44817

National Library of Canada — Bibliothèque nationale du Canada

Canadian Theses Division — Division des thèses canadiennes

Ottawa, Canada
K1A 0N4

PERMISSION TO MICROFILM — AUTORISATION DE MICROFILMER

- Please print or type — Écrire en lettres moulées ou dactylographier

Full Name of Author — Nom complet de l'auteur

ALLEN WILLIAM SHOSTAK

Date of Birth — Date de naissance	Country of Birth — Lieu de naissance
2 SEPTEMBER 1955	CANADA

Permanent Address — Résidence fixe

4809-ADA BLVD
EDMONTON ALBERTA
T5W 4M9

Title of Thesis — Titre de la thèse

SURVIVAL OF FIRST-STAGE LARVAE OF PARELAPHOSTRONGYLUS ODOCOILEI AND PARELAPHOSTRONGYLUS TENUIS (NEMATODA: METASTRONGYLOIDEA).

University — Université

UNIVERSITY OF ALBERTA

Degree for which thesis was presented — Grade pour lequel cette thèse fut présentée

M. Sc.

Year this degree conferred — Année d'obtention de ce grade	Name of Supervisor — Nom du directeur de thèse
1980	DR. W. M SAMUEL

Date	Signature
4 February 1980	Allen W Shostak

THE UNIVERSITY OF ALBERTA

SURVIVAL OF FIRST-STAGE LARVAE OF PARELAPHOSTRONGYLUS ODOCOILEI AND PARELAPHOSTRONGYLUS TENUIS (NEMATODA:METASTRONGYLOIDEA)

by

© ALLEN WILLIAM SHOSTAK

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

DEPARTMENT OF ZOOLOGY

EDMONTON, ALBERTA

SPRING, 1980

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "Survival of first-stage larvae of Parelaphostrongylus odocoilei and Parelaphostrongylus tenuis (Nematoda:Metastrongyloidea)." submitted by Allen William Shostak in partial fulfilment of the requirements for the degree of Master of Science.

William M. Samuel
Supervisor

[signature]

B. A. Henry

W. C. Mackay

Date 9 JAN. 1980

ABSTRACT

The survival of first-stage larvae of *Parelaphostrongylus odocoilei* and *Parelaphostrongylus tenuis* was determined in a variety of temperature and moisture conditions. Following treatment in some of those conditions, the infectivity of surviving first-stage larvae to the experimental intermediate host *Triodopsis multilineata* was determined.

The survival of larvae of *P. odocoilei* was strongly influenced by both moisture and temperature conditions. Hydrated larvae, and larvae desiccated at low relative humidity, had the lengthiest survival. Desiccation enhanced survival of larvae at high temperature, but reduced their survival while frozen. A major loss of infectivity to the intermediate host was observed for larvae which survived desiccation.

Repeated temperature changes above freezing did not alter survival of larvae of *P. odocoilei*. Repeated freezing or repeated desiccation resulted in reduced survival of the larvae of *P. odocoilei* and *P. tenuis*. The reduction in survival was proportional to the number of treatments administered.

The survival of larvae of *P. odocoilei* from two sources, Vancouver Island and Jasper National Park, did not differ following storage at various temperature and moisture conditions. Differences were noted between species in both their survival, and in their infectivity to the intermediate host. Larvae of *P. odocoilei* survived better than *P. tenuis* following repeated freezing, while larvae of *P. tenuis* survived better than *P. odocoilei* following

repeated desiccation. Following freezing, a greater proportion of the surviving first-stage larvae of P. odocoilei were infective to the intermediate host than were larvae of P. tenuis, when compared to the infectivity of the larvae of the two species which had not been previously frozen.

The results are discussed in light of current concepts regarding survival of the free-living stages of parasitic nematodes, and in light of the current distribution of Parelaphostrongylus spp. in North America. It is suggested that differences in the tolerance of first-stage larvae of P. odocoilei and P. tenuis to temperature and moisture conditions provide a means by which climatic factors can differentially influence the survival of the free-living stage of these two species, and thereby their distributions.

