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# UMINGMAKSTRONGYLUS PALLIKUUKENSIS (NEMATODA: PROTOSTRONGYLIDAE) IN GASTROPODS: LARVAL MORPHOLOGY, MORPHOMETRICS, AND DEVELOPMENT RATES

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**ABSTRACT:** Morphological and morphometric aspects of larval development of *Umingmakstrongylus pallikuukensis* in *Deroceras laeve* and the effects of temperature on development rates in *D. laeve* and *Deroceras reticulatum* were investigated in the laboratory. Larval stages were best differentiated by separation of cuticular sheaths, tail structure, and viability following digestion. Growth in body and esophagus width was observed during the first-stage within the intermediate host, but the major increases in body length and width occurred immediately following the second molt. Larval development in *D. laeve* and *D. reticulatum* occurred more rapidly at warmer temperatures. The calculated threshold temperatures were 8.5 and 9.5 C in *D. laeve* and *D. reticulatum*, respectively, and 167 degree-days were required for development to third-stage larvae (L3) in both hosts. These thresholds are higher than those calculated from published data for the closely related *Muellerius capillaris* (4.2 C) but are similar to those for the more distantly related northern protostrongylid, *Elaphostrongylus rangiferi* (8.3–10.3 C). Conversely, degree-days required for development to infective L3 were more similar among the Muelleriinae than between this group and the Elaphostrongylinae. Developmental parameters for protostrongylid larvae may be influenced both by the environment and by features of the parasites and the intermediate hosts, including phylogeny.

*Umingmakstrongylus pallikuukensis* is a protostrongylid lungworm in the subfamily Muelleriinae (Carreno and Hoberg, 1999). It is found only in muskoxen (*Ovibos moschatus*) from the mainland of the west central Canadian Arctic, primarily in the region west of Kugluktuk, Nunavut (67°49'N, 115°08'W) (Gunn and Wobeser, 1993; Hoberg et al., 1995; S. J. Kutz, unpubl. obs.). Adult parasites live and reproduce in cysts in the lung parenchyma (Hoberg et al., 1995), and up to 258 cysts have been recovered from a single, naturally infected muskox (Gunn and Wobeser, 1993). Despite a harsh arctic environment, *U. pallikuukensis* appears to be a successful parasite with a 93% prevalence in hunter-killed muskoxen west of Kugluktuk (Gunn and Wobeser, 1993).

As a member of the Protostrongylidae, *U. pallikuukensis* requires gastropod intermediate hosts for development from the first (L1) to third (L3) larval stage (Hoberg et al., 1995). Development of protostrongylid larvae in gastropods is affected by a variety of factors including temperature, humidity, and intensity of infection, and the species, age, and physiological condition of the intermediate hosts (Gerichter, 1948; Rose, 1957; Skorping, 1984; Samson and Holmes, 1985; Cabaret, 1987; Solomon et al., 1996). Temperature has a critical influence on rates of larval development. For all species there is a threshold temperature, below which development is minimal (Samson and Holmes, 1985) or does not occur (Gerichter, 1948; Halvorsen and Skorping, 1982). As temperatures increase, larval development rates increase (Rose, 1957; Halvorsen and Skorping, 1982). Threshold temperatures and development rates may differ among species of protostrongylids, and even within a species, these parameters may vary depending on intermediate host species (Gerichter, 1948; Halvorsen and Skorping, 1982).

The objectives of the present study were (1) to describe morphological and morphometric changes as L1 of *U. pallikuuk-*

*ensis* develop to L3 in *Deroceras laeve*; (2) to determine the effect of temperature on rates of larval development in *D. laeve* and *Deroceras reticulatum*; and (3) to determine the threshold temperature(s) ( $T_0$ ) and degree-days (DD) required for development to L3 in both gastropod species. It was hypothesized that the developmental parameters of *U. pallikuukensis* (growth rate,  $T_0$ , DD) would be influenced primarily by extrinsic environmental factors and secondarily by intrinsic constraints on the parasite, including phylogeny.

## MATERIALS AND METHODS

In total, 12 experiments were used to investigate larval development rates at various temperatures (Table I). One experiment (no. 12) was also used as the basis for morphological and morphometric descriptions of larval stages.

### Sources of larvae and gastropods

L1 of *U. pallikuukensis* were obtained from feces of an experimentally infected muskox (Kutz et al., 1999). Feces had been frozen for up to 20 mo at  $-13 \pm 2$  C. Larvae for gastropod infections were isolated from feces using methods described in Kutz et al. (1999) or Forrester and Lankester (1997) and concentrated to 2–4 ml in tap water.

Specimens of *D. reticulatum*, a slug of temperate climates, were obtained from captive-bred protostrongylid-free colonies (see Kutz et al., 1999) and used for experiments 1–6. Specimens of *D. laeve*, the only slug species native to the North American Arctic (Pilsbry, 1946, 1948), were from 2 sources. Wild *D. laeve*, collected along the Coppermine River near the town site of Kugluktuk, were used for experiments 10 and 11. This collection site was not used by muskoxen and sightings of caribou were infrequent, thus natural infections with protostrongylids were unlikely. Because it proved difficult to maintain breeding colonies of the wild-caught specimens, a laboratory colony of *D. laeve* that originated from a Saskatoon greenhouse, and survived and reproduced well in captivity, was used for experiments 7–9 and 12.

### Infection of gastropods

Prior to experimental infections, the feet of the wild-caught *D. laeve* were examined for lesions indicative of pre-existing protostrongylid infections (Kutz et al., 2000). During 2 yr of related field studies, no lesions were detected and no larvae were recovered from more than 100 *D. laeve* collected from the Coppermine River site.

Slugs were infected with L1 of *U. pallikuukensis* as described by Hoberg et al. (1995) (Table I). In preliminary studies, a greater proportion of larvae established in *D. laeve* than in *D. reticulatum*, and there-

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TABLE I. Experimental infections of *Deroceras laeve* and *Deroceras reticulatum* with *Umingmakstrongylus pallikuukensis*.

