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THE OVER-WINTER SURVIVORSHIP OF *PARELAPHOSTRONGYLUS TENUIS* FIRST-STAGE LARVAE

BY

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ABSTRACT

This study examined the ability of first-stage *Parelaphostrongylus tenuis* larvae to survive winter conditions at the northern limits of white-tailed deer (*Odocoileus virginianus*) range. Fecal pellets freshly deposited by infected white-tailed deer were collected and exposed to winter conditions at the beginning of each winter month (9 December 1996 to 11 March 1997) in a traditional white-tailed deer wintering area located near Grand Marais, Minnesota. A portion of the pellets from each fecal sample was examined immediately and the initial number of larvae per gram of dry feces (larvae/g^d) was determined before exposing the remaining sub-samples to winter conditions. The temperature experienced by larvae was monitored and survivorship was calculated each month.

The mean intensity (larvae/g^d) of first-stage larvae in fecal samples varied considerably over winter. Lowest numbers were passed in December (289 larvae/g^d) and January (188 larvae/g^d) while peak numbers occurred in March (1127 larvae/g^d). Intensity then declined in April (656 larvae/g^d).

Results reported here indicate that first-stage *P. tenuis* larvae are not particularly well adapted to survive winter conditions in the boreal forest. Overall survivorship from December to April was only 27%. Winter temperatures experienced in and beneath the snow layer were moderated and resulted in lower

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larval survivorship compared to the levels observed at lower constant temperatures in the laboratory. The increased number of larvae produced in the March spring rise was similarly affected. Nonetheless, the meningeal worm still successfully infects up to 82% of white-tailed deer in northern areas and it is those first-stage larvae produced during the snow-free period while deer are dispersed over their summer habitat that probably play the biggest role in transmission.

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INTRODUCTION

Parelaphostrongylus tenuis is a parasitic nematode found in the central nervous system of white-tailed deer (*Odocoileus virginianus*) in eastern North America. In this host the meningeal worm is relatively benign but can cause severe neurological disease when transmitted to other native cervids, antelope, exotic bovids and camelids (Anderson and Prestwood 1981; Anderson 1992). The importance of this parasite in the management of sustainable cervid populations is not fully understood (Lankester and Samuel 1997). Much remains to be learned about its transmission, particularly in the eastern boreal forest where white-tailed deer reach their northern-most distribution and cohabit with moose (*Alces alces*) which are susceptible to parelaphostrongylosis (Anderson 1992; Lankester and Samuel 1997).

Adult *P. tenuis*, living in the cranial venous blood sinuses and subdural space of deer, deposit eggs into the blood system that are swept to the lungs where they develop into first-stage larvae. Larvae move into the terminal air-ways, are carried upwards by the muco-ciliary escalator to the pharynx, are then swallowed and passed out on fecal pellets (Anderson 1963 and 1965). Most first-stage larvae are located on or in the thin layer of mucus covering the pellet and are easily removed by water (Lankester and Anderson 1968; Forrester and Lankester 1997a). Terrestrial gastropods are required intermediate hosts and rather than having to be

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attracted to deer feces, probably encounter larvae that have been washed by rains into the soil (Lankester and Peterson 1996). Larvae penetrate the foot of gastropods, moult twice, and develop into the infective third-stage. Ruminants acquire the infection by accidently eating infected gastropods adhering to vegetation.

Meningeal worm probably evolved in the temperate-zone eastern forest with white-tailed deer (Anderson 1972; Carreno and Lankester 1994). However, its present day success in infecting up to 82% of white-tailed deer in northern regions (Bogaczyk *et al.* 1993; Slomke *et al.* 1995; Gogan *et al.* 1997) suggests that it can survive fairly severe winter conditions. Some supporting evidence from laboratory experiments have demonstrated that first-stage larvae of *P. tenuis* can survive constant low temperature freezing from -15°C to -25°C for extended periods (Lankester and Anderson 1968; Shostak and Samuel 1984). This suggests that larvae are resistant to winter freezing. On the other hand, the production of larvae varies throughout the year with the lowest output in the fall and early winter and the highest level in spring (Peterson and Lankester 1991; Slomke *et al.* 1995; Peterson *et al.* 1996). This so called "spring rise" (Taylor 1935) in larval production might suggest instead that the optimum time for the release of larvae is after winter, at a time when terrestrial gastropods are expected to become active.

The importance of larval survivorship over winter is relevant to

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understanding the transmission of the parasite within deer wintering yards. In areas where snow depth regularly exceeds 30-40 cm, deer may aggregate in wintering yards at high densities (eg. $50/km^2$) for 4-5 mo, but are found at lower densities for the remainder of the year (eg. $4/km^2$) (Lankester and Peterson 1996). If the survivorship of larvae passed during winter is high, deer yards may serve as important foci of infection. Two recent field studies demonstrated that the prevalence of *P. tenuis* infection in gastropods can be twice as high in wintering yards as on summer range but levels of infection in both areas were still below 1% (Lankester and Peterson 1996; Whitlaw *et al.* 1996). The relatively small difference in the prevalence of infected gastropods between wintering yards and summer areas suggests that larvae produced over winter may not enjoy particularly high survivorship.

I addressed these conflicting suggestions by examining the ability of first-stage *P. tenuis* larvae to survive winter conditions in the field near the northern limits of white-tailed deer range. Freshly deposited white-tailed deer feces containing first-stage larvae were exposed to winter conditions on a deer wintering yard in northeastern Minnesota every month starting in December and ending in March. The temperature experienced by larvae was monitored and survivorship was determined monthly.

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MATERIALS AND METHODS

Freshly deposited white-tailed deer fecal pellets containing first-stage *P. tenuis* larvae were collected at the beginning of each month from 9 December 1996 to 12 March 1997. Larvae on pellets were exposed to winter conditions on a traditional white-tailed deer wintering area located 20 km southwest of Grand Marais, Minnesota, USA (47°41'N, 90°35'W) and survivorship was calculated monthly. Each month, 10 fecal samples, each comprising ≤ 50 pellets from individual deer, were collected and split into sub-samples of 10 pellets. One sub-sample was examined immediately to determine the initial mean number of larvae per gram of dry feces (larvae/g^d) following a method described by Forrester and Lankester (1997a). The others (enough for one sub-sample to be retrieved and examined after monthly intervals of winter exposure up to 3 April) were enclosed in envelopes made of fibre glass window screening. Each envelope was labelled and placed out in a fenced area on the wintering yard, initially (9 December) on the ground, and later on the surface of the snow existing at the time. A small amount of snow (2-5 cm) was dusted over the feces to approximate the position in which they were originally found and to reduce possible effects of short term desiccation. A temperature recording device (Stowaway, Onset Computer Corporation, Pocasset, MA) was positioned near the sub-samples. Thereafter, the pellets and temperature recorders were allowed to become covered with naturally

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accumulating snow. Air temperature was similarly recorded nearby at a height of 2 m. Daily snowfall and the weekly snow depth at Grand Marais were recorded by Mr. Bill Peterson, Department of Natural Resources, Grand Marais, Minnesota.

