim. Behav.* **56**, 265-74.

LANGVATN, R., ALBON, S. D., IRVINE, R. J., HALVORSEN, O & ROPSTAD, E. (1999) Parasitter, kondisjon og reproduksjon hos svalbardrein. In Svalbardtundraens Økologi. (Eds. S. A. Bengstron, F. Mehlum og T. Severinsen). Norsk Polarinstitut, Tromsø, Norway. pp 139-148.

LEATHWICK, D. M., BARLOW, N. D. & VLASSOF, A. (1992). A model for nematodiasis in New Zealand lambs. *International Journal for Parasitology* **22**, 789-799.

LEBRETON, J. -D. (1989). Statistical methodology for the study of animal populations. *Bulletin of the International Statistical Institute* **53**, 267-282.

LICHTENFELS JR, HOBERG EP. (1993). The systematics of nematodes that cause ostertagiasis in domestic and wild ruminants in North America; an update and a key to species. *Vet Parasitol.* **46**, 33-53.

LICHTENFELS, J. R., HOBERG, E. P. & ZARLENGA, D. S. (1997) Systematics of gastrointestinal nematodes of domestic ruminants: advances between 1992 and 1995 and proposals for future research. *Veterinary Parasitology* **72**, 225-238.

LICHTENFELS, J. R., PILITT, P. A. & FRUETEL, M. (1990). Cuticular ridge pattern in *Ostertagia gruehneri* and *Ostertagia arctica* (Nematode: Trichostrongyloidea) from caribou, *Rangifer tarandus*. *J. Helminthol. Soc. W.* **57**, 61-8.

LLOYD, S. (1995). Environmental influences on host immunity. In Ecology of Infectious Diseases in Natural Populations (ed. Grenfell, B. T. & Dobson, A. P.), pp. 144-176. Cambridge University Press, Cambridge.

MCCULLAGH, P & NELDER, J. A. (1989). Generalised Linear Models. Monographs on Statistics and Applied Probability 37. Chapman & Hall, London.

MCDONALD, P., EDWARDS, R. A. & GREENHALGH, J. F. D. (1984). Animal Nutrition: Chapter 8, Digestion. pp. 121-145. Longman, London.

- MCKEAND, J. B. (1998) Molecular diagnosis of parasitic nematodes. *Parasitology* **117**, S87-S96
- MACKINTOSH, C. G., WALDRUP, K., LABES, R. & TAYLOR, M. (1993) Efficacy of ivermectin injections and moxidectin pour-on formulations in young red deer. Proceedings of a Deer Course for Veterinarians, Deer Branch of the New Zealand Veterinary Association, Palmerston North July 6 1993. pp 143-150.
- MAFF/ADAS. (1986). Manual of Veterinary Parasitological Techniques. Reference Book 418. HMSO, London.
- MARGOLIS, L., ESCH, G. W., HOLMES, J. C., KURIS, A. M. & SCHAD, G. A. (1982). The use of ecological terms in parasitology (report of an ad hoc committee of the American Society of Parasitology). *J. Parasitol.* **68**, 131-133.
- MAY, R. M. & ANDERSON, R. M. (1978). Regulation and stability of host-parasite population interactions. II. Destabilising processes. *Journal of Animal Ecology* **47**, 249-267.
- MICHAEL, E. & BUNDY, D. A. P. (1989). Density dependence in establishment, growth and worm fecundity in intestinal helminthiasis: the population biology of *Trichuris muris* (Nematoda) infection in CBA/Ca mice. *Parasitology* **98**, 451-458.
- MICHEL JF, LANCASTER MB, HONG C. 1979The effect of age, acquired resistance, pregnancy and lactation on some reactions of cattle to infection with *Ostertagia ostertagi*. *Parasitology* **79**, 157-68.
- MICHEL, J.F. (1969).Some observations on the worm burdens of calves infected daily with *Ostertagia ostertagi*. *Parasitology* **59**, 575-595.
- MICHEL, J.F. (1974). Arrested development of nematodes and some related phenomena. In Advances in Parasitology Volume 12 Chapter 136 (ed Ben Dawes) 279-344.
- MICHEL, J.F. (1970). The regulation of populations of *Ostertagia ostertagi* in calves. *Parasitology* **61**, 435-447.
- MOSS, R., WATSON, A., TRENHOLM, I. B., & PARR, R. (1993) Caecal threadworms *trichostrongylus-tenuis* in red grouse *lagopus-lagopus-scoticus* - effects of weather and host density upon estimated worm burdens. *Parasitology* **107**, 199-209.
- NEWTON, L. A., CHILTON, N. B., BEVERIDGE, I. & GASSER, R. B. (1998) Genetic evidence indicating that *Cooperia surnabada* and *Cooperia oncophora* are one species. *International Journal for Parasitology* **28**, 331-336.
- NILSSEN, A. C., ÅSBAKK, K., HAUGERUD, R. E., HEMMINGSEN, W. & OKSANEN, A. (1999) Treatment of reindeer with ivermectin - effect of treatment on insect fauna. *Rangifer* **19**, 61-69
- NILSSEN, A. C., HAUGERUD, R. E. & FOLSTAD, I. (1998). No interspecific covariation in intensities of macroparasites of reindeer, *Rangifer tarandus* (L.). *Parasitology* **117**, 273-281.