Experiment no.	Temp. (°C)	Gastropod species	L1/slug	Slug weight (mg) mean ± 1 SD	Total no. of slugs	Days postinfection slugs examined	No. of slugs examined each day	No. of larvae examined/slug	Median intensity (range)
1	10	<i>D. reticulatum</i>	225	—	15	15, 25, 47, 49, 63, 66, 81	1–3	Up to 5*	<10†
2	12	<i>D. reticulatum</i>	225	—	5	31, 47, 66, 69	1 or 2	All	5 (1–21)
3	15	<i>D. reticulatum</i>	1,000	780 ± 180	35	Every 3 days from 4 to 28, then 32, 35, 37	3	Up to 5	<10
4	20	<i>D. reticulatum</i>	1,000	800 ± 210	21	4, eod‡ from 11 to 21	3	Up to 5	<10
5	20	<i>D. reticulatum</i>	1,000	770 ± 190	9	7, 11, 20	2 or 3	Up to 5	<10
6	25	<i>D. reticulatum</i>	1,000	710 ± 160	16	Eod from 3 to 11, then 12	2 or 3	Up to 5	<10
7	8.5	<i>D. laeve</i>	225	114 ± 39	35	Every 7 days from 49 to 91	3	All	6 (0–18)
8	11.5	<i>D. laeve</i>	225	138 ± 41	35	Every 7 days from 48 to 76	3 or 5	All	9 (3–21)
9	11.5	<i>D. laeve</i>	225	107 ± 35	24	25, 35, 42, 49	3	All	8 (2–25)
10	15	<i>D. laeve</i>	225	40 ± 20	17	8, 10, 12, 18, 21, 24	3	All	6 (2–29)
11	20	<i>D. laeve</i>	225	40 ± 20	26	Eod from 4 to 12, then 13, 14, 16	3	All	5 (1–18)
12	23.4	<i>D. laeve</i>	300	180 ± 70	40	Daily from 3 to 13, then 15, 36, 38	3	Up to 5	23 (3–72)

\* Some slugs contained fewer than 5 larvae.

† Number of larvae estimated.

‡ Every other day.

fore, lower numbers of L1 were used to infect *D. laeve*. Also, to minimize potential slug mortality, relatively low numbers of L1 were used in the long-duration, low-temperature experiments (10 and 12 C) with *D. reticulatum*. After infection, all slugs were fed and housed as described by Kutz et al. (1999). They were kept in darkness in temperature-controlled refrigerated incubators and fed weekly (experiments at 15–25 C) or every 2 wk (experiments at <15 C).

#### Digestion of gastropods and examination of larvae

Subsets of slugs were examined for larvae at various days postinfection (DPI), determined on the basis of temperature of the experiment (see Table I). Slugs were weighed and then digested in a pepsin/hydrochloric acid solution (Hoberg et al., 1995). Larvae recovered were heat-killed by placing them in a drop of water on a glass slide and passing the slide quickly through a Bunsen burner flame 7 times. Larvae were examined at 400× and identified as L1, second-stage larvae (L2), early L3, intermediate L3, late L3, or emerged L3, based on morphological characteristics (described below), the age of infection (DPI), and behavior.

In experiment 12, a subset of 15 live L1 were removed from the petri dishes used for slug infection, heat-killed as above, and measured at 400× using an ocular micrometer. Fifteen larvae were examined and measured on each sampling day thereafter with the exception of emerged L3, of which only 9 were recovered and examined.

#### Data analysis

Statview SE<sup>®</sup> SE + Graphics (1988 Abacus Concepts Inc., San Francisco, California) was used for data analysis. Morphometric data for each morphologically defined category were summarized and compared using a 1-way analysis of variance and Scheffe's test ( $P = 0.05$ ). The theoretical  $T_0$  and DD required for development of *U. pallikuukensis* to intermediate L3 in *D. laeve* and *D. reticulatum* were calculated using a regression analysis of temperature on larval development rates (1/ days to intermediate L3) ( $y = a + bx$ ,  $T_0 = -a/b$ , and  $DD = 1/b$ )

(Campbell et al., 1974; Samson and Holmes, 1985). The number of days to the first intermediate L3 was used as the end point to determine development rates. This stage of development was the most comparable to those defined by Rose (1957) for *Muellerius capillaris*, Halvorsen and Skorpning (1982) for *Elaphostrongylus rangiferi*, and Samson and Holmes (1985) for *Protostrongylus* spp. as infective L3. To compare the  $T_0$  and DD values among these protostrongylids, regression analyses were performed using data for the development of *M. capillaris* to infective L3 in *D. reticulatum* or *D. agrestis* (from Table I in Rose [1957]; gastropod species not specified for individual experiments) and for the development of *E. rangiferi* to infective L3 in *Arianta arbustorum* and *Euconulus fulvus* (Figures 1 and 2 in Halvorsen and Skorpning [1982]).

## RESULTS

### Larval development: Morphology

Morphological characteristics of L1 and L3 of *U. pallikuukensis* have been described in detail (Hoberg et al., 1995), thus the following descriptions of these stages are limited to observations that facilitate their differentiation from L2. Within the continuum of larval development in *D. laeve* at 23 C (experiment 12), on the bases of morphology, age of infection, and behavior, 6 categories of larvae were identified: L1 on 0, 3, and 4 DPI; L2 on 4–9 DPI; early L3 on 9–11 DPI; intermediate L3 on 12, 13, and 15 DPI; late L3 on 36 and 38 DPI; and emerged L3 from 22 to 38 DPI. Morphological characteristics useful for defining the stages of development are summarized in Table II.

**L1 (0 DPI):** Larvae consistent with those described by Hoberg et al. (1995) with limited variability among larvae.

**L1 (3 DPI):** Cuticle and cuticular sheaths: homogenous refractile granules, numerous, small, uniformly ovoid, situated

TABLE II. Morphological characteristics of developing larvae of *Umingmakstrongylus pallikuukensis*.

Character*	L1†	L1–L2	L2	L2–L3	aL3	bL3	c/eL3
Live‡	+	–	–	–	–	+	+
Visibility/condition of characters§	**/**	*	*	*	**	***	***
CS1 separated‡	–	+	+	+	+	+	+
CS2 separated‡	na	na	–	+	+	+	+
Fine granules underlying cuticle‡	+/-	+	–	+	na	na	na
GP number of cells visible	2	2	2–4	2–4	6–8	6–8	6–8
Larval form (heat killed/digested)	a, c	a	a, b	a, b, c	a, c	c	c

\* CS1, first cuticular sheath; CS2, second cuticular sheath; GP, genital primordium.

† L1, first-stage larvae (in slugs); L1–L2, transition from L1 to L2; L2, second-stage larvae; L2–L3, transition from L2 to aL3; aL3, early third-stage larvae; bL3, intermediate L3; c/eL3, late and emerged L3.

‡ +, Yes; –, no; na, not applicable.

§ \*, Poor; \*\*, good; \*\*\*, excellent.

|| a, Comma form; b, circle form; c, “c” shape.

immediately beneath cuticle in single line extending from base of esophageal–intestinal junction (EIJ) to near anus (Fig. 1). Intestine: prominent walls, large round nuclei present, lumen variable size. Digestion: larvae motile following digestion (and considered to be alive). General: some larvae bulge at anus and excretory pore.