A parallel study of winter larval survivorship was conducted in a fenced area on Lakehead University campus, Thunder Bay, Ontario, Canada (48°25'N, 89°12'W). Fecal samples from two white-tailed deer experimently infected with *P. tenuis* were collected over a one week period and stored at -14°C for 2 mo before use. After thoroughly mixing the pellets, 50 sub-samples, each comprising 10 pellets were removed. Ten sub-samples were immediately examined to determine the initial mean number of larvae/g^d and the remaining 40, enclosed in screen envelopes, were put in individual aluminum trays (15 cm diameter) and placed on the ground on 4 December 1996. Sub-samples were grouped in lots of 10 and their position marked by a stake so that 10 could be uncovered each month and examined for live larvae without disturbing the snow covering the remaining samples. Aluminum trays were placed beneath each fecal sample to trap any larvae that might be washed from the pellets by melting snow or rain. All snow, and any liquid found in the trays when samples were retrieved, was collected and placed in a 1000 ml beaker, allowed to melt and settle, and examined for larvae. The temperature and relative humidity was recorded at ground level beneath the snow and nearby in an instrumentation shelter at a height of 2 m throughout

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winter.

To determine the effects of constant, low-temperature freezing on the survivorship of larvae, six fecal samples of 20-26 pellets containing first-stage larvae were collected at Grand Marais on 9 December 1996 and each sample was divided in half. Half of the pellets in each sample were used to determine the initial number of larvae/g^d. The remainder were stored in sealed plastic bags and placed in a freezer at -14°C. After 4 mo, the samples were retrieved and the number of surviving larvae was determined.

In a preliminary study, the number of larvae/g^d before and after monthly intervals of winter exposure was determined by extracting live larvae from feces using the Baermann funnel technique (Baermann 1917). The technique involved placing feces over a porous filter of cheese cloth or cellulose tissue in a water-filled funnel. Larvae that left the feces, passed through the filter, and sank into the funnel stem, were drained into a Petri dish after 24 hr and counted using a stereomicroscope at a magnification of 16 X. Subsequently, however, Forrester and Lankester (1997a) found that the sloping sides of glass Baermann funnels retained large numbers of larvae and the number drained from the funnels did not correlate with total numbers initially present in feces. Consequently, results generated in the preliminary survivorship study during the winter of 1995-96 using the unreliable Baermann technique were discarded.

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An alternative method for determining the number of larvae in fecal samples was developed and tested (Forrester and Lankester 1997a) and was used routinely to produce results reported herein. Ten fecal pellets containing larvae were weighed and placed in screen envelopes (12 x 12 cm) made from a folded piece of fibre glass window screening; the open sides were stapled to contain the pellets. Two or three pellets from the sample were weighed before and after being placed in a drying oven at 100°C for 24 hr. This allowed the number of larvae recovered from test pellets to be expressed on a fecal dry weight basis (larvae/g^d). The percent moisture content of feces was also calculated and used as an estimate of the degree of desiccation. Screen envelopes containing feces were submerged in 250 ml beakers filled with tap water at room temperature and removed after 24 hr. Beakers were allowed to settle for an additional 1 hr before the top 200 ml of solution was siphoned off and the residual volume of 50 ml poured into a plastic Petri dish and examined for live larvae using a stereomicroscope at a magnification of 16 X. A 0.8 x 0.8 cm grid, etched manually on the bottom of the Petri dish using a dissecting pin, aided in counting larvae. Forrester and Lankester (1997a) demonstrated that this method consistently extracted 87% of live larvae present in fecal samples.

The percent larval survivorship was calculated by dividing the number of live larvae/g^d after monthly exposure to winter conditions by the number of live

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larvae/g^d extracted before exposure x 100. Larvae observed motionless and shaped like a "C" or "J" were gently stroked with a piece of hair. If no movement was observed, they were considered dead and not counted. All glassware was washed between trials with soap and water and rinsed with 95% ethyl alcohol (Whitlaw and Lankester 1995).

Data were analysed using the Statistical Package for the Social Sciences (SPSS) (SPSS Inc., Chicago, Illinois, USA) and normalized using a natural log transformation. To determine significant monthly differences in the number of live larvae/g^d, four separate repeated measures ANOVA (F-test) were used for samples placed out in December, January, February and March and exposed until April. When data that did not meet the assumption of homogeneity of covariance, a Greenhouse-Geisser Epsilon was applied to the degrees of freedom to legitimize the F-test (Howell 1997). Multiple comparisons of mean number of larvae/g^d between exposure times were performed using multiple paired t-tests with the Bonferroni correction (Howell 1997).

To determine if larval survivorship at the end of the test period (early April) depended upon the length of exposure to winter conditions, the difference between the initial number of larvae/g^d (at the time samples were collected each month) and the number finally extracted in April was compared. A new set of variables called "change scores" was produced for each comparison by subtracting the natural log of the number of larvae/g^d extracted in April from the natural log of the number of

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larvae/g^d before winter exposure. A one way analysis of variance was performed on the change scores (Jamieson 1994; Wainer 1991) with a least significant difference (LSD) multiple comparison test (Zar 1984). P-values ≤ 0.05 were considered significant.

RESULTS

Weather and subnivean conditions

Snow began to fall at Grand Marais on 18 November 1996 and reached a maximum depth of 70 cm on 10 March 1997 (Fig. 1). Daily snowfall totalled 57 cm in December, 91 cm in January, 13 cm in February, and 47 cm in March for a total winter snow fall of 208 cm (Fig. 2). Bare patches of ground first appeared 22 April and most of the snow had melted by 28 April. Light to medium rain (< 20 mm) fell on 28 March and 7 different days in April.

The mean ± SE air temperature between 9 December 1996 and 3 April 1997, the period over which fecal samples were exposed, was -8.2 ± 0.6°C (Max. 6.5°C; Min. -24.0°C), while the mean temperature beneath the snow during the same time period was -1.1 ± 0.1°C (Max. -0.2°C; Min. -2.5°C) (Fig. 1). Air temperatures fluctuated widely while temperatures beneath the snow were fairly stable. As an example, the air temperature at Grand Marais fluctuated by as much as 30°C during the first 18 days of January and by as much as 20°C in a 24 hr

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period (Fig. 3). During the same 18 day period, the temperature beneath 32 to 49 cm of snow fluctuated only 1.3°C and the maximum fluctuation in a single day was only 0.8°C. The mean air relative humidity at Grand Marais between 9

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Fig. 1. Snow accumulation at Grand Marais, Minnesota from 18 November 1996 to 10 March 1997. Insets show the fluctuations in air and subnivean temperatures during winter.