- OKSANEN, A., NIEMINEN, M., SOVERI, T. & KUMPULA, K. (1992) Oral and parenteral administration of ivermectin to reindeer. *Veterinary Parasitology* **41**, 241-247
- OKSANEN, A. & NIEMINEN, M. (1998) Moxidectin as an endectocide in reindeer. *Acta Veterinaria Scandinica* **39**, 483-489
- ØRITSLAND NA, ALENDAL E. Svalbardreinen. Bestandens størrelse og livsgrunnlag (in Norwegian). In: Øritsland NA, editor. Svalbardreinen og dens livsgrunnlag. Oslo: Universitetsforlaget, 1986; 52-60.
- PACCALA, S. W. & DOBSON, A. P. (1988). The relation between the number of parasites /host and host age: population dynamic causes and maximum likelihood estimation. *Parasitology* **96**, 197-210.
- PATERSON, S., WILSON, K., & PEMBERTON, J.M. (1998). Major histocompatibility complex variation associated with juvenile survival and parasite resistance in a large unmanaged ungulate population (*Ovis aries* L.). *P. Natl. Acad. Sci. USA* **95**, 3714-3719
- POULIN, R. (1999). The functional importance of parasites in animal communities: many roles at many levels? *International Journal for Parasitology* **29**, 903-914.
- QUINNEL, R. J., MEDLEY, G. F. & KEYMER, A. E. (1990). The regulation of gastrointestinal helminth populations. *Philosophical Transactions of the Royal Society of London B* **330**, 191-201.
- REID, J. F. S. & ARMOUR, J. (1972). Seasonal inhibition and inhibited development of gastro-intestinal nematodes of sheep. *Research in Veterinary Science* **13**, 225-229
- REIMERS, E. (1977). Population dynamics of two sub-populations of reindeer in Svalbard. *Arctic and Alpine Research* **9**, 369-381.
- REIMERS, E. & NORDBY, O. (1968). Relationship between age and tooth cementum layers in Norwegian reindeer. *Journal on Wildlife Management* **32**, 957-961.
- RETTIE, W. J. & MESSIER, F. (2000). Hierarchical habitat selection by woodland caribou: its relationship to limiting factors. *Ecography* **23**, 466-478.
- ROED, K. H. & WITTEN, K. R. (1986). Transferrin variation and evolution of Alaskan reindeer and caribou, *Rangifer tarandus* L. *Rangifer* (Special Issue) **1**; 247-251.
- ROOS, M. H. & GRANT, W. N. (1993). Species-specific PCR for the parasitic nematodes *Haemonchus contortus* and *Trichostrongylus colubriformis*. *International Journal for Parasitology* **23**, 419-21.
- ROUSSET, F. (1996). Inference of parasite-induced host mortality from distributions of parasite loads. *Ecology* **77**, 2203-2211.
- SALISBURY, J. R. & ARUNDEL, J. H. (1970). Peri-parturient deposition of nematode eggs by ewes and residual pasture contamination as sources of infection for lambs. *Aust. Vet. J.* **46**, 523-529.

