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Susan J. Kutz

University of Saskatchewan, susan.kutz@usask.ca

Eric P. Hoberg

United States Department of Agriculture, Agricultural Research Service, geocolonizer@gmail.com


John Nishi

Department of Resources, Wildlife and Economic Development, Government of Northwest Territories, Fort Smith, Canada

Lydden Polley

University of Saskatchewan, lydden.polley@usask.ca

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Development of the muskox lungworm, *Umingmakstrongylus pallikuukensis* (Protostrongylidae), in gastropods in the Arctic

Susan J. Kutz, Eric P. Hoberg, John Nishi, and Lydden Polley

Abstract: Development of the muskox protostrongylid lungworm, *Umingmakstrongylus pallikuukensis*, in its slug intermediate host, *Deroceras laeve*, was investigated under field conditions in the Arctic. Every 2 weeks, from 19 June to 28 August 1997, groups of 10 experimentally infected slugs were placed in tundra enclosures in a mesic sedge meadow near Kugluktuk, Nunavut, Canada. First-stage larvae (L1) infecting slugs on or before 17 July developed to third-stage larvae (L3) in 4–6 weeks. Intensity of L3 in slugs peaked at 6–8 weeks post infection (PI) and then progressively declined by 10, 12, and 48–50 weeks PI. Abundance of L3 in slugs was greatest during mid to late August. L1 infecting slugs on 31 July or later did not develop to L3 before the end of September but overwintered in slugs on the tundra as L1 or as second-stage larvae, completing development to L3 the following summer. The years 1997 and 1998 were exceptionally warm and, in cooler years, rates of larval development may be slower and patterns of availability may differ. The amount of heating (degree-days) accumulated during each trial was calculated using the 8.5°C threshold determined in the laboratory, a 21°C maximum, and either surface, soil, or air temperature. Only degree-days accumulated at the surface were sufficient to correspond to the observed rates of larval development. This enclosure-based system and associated degree-day calculations may be used for predicting the effects of climate and climate change on patterns of parasite development and transmission in the Arctic.

Résumé : Nous avons étudié le développement du ver du poumon du boeuf-musqué, *Umingmakstrongylus pallikuukensis*, chez son hôte intermédiaire, la limace *Deroceras laeve*, en nature dans l'Arctique. Toutes les deux semaines, du 19 juin au 28 août 1997, nous avons mis 10 limaces infectées expérimentalement dans des enceintes de la toundra, dans une prairie à laiches mésique, près de Kugluktuk, Nunavut, Canada. Les larves de premier stade (L1) qui ont infecté les limaces jusqu'au 17 juillet inclusivement sont parvenues au troisième stade larvaire (L3) en 4–6 semaines. L'intensité des infections des limaces par les larves L3 a atteint un sommet 6–8 semaines après l'infection (PI) pour décliner par la suite, tel qu'observé aux semaines 10, 12 et 48–50 PI. L'abondance des larves L3 chez les limaces a été maximale de la mi-août à la fin d'août. Les larves de premier stade qui ont infecté les limaces après le 30 juillet n'ont pas atteint le troisième stade à la fin de septembre, mais elles ont passé l'hiver dans les limaces de la toundra sous forme de larves de premier ou de deuxième stade et ne sont parvenues au stade L3 que l'été suivant. Les années 1997 et 1998 ont été exceptionnellement chaudes et il se peut qu'au cours d'années plus fraîches, le développement soit plus lent et les patterns de disponibilité, différents. La quantité de chaleur accumulée (degrés-jours) à chaque expérience a été calculée en tenant compte du seuil de 8,5°C déterminé en laboratoire, de la température maximale de 21°C, et de la température de la surface, du sol ou de l'air. Seul le nombre de degrés-jours en surface est suffisamment élevé pour expliquer les taux observés de développement larvaire. Le système à enceintes et le calcul des degrés-jours peuvent servir à prédire les effets du climat et des changements climatiques sur les patterns de développement des parasites et sur leur transmission dans l'Arctique.

[Traduit par la Rédaction]

Introduction

Umingmakstrongylus pallikuukensis is a protostrongylid lungworm unique to muskoxen (*Ovibos moschatus*) in the Canadian Arctic (Hoberg et al. 1995; Kutz et al. 1999, 2001a).

It is well established in the west-central mainland muskox populations, with prevalence approaching 100% in adult animals, but is apparently absent from all other muskox populations in Canada, Alaska, and Greenland (Gunn and Wobeser 1993; Hoberg et al. 1995; M. Kapakatoak, S. Kutz, J. Nagy,

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S.J. Kutz¹ and L. Polley. Department of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK S7N 5B4, Canada.

E.P. Hoberg. U.S. National Parasite Collection, and Parasite Biology, Epidemiology and Systematics Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD 20705, U.S.A.

J. Nishi. Department of Resources, Wildlife and Economic Development, Government of the Northwest Territories, Fort Smith, NWT X0E 0P0, Canada.

¹Corresponding author (e-mail: susan.kutz@usask.ca).

J. Nishi, and A. Veitch, unpublished observation). The life cycle of *U. pallikuukensis* requires a gastropod intermediate host (IH) for development from first-stage larvae (L1) to infective third-stage larvae (L3) (Hoberg et al. 1995; Kutz et al. 1999) and, on the mainland tundra, the slug *Deroceras laeve* is a suitable and common IH (Kutz 2000). Larval development is temperature dependent; for example, in *D. laeve*, development from L1 to infective L3 requires only 12 days at 20°C but, at 12°C, takes 49 days (Kutz et al. 2001b). The threshold temperature (temperature below which minimal or no larval development occurs) is estimated to be 8.5°C, and 167 degree-days (amount of accumulated heat over the threshold temperature) are necessary for development from L1 to L3 (Kutz et al. 2001b). Given the close link between larval development and temperature, it follows that past and present climatic conditions are important factors influencing the geographic distribution and abundance of *U. pallikuukensis* in muskoxen.