ACKNOWLEDGEMENTS

I wish to express my deep gratitude to my supervisor, Dr. W.M. Samuel, for his advice, encouragement, and interest in this study. I would like to thank the members of my supervisory and examining committees, Drs. J.C. Holmes, W.M. Samuel, and F.C. Zwickel, and Drs. B. Heming, J.C. Holmes, W.C. Mackay, and W.M. Samuel, respectively, for their helpful comments.

Dr. J.M. Mayo, Dr. D.W. Whitfield, and Mr. J. Richards, Department of Botany, provided helpful comments dealing with the methods of this study. Ms. M. Barker, Department of Zoology, and the staff of the University of Alberta Vivarium, particularly Mr. K. Taylor, provided excellent technical assistance in maintenance of the experimental animals.

I am also indebted to the members of the parasitology group at the University of Alberta, for interesting discussions and probing questions.

Financial support was provided by the Alberta Fish and Wildlife Division, with special thanks to W. Wishart and D. Neave, the Natural Sciences and Engineering Council of Canada (Operating Grant No. A-6603 to W.M.S., and Postgraduate Scholarship to A.W.S.), the Boreal Institute for Northern Studies, the Alberta Fish and Game Association, the Saskatchewan Department of Tourism and Renewable Resources, and the University of Alberta (Teaching Assistantship to A.W.S.).

Finally, I would like to thank Shirley Hilger, whose love, understanding, and encouragement during this study will always be remembered.

TABLE OF CONTENTS

LIST OF TABLES

List of Tables - continued

LIST OF FIGURES

List of Figures - continued

I. INTRODUCTION

Three species of the genus *Parelaphostrongylus* (Nematoda: Metastrongyloidea: Elaphostrongylinae) have been reported in North America. *Parelaphostrongylus tenuis* (Dougherty 1945), the meningeal worm, is found in its normal host, the white-tailed deer (*Odocoileus virginianus*), throughout eastern North America (Fig. 1) (Dougherty 1945; DeGiusti 1955; Anderson 1956; Alibasoglu et al. 1961; Karns 1967; Smith and Archibald 1967; Behrend and Witter 1968; Prestwood and Smith 1969; Samuel and Trainer 1969; Bindernagel and Anderson 1972; Carpenter et al. 1972; Pursglove 1977; Thurston and Strout 1978). *Parelaphostrongylus andersoni* Prestwood 1972, a muscleworm of white-tailed deer, has been reported in that host from the southeastern United States (Prestwood et al. 1974; Pursglove 1977) and in southeastern British Columbia (M.J. Pybus, pers. comm.) (Fig. 1). Another muscleworm, *Parelaphostrongylus odocoilei* (Hobmaier and Hobmaier 1934), has been reported from Columbian black-tailed deer (*Odocoileus hemionus columbianus*) and California mule deer (*Odocoileus hemionus californicus*) in northcentral California (Hobmaier and Hobmaier 1934; Brunetti 1969), in *O. h. columbianus* from Vancouver Island, British Columbia (Platt and Samuel, unpub.), and from mule deer (*Odocoileus hemionus hemionus*) in westcentral Alberta (Platt and Samuel 1978*a*; Samuel, unpub.) (Fig. 2).

The life cycle of all three species of *Parelaphostrongylus* involves the adult occupying an extraintestinal site in a cervid definitive host, the first-stage larva (L1) shed in the feces of the

Figure 1. Approximate distributions of *Parelaphostrongylus tenuis* and *Parelaphostrongylus andersoni* in *Odocoileus virginianus* in North America. Star indicates a report of *P. tenuis* in Angora goats, outside of known *P. tenuis* range in *O. virginianus* (Guthery and Beasom 1979). Deer distribution is from Stock (1978).