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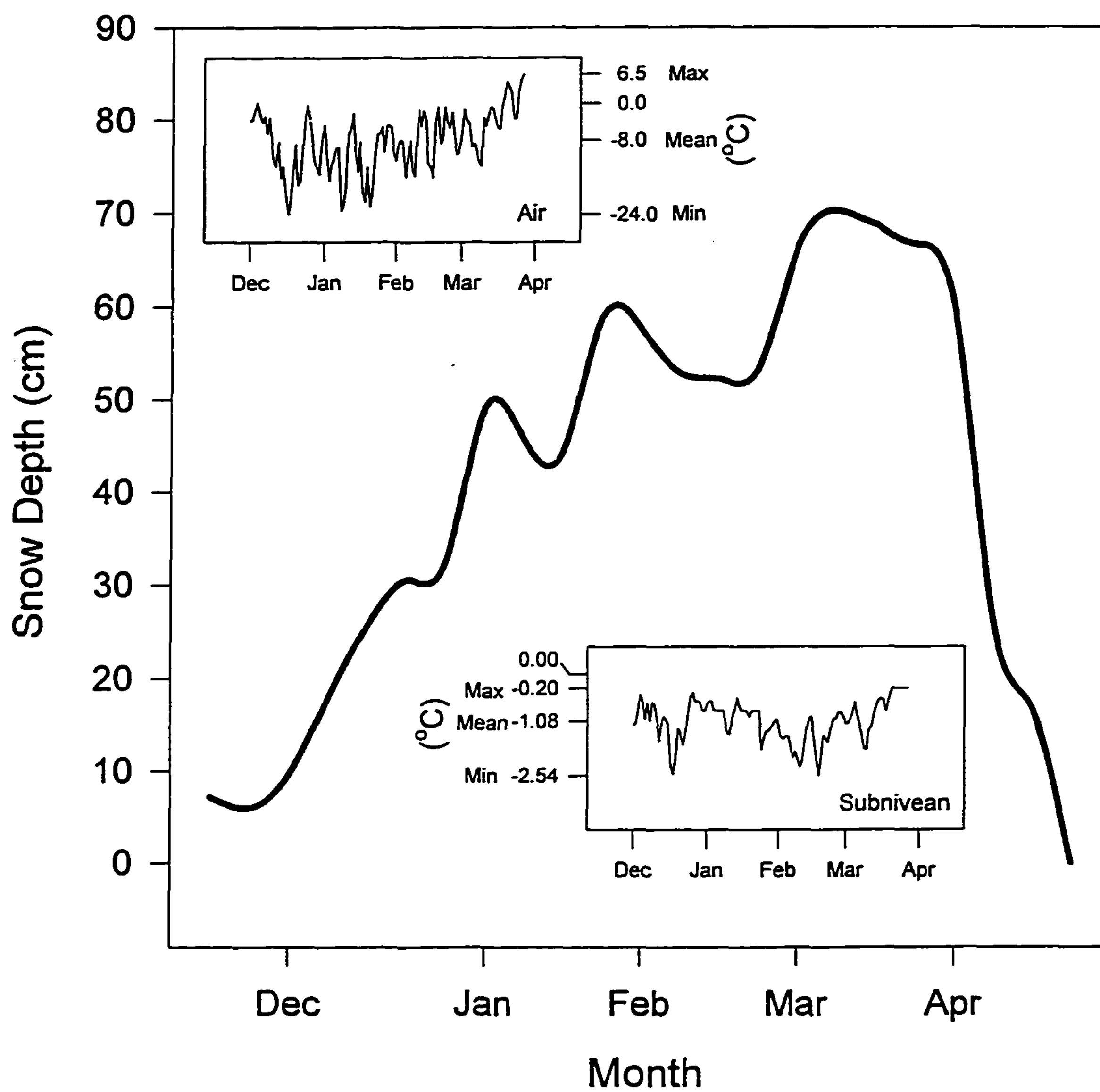

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Fig. 2. Daily snowfall at Grand Marais, Minnesota from 1 December 1996 to 31 March 1997

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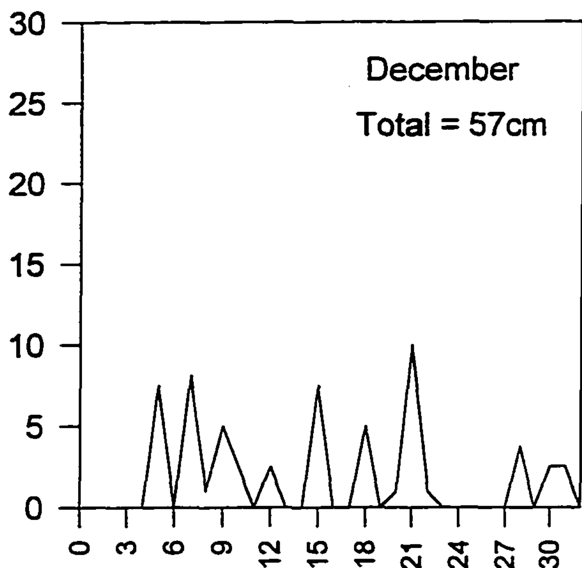

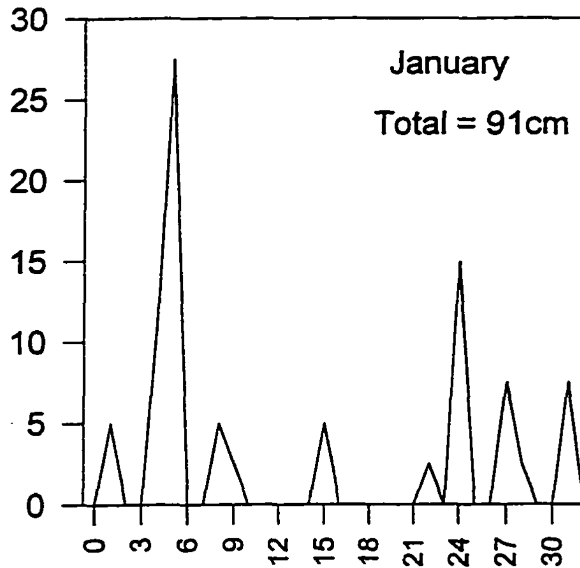

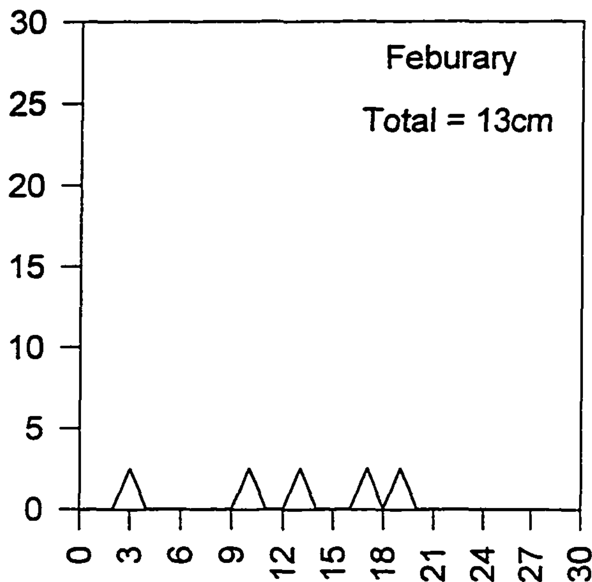

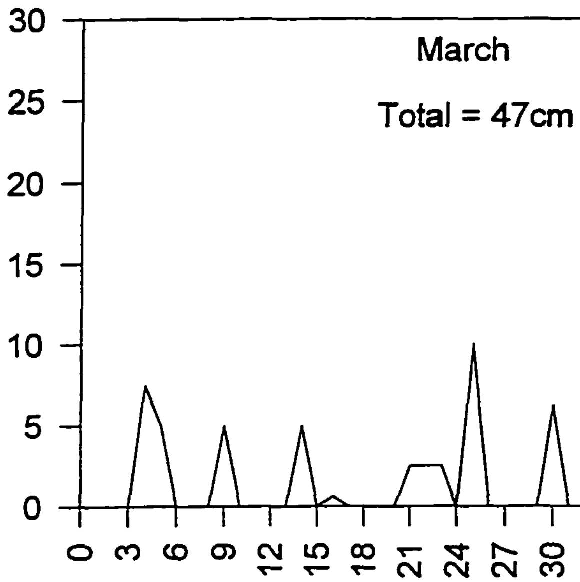

Daily Snowfall (cm)

Days

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Fig. 3. Air (A) and subnivean (B) temperatures measured every 2 hr at Grand Marais 1-18 January 1997.