Global climate change is predicted to alter the range, epidemiological patterns, and disease emergence of a variety of pathogens (Patz et al. 1996; Hoberg 1997; Daszak et al. 2000; Marcogliese 2001; Harvell et al. 2002). Arctic ecosystems are especially constrained by climatic conditions (Danks 1992), and several northern studies demonstrate or predict links between a warmer climate and changes in the geographic distribution of pathogens and disease occurrence (Halvorsen 1986; Handeland and Slettbakk 1994; Lindgren et al. 2000; Randolph and Rogers 2000; Hoberg et al. 2001; Kutz et al. 2001a). In Canada, the Mackenzie District and the Arctic Tundra are undergoing a period of accelerated warming, 2.0 and 1.1°C, respectively (Environment Canada at http://www.msc-smc.gc.ca/ccrm/bulletin/annual01/ttabsumm_e.html (accessed on 10 Dec. 2002)). This warming trend, and associated changes in climate and habitat and host ecology, may significantly alter the geographic distribution, as well as patterns of transmission and disease occurrence, associated with *U. pallikuukensis* and other parasites. To understand the epidemiology of *U. pallikuukensis* under current and changing climatic conditions, field and laboratory studies investigating the role of temperature on larval development are necessary.

In the present study, we used field experiments to investigate the development and temporal availability of *U. pallikuukensis* in *D. laeve* on the arctic tundra. The specific objectives of this research were (i) to determine the time required for development of *U. pallikuukensis* from L1 to L3 in experimentally infected *D. laeve*; (ii) to determine the temporal pattern of L3 availability in experimentally infected *D. laeve*; (iii) to determine if larvae can overwinter in gastropods and resume development the following summer; and (iv) to determine whether laboratory data on the threshold temperature and degree-days, together with measurements of ambient temperature (soil, surface, or air), can be used to accurately predict rates of larval development in the field. This work contributes to the development of techniques to predict and understand biotic responses to climate change in northern ecosystems.

Materials and methods

Study site

The study site was located in the low Arctic mainland, at

67°45.60'N, 115°15.00'W, near the hamlet of Kugluktuk, Nunavut, Canada. This hamlet borders on the Mackenzie District and the Arctic Tundra region as defined by Environment Canada. In Kugluktuk, from 1977 to 1992, mean daily temperatures exceeded 0°C only from June through September and monthly averages across this period were 4.3 (June), 10.0 (July), 8.3 (August), and 2.8°C (September). Average total rainfall was 13.4 (June), 34.5 (July), 44.5 (August), and 31.8 mm (September) (Environment Canada, Atmospheric Environment Service, Climate Research Branch, Saskatoon, Sask., Canada).

The study site was a 30 × 50 m mesic sedge meadow dominated by sedges, grasses, and mosses, with a few willows and forbs (for descriptions of important habitat types for gastropods see Kutz 2000), and was surrounded by a solar-powered electric fence to deter wildlife. Depending on the time of year and recent precipitation, the study site was 5–15% covered by standing water.

Study design

Six consecutive trials on the larval development of *U. pallikuukensis* in the slug IH, *D. laeve*, were established at bi-weekly intervals from 19 June to 28 August 1997. At the start of each trial, 10 laboratory-infected slugs were placed in each of a series of enclosures and 10 uninfected slugs were placed in each of a series of separate enclosures as controls. Enclosures were randomly placed in the tundra at the study site. Three enclosures with infected slugs from each trial were collected biweekly until 11 or 25 September 1997, and slugs recovered from the enclosures were examined for larvae of *U. pallikuukensis*. Control enclosures were collected every 4 weeks. Enclosures from trials 1 to 5 were left in place in the tundra over the winter and examined in 1998. On 15 June 1998, six, four, and six enclosures were examined from trials 1, 2, and 3, respectively. Three enclosures from trial 4 were examined on each of 1 and 8 July 1998, and three from trial 5 were examined on each of 8 and 22 July 1998.

Enclosures

For each enclosure, a columnar section of turf, approximately 10–13 cm deep and 18–20 cm in diameter, was collected and placed in a white-plastic pail. The pail was filled to the brim with water and left on the tundra for 24–48 h to flood out resident gastropods. Excess water was then drained and the turf was placed in a modified white-plastic pail. The effectiveness of this flooding technique was confirmed in June 1998 by flooding nine additional turf sections in pails and subsequently examining them in the laboratory for gastropods.

The pails were 15 and 19 cm high for trials 1–3 and 4–6, respectively. The top inside diameter of all pails was 20.3 cm. Pails were modified by drilling 30 holes (diameter 1–2 mm) in the base and 20 holes around the side within 4 cm of the base. To prevent slugs from escaping, the insides of the pails were lined with one layer of white-polyester bridal netting and the tops were covered with two layers of netting pulled tight and fixed in place with the pail lid; the center had been removed from the lid, leaving only a narrow rim. Each pail was then replaced in the hole from which the turf had been

removed. From 4 to 5 cm of the pail remained above ground (Fig. 1). Enclosures were prepared in late June and early July.

Slug source and infection

During the week prior to the start of each trial, *D. laevis* were collected once or twice daily from a mesic sedge meadow along the Coppermine River near Kugluktuk. Wet masonite boards (30 × 30 cm) were placed arbitrarily and rough side down; the undersurface of the boards and underlying vegetation were examined for slugs after 2–12 h. Slugs were classified on the basis of mass as small (<15 mg), medium (15–44 mg), or large (>44 mg) and maintained at 1–4°C in Rubbermaid® containers with small pieces of turf and moist paper towel until they were used.