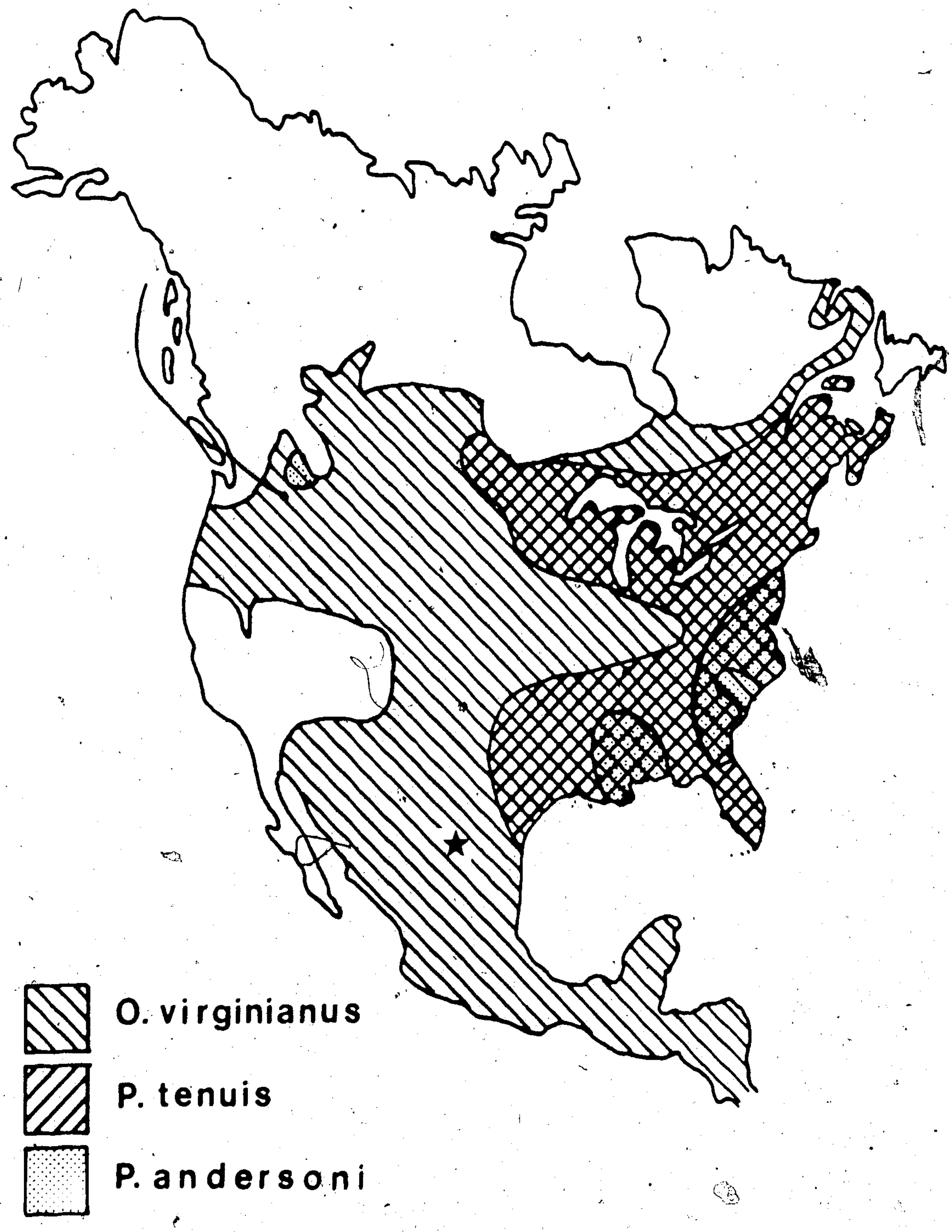