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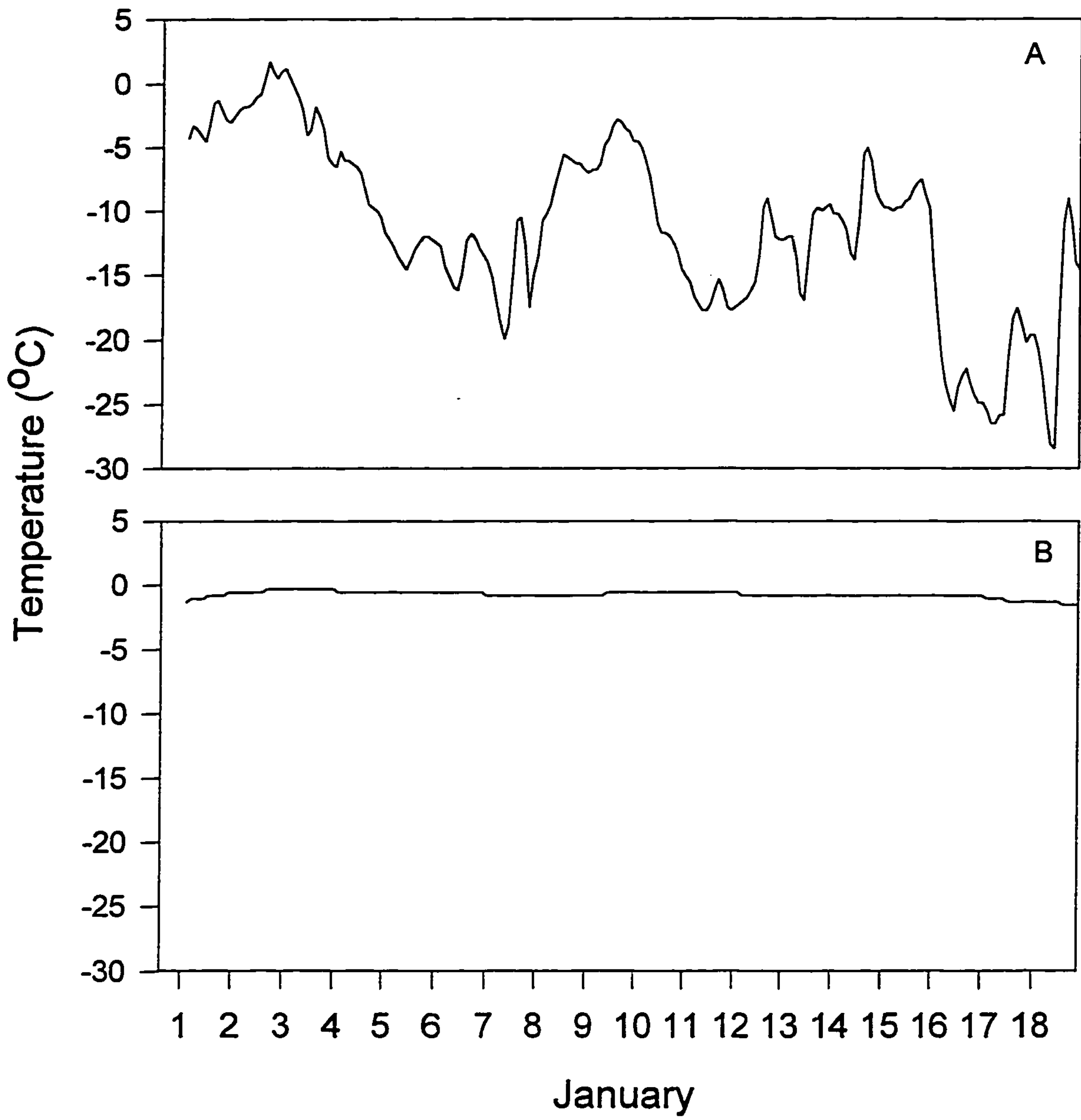

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Fig. 4. Air relative humidity (%) at Grand Marais, Minnesota from 9 December 1996 to 3 April 1997

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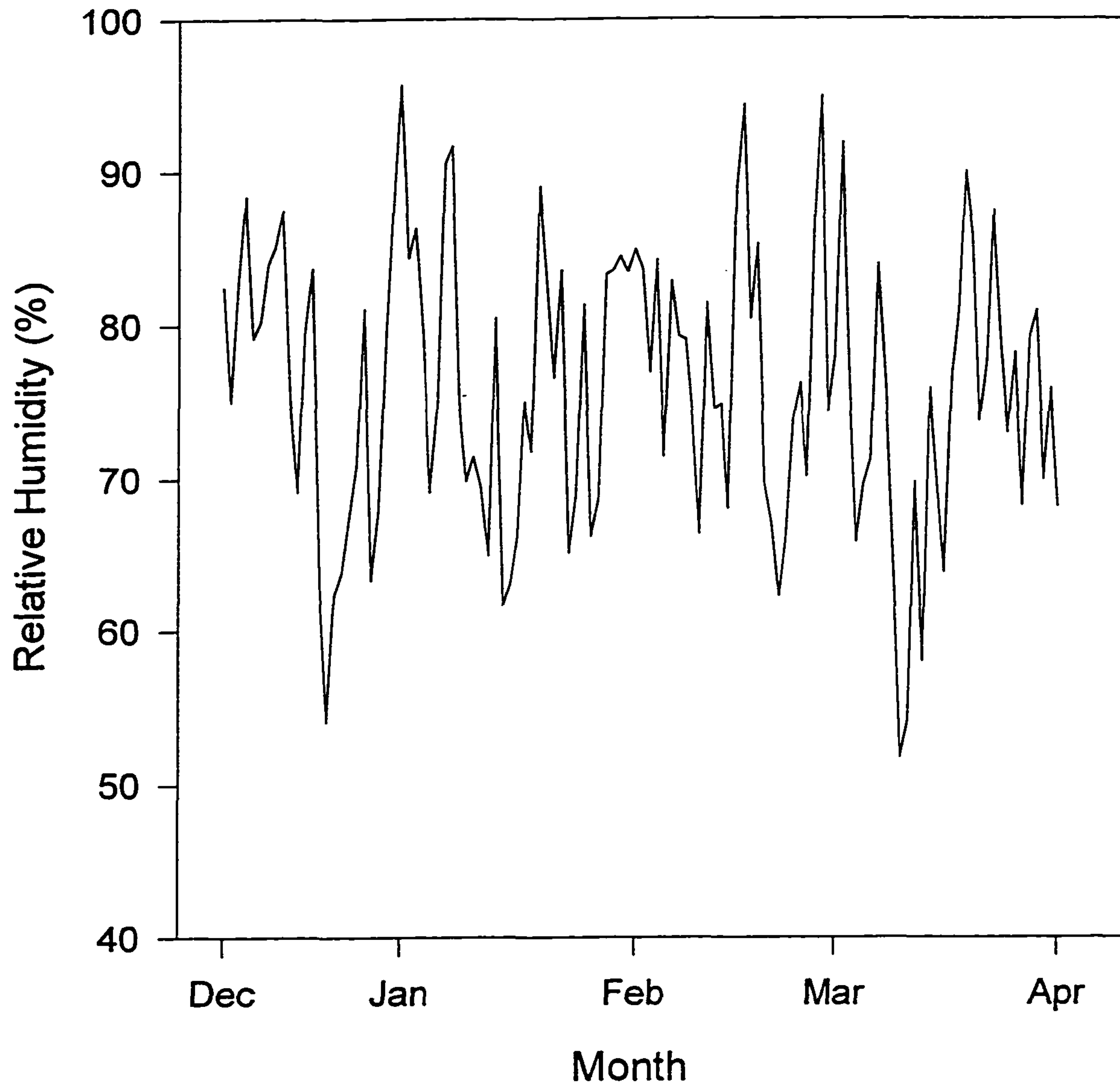