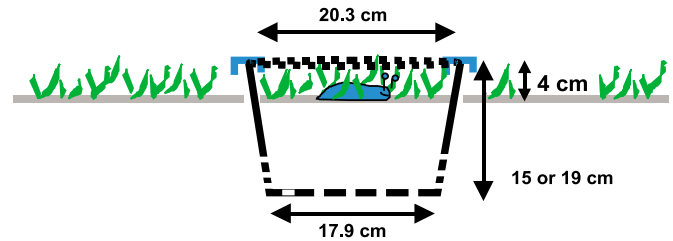
L1 used to infect slugs had been obtained from the feces of an experimentally infected muskox (Kutz et al. 1999) and kept frozen for 2–4 months. Larvae were recovered either by a funnel (Kutz et al. 1999) or by the beaker–Baermann technique, modified from Forrester and Lankester (1997). The sediment containing L1 was held at 1–4°C for a maximum of 48 h before being used to infect slugs.

Slugs were exposed to L1 on filter paper in petri dishes (Hoberg et al. 1995). Twelve slugs were placed in each petri dish and exposed to a total of approximately 2700 L1 for 3 h. Control slugs were handled in the same manner, except that no L1 were placed in the petri dishes. After exposure to L1, slugs were distributed among the enclosures in the tundra. For each trial, to approximate the population of slugs that would be exposed to natural infection at that time, slugs were assigned to each enclosure in the same size ratios (small:medium:large) in which they had been collected. Additionally, when each trial was established, 10 infected and 10 control slugs were maintained in separate Rubbermaid® containers at ambient temperatures (approximately 20–27°C) in the laboratory and examined for larvae after 2 weeks. This ensured the viability of the larvae used to infect slugs for each trial and confirmed that wild-caught slugs had no pre-existing infections with protostrongylid larvae (control slugs).

Temperature measurements

Temperatures were monitored inside and outside two or three additional enclosures from 25 June 1997 to 28 July 1998. In 1997, three enclosures were prepared in a manner identical to those used for development trials (except no slugs were put in these) and placed arbitrarily in the study site. On 25 June, the sensors from two temperature monitors (Hobo XT Temperature Logger, Onset Computer Corporation, Pocasset, Mass.) were placed in each of these three enclosures, one at the surface of the soil–leaf litter (surface) and the other inserted vertically 1 cm into the soil–leaf litter (soil). On 8 July, two additional sensors (one surface and one soil) were placed at a central location within the study site but not in an enclosure. On 26 July, one set of probes was removed from an enclosure and arbitrarily placed at surface and soil at another site (not in an enclosure) in the study area. The monitors were programmed to record mean temperatures every 30 min, 24 h each day, except from November to May, when mean temperatures were recorded every 4 h. As a result of technical difficulties and destruction of

Fig. 1. Enclosure used in the field trials.



monitors by small mammals, only one set of inside-enclosure and one set of outside-enclosure temperatures were consistently measured during 1997. In 1998, from 10 June to 28 July, temperatures were recorded from one surface and one soil site inside and outside each of three randomly placed modified enclosures (except from 20–25 June, when only two of three outside-enclosure soil temperatures were recorded). Air temperatures were obtained from the Kugluktuk airport weather station (67°49'N, 115°08'W), approximately 5 km east of the study site.

Slug recovery and examination

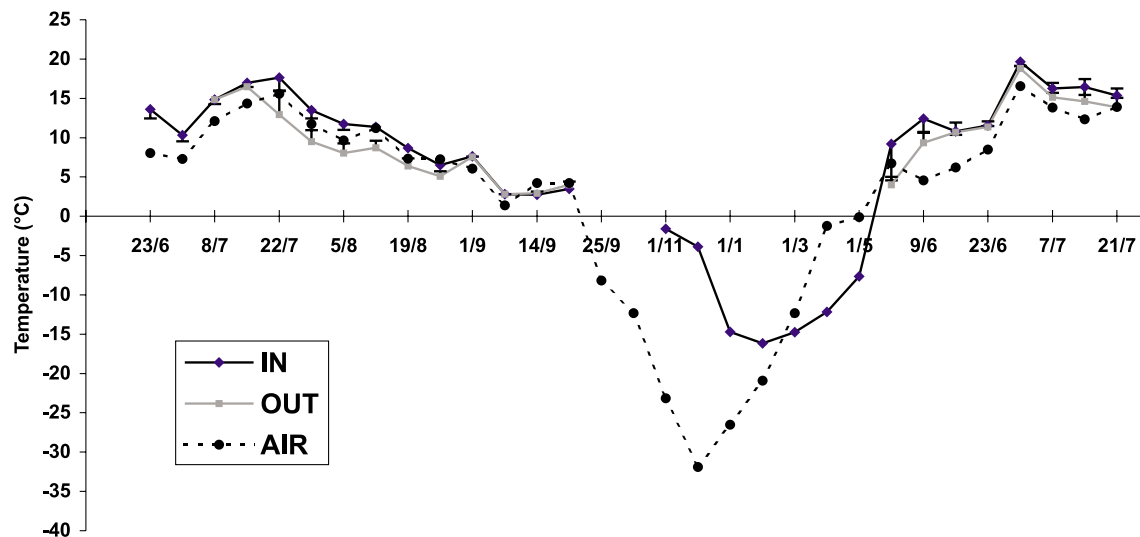
Enclosures from each trial were collected every 2 weeks from 3 July to 11 or 25 September 1997 and then in June or July of the following summer. The turf sections were removed, together with the netting lining the enclosures, and placed in unperforated pails. Cold water was added to these pails over a 3-day period and slugs were collected once daily as they were forced on to the vegetation, sides, and lids to avoid the water (Kralka 1986). Enclosures and the slugs recovered were held at 1–4°C during the flooding and until examination, to prevent further larval development. Slugs were weighed, their feet examined for lesions (Kutz et al. 2001b), and then digested in a pepsin – hydrochloric acid solution (Hoberg et al. 1995) at 37–40°C for 1–2 h. The digest was examined for larvae, which were then counted, classified to stage of development (Kutz et al. 2001b), and preserved in steaming ethanol (70%) and glycerin (5%). Control enclosures were processed in the same manner.