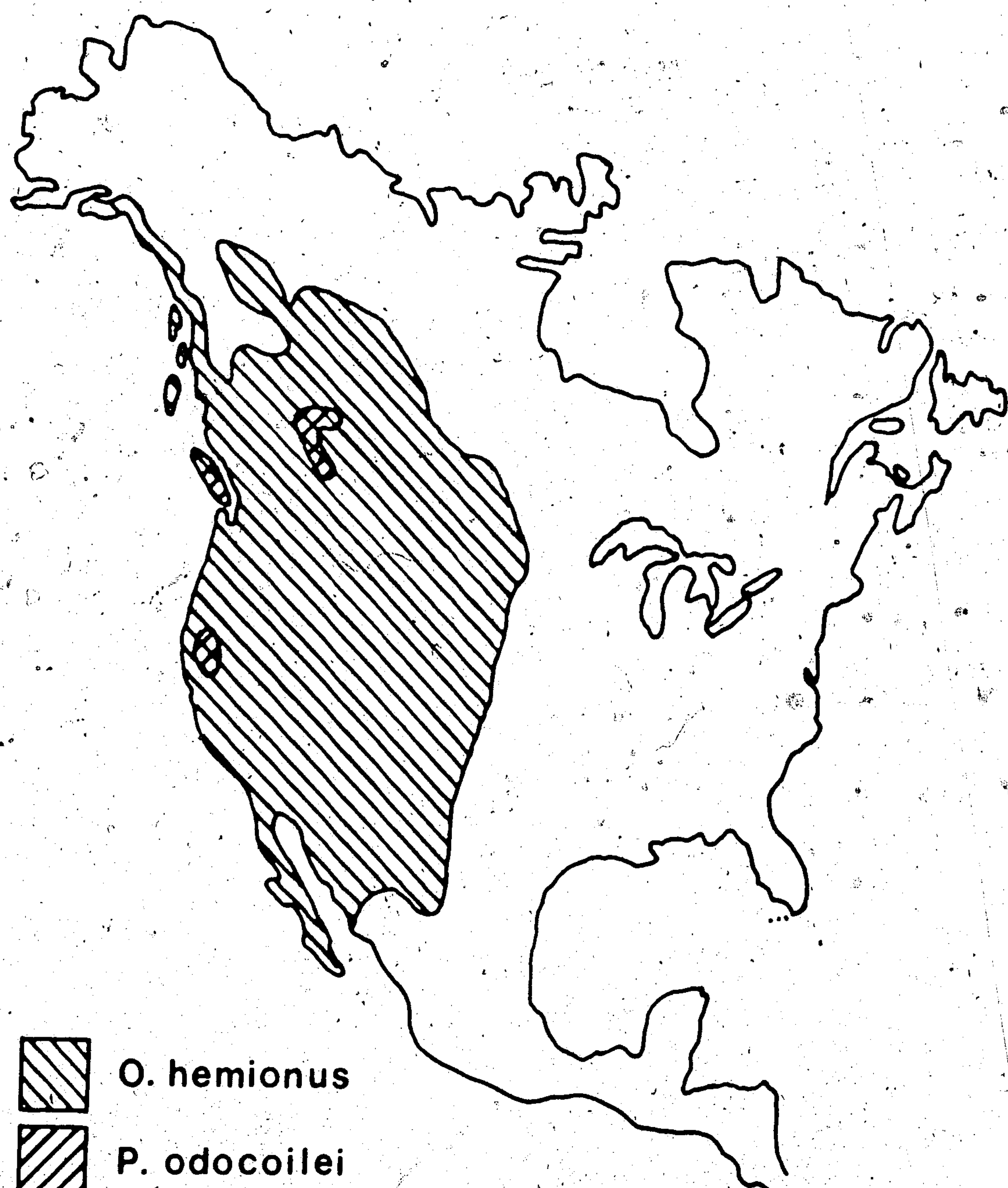