**L1 to L2 transition (4 DPI):** Both L1 and L2 present at 4 DPI (8 L1, 7 L2). L1: As described at 3 DPI. L2: Cuticle and cuticular sheaths: homogenous refractile granules present under first cuticular sheath (CS1); CS1 usually separated at tail and separated or thickened at cephalic extremity (Fig. 2). Intestine: lumen usually wide; intestinal cells with large round nuclei. Tail: short ventral point, consistent with first section of L1 tail, visibly separating from CS1. Digestion: larvae nonmotile following digestion (and considered to be dead). General: notable bulges at anus and excretory pore of most larvae.

**L2 (5–9 DPI):** Cuticle and cuticular sheaths: separation of CS1 complete but cuticle still intact; cuticular lining of esophagus, excretory duct, and rectum separated (Fig. 3); homogenous refractile granules absent under CS1 by 5 DPI but increase in number under cuticle of L2 from 6 to 9 DPI. Intestine: cells granular from EIJ to genital primordium (GP), often obscuring cellular structure; large round nuclei occasionally visible in intestinal cells; intestine dark and lumen narrow. Tail: short ventral point, consistent with first section of L1 tail (Fig. 4); point absent from some larvae by 8 DPI. Digestion: larvae dead. General: larvae with considerable morphological variation, initially (5–7 DPI) thin, granular, later wider, and often a marked bulge between EIJ and GP; morphological characters often obscure; excretory gland prominent, often extending posterior to EIJ.

**Early L3 (9–11 DPI):** Cuticle and cuticular sheaths: CS1 often broken at cephalic end, occasionally absent; second cuticular sheath (CS2) present but separated (Fig. 4); distortion of third-stage cuticle observed in some larvae. Intestine: walls thick and dark, but density decreased compared to late L2; lumen narrow. Tail: bluntly rounded. Digestion: larvae dead. General: L3 first observed at 9 DPI (11 of 15 larvae examined); differentiation of internal structures and organ systems apparent, features more distinct, and larvae larger than L2 at 8 and 9 DPI.

**Intermediate L3 (12, 13, and 15 DPI):** Cuticle and cuticular sheaths: CS1 usually absent; CS2 broken at cephalic extremity (Fig. 5); third cuticle thick. Intestine: clearly defined lumen,

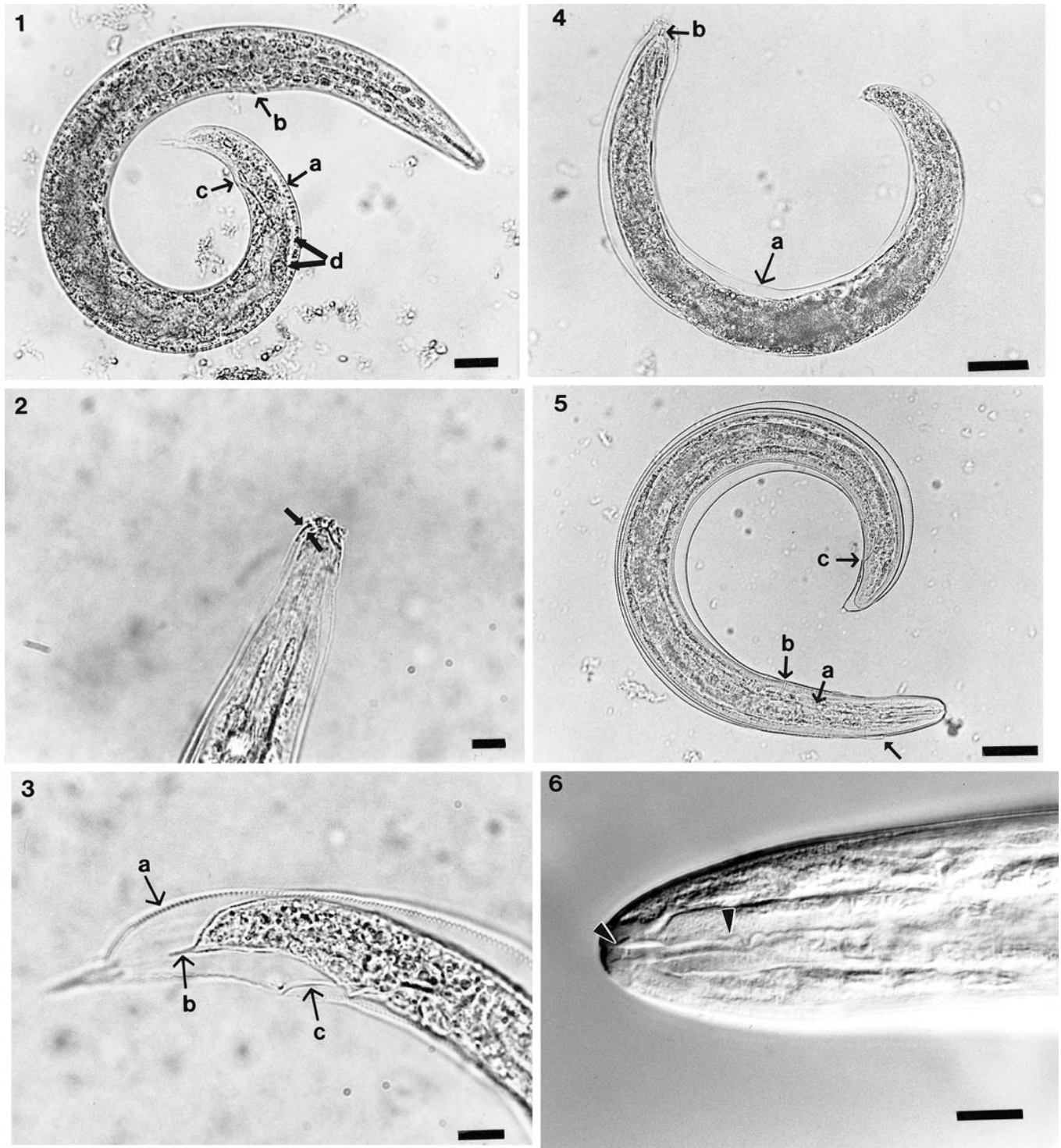
occasionally containing small, heterogeneous, refractile granules; intestinal cells pale, agranular, and clearly defined. GP: increased in size. Tail: bluntly rounded. Digestion: larvae alive. General: cephalic and buccal structures well developed, 6 papillae of inner circle and lateral amphids prominent, surrounding buccal opening; buccal capsule containing cuticularized, stylet-like organ with prominent barblike points in anterior, extending from near buccal opening posterior into insertion in upper esophagus (Fig. 6); excretory gland large, compressing esophagus dorsally.