Slugs weighing less than 10 mg with no foot lesions and recovered 6 or more weeks after the start of the trial were considered to be recently hatched (either from eggs present prior to flooding of the pails or from eggs deposited by experimental slugs), and therefore, were not considered as part of the experiments. To confirm this assumption, these slugs were digested, either individually or in groups, and examined for larvae.

Statistical analyses

L3 were considered present in a trial on the date on which at least one slug from at least one enclosure contained a mobile L3 (see Kutz et al. 2001b). A Kruskal–Wallis test followed by a multiple comparisons test was used to compare the mean number of L3 (intensity) per slug in different enclosures within a trial on a single sampling day and also to compare sampling days of a trial (StatView™ SE + Graphics, Abacus Concepts Inc., 1988, San Francisco, Calif.; Siegel and Castellan 1988; Sokal and Rohlf 1995). The probability of significance was set at $P = 0.05$. The total number of “L3 in slugs” contributed by each trial (mean no. of L3 per

Fig. 2. Surface temperatures inside and outside enclosures and air temperatures from the Kugluktuk airport, for June 1997 – July 1998. The temperatures for June–September 1997 and June–July 1998 are weekly means and those for October 1997 – April 1998 are monthly means. The mean surface temperatures reported for May are based on temperatures from 1 to 11 May; data were not available for 12–29 May. Error bars = 1 SD.



slug \times number of slugs recovered) was summed among all trials for each sampling day, to assess temporal patterns of L3 availability in slugs in 1997.

Degree-day calculations

The degree-days accumulated for each sampling day in each trial were calculated separately for soil and surface temperatures inside and outside the enclosures, as well as for air temperatures. The number of sites at which temperatures were measured varied from one to three throughout the study period, and when measurements from multiple sites were available, they were averaged to calculate degree-days. Degree-days for surface and soil sites were calculated using 30-min mean temperatures. The minimum threshold temperature was set at 8.5°C (Kutz et al. 2001b) and the maximum temperature was set at 21°C (i.e., temperatures above 21°C were automatically converted to 21°C). The threshold temperature was then subtracted from each 30-min mean temperature (corrected for temperatures >21°C) and negative results were converted to 0. The degree-day values calculated for each 30-min period during a single day were averaged to determine the degree-days for that day. Cumulative degree-days for each experiment were calculated by adding the daily degree-days. Degree-days for the air were calculated in a similar manner, using hourly mean temperatures.

Results

Enclosures and gastropod recovery

Flooding turf to remove resident gastropods before the start of each trial was effective for *D. laevis*. After flooding, some pails still contained the small snails *Vertigo* cf. *modesta* but no slugs remained. Recovery rates of infected and control slugs from the enclosures changed over time. At 2 weeks post infection (PI), slug recovery ranged from 60 to 90% but dropped to 30–45% by 8 weeks and to 0–7% by the following summer.

Temperatures

The summer of 1997 ranked fifth warmest for the Mackenzie District (1.4°C above the 1948–2000 average) and the summer of 1998 was the warmest on record for both the Mackenzie District and the Arctic Tundra region (by 2.5 and 3.0°C, respectively; Environment Canada at http://www.msc-smc.ec.gc.ca/ccrm/bulletin/summer02/national_e.cfm, (accessed on 10 Dec. 2002)). In the summers of 1997 and 1998, the monthly summer air temperatures at the Kugluktuk airport (4.7 and 6.2°C (June), 12.1 and 14.2°C (July), 9.3 and 12.1°C (August), and 4.7 and 5.4°C (September) for 1997 and 1998, respectively) were considerably warmer than the 1977–1992 average, ranging from a minimum of 0.4°C above normal for June 1997 to 4.2°C above normal in July 1998.

In 1997, temperatures measured inside the enclosures were similar to those outside the enclosures during early and mid-July and all of September, but from the end of July and throughout August, temperatures were 2–5°C warmer inside the enclosures (Fig. 2). In 1998, temperatures measured inside the modified enclosures in early June were warmer than those outside the enclosures, but after mid-June, the difference was less marked (Fig. 2).

Larval development

In 1997, mobile L3 were first found at 6, 4, and 4 weeks PI in slugs from trials 1, 2, and 3, respectively (Fig. 3). At 6 weeks PI in trial 1, all slugs ($n = 9$) from all three enclosures contained at least one L3. At 4 weeks PI in trial 2, L3 were present in 6 of 12 slugs from two enclosures and in 0 of 3 slugs from the third enclosure but, at 6 weeks PI, all slugs ($n = 10$) from all three enclosures contained at least one L3. At 4 weeks PI in trial 3, L3 were present in 8 of 9 slugs from all three enclosures, and at 6 weeks PI, all slugs ($n = 11$) in all three enclosures contained L3. Mobile L3 were not present in slugs from trials 4, 5, and 6 in 1997 but were isolated in the few slugs recovered from trials 4 and 5 in July 1998 (Fig. 3). No enclosures from trial 6 were available for examination in 1998.