O. virginianus
P. tenuis
P. andersoni

Figure 2. Approximate distribution of *Parelaphostrongylus odocoilei* in *Odocoileus hemionus* in North America. Deer distribution is from Stock (1978).

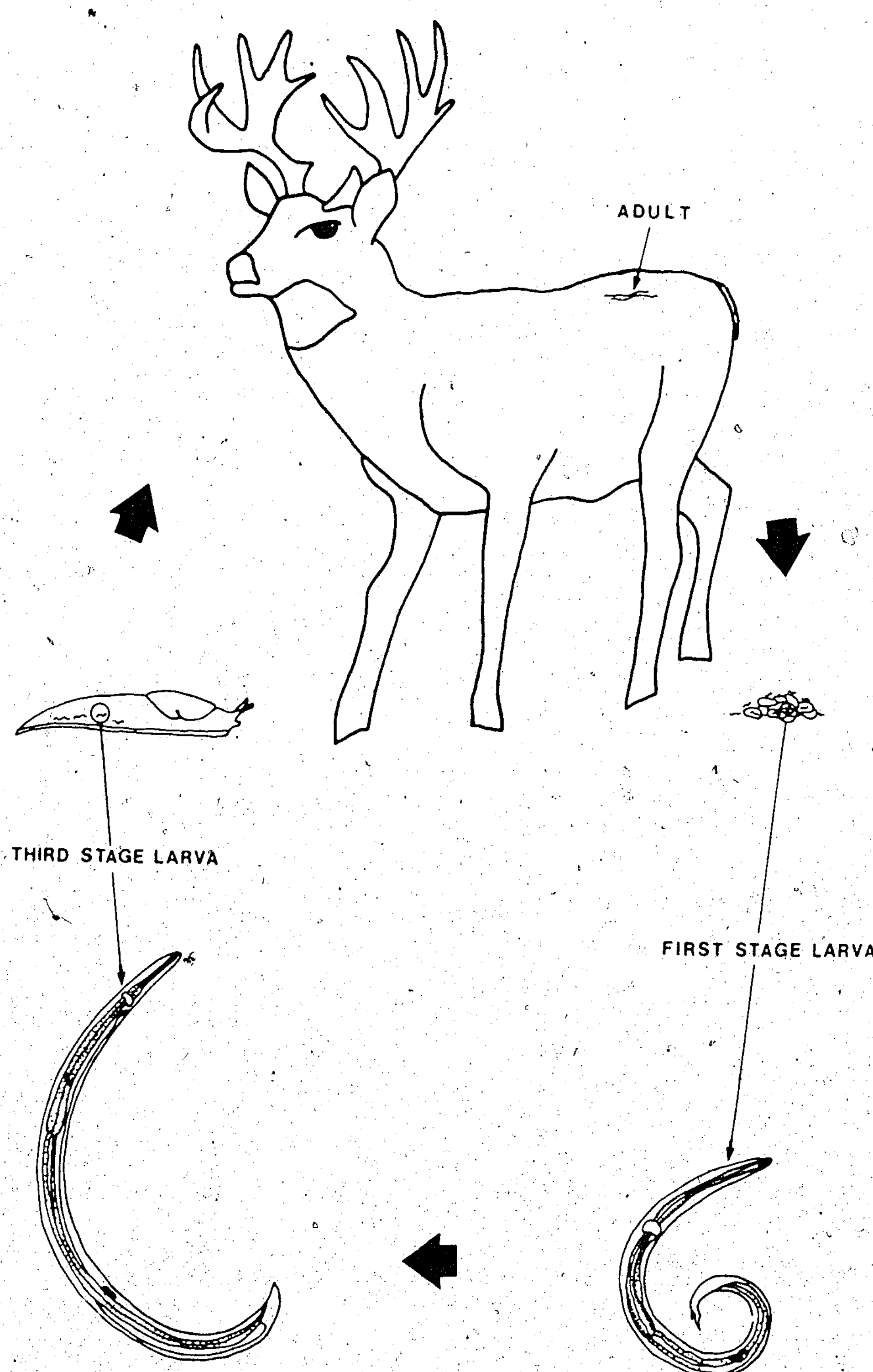

O. hemionus
P. odocoilei

definitive host, and the penetration of the foot of a terrestrial gastropod by the L1. Development to the infective, third-stage larva occurs in the foot, and the life cycle is completed when the gastropod infected with third-stage larvae is accidentally ingested by another cervid.