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December 1996 and 3 April 1997 was 76% (Max. 96.0%; Min. 51.7%) (Fig. 4). The relative humidity of the subnivean environment remained at 100%.

Larval production and survivorship

The numbers of *P. tenuis* larvae produced by white-tailed deer varied over winter. Mean ± SE numbers present in freshly deposited feces increased from 289 ± 101 larvae/g^d in early December to a peak of 1127 ± 237 larvae/g^d in early March (Fig. 5, Table 1) and thereafter declined to 657 ± 139 larvae/g^d in early April (Table 1).

Only 27 % of the total larvae initially present in deer feces were recovered when examined in April. Of those larvae on pellets placed on the ground in early December, only 16% were recovered in April (Table 1). The greatest decline occurred within the first 2 months (Fig. 5 and 6). These pellets remained covered with up to 70 cm of snow during the study period. They experienced relatively warm ($\bar{x}$ = -1.1 °C) and constant temperatures (-0.2 to -2.5 °C) (Fig. 5). The percent moisture content of pellets retrieved over winter did not differ from that of freshly deposited pellets. Beginning in February, they became covered with an increasingly dense growth of an unidentified white mould.

Numbers of larvae on pellets placed on snow in January and in February similarly declined substantially within the first two months (Fig. 5 and 6); and

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Fig. 5. Mean numbers of *Parelaphostrongylus tenuis* larvae/g[d] of feces after each winter month for samples deposited in A. December B. January C. February and D. March. The recorded temperatures adjacent to pellets are also shown. Vertical scales are not the same for A,B,C, and D.

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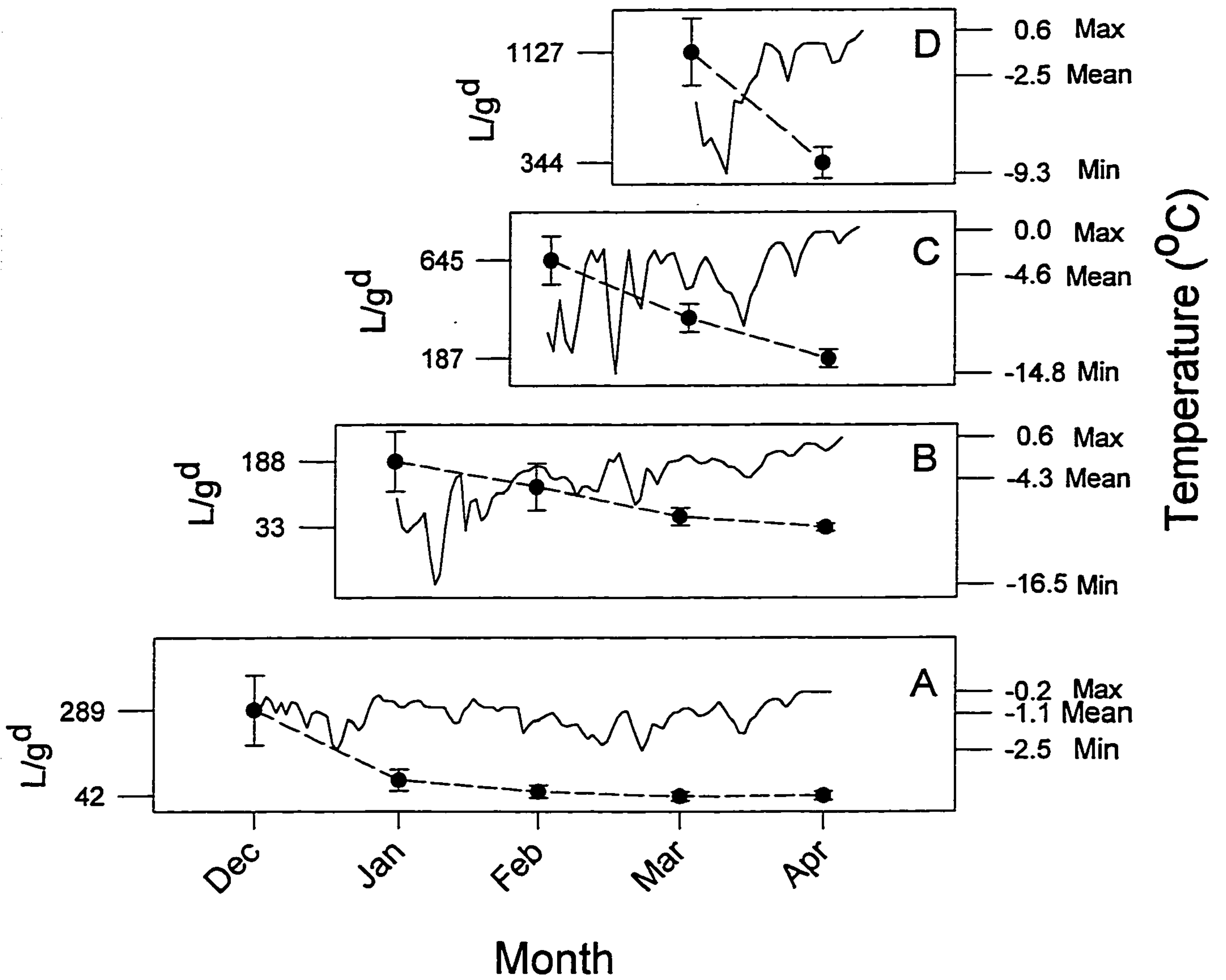

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Table 1. Monthly determination of the mean number of *Parelaphostrongylus tenuis* larvae/g^{d}* surviving on white-tailed deer feces exposed to winter conditions at Grand Marais, Minnesota.