Fig. 3. Gastropod and larval recoveries from each enclosure in trials 1–6 at each sampling date. Bars indicate the mean proportion of each larval stage in all infected slugs from an individual enclosure. Numbers above the bars indicate the number of slugs recovered from that enclosure. Where no bar is associated with a number, either no slugs were recovered from the enclosure (indicated by a 0) or the slugs that were recovered contained no larvae. Mean monthly temperatures for July, August, and September are given below the figure. L1, first-stage larvae; L2, second-stage larvae; aL3, non-mobile third-stage larvae; bL3, mobile third-stage larvae.

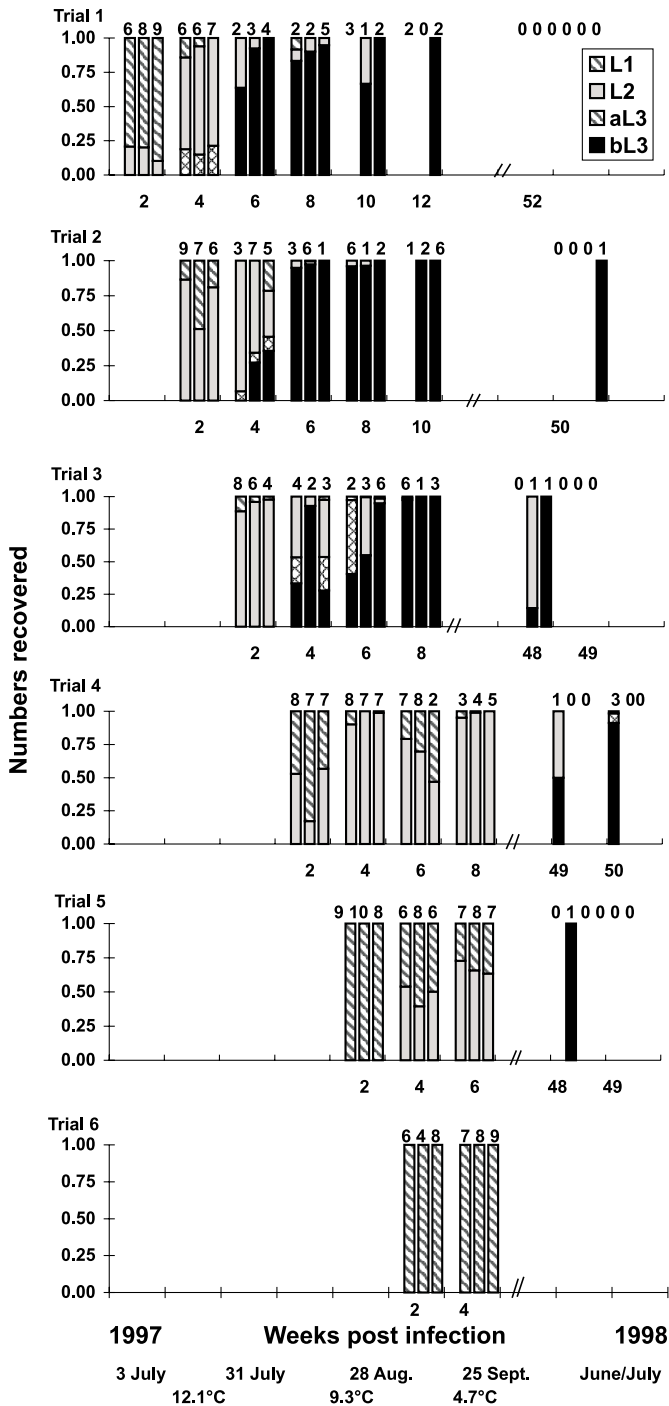


Fig. 4. The mean intensity of L3 in slugs in trials 1–3 at different times post infection (PI). Error bars = 1 SD.

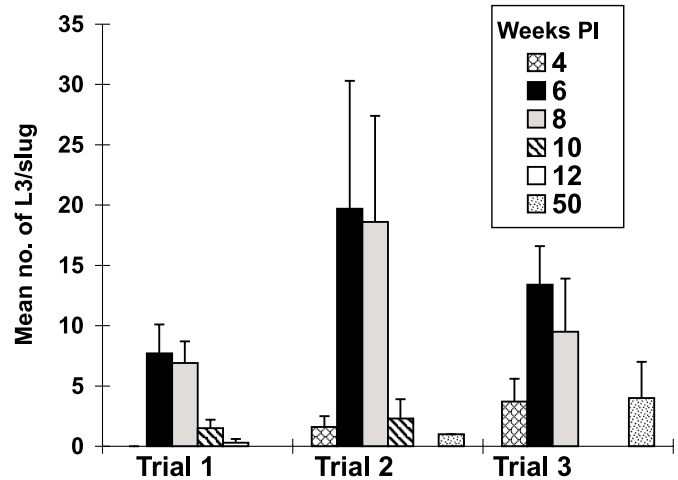
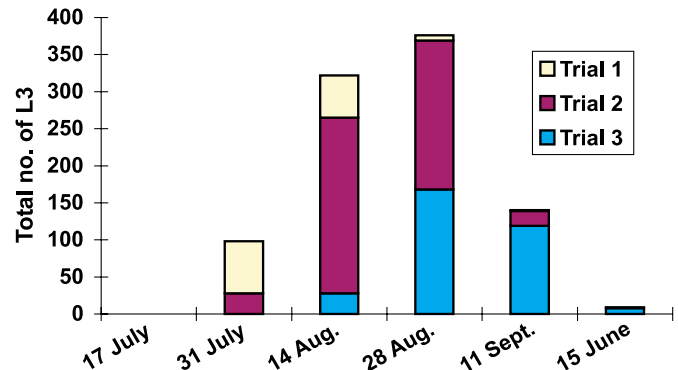