The maintenance of Parelaphostrongylus in a area is dependent upon both suitable intermediate and definitive host conditions, and sufficient resistance to the external environment by the free-living stage (the L1). Infective conditions require suitable densities of both hosts, and behavioral characteristics which facilitate each host acquiring the appropriate stage of the parasite. The intermediate host must come in contact with the first-stage larva, and subsequently must be available in sufficient numbers to the definitive host as it grazes. The free-living larval stage must resist environmental extremes of moisture, temperature, and solar radiation, either through physiological adaptation or avoidance.

Many aspects of the relationship between the parasite Parelaphostrongylus and its normal intermediate and definitive hosts have been studied. Ecological aspects of the parasite- intermediate host relationship have been studied (Lankester and Anderson 1968; Kearney and Gilbert 1978; Platt 1978), as has the life cycle in the intermediate host (Lankester and Anderson 1968; Platt 1978). Work on the definitive host has provided information not only on the prevalence and distribution (as previously cited) and pathology (reviewed by Anderson 1971), but also on such aspects as prepatent periods (Anderson 1963; Nettles and Prestwood 1976; Platt and Samuel 1978b), effect of size,

Figure 3. Generalized life cycle of genus *Parelaphostrongylus*.


ADULT
THIRD STAGE LARVA
FIRST STAGE LARVA

and frequency of administration, of infective inocula (Nettles and Prestwood 1976; Prestwood and Nettles 1977; Platt and Samuel 1978b), and larval output (Nettles and Prestwood 1976; Platt and Samuel 1978b).

The relationship between the first-stage larva and the environment has not been as well studied as that between the parasitic stages and their hosts. For example, although the range of gastropods and ungulates which can maintain the parasitic stages of *Parelaphostrongylus* has been extensively documented (Lankester and Anderson 1968; review by Brown et al. 1978; Platt 1978; Platt and Samuel 1978b), the range of environmental conditions that can support the free-living stage is almost unknown.

The importance of environmental influences on the free-living stages of parasitic nematodes has long been recognized in epizootiological studies on parasites of domestic animals (reviewed by Gordon 1948; Levine 1963; Rogers and Sommerville 1963; Kates 1965; Gibbs 1973), but Lankester and Anderson (1968) have been the only investigators to attempt documenting the environmental resistance of *Parelaphostrongylus* free-living larvae. Their study, using an extremely limited range of conditions, established that the first-stage larvae of *P. tenuis* are somewhat resistant to desiccation and freezing.

This study was initiated to expand upon the pioneering work of Lankester and Anderson (1968) on the environmental resistance of first-stage larvae of *Parelaphostrongylus*. The objectives of this study were threefold: 1) to determine the range of two major climatic factors, temperature and moisture, over which the first-stage larvae of P. odocoilei could survive; 2) to determine if those first-stage larvae of P. odocoilei which survived temperature or moisture stress retained their infectivity to the intermediate host, and; 3) to determine whether or not temperature or moisture stress equally affected the survival and infectivity of two of the species of Parelaphostrongylus, P. odocoilei and P. tenuis.

II. MATERIALS AND METHODS

A. Definitions

The following are definitions of terms used throughout the text which have various meanings in the literature:

1. Surviving larva: one that is living following storage under specified conditions. The specific criterion used was that a surviving larva must move on its own, or exhibit active motion following prodding with a sharp probe. A larva was assumed dead or moribund if decayed or if not exhibiting active motion even after prodding. Survival of desiccated larvae was monitored after they had been given a minimum of three hours in water to revive. Survival of frozen larvae was monitored no sooner than one hour after thawing.