**Late L3 and emerged L3 (36, 38 DPI, and 22–38 DPI, respectively):** Cuticle and cuticular sheaths: CS1 absent, CS2 usually absent, and when present is broken at cephalic end. Intestine: fully differentiated, containing few small, heterogeneous refractile granules in lumen. Digestion: larvae alive following digestion or emergence. General: characters consistent with intermediate L3 stages.

### Larval development: Morphometrics

Morphometric data for each day of examination of larvae of *U. pallikuukensis* in *D. laevis* at 23.4 C are presented in Figure 7a–c. Fifteen larvae were examined each day, but not all characters were visible in all larvae at all stages of development (particularly the L2). This resulted in the sample size (n) for each character varying between days of examination. The range of n for GP measurements was 2–15; L2 had the smallest n for this character. On 4 and 9 DPI, larvae were a mixed population (L1 and L2 on day 4, L2 and L3 on day 9), and the n for the different characters were 8 (L1, 4 DPI), 4–7 (L2, 4 DPI), 0–2 (L2, 9 DPI), and 6–11 (L3, 9 DPI). The n for each character on remaining days ranged from 8 to 15, and was 10 or greater for most characters.

Larval growth from 0 to 4 DPI consisted primarily of an increase in body width (BW) and esophagus width (EW), and a decrease in the ratio of the esophagus length to total body length (%E) (Fig. 7b, c). From 4 to 9 DPI, the GP became located more posteriorly, the tail became shorter, and BW and %E were highly variable. From 8 to 10 DPI, total body length (BL), distance to GP (GPD), and GP length (GPL) increased and %E decreased. The large standard deviations of measurements on these days reflect the variability observed among these larvae. Between 11 and 38 DPI variability between larvae



FIGURES 1–6. Larvae of *Umingmakstrongylus pallikuukensis* from *Deroceras laeve* at various days postinfection (DPI). **1.** First-stage larva at 3 DPI. Small refractile granules are visible under the cuticle (a). Excretory pore (b) and anus (c) are prominent. Nuclei of intestinal cells (d) (bar = 20  $\mu$ m). **2.** First- to second-stage larval transition at 4 DPI. The cuticle is thickened at the cephalic extremity (arrows) (bar = 20  $\mu$ m). **3.** Second-stage larva at 5 DPI. The first cuticular sheath (CS1) (a) is completely separated from the larva. The tail has a short, ventral point (b). The lining of the rectum has molted (c) (bar = 20  $\mu$ m). **4.** Early third-stage larva (aL3) at 10 DPI. CS1 is absent, the second cuticular sheath (CS2) is present (a) but separated from the larva. The lining of the esophagus is being shed (b) (bar = 50  $\mu$ m). **5.** Intermediate third-stage larva (bL3) at 12 DPI. The CS1 is absent and CS2 is broken at cephalic extremity (arrow). Features more distinct than in earlier larval stages (nerve ring (a), excretory pore (b), and anus (c) are clearly visible) (bar = 40  $\mu$ m). **6.** Late L3. Cuticularized stylet-like organ with barblike points in buccal capsule (arrows pointing at either end of structure) (bar = 10  $\mu$ m).

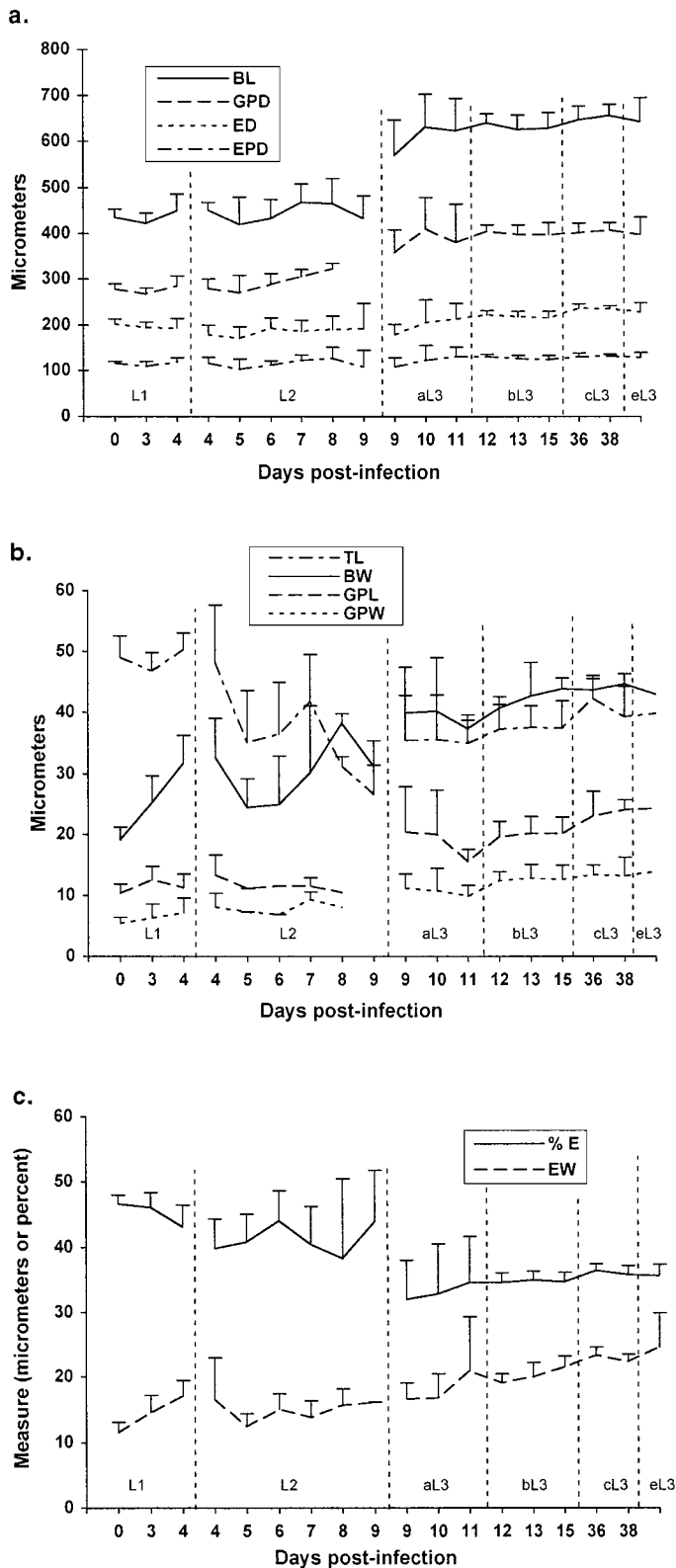


FIGURE 7. (a-c) Mean measurements of larvae of *Umingmakstrongylus pallikuukensis* from *Deroceras laeve* at 23.4 C at various days postinfection (DPI). L1—first-stage larvae, L2—second-stage larvae, aL3—early thirdstage larvae (L3), bL3—intermediate L3, cL3—late L3, eL3—emerged L3. The 2 sets of data for 4 DPI represent measurements from L1 and L2, and for 9 DPI represent measurements from L2

progressively decreased and tail length (TL), BW, EW, and GPL increased.