Samples collected and exposed	Mean number of live larvae/g^{d} extracted from fecal pellets					Mean % survival as of 3 Apr
	9 Dec	11 Jan	12 Feb	12 Mar	3 Apr	
9 Dec	289±101[a†]	86±31[b]	53±18[c]	39±13[c]	42±13[c]	16 %
11 Jan	-	188±71[d†]	128±55[e]	58±21[f]	33±9[f]	25 %
12 Feb	-	-	645±111[g†]	376±66[g]	187±42[h]	36 %
12 Mar	-	-	-	1127±237[i†]	344±110[j]	30 %
3 Apr	-	-	-	-	657±139[†]	N/A

Note: Mean ± standard error values superscripted with the same letter are not significantly different from each other based on paired t-tests with the Bonferroni correction at $P \geq 0.05$ (Howell 1997).

*larvae/g^{d} = mean number of larvae per gram of dry feces

† initial mean number of larvae/g^{d} of feces before exposure

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Fig. 6. Comparisons of the monthly mean percent survivorship of *Parelaphostrongylus tenuis* larvae in feces deposited in (●) December (○) January (■) February and (□) March and exposed until April.

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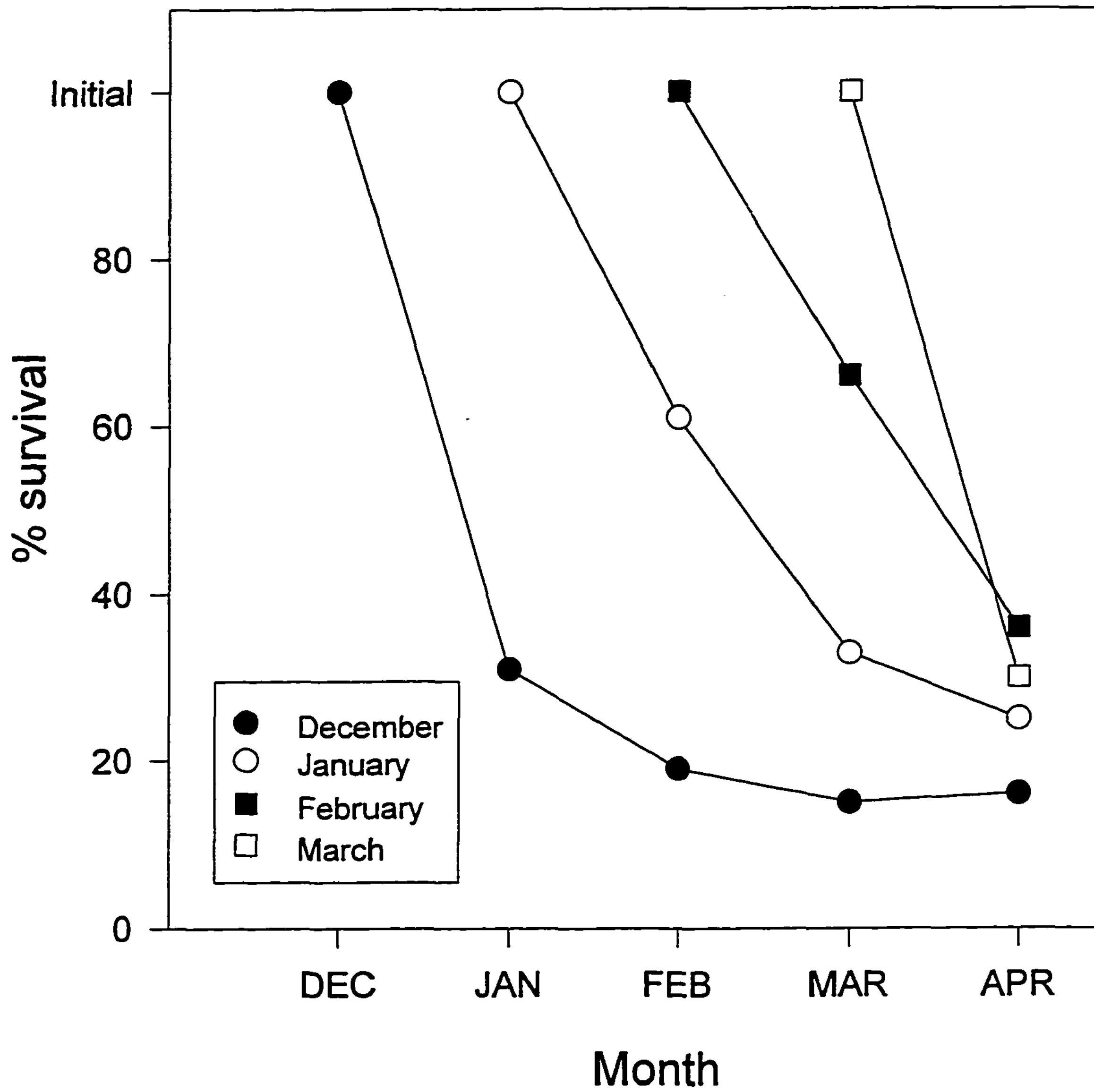

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only 25% and 36%, respectively, were recovered in April. Pellets were placed on top of 45-50 cm of snow, became covered with an additional 20 cm of snow and experienced slightly colder (ca. $\bar{x}$ = - 4.5 °C) and greater fluctuations in temperature (0.6 to -16.5 °C) (Fig. 5). These pellets did not lose their moisture content and no mould growth was evident.

In early March, deer passed the greatest numbers of larvae but after 23 days of exposure, only 30% were recovered (Table 1, Fig. 6). Samples were placed on 68 cm of snow and experienced one day of rain on 28 March and a mean temperature of - 2.5 °C (range 0.6 to -9.3 °C) throughout the month (Fig. 5). Pellets were not covered with snow when retrieved 3 April and had lost over half of their initial moisture content (declined from 72% to 33%). No mould growth was evident.

Although it was planned that the winter survivorship tests terminate 3 April, samples of pellets deposited by deer on that date were placed on the snow and examined 1 mo later. Pellets initially contained a mean of 657 ± 139 larvae/g^d but only 50 ± 18 larvae/g^d (8%) were recovered 30 days later. Pellets remained free of snow and were exposed to rain and air temperatures ranging from 6.7 to -2.2 °C. When retrieved, the pellets were partially disintegrated, powder dry and most had lost their dark-coloured outer coating.

By early April, there was an evident trend toward decreased percent larval

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recovery with increased length of exposure to winter conditions; 16% recovery of larvae exposed for 4 mo since December, 25% after 3 mo since January, and 36% after 2 mo since February, but none of these values differed statistically based on a LSD multiple comparison test on change scores. Larvae exposed for only 23 days since early March, were not included because pellets were disintegrating when retrieved.

The parallel experiment on campus at Lakehead University was designed to determine if larvae that were not recovered from fecal pellets might have left the pellets rather than died. Percent recovery of larvae was similar to that of pellets placed out in December at Grand Marais with 33% after 1 mo, 11% after 2 mo, 11% after 3 mo, and 12% after 4 mo. Mould was observed on fecal pellets retrieved on the campus after 2 mo. No larvae were found in the snow retained by 30 pie plates beneath pellets exposed for up to 3 mo and only 5 larvae of a total of 179 survivors were found in 10 plates examined after 4 mo (April). Pellets at the campus location were covered with a maximum of 68 cm of snow which had dropped to approximately 35 cm when samples were retrieved in early April. Samples experienced a mean subnivean temperature of -1.6 °C ± 0.1 (Max. 0.0°C; Min. -5.8 °C) for the entire winter. The mean air temperature during winter was -10.8°C ± 0.2 (Max. 9.0°C; Min. -33.6°C) and the mean air relative humidity was 84.4% ± 0.4% (Max. 100.0%; Min. 29.5%).