2. Infective larva: one possessing the ability to enter and develop further in the next host of the life cycle.

3. Sample: a group of 100 first-stage larvae (unless otherwise specified) in a 60 mm Petri dish, used for testing of survival.

4. Replicate: one in a group of samples prepared at the same time from a common source of larvae, and used for the same experimental treatment.

5. Varying: treatment conditions which are changed between two levels on a regular basis.

6. Non-varying: treatment conditions maintained at a constant level throughout an experimental period.

7. Hydrated: a larva in water.

8. Desiccated: a larva in air.

B. Source of Experimental Animals

First-stage larvae of Parelaphostrongylus odocoilei were obtained from experimentally infected mule deer (Odocoileus hemionus hemionus) and black-tailed deer (Odocoileus hemionus columbianus) housed at the University of Alberta Vivarium, Ellerslie, Alberta. The majority of the larvae used in this study was from mule deer infected with P. odocoilei originating from a population of mule deer in Jasper National Park, Alberta. A smaller number of larvae, used in only a few experiments, was from a black-tailed deer infected with P. odocoilei originating from a population of black-tailed deer on Vancouver Island, British Columbia.

First-stage larvae of Parelaphostrongylus tenuis were obtained, frozen on feces, from naturally infected white-tailed deer (Odocoileus virginianus) from the Rachelwood Wildlife Research Preserve in Pennsylvania. A white-tailed deer from Alberta was exposed to larvae from this source, and P. tenuis was the only helminth recovered at necropsy (D.R. Anderson, pers. comm.). This deer, housed in isolation at the University Vivarium, provided an additional supply of larvae.

All first-stage larvae were left on fecal pellets until required. P. odocoilei (Vancouver Island source) larvae were obtained fresh and were refrigerated (8^{o}C) until required. Larvae of P. odocoilei (Jasper source) and P. tenuis were available from feces both fresh and previously frozen (-25°C).

In experiments involving a comparison of larvae from two sources, it was ensured that both groups of larvae had similar prior treatments, to minimize the influence this might have on experimental outcomes. If fresh larvae were available, they were used preferentially over previously frozen larvae. The sources of larvae used in each experiment are itemized in Appendix I.