- SCHNIEDER, T., HEISE, M. & EPE, C. (1999) Genus-specific PCR for the differentiation of eggs or larvae from gastrointestinal nematodes of ruminants. *Parasitol Res.* **85**, 895-898.
- SCOTT, M. E. & LEWIS, J.W. (1987). Population dynamics of helminth parasites in wild and laboratory rodents. *Mammal Review* **17**, 95-103.
- SHAW J. L. & MOSS, R. (1989). The role of parasite fecundity and longevity in the success of *Trichostrongylus tenuis* in low density red grouse populations. *Parasitology* **99**, 253-258.
- SHAW, D. J., GRENFELL, B. T. & DOBSON, A. P. (1998). Patterns of macroparasite aggregation in wildlife host populations. *Parasitology* **117**, 597-610.
- SHOOP, W. L., MROZIK, H. & FISHER, M. H. (1995) Structure and activity of avermectins and milbemycins in animal health. *Veterinary Parasitology* **59**, 139-156
- SKOGLAND, T. (1984). The effects of food and maternal condition on fetal growth and size in wild reindeer. *Rangifer* **4**, 39-46
- SKOGLAND T. (1990). Villreinens tilpasning til naturgrunlaget (in Norwegian). *Villreinen*, 49-64.
- SLOCOMBE J. O. D. (1974). Overwintering of bovine gastrointestinal nematodes in southwestern Ontario. *Can J Comp Med Vet Sci.* **38**, 90-93.
- SMEAL, M. G., FRASER, G. C. & ROBINSON, G. C. (1980). Seasonal changes in the structure nematode populations of cattle in New South Wales in relation to inhibited larval development. *Australian Veterinary Journal* **56**, 80-86.
- SMITH G. (1988). The population biology of the parasitic stages of *Haemonchus contortus*. *Parasitology* **96**, 185-195.
- SMITH G, GRENFELL BT, ANDERSON RM. (1986). The development and mortality of non-infective free-living stages of *Ostertagia ostertagi* in the field and in laboratory culture. *Parasitology* **92**, 471-82.
- SMITH, G. & GRENFELL, B. T. (1985). The population biology of *Ostertagia ostertagi*. *Parasitology Today* **1**, 76-81.
- SMITH G. (1994). Population biology of the parasitic phase of trichostrongylid nematode parasites of cattle and sheep. *International Journal for Parasitology* **24**, 167-78.
- SMITH, G. (1995). Macroparasites Group Report. In Ecology of Infectious Diseases in Natural Populations (ed. Grenfell, B. T. & Dobson, A. P.), pp. 209-229. Cambridge University Press, Cambridge.
- SMITH, H. J., & ARCHIBALD, R. McG. (1969) On the survival of overwintering bovine gastrointestinal nematode larvae during the subsequent grazing season. *Canadian Journal of Comparative Medicine & Veterinary Science* **33**, 44-47.
- SOULSBY, E. J. L. (1982). Helminths, Arthropods and Protozoa of Domesticated Animals, 7th edn. Baillière Tindall, London.

- STAALAND H. (1986). Svalbardreinens ernæring (in Norwegian). In: Øritsland NA, editor. Svalbardreinen og dens livsgrunnlag. Oslo: Universitetsforlaget. 72-91.
- STEAR, M. J., BISHOP, S.C., DOLIGALSKA, M., DUNCAN, J.L., HOLMES, P.H., IRVINE, J., McCRIRIE, L., McKELLAR, Q. A., SINSKI, E. & MURRAY, M. (1995). Regulation of egg production, worm burden, worm length and worm fecundity by host responses in sheep infected with *Ostertagia circumcincta*. *Parasite Immunology* **17**, 643-652.
- STROMBERG, B. E. (1997). Environmental factors influencing transmission. *Vet. Parasitol.* **72**, 247-256.
- STEAR, M. J., PARK, BISHOP, S.C. (1996). The key components of resistance to *Ostertagia circumcincta* in lambs. *Parasitology Today* **12**, 438-441
- STEVENSON, L. A., GASSER, R. B. & CHILTON, N. B. (1996) The ITS-2 rDNA of *Teladorsagia circumcincta*, *T. trifurcata* and *T. davtiani* (Nematoda: Trichostrongylidae) indicates that these taxa are one species. *International Journal for Parasitology* **26**, 1123-1126.
- SUAREZ VH, CABARET J, GRUNER L. (1995). Morphological polymorphism in the nematode *Teladorsagia circumcincta* in relation to age of larvae, infection mode and lamb characteristics in experimental conditions. *Int. J. Parasitol.* **25**, 1173-7.
- SUAREZ, V. H. & CABARET, J. (1991) Similarities between species of the Ostertagiinae (Nematoda: Trichostrongyloidea) in relation to host-specificity and climatic environment. *Systematic Parasitology* **20**, 179-185.
- SYMONS, L. (1983). Anorexia: occurrence, pathophysiology, and possible causes in parasitic infections. *Advances in Parasitology* **24**, 103-133.
- TAYLOR, S. M., EDGAR, H. & KENNY, J. (1993) Prophylactic efficacy of moxidectin for per parturient ewes and mid-summer lambs. *Veterinary Research* **133**, 270-271
- THARALDSEN, J. (1976). The epidemiology of trichostrongylid infections in young cattle in Norway. *Acta Vet Scand.* **17**, 1-21.
- THOMAS, D. C. (1982). The relationship between fertility and fat reserves in Peary Caribou. *Can J. Zool.* **60**, 597-602.
- THOMAS, D. C. & KILLIAN, H. P. L. (1990). Warble infestation in some Canadian caribou and there significance. *Rangifer*, Special Issue **3**; 409-417.
- THOMPSON, J. D., GIBSON, T. J., PLEWNIAK, F., JEANMOUGIN, F. & HIGGINS D. G. (1997). The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* **25**, 4876-82.
- TOMPKINS, D. M. & HUDSON, P. J. (1999). Regulation of nematode fecundity in the ring necked pheasant (*Phasianus colchicums*): not just density dependence. *Parasitology* **118**, 417-423.