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The recovery of *P. tenuis* larvae from pellets placed in a freezer at -14 °C was compared to that of larvae exposed for the same length of time beneath the snow. Seventy-six percent of those in the freezer since December were recovered 4 mo later compared with only 16% of those exposed beneath the snow.

DISCUSSION

The numbers of first-stage *P. tenuis* larvae in feces passed by white-tailed deer varied considerably over the winter months. Lowest in December and January, they more than tripled in number in early March, only to decline by almost half in early April. Peak larval numbers in early March were also recorded by Peterson *et al.* (1996) who studied *P. tenuis* infected deer near Grand Marais over a period of 8 years. Similar results were reported by Slomke *et al.* (1995).

A spring rise in the number of first-stage *P. tenuis* larvae in white-tailed deer feces is a feature of several other protostronglyid nematodes of cervids. Samuel *et al.* (1985) found that mule deer (*Odocoileus hemionus*) passed the maximum number of *Parelaphostrongylus odocoilei* larvae in March and caribou (*Rangifer tarandus caribou*) infected with *P. andersoni* passed more larvae in March-early April than in December (Lankester and Hauta 1985). Uhazy *et al.* (1973) also reported a peak in larval production in March for *Protostrongylus* spp. in bighorn sheep feces while Forrester and Senger (1964) reported highest numbers during April and May.

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Halvorsen *et al.* (1985) presented evidence suggesting that seasonal changes in the output of protostrongylid larvae might be regulated by the immune response. A spring rise in *Elaphostrongylus rangiferi* larvae was seen most strongly in female reindeer (*R. t. tarandus*) whose immune response, it was suggested, might be compromised by the stress of calving while the highest output from males coincided with the fall rut season. Peterson *et al.* (1996) found no evidence that the seasonal output of *P. tenuis* by white-tailed deer differed between the sexes but output was negatively correlated with deer age. Deer of all ages passed the most larvae in spring (March-May) but almost twice as many larvae were passed by 9- to 10-month-old fawns than by older animals (Slomke *et al.* 1995, Peterson *et al.* 1996). Anderson (1963) suggested that high larval output by fawns reflected the infection of immunologically naive animals.

The spring rise in protostrongylids may be an adaptation designed to ensure that the maximum numbers of larvae are produced at a time when survivorship is greatest and when maximum opportunity exists for infection of terrestrial gastropod intermediate hosts. Over much of the historical range of white-tailed deer in eastern North America, a spring rise in March coincides reasonably well with the usually warmer and wet spring period. But in those areas of the eastern boreal forest where deer reach their most northern limits of distribution, winter is prolonged.

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Winter in northeastern Minnesota and northwestern Ontario extends from about mid-November to late April, during which time the ground is snow-covered. Larvae of *P. tenuis* deposited during this period experienced high mortality, including those produced during the spring rise. Mortality was highest during the first 2 months of exposure, regardless of when they were placed out. Overall, only 27 % of larvae initially present could be recovered from samples retrieved in April. My assumption that unrecoverable larvae died rather than left the pellets is supported by the experiment in which an aluminum tray was positioned beneath each fecal sample. No larvae were found in snow held by the trays from December to March and only 5 larvae (3% of total) were recovered from the trays in April. Water is usually not evident as the snow slumps and disappears in April. As well, larvae on pellets surrounded by snow are likely immobile at near zero temperatures and may be unable to free themselves from the pellet surface. Hansson (1974) found that *Skrjabingylus nasicola* larvae left polecat (*Mustela putorius* L.) feces more slowly when they were submerged in water near 0°C than at room temperature. Although first-stage larvae of *P. tenuis* quickly wiggle free from the surface of pellets submerged in water at room temperature (Lankester and Anderson 1968; Forrester and Lankester 1997a), their removal from feces in early spring may require a medium to heavy rainfall.

Differences in survivorship among winter-exposed samples retrieved in

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April were small (16 to 36%) and were not statistically different but results hint at the possible importance of exposure temperature, duration, and the degree of desiccation in determining larval survivorship. Pellets placed out in December remained on the ground throughout winter until early April and experienced the warmest and steadiest temperatures. However, only a mean of 19% survived after the first 2 mo and 16% after 4 mo. Pellets placed out in January and February on accumulated snow, experienced lower and fluctuating temperatures and more survived (33% and 36%, respectively) after the first 2 mo. This suggests that larval survivorship is increased at lower temperatures. Interestingly, 76% of larvae on pellets kept at a constant temperature of -14°C survived after 4 mo. Shostak and Samuel (1984) investigated the effects of a constant freezing temperature compared to repeated freeze-thaw cycles and found that both *P. tenuis* and *P. odocoilei* larvae survived well at - 25 °C but *P. tenuis* was the more susceptible to fluctuating temperatures. Generally, *P. odocoilei* larvae survived best when relative humidity and temperature were low.

Mould growth was observed only on deer pellets positioned directly on the ground in December, both at Grand Marais and at the campus site. It was probably promoted by the warmer temperature and high relative humidity of the subnivean environment. Although fewer larvae (16%) were recovered from mouldy feces than from those without mould, differences were not significant. The presence of

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mould on bighorn sheep feces was not noted by Uhazy *et al.* (1973) who recovered 61% of *Protostrongylus* spp. larvae exposed for 156 days beneath snow. This species may simply be more resistant to subnivean conditions than *P. tenuis* or part of the difference may be due to the position of *Protostrongylus* spp. larvae in feces. Forrester and Lankester (1997b) found that most larvae of *Protostrongylus* spp. are in the interior of the pellet where they may be protected somewhat from mould and the conditions that promote its growth.

The duration of exposure to winter conditions was most important within the first 2 months when the mortality of larvae was greatest; larval numbers declined more slowly thereafter. However, the large decline in larval numbers on pellets placed on top of the snow for only 23 days in March seemed unusual, particularly since winter conditions by then were abating somewhat. Although some new snow fell in March, there was a net decline in snow depth and pellets were periodically exposed to sunlight. They also experienced one day of light rain. Some larvae may have been washed off but the air temperature that day (2-5°C) probably was low enough to limit larval mobility. Larvae in water stored in the fridge at 4-5°C are inactive (personal observation). The effects of direct sunlight on *P. tenuis* larvae is unknown but when larvae of the sheep lungworm, *Muellerius capillaris* were exposed to bright light, 85% died within 1 hr (Rose 1957).