Larval categories established on the basis of morphological characteristics corresponded with statistically significant morphometric differences with the exception of late L3 and emerged L3 (Table III). Late L3 and emerged L3 were, therefore, combined for further analyses. L1 significantly differed from intermediate L3 and late/emerged L3 for all characters except distance to nerve ring (NRD). L2 differed significantly from intermediate L3 and late/emerged L3 in all characters except NRD, TL, and %E. There were also significant differences between early L3 and intermediate L3 (distance to base of the esophagus [ED] and EW), between early L3 and late/emerged L3 (ED, EW, TL, GPL, and GPW), and intermediate L3 and late/emerged L3 (EW and GPL).

### The effect of temperature on larval development

Larval development occurred more rapidly at warmer temperatures in both gastropod species. Detailed results for *D. laeve* are presented in Figure 8a-d. In *D. reticulatum*, development of L1 to intermediate L3 took 66, 32, 15, and 11 days at 12, 15, 20, and 25 C respectively. In general, transition from one larval stage to the next was both more synchronous among slugs and quicker at warmer temperatures. Similar patterns of larval development were seen in both gastropod species, but transition periods between larval stages were longer in *D. reticulatum*.

Using data for development of *U. pallikuukensis* to the first intermediate L3, a regression of temperature on development rate was calculated. The regression equation for development in *D. laeve* was  $y = -0.051 + 0.006x$ ,  $r^2 = 0.97$ ,  $P = 0.015$ ,  $T_0 = 8.5$  C and  $DD = 167$  and in *D. reticulatum* was  $y = -0.057 + 0.006x$ ,  $r^2 = 0.99$ ,  $P = 0.003$ ,  $T_0 = 9.5$  C and  $DD = 167$ . The regression for development of *M. capillaris* in *D. reticulatum* or *D. agrestis* (data from Rose [1957]) was  $y = -0.025 + 0.006x$ ,  $r^2 = 0.97$ ,  $P = 0.003$ ,  $T_0 = 4.2$  C and  $DD = 167$ . The regression for development of *E. rangiferi* in *A. arbustorum* was  $y = -0.041 + 0.004x$ ,  $r^2 = 1$ ,  $P = 0.002$ ,  $T_0 = 10.25$  C and  $DD = 250$  and for *E. rangiferi* in *E. fulvus* was  $y = -0.033 + 0.004x$ ,  $r^2 = 1$ ,  $P < 0.001$ ,  $T_0 = 8.25$  C and  $DD = 250$ . The data for *E. rangiferi* at 28 C in *E. fulvus* were not used because rate of development at this temperature did not differ from that at 24 C and was in the nonlinear range of the curve (Range III as described by Li [1998]).

## DISCUSSION

### Larval development: Morphology and morphometrics

The characters most useful for identifying the stage of larval development of *U. pallikuukensis* in *D. laeve* were separation

←  
and aL3. All measurements in micrometers except for esophagus as a percentage of total body length (%E) (error bars = 1 SD). a. Total body length (BL) (not including separated cuticular sheaths) and distances from the cephalic extremity to genital primordium (GPD), to base of esophagus (ED), and to excretory pore (EPD). b. Tail length measured from caudal extremity (TL), body width measured at base of esophagus (BW), GP length (GPL), and width (GPW). c. %E and width at base of esophagus (EW).

TABLE III. Measurements\* of the different larval stages of *Umingmakstrongylus pallikuukensis* digested or emerged from slugs.

Character	This study					Hoberg et al. (1995)
	L1	L2	aL3	bL3	c/eL3	cL3
Body length	n = 23 431 ± 30 (400–508)‡	n = 63 446 ± 50 (291–552)	n = 38 612 ± 72 (448–769)	n = 46 630 ± 29 (547–723)	n = 29 648 ± 35 (545–691)	n = 10 560 ± 34 (514–600)
Body width	n = 23 28 ± 4 (21–42)	n = 48 30 ± 8.9 (16–58)	n = 37 40 ± 6.2 (23–55)	n = 46 43 ± 3.8 (37–58)	n = 29 44 ± 1.8 (42–46)	n = 10 47 ± 7 (39–60)
Nerve ring†	n = 22 109 ± 9 (83–120)	n = 47 110 ± 19.7 (69–164)	n = 31 101 ± 19 (69–157)	n = 46 104 ± 6.6 (90–118)	n = 29 107 ± 6.7 (83–118)	n = 10 99 ± 4.2 (93–106)
Excretory pore†	n = 23 113 ± 10 (83–134)	n = 57 117 ± 19.2 (53–173)	n = 38 119 ± 23 (74–182)	n = 46 127 ± 7.2 (111–150)	n = 29 130 ± 7.9 (104–146)	n = 10 118 ± 5 (109–127)
Esophagus†	n = 23 194 ± 15 (169–238)	n = 57 184 ± 25.6 (120–238)	n = 38 197 ± 31 (141–298)	n = 46 219 ± 12 (194–249)	n = 29 233 ± 13 (201–263)	n = 10 200 ± 12 (181–214)
Esophagus width	n = 23 15 ± 2.9 (12–23)	n = 41 15 ± 3.4 (9.2–28)	n = 35 17 ± 3.4 (12–23)	n = 46 20 ± 2.2 (16–25)	n = 29 23 ± 3.1 (18–35)	n = 10 23 ± 3.3 (18–26)
% Esophagus	n = 23 45 ± 3 (38–49)	n = 57 41 ± 4.9 (33–55)	n = 38 32 ± 5.8 (22–47)	n = 46 35 ± 1.7 (31–39)	n = 29 38 ± 1.4 (35–41)	n = 10 — (33–39)
Genital primordium†	n = 23 27 ± 18 (247–326)	n = 28 287 ± 32 (178–342)	n = 32 380 ± 66 (182–547)	n = 46 399 ± 20 (344–469)	n = 29 402 ± 26 (316–432)	n = 10 361 ± 23 (318–388)
GP‡ length	n = 23 12 ± 2 (9–14)	n = 17 12 ± 2.5 (6.9–16)	n = 26 19 ± 5.8 (12–35)	n = 44 20 ± 2.4 (14–23)	n = 29 24 ± 2.9 (16–30)	— — —
GP‡ width	n = 23 7 ± 2 (5–12)	n = 17 8 ± 1.8 (4.6–12)	n = 26 11 ± 1.9 (7–14)	n = 44 13 ± 1.8 (9–18)	n = 29 13 ± 2.3 (12–21)	— — —
Tail§	n = 23 48 ± 4 (42–55)	n = 56 37 ± 9.6 (14–55)	n = 38 35 ± 5.8 (23–44)	n = 46 37 ± 3.7 (32–49)	n = 29 40 ± 4.5 (32–49)	n = 10 31 ± 2.9 (26–34)