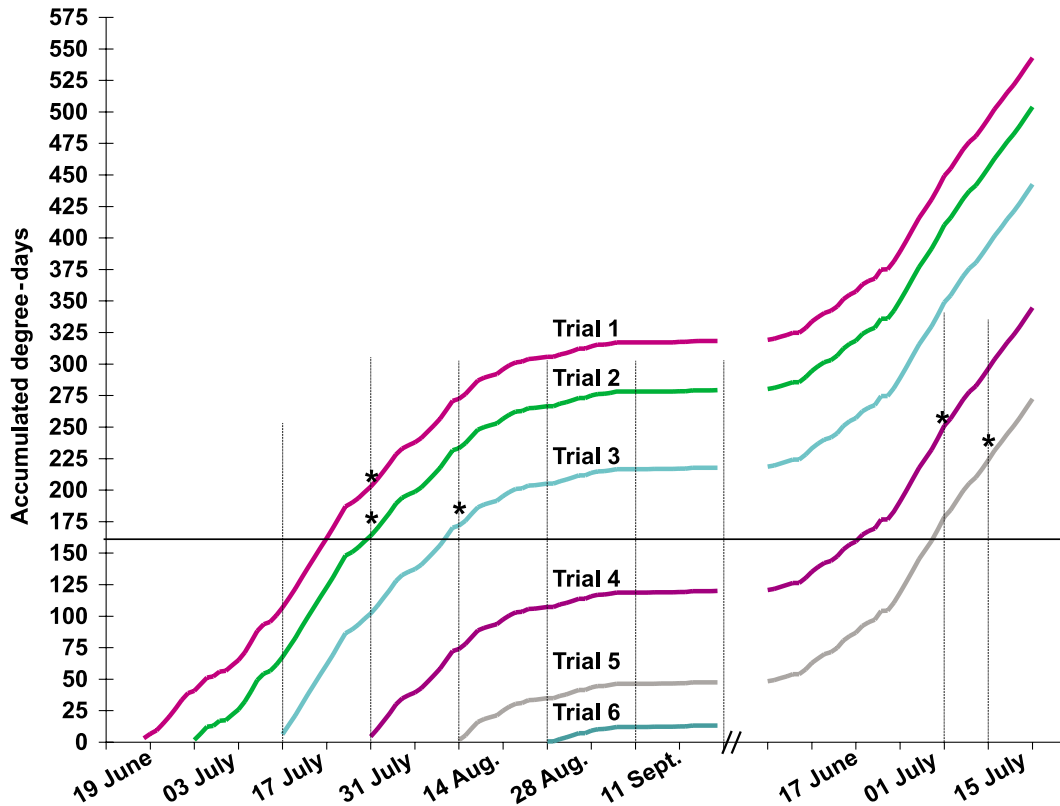
Fig. 5. The total number of L3 in slugs contributed at different times of the year in trials 1–3.



The mean intensities of L3 in slugs within a trial on a given sampling day did not differ significantly between enclosures (Kruskal–Wallis, $P > 0.05$ for all trials). For trials 1–3, the mean intensities of L3 in slugs were compared among sampling days within a trial (Fig. 4). The intensities of L3 significantly increased between 4 and 6 weeks PI, reached a plateau at 6–8 weeks PI, and then declined by 10 weeks PI (trials 1 and 2) or by the following year (trial 3) (Kruskal–Wallis, $P < 0.05$). Additionally, in trial 2, there were more L3 at 4 and 10 weeks PI than at 50 weeks PI. The total number of L3 in slugs contributed by trials 1, 2, and 3 (pooled data) was greatest from mid to late August 1997 (Fig. 5).

Degree-days calculated from soil and surface temperatures both inside and outside the enclosures, and air temperatures, differed, accumulating most rapidly at the surface. Based on the soil and surface temperatures inside enclosures, and air temperatures from the Kugluktuk airport, when the first L3 in trials 1 and 2 were observed on 31 July, 131, 104, and 203 degree-days had accumulated in the air, soil, and at the surface, respectively, for trial 1 and 112, 95, and 164 degree-days had accumulated in the air, soil, and at the surface, respectively, for trial 2. For trial 3, the first L3 were observed on 14 August, at which time the degree-days accumulated were 137 in air, 100 in soil, and 172 at the surface. L3 were not

Fig. 6. Degree-days accumulated based on surface temperatures inside enclosures for trials 1–6. The horizontal line at 167 degree-days represents the accumulated degree-days required for development to mobile L3 (Kutz et al. 2001b). Vertical dotted lines represent sampling days. Asterisks (*) mark the sampling day that the first mobile L3 were observed in each trial.



present in trials 4 and 5 until 1998. In trial 4, by 8 July 1998, 162, 141, and 251 degree-days had accumulated in the air, soil, and at the surface, respectively, whereas in trial 5, 139, 144, and 224 degree-days had accumulated in the air, soil, and at the surface, respectively, by 15 July 1998. In trial 6, L1 that infected slugs on 28 August 1997 had not developed past L1 by 25 September 1997; only 11, 0, and 13 degree-days had accumulated in the air and soil and at the surface, respectively, by that date.

The majority of larvae in infected slugs kept in the laboratory at ambient temperatures reached the L3 stage by 2 weeks PI (when approximately 161–259 degree-days had accumulated). Because laboratory temperatures could not be controlled (i.e., frequently too warm), slug survival for some trials was poor. No foot lesions typical of infection with *U. pallikuukensis* (see Kutz et al. 2001b) were seen and no larvae were recovered from any of the uninfected control slugs from either the laboratory or field trials. Also, no larvae were found in slugs that were recovered from trials 1–5 at 6 or more weeks PI, weighed less than 10 mg, and had no foot lesions.