- TYLER, N. J. C. (1987). Natural limitation of the abundance of the High Arctic Svalbard Reindeer. (PhD thesis) University of Cambridge.
- TYLER NJC. (1987). Body composition and energy balance of pregnant and non-pregnant Svalbard reindeer during winter. *Symp. Zool. Soc. Lond.* **57**, 203-29.
- TYLER, N. & ØRITSLAND, N. A. (1989). Why don't Svalbard reindeer migrate? *Holarctic Ecology* **12**, 369-376.
- TYLER, N. & ØRITSLAND, N. A. (1999). Varig ustabilitet og bestandsregulering hos svalbardrein. (Eds. S. A. Bengstron, F. Mehlum og T. Severinsen). Norsk Polarinstitut, Tromsø, Norway. Pp 125-138.
- VAN HOUTERT, M. F. J., SYKES, A. R. (1996). Implications of nutrition for the ability of ruminants to withstand gastrointestinal nematode infections. *International Journal for Parasitology* **26**, 1151-1168.
- VAN DER WAL R, KUNST P, DRENT R. (1998). Interactions between hare and Brent goose in a salt marsh system: evidence for food competition? *Oecologia* **117**, 227-234.
- VAN DER WAL, R., IRVINE, J., STIEN, A., SHEPHERD, N. & ALBON, S. D. (2000). Faecal avoidance and the risk of infection by nematodes in a natural population of reindeer. *Oecologia* **124**: 19-25.
- WAHL, G. M., STERN, M. & STARK, G. R. (1979). Efficient transfer of large DNA fragments from agarose gels to diazobenzyloxymethyl-paper and rapid hybridization by using dextran sulphate. *P. Natl. Acad. Sci. USA* **76**, 3683.
- WALLER, P. J., DONALD, A. D., & DOBSON, R. J. (1981). Arrested development of intestinal *Trichostrongylus* spp. in grazing sheep and seasonal changes in the relative abundance of *T. colubriformis* and *T. vitrinus*. *Res. Vet. Sci.* **30**, 213-216.
- WILSON, K., & GRENFELL, B. T. (1997). Generalised linear modelling for parasitologists. *Parasitology Today* **13**, 33-38.
- WILSON, K., GRENFELL, B. T. & SHAW, D. J. (1996). Analysis of aggregated parasite distributions: A comparison of Methods. *Functional Ecology* **10**, 592-601
- YOUNG, R. R., NICHOLSON, R. M., TWEEDIE, R. L. & SCHUH, H. J. (1980). Quantitative modelling and prediction of development times of the free-living stages of *Ostertagia ostertagi* under controlled and field conditions. *Parasitology* **81**, 493-505.
- YOUNG, R. R., & ANDERSON, N. (1981). The ecology of the free-living stages of *Ostertagia ostertagi* in a winter rainfall region. *Australian Journal of Agricultural Research* **32**, 371-388.
- ZARLENGA, D. S., HOBERG, E. P., STRINGFELLOW, F. & LICHTENFELS, J. R. (1998) Comparisons of two polymorphic species of *Ostertagia* and phylogenetic relationships within the *Ostertagiinae* (Nematoda: Trichostrongyloidea) inferred from ribosomal DNA repeat and mitochondrial DNA sequences. *Journal of Parasitology* **84**, 806-812.