\* In micrometers except % Esophagus, which is esophageal length as a percentage of total body length. L1, first-stage larvae (in slugs); L2, second-stage larvae; aL3, early third-stage larvae; bL3, intermediate L3; c/eL3, late and emerged L3 combined.

† Measurements are from the cephalic extremity.

‡ Genital primordium.

§ Anus to caudal extremity.

‡ Mean ± 1 SD (range).

of cuticular sheaths, tail structure, and viability of larvae following digestion. Small, uniform refractile granules immediately beneath CS1 and CS2 appeared to be indicative of an impending molt to L2 and L3, respectively.

Larval measurements of *U. pallikuukensis* changed significantly from late L2 through to early L3 stages (8–11 DPI), and there was notable variation in morphology and morphometrics among the larvae. As larvae reached intermediate and late/emerged L3, the variability decreased. Similar observations for variability in the mid to late L2 have been reported for *Protostrongylus boughtoni* (Kralka and Samuel, 1984). Although, in the present study, digestion may have caused distortion of L2 and early L3, similar variability of L2 was seen in preliminary studies with *U. pallikuukensis* when larvae were recovered from slug tissue by dissection instead of digestion. We believe, therefore, that the variability we observed is a real characteristic of L2 and early L3 stages.

Early L3 of *U. pallikuukensis* exhibited similar morphol-

ogy and survival characteristics after digestion as those described as preinfective L3 for *M. capillaris* (Gerichter, 1948; Rose, 1957; Beresford-Jones, 1966) and *E. rangiferi* (Halvorsen and Skorping, 1982). Small, but statistically significant, increases in esophagus width and GP length occurred from intermediate L3 to late/emerged L3, suggesting that larvae may continue to grow after reaching the intermediate L3 stage.

The late L3 in the present study were larger than those reported by Hoberg et al. (1995). This may be accounted for by differences in intermediate host species (*D. laeve* in present study, *D. reticulatum* in Hoberg et al. [1995]), intensities of infection in gastropods (low to medium vs. very high), or methods of preservation (heat killed over a flame and examined immediately vs. heat killed in ethanol and examined cleared in glycerin). Alternatively, they may be a result of natural variation in the larval populations. Ash (1970) postulated that differences in intensity of infection as well as differences in ages

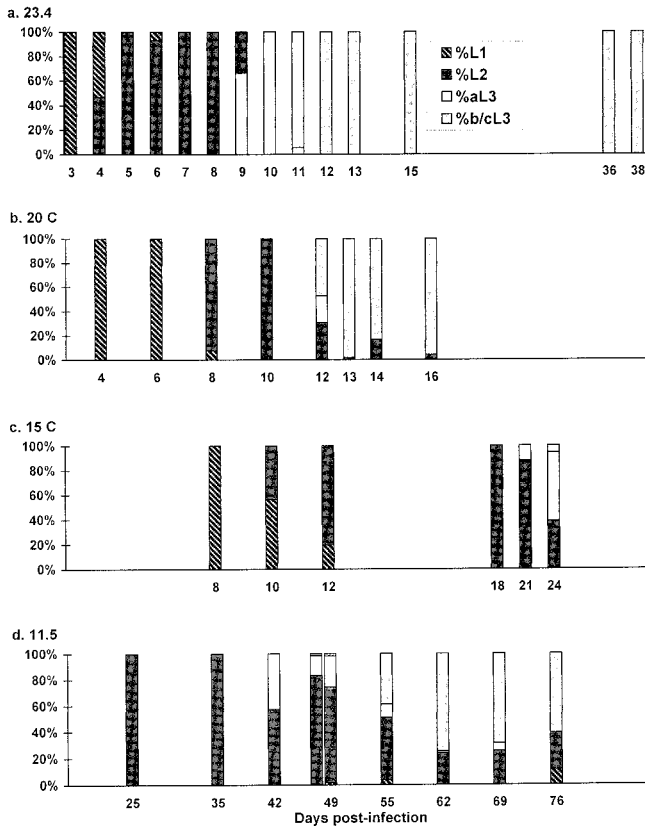


FIGURE 8. (a–d) Distribution of larval stages (as a percentage of total larvae recovered) of *Umingmakstrongylus pallikuukensis* in *Deroceras laeve* on different days postinfection at different temperatures. Bars represent the average proportion of the different larval stages recovered from slugs on each day. L1—first-stage larvae, L2—second-stage larvae, aL3—early third-stage larvae (L3), b/cL3—intermediate or late L3. **a.** 23.4 C. **b.** 20 C. **c.** 15 C. **d.** 11.5 C. Results for the experiment at 11.5 C on days 25, 35, 42, and 49 are based on data from experiment 9; remaining days are based on data from experiment 8.

of larvae within the gastropods may affect the size of L3 of *Angiostrongylus cantonensis*.

Limited published data and the present study suggest that patterns of larval growth may differ among the Protostrongylidae. Within the Muellieriinae, most larval growth appears to occur during the L1 and then immediately following the second molt. Gerichter (1948) reported considerable growth of *Cystocaulus ocreatus* and *M. capillaris* in the late L1 stage, little growth during the L2 stage, and that preinfective L3 were longer and thinner than the L2, suggesting that growth occurred during this phase of development. Beresford-Jones (1966) came to similar conclusions for *M. capillaris* and noted that L3 that had exsheathed in the intermediate host were, on average, longer than those that had not. The pattern of larval growth observed for *U. pallikuukensis* is consistent with the findings for these Muellieriinae.