Discussion

In this study we used an enclosure-based system to investigate the development and availability of a protostrongylid parasite in its gastropod IH on the arctic tundra. We then applied a simple degree-day formula to soil, surface, and air temperatures and found that degree-days calculated from surface

temperatures inside the enclosures most closely estimated the rates of larval development in the field. We observed that L1 infecting *D. laeve* early in the summer developed to L3 within 4–6 weeks, and that late August was the period with the greatest total number of L3 in slugs. L3 overwintered in slugs but slug mortality was high and the intensity of infection in the few surviving slugs was significantly lower than in the previous summer. L1 infecting slugs later in the summer overwintered in slugs as L1 or second-stage larvae (L2) and resumed development the following year. Again, survival of the slugs and the intensity of infection were low. This study took place during two exceptionally warm summers and the results must be interpreted within this context. This work contributes critical information on the development and availability of the free-living stages of *U. pallikuukensis* and establishes a framework for understanding the epidemiology of this parasite and for comparing it with other protostrongylids. It also provides the basis for generating, and the tools for testing, predictions about the effect of climate and climate change on the transmission rates and geographic distribution of *U. pallikuukensis* in muskox populations.

An objective of this study was to identify the temperature measurement that would most closely reflect the average conditions that the slugs experience, and therefore, the thermal conditions under which the larvae develop. Because of the substantial vertical and horizontal variation in the microclimate at the level of the gastropod IH on the tundra (see Hansen 1973; Coulson et al. 1993; Young et al. 1997), we

measured air, soil, and surface temperatures, to encompass as much as possible of the full thermal range of the microhabitat. We then calculated degree-days accumulated at all three levels. These calculations incorporated the threshold temperature (8.5°C; see Kutz et al. 2001b) and considered slug behavior (avoidance of temperatures above 21°C; Dainton 1989; Rollo 1991). Predictions from surface temperatures inside enclosures consistently and closely approximated the observed larval development (167 degree-days had accumulated by the time the first L3 were observed), whereas degree-days calculated from air and soil temperatures frequently overestimated the time required for development to L3. These simple measurements and calculations can now serve as the basis for understanding and estimating larval development rates throughout the year, in different geographic regions, and under changing climatic conditions.

In the Arctic, the window when temperatures are sufficiently warm for development of *U. pallikuukensis* is narrow. For example, in 1997, L1 had to infect slugs on or before 17 July (trials 1–3) to produce infective L3 by the end of the summer. L1 infecting slugs on 31 July or later (trials 4, 5) developed only to L2 by the end of September. The estimated degree-days accumulated in early to mid-June and in September are negligible (see Fig. 6), indicating that the maximum window when larvae can develop is 10 weeks (late June to the end of August). However, because of unusually warm temperatures, the “normal” window for development may be even narrower. First, in 1997, the mean air temperatures at Kugluktuk in June, July, August, and September were 0.4, 2.1, 1.0, and 1.9°C, respectively, above average. Second, surface temperatures recorded inside enclosures were on average warmer than those outside the enclosures. Consequently, larvae in the 1997 trials probably began development earlier, developed faster, and continued development later in the year than would have been the case outside the enclosures in a year with normal summer temperatures. To estimate the effect of the warmer temperatures inside the enclosures on the rate of larval development, we calculated accumulated degree-days based on surface temperatures outside the enclosures and found that L1 would have had to infect slugs by 10 July to develop to L3 by the end of the summer. In cooler years this date would be even earlier and, in some years, larval development may not be completed within a single year.

Several authors have reported or suggested that protostrongylid larvae overwinter in gastropods (Halvorsen and Skorping 1982; Samson and Holmes 1985; Samuel et al. 1985) and that survival of these larvae varies depending on the stage in which they enter winter (Schjetlein and Skorping 1995). Our results confirmed that larvae of *U. pallikuukensis* also overwinter. These larvae are an available source of L3 earlier in the summer than is the cohort developing from L1 infecting gastropods in the spring. The abundance of over-wintered L3 in slugs was, however, very low compared with L3 abundance the preceding August. Likewise, in the Rocky Mountains of Alberta, Canada, the abundance of over-wintered *Parelaphostrongylus odocoilei* in *D. laeve* is relatively low, as is the predicted abundance of over-wintered *Protostrongylus stilesi* in its IH (Samson and Holmes 1985; Samuel et al. 1985).

Overwintering of protostrongylids in IHs may not be of great quantitative significance, but perhaps more important is the ecological role of over-wintered larvae in maintaining the parasite in the host population when temperatures during the preceding summer(s) are insufficient for substantial larval development to L3 within a single summer.