Desiccation may also affect survivorship of larvae but only circumstantial

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evidence is provided by this study. Even though the pellets placed out in early March were rained on, they had still lost more than half their initial moisture content when retrieved in early April. As winter begins to wane during April, recently deposited feces increasingly become exposed to drying winds as snow depth declines. Feces may also be exposed alternately to rapid drying and intermittent rain. Pellets placed out in early April had lost their black-coloured outer coating, were disintegrating and had dried almost to a powder by the time they were retrieved 3 May. Only 8% of the larvae could be recovered. Some may have died from desiccation or exposure to sunlight but loss of the fecal surface coating suggests that most larvae were probably washed off onto the surrounding snow and ground litter. The pellet surface contains up to 87% of first-stage *P. tenuis* larvae (Forrester and Lankester, 1997a)

Results reported here help to explain why deer wintering yards do not have as many gastropods infected with *P. tenuis* as might be expected from their concentrated use by deer. Deer existed at the Grand Marais wintering yard at densities of 50/km^2 for nearly 5 mo and on summer range at 4/km^2 for 7 mo, but in over 12,000 snails and slugs examined, only 7 from the wintering yard and 3 from an equal area of summer range had third-stage *P. tenuis* larvae (Lankester and Peterson 1996). Using monthly mean larval output data presented here and by Peterson *et al.* (1996) (and assuming constant seasonal deer defecation rates), I

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estimate that 16 times as many first-stage larvae are initially deposited in wintering yards (/km^2) compared to summer range. However, considering that only 27% of larvae deposited during the snow-covered period survived, this difference is reduced to a factor of 4 by the time snow starts to disappear in spring. But a discrepancy still exists in that infected gastropods are only twice as abundant in the Grand Marais wintering yard as on the summer range. Possibly those larvae that survive winter have a reduced capacity to infect gastropods and develop to the infective stage. Shostak and Samuel (1984) found that freezing first-stage *P. tenuis* larvae at -25°C for 30 days, reduced the number of larvae capable of developing in snails by 96%. Although loss of infectivity by larvae protected beneath snow may be less, it nonetheless becomes clear why deer wintering yards are not the foci of infection that they might be.

In summary, my results indicate that first-stage *P. tenuis* larvae are not particularly well adapted to survive winter conditions in the boreal forest where white-tailed deer reach their most northerly distribution. Overall larval survivorship from December to April was 27%. Winter temperatures experienced by larvae in and beneath the snow layer were moderated but appeared to increase rather than decrease larval mortality compared to levels observed at lower constant temperatures in the laboratory. The increased number of larvae produced in the March spring rise were similarly affected by high mortality. At this time, the

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ground was still snow-covered and deer remained aggregated in winter yarding areas. Despite these productivity losses, the meningeal worm still successfully infects up to 82% of its deer hosts in northern areas and remains a threat to co-habiting susceptible cervids. Although little is known about the survivorship of larvae produced during the snow-free period while deer are dispersed over their summer habitat, these larvae probably play the biggest role in transmission. Slomke *et al.* (1995) found that 79% of white-tailed deer become infected during their first summer and fall of life. In any event, wintering yards still represent a greater risk of infection to the few deer and moose that reside there over summer but these areas still comprise a relatively small part of their total habitat.

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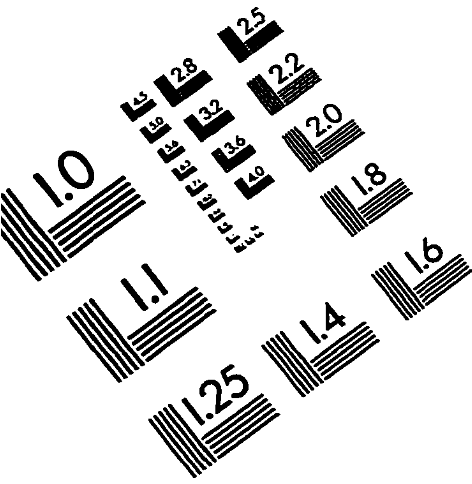

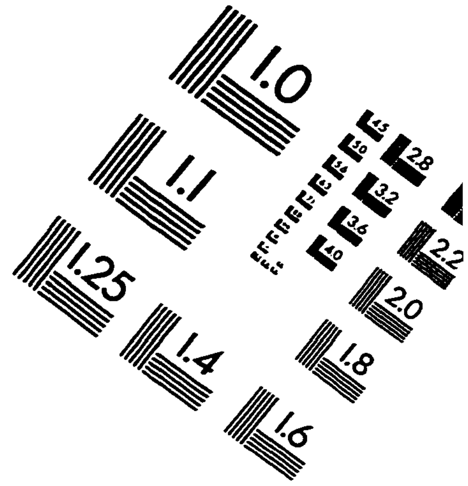

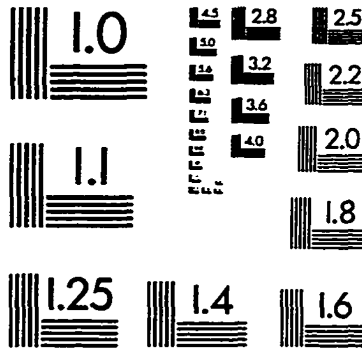

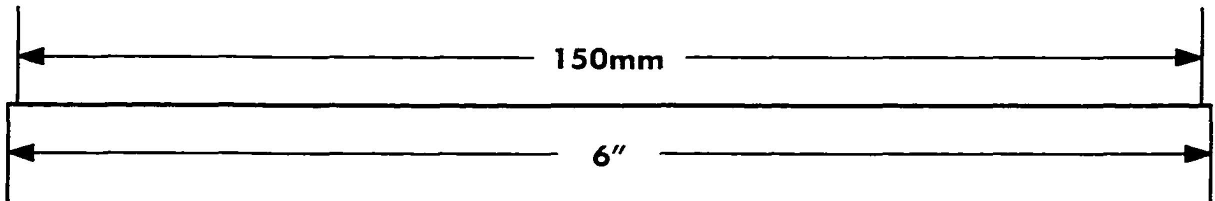

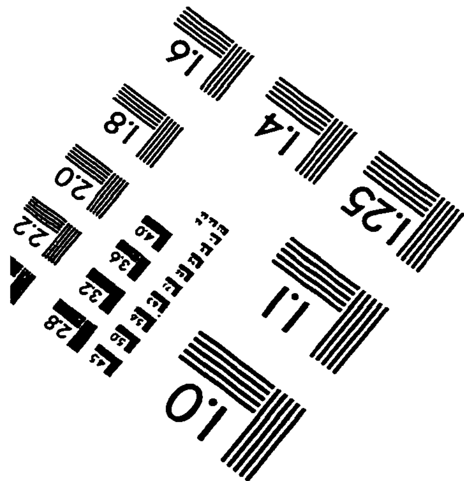

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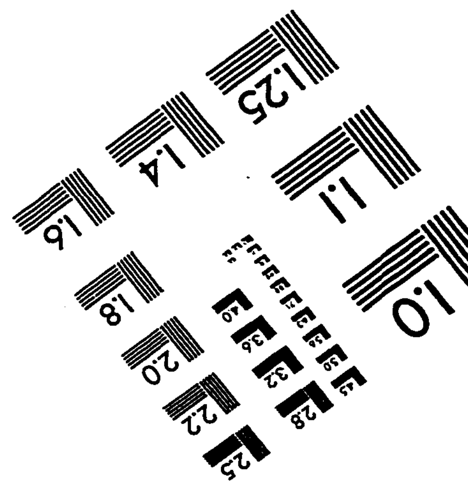

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