In the Elaphostrongylinae, the putative sister group of the Muellieriinae (Carreno and Hoberg, 1999), growth in total length appears to be greatest during the L1 and L2 stages (e.g., *E. rangiferi*, Halvorsen and Skorping, 1982). However, Halvorsen and Skorping (1982) did not distinguish preinfective L3 from L2, so it is unclear whether growth occurred during the

L2 phase or at/shortly after the second molt. In the Protostrongylinae, development, based on length and width, appears to be greatest during the L2 phase (e.g., *P. boughtoni*, Kralka and Samuel [1984]).

There are few published descriptions of protostrongylid larval development and no standardized format for describing and differentiating these stages. There is also great variation in experimental design and intermediate host species used. Comparisons of patterns of larval development among the Protostrongylidae should, therefore, be interpreted with caution. It is apparent, however, that the potential influence of phylogenetically determined or linked patterns for larval growth, differentiation, and life history patterns deserves further investigation.

### Larval feeding and stomal morphology

For some parasitic nematodes with direct life cycles, e.g., *Ancylostoma tubaeforme*, feeding of L1 is critical for subsequent growth to the infective stage (Croll, 1972). Little is known, however, about the feeding behavior of larval protostrongylids. Food particles in the intestine or droplets or granules associated with the intestinal cells have been described in L1 and L2 of the Muellieriinae, Elaphostrongylinae, and Protostrongylinae. These particles typically decrease in number by the L3 phase (Gerichter, 1948, 1951; Rose, 1957; Kralka and Samuel, 1984; Lankester et al., 1998). Some authors have implied that developing protostrongylid larvae feed within the gastropods (Svarc and Zmoray, 1974; Svarc, 1978; Skorping, 1984). Increase in dimensions of the body and esophagus and increased granularity of the intestinal region of late L1 of *U. pallikuukensis* support the contention that L1 of this species may feed within gastropods. The L2 were always enclosed in CS1 and thus were presumably unable to feed. For the L3, the absence of sheaths (or broken sheaths), the presence of a stylet within the buccal cavity, and granules in the intestinal lumen of late and emerged L3, indicate that these larvae may feed.

The cuticularized stylet-like organ in L3 of *U. pallikuukensis* has not been described for the Protostrongylidae (e.g., Carreno and Hoberg, 1999); however, the L2 and L3 of some Strongylida have a similar structure that simulates a stomatostyl (Chitwood and Chitwood, 1974; Maggenti, 1981; Poinar, 1983). Taxa considered close to the Strongylida, including the Diplogasterida and the Rhabditida (Blaxter et al., 1998), possess stylets that may be well developed in larval forms but degenerate in the adult stages (Chitwood and Chitwood, 1974). The stylet in L3 of *U. pallikuukensis* may function for feeding both within gastropods and, subsequent to emergence, in the external environment. It may also play a role in L3 emergence from gastropods and penetration of the gastrointestinal mucosa of the definitive host. Larval emergence is a common and possibly epidemiologically important feature of *U. pallikuukensis* (Kutz et al., 2000). It might be expected, therefore, that emerged larvae are capable of feeding, which could contribute to long-term survival of larvae in the environment and perhaps influence patterns of transmission.

### The effects of temperatures on larval development

Rates and synchrony of larval development for *U. pallikuukensis* were highly temperature dependent in both *D. laeve* and *D. reticulatum*. At lower temperatures not only did larvae de-



velop more slowly, but the transition period from L2 to intermediate/late L3 was longer. Under natural conditions, this may result in L3 in gastropods becoming available later and over a more extended period at cooler average temperatures than at warmer temperatures. The temperature-related differences in the synchrony of larval development may result in differences in the patterns of larval acquisition by muskoxen and, in turn, influence adult parasite population structure, pathology, and epidemiology (Kutz et al., 1999).

A high mortality rate of *D. reticulatum* at low temperatures (10 and 12 C) and the length of these experiments resulted in the examination of few slugs many days apart. It is possible, therefore, that L3 were present before they were detected. For example, at 12 C, L2 were first observed at 47 DPI; by the next sampling period at 66 DPI, live L3 were present. If these L3 were present earlier, for example by 50 DPI, the calculated  $T_0$  would have been lower (8.3 C), but the DD would have remained the same.

It is interesting to compare the  $T_0$  and DD determined for *U. pallikuukensis* with those for related species. Within the Muelleriinae, using the same genus of intermediate host *Deroceras*, the same DD (167) were calculated for *U. pallikuukensis* and *M. capillaris*, although the  $T_0$  differed considerably (8.5 C in *D. laeve* or 9.5 C in *D. reticulatum* for *U. pallikuukensis*, 4.2 C in *Deroceras* spp. for *M. capillaris*). In contrast, the  $T_0$  for *U. pallikuukensis* were similar to that of the more distantly related, but northern elaphostrongyline, *E. rangiferi* (10.25 C in *A. arbustorum* or 8.25 C in *E. fulvus*), whereas the DD differed (250 for *E. rangiferi* in both *A. arbustorum* and *E. fulvus*). Finally, whereas the DD in different intermediate host species were the same for *E. rangiferi*, the  $T_0$  differed between species. Schjetlein and Skorping (1995) suggested that environment may be an important factor determining  $T_0$ , with protostrongylids from higher latitudes having higher development thresholds. The data from the present study together with those from work on *M. capillaris* are consistent with this hypothesis. Parameters for larval development appear, however, to be a result of complex interactions between the environment and intrinsic pathways for ontogeny that, in part, may be phylogenetically determined for parasites and intermediate hosts. Based on the present study, it appears that  $T_0$  may be related to environment, whereas DD is a function of parasite phylogeny. Gastropod phylogeny may influence both parameters. To make more meaningful comparisons of development patterns among the Protostrongylidae, detailed studies, at the level of the species, genera, and subfamilies, are required.

The development parameters obtained in the laboratory for *U. pallikuukensis* in a natural intermediate host, *D. laeve*, coupled with appropriate microhabitat temperature measurements, may permit us to predict larval development rates in the Arctic. This information, verified by field experiments, is being used as the foundation for a model system to predict effects of climate, including global climate change, on geographic distribution, and the dynamics of transmission in this complex host-parasite system in the Arctic.