The highest intensity of infection of L3 in slugs occurred in mid to late August and then substantially declined. This reduction in larval intensity may be caused by mortality of L3 or the emergence of L3 from live and (or) dead slugs. Laboratory studies have demonstrated that L3 emergence from live slugs is a common phenomenon for *U. pallikuukensis*; for example, after 60 days PI at 20°C, 20–100% of L3 emerged from *D. laeve* (Kutz et al. 2000). Emergence also occurs in the field; live L3 were recovered from the vegetation in the enclosures of trials 1 and 2 in September 1997 (2 and 27 L3, respectively), and live (3) and dead (4) L3 were recovered from the vegetation of trials 2 and 3 in June 1998 (Kutz et al. 2000).² Larval emergence may be important for extending transmission beyond the daily and seasonal periods when the gastropod IHs are active (Kutz et al. 2000). Emergence has been reported for other protostrongylids (e.g., *Cystocaulus ocreatus* from domestic sheep, *Protostrongylus boughtoni* from snowshoe hare (*Lepus americanus*), *P. stilesi* from bighorn sheep (*Ovis canadensis*), and *P. odocoilei* from Dall's sheep (*Ovis dalli*)) but detailed field and laboratory studies describing patterns, frequency, and cause of emergence are lacking (Monson and Post 1972; Boev 1975; Kralka and Samuel 1984; Kutz et al. 2001c). In a 2-year study on the natural prevalence of *P. odocoilei* in *D. laeve*, the results in 1 year showed a decline in L3 intensity in the autumn (Samuel et al. 1985). Another study examining larval development of *Neostromylus linearis* in *Cernuella cespitum arizonis* under natural conditions also showed a decline in infection intensity over time (Morrondo-Pelayo et al. 1987). Neither study includes comments on the possible cause or significance of these declines; however, larval emergence is a factor that could be considered.

Development strategies of protostrongylids in their gastropod IHs change from southern to northern latitudes. Field studies in Spain demonstrated that *N. linearis* develops year-round (Morrondo-Pelayo et al. 1987). In temperate climates protostrongylid larval development becomes seasonally restricted but still occurs within a single year, for example, *P. stilesi* and *P. odocoilei* in the Rocky Mountains, Alberta (Samson and Holmes 1985; Samuel et al. 1985), and *Elaphostrongylus rangiferi* in Newfoundland, Canada (Ball et al. 2001). This window for development remains quite broad; in the Rocky Mountains, Canada, development of *P. stilesi* can occur over a 6-month period from April through September (Samson and Holmes 1985). At more northern latitudes, however, the window for development narrows (a maximum of 10 weeks in our study) and larvae may more frequently require 2 years to develop to L3 (e.g., *E. rangiferi* in Norway; Halvorsen and Skorping 1982).

The stability of these protostrongylid–host systems, particularly in northern regions, is very sensitive to climatic conditions. For example, in Norway, outbreaks of cerebrospinal elaphostrongylosis (CSE) in reindeer, caused by *E. rangiferi*,

²Although these L3 may have emerged from dead slugs there were no remains of slugs on the vegetation.

are more likely to occur after summers (June–August) with mean temperatures that are 1.5°C above normal (Handeland and Slettbakk 1994). It is thought that these outbreaks occur because of the rapid and mass larval development within a single year, resulting in unusually high numbers of L3 available to young susceptible reindeer before the winter. In contrast, Ball et al. (2001) suggest that, in Newfoundland, summer temperatures are typically sufficient for development of *E. rangiferi* within a single season, and it is the length of the period of transmission that is the critical factor in outbreaks of CSE. They concluded that outbreaks occur in years with long snow-free seasons (temperatures are above 0°C), because the slugs are active over a longer period of time in these years, and therefore, caribou are exposed to L3 for an extended period. Although not as well defined and probably primarily through the effects on gastropod abundance and activity, precipitation also plays a role in the development and availability of L3 and the subsequent intensity of infection by protostrongylids in host populations (Forrester and Littell 1976; Handeland and Slettbakk 1994; Ball et al. 2001).

For *U. pallikuukensis* on the arctic tundra, our results suggest that summer temperatures and the length of the window for development are important factors limiting the availability of L3 and the geographic distribution of this parasite. The length of time that gastropods are available (beyond the time when larval development can occur) may not be as important for the transmission of *U. pallikuukensis* as it is for *E. rangiferi* in Newfoundland because L3 emergence may extend the period of transmission well beyond the time that slugs are active (Kutz et al. 2000; Ball et al. 2001). The pattern of availability of L3 of *U. pallikuukensis* observed in 1997, when the mean summer temperature (June–August) was 1.2°C above normal, indicated rapid and mass development of L3 in a single summer. This parallels the hypothesized pattern of L3 availability of *E. rangiferi* in Norway preceding disease outbreaks (Handeland and Slettbakk 1994). Abnormally warm years, such as 1997 and 1998, with early springs and warm summers, are probably important for amplifying *U. pallikuukensis* in the muskox population. However, because of the remoteness of the affected muskox population, our ability to monitor and detect changes in prevalence, intensity, and disease occurrence associated with different climatic conditions is limited.

Our knowledge of the epidemiology of protostrongylids at temperate and northern latitudes continues to expand (e.g., see Forrester and Lankester 1998; Lankester 2001; Ball et al. 2001; Kutz et al. 2001a, 2001c; Hoberg et al. 2003). These host–parasite systems are extremely sensitive to temperature and, as the climate changes, with accelerated warming in the north, we can expect changes in epidemiology, geographic distribution, and patterns of disease occurrence. The west-central Canadian Arctic is currently experiencing one of the most significant warming trends in the country (Environment Canada at http://www.msc-smc.ec.gc.ca/ccrm/bulletin/annual01/ttabsumm_e.html (accessed on 10 Dec. 2002)). In this region many parasites historically may have been limited, quantitatively and (or) geographically, by climatic conditions (Hoberg et al. 2001). We anticipate that changing climatic conditions in the ecologically sensitive arctic and sub-arctic environments will disrupt the balance of many host–parasite systems, re-

sulting in the emergence of parasites and parasite-induced diseases (Dobson and Carper 1992; Hoberg et al. 2003). The enclosure-based system described in this paper, the application of simple degree-day calculations, and additional research on related protostrongylid parasites in temperate and Arctic regions, bring us closer to understanding protostrongylid epidemiology in the Arctic and improve our ability to monitor and predict the effects of climate change on these parasites and their hosts.

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