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#### LITERATURE CITED

- ASH, L. R. 1970. Diagnostic morphology of the third-stage larvae of *Angiostrongylus cantonensis*, *Angiostrongylus vasorum*, *Aelurostrongylus abstrusus*, and *Anafilaroides rostratus* (Nematoda: Metastrongyloidea). *Journal of Parasitology* **56**: 249–253.
- BERESFORD-JONES, W. P. 1966. Observations on *Muellerius capillaris* (Muller, 1889), Cameron, 1927 I.—The bionomics and development in *Trichia hispida* (Linnaeus) of larvae obtained from sheep grazed on permanent pasture. *Research in Veterinary Science* **7**: 61–66.
- BLAXTER, M. L., P. DE LAY, J. R. GAREY, L. X. LIU, P. SCHELDAMAN, A. VIERSTRAETE, J. R. VANFLETEREN, L. Y. MACKAY, M. DORRIS, L. FRISSE, J. T. VIDA, AND W. K. THOMAS. 1998. A molecular evolutionary framework for the phylum Nematoda. *Nature* **392**: 71–75.
- CABARET, J. 1987. Age susceptibility of molluscan intermediate hosts to protostrongylid nematodes. *American Society of Parasitologists* **73**: 857–858.
- CAMPBELL, A., B. D. FRAZER, N. GILBERT, A. P. GUTIERREZ, AND M. MACKAUER. 1974. Temperature requirements of some aphids and their parasites. *Journal of Applied Ecology* **11**: 431–438.
- CARRENO, R. A., AND E. P. HOBERG. 1999. Evolutionary relationships among the Protostrongylidae Leiper, 1926 (Nematoda: Metastrongyloidea) as inferred from morphological characters, with consideration of parasite–host coevolution. *Journal of Parasitology* **85**: 638–648.
- CHITWOOD, B. G., AND M. B. CHITWOOD. 1974. Introduction of nematology. University Park Press, Baltimore, Maryland, 334 p.
- ROLL, N. A. 1972. Feeding and lipid synthesis of *Ancylostoma tubaeforme* preinfective larvae. *Parasitology* **64**: 369–378.
- FORRESTER, S. G., AND M. W. LANKESTER. 1997. Extracting protostrongylid nematode larvae from ungulate feces. *Journal of Wildlife Diseases* **33**: 511–516.
- GERICHTER, C. B. 1951. Studies on the lung nematodes of sheep and goats in the Levant. *Parasitology* **41**: 166–182.
- . 1948. Observations on the life history of lung nematodes using snails as intermediate hosts. *American Journal of Veterinary Research* **9**: 109–112.
- GUNN, A., AND G. WOBESER. 1993. Protostrongylid lungworm infection in muskoxen, Coppermine, N.W.T., Canada. *Rangifer* **13**: 45–47.
- HALVORSEN, O., AND A. SKORPING. 1982. The influence of temperature on growth and development of the nematode *Elaphostrongylus rangiferi* in the gastropods *Arianta arbustorum* and *Euconulus fulvus*. *Oikos* **38**: 285–290.
- HOBERG, E. P., L. POLLEY, A. GUNN, AND J. S. NISHI. 1995. *Umingmakstrongylus pallikuukensis* gen. nov. et sp. nov. (Nematoda: Protostrongylidae) from muskoxen, *Ovibos moschatus*, in the central Canadian Arctic, with comments on biology and biogeography. *Canadian Journal of Zoology* **73**: 2266–2282.
- KRALKA, R. A., AND W. M. SAMUEL. 1984. Experimental life cycle of *Protostrongylus boughtoni* (Nematoda: Metastrongyloidea), a lungworm of snowshoe hares, *Lepus americanus*. *Canadian Journal of Zoology* **62**: 473–479.
- KUTZ, S. J., E. P. HOBERG, AND L. POLLEY. 1999. Experimental infections of muskoxen (*Ovibos moschatus*) and domestic sheep with *Umingmakstrongylus pallikuukensis* (Nematoda: Protostrongylidae): Parasite development, population structure and pathology. *Canadian Journal of Zoology* **77**: 1562–1572.
- , ———, AND ———. 2000. Emergence of third-stage larvae of *Umingmakstrongylus pallikuukensis* from three gastropod intermediate host species. *Journal of Parasitology* **86**: 743–749.
- LANKESTER, M. W., I. C. OLSSON, M. STEEN, AND A. A. GAJADHAR. 1998. Extra-mammalian larval stages of *Elaphostrongylus alces* (Nema-

- toda: Protostrongylidae), a parasite of moose (*Alces alces*) in Fennoscandia. *Canadian Journal of Zoology* **76**: 33–38.
- LI, D. Q. 1998. A linear model for description of the relationship between the lower threshold temperature and thermal constant in spiders (Araneae: Arachnida). *Journal of Thermal Biology* **23**: 23–30.
- MAGGENTI, A. 1981. *General nematology*. Springer-Verlag, New York, New York, 372 p.
- PILSBRY, H. A. 1946, 1948. *Land Mollusca of North America (north of Mexico)*, Vol. 2. George W. Carpenter Fund for the Encouragement of Original Scientific Research, Philadelphia, Pennsylvania, 1,113 p.
- POINAR, G. W., JR. 1983. *The natural history of nematodes*. Prentice-Hall Inc., Englewood Cliffs, New Jersey, 323 p.
- ROSE, J. H. 1957. Observations on the larval stages of *Muellerius capillaris* within the intermediate hosts *Agriolimax agrestis* and *A. reticulatus*. *Journal of Helminthology* **31**: 1–16.
- SAMSON, J., AND J. C. HOLMES. 1985. The effect of temperature on rates of development of larval *Protostrongylus* spp. (Nematoda: Metastrongyloidea) from bighorn sheep, *Ovis canadensis canadensis*, in the snail *Vallonia pulchella*. *Canadian Journal of Zoology* **63**: 1445–1448.
- SCHJETLEIN, J., AND A. SKORPING. 1995. The temperature threshold for development of *Elaphostrongylus rangiferi* in the intermediate host: An adaptation to winter survival? *Parasitology* **111**: 103–110.
- SKORPING, A. 1984. Density-dependent effects in a parasitic nematode, *Elaphostrongylus rangiferi*, in the snail intermediate host. *Oecologia* **64**: 34–40.
- SOLOMON, A., I. PAPERNA, AND A. MARKOVICS. 1996. The influence of aestivation in land snails on the larval development of *Muellerius* cf. *capillaris* (Metastrongyloidea: Protostrongylidae). *International Journal for Parasitology* **26**: 363–367.
- SVARC, R. 1978. Ecomorphological and pathological phenomena in the bionomy of *Cystocaulus ocreatus* (Railliet and Henry, 1907) Mikacic, 1939. *Biologické Práce* **24**: 1–89.
- , AND I. ZMORAY. 1974. The development of *Muellerius tenuispicularis* Gebauer, 1932 in the intermediate host under experimental conditions. *Biologia (Bratisl)* **29**: 121–127.