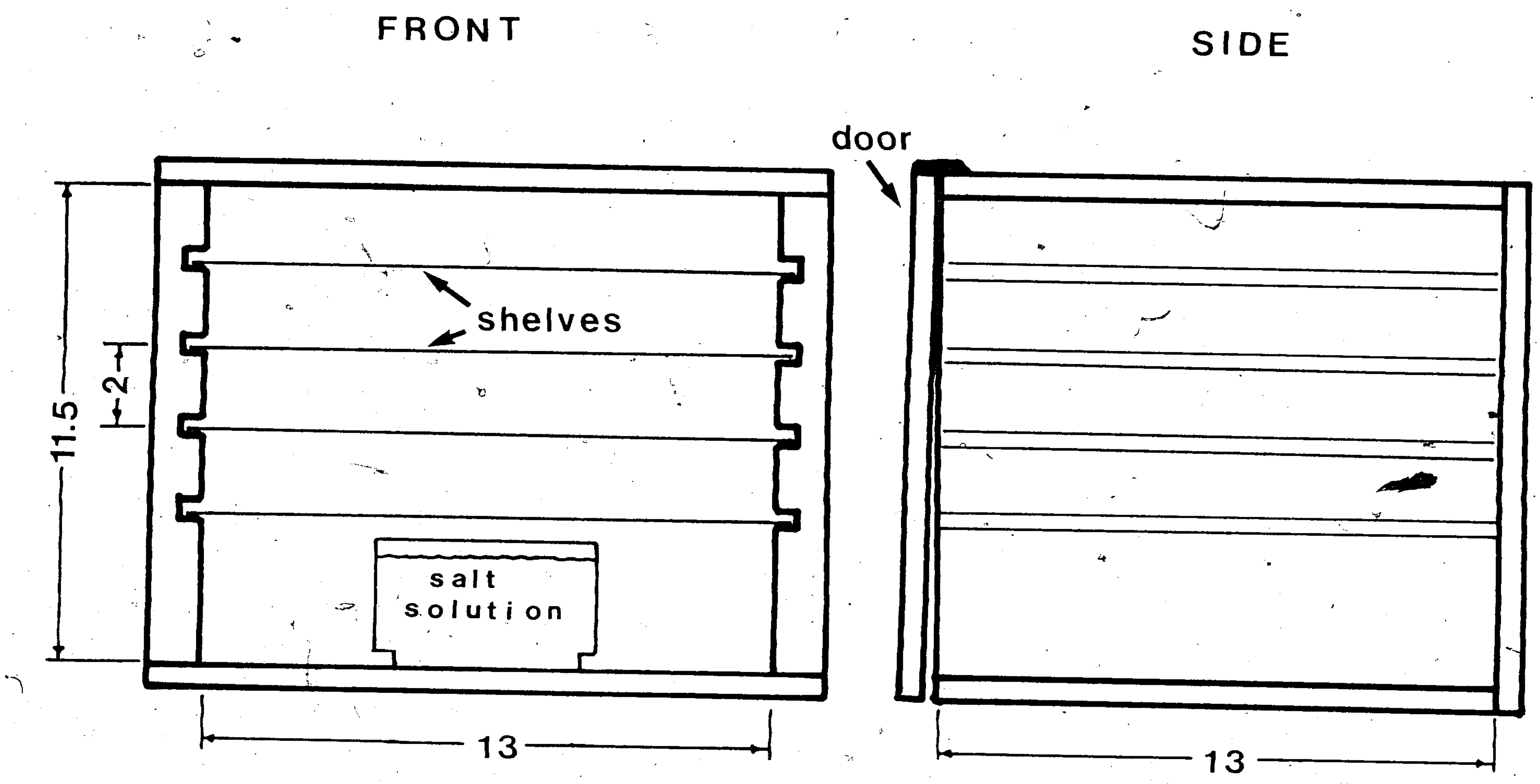
The snail Triodopsis multilineata (Say) was used as the experimental intermediate host. These snails have been maintained in a laboratory colony at the University of Alberta for several years. The original stock of the colony was from Nebraska.

C. Temperature and Humidity Control

Temperatures other than room temperature (18°C) were provided by a variety of incubators, coolers, environmental chambers, and a freezer. Use of some of these facilities for other purposes placed minor constraints on the choice of temperatures for experiments.

Relative humidities were maintained at desired levels by the use of saturated salt solutions (Winston and Bates 1960) in small chambers (Fig. 4). The chambers, constructed of plywood, had four shelves (5 mm mesh, acrylic-coated galvanized metal) capable of holding a total of 16 samples. Each chamber was enclosed in plastic to reduce transfer of moisture through the walls. Forty to 50 ml of appropriate saturated salt solution with precipitate, in a 50 mm diameter glass dish, were placed in the bottom of each chamber.

Figure 4. Diagrammatic representation of humidity control chamber. Measurements are in centimeters.


FRONT
SIDE
door
shelves
salt solution
11.5
2
13
13

Temperatures were monitored several times throughout the course of each experiment. The relative humidity in several chambers was measured in the early stage of the study to confirm the effectiveness of the apparatus. An electronic probe (Brady-Array Humidity Module, model PC 2000, from Thunder Scientific in Albuquerque, N.M.) with a stated accuracy of ± 2%, calibrated shortly prior to measurement, was used to measure relative humidity (RH). Chambers were allowed to equilibrate for two days before measurements of relative humidity were made. The results are given in Table I. The discrepancy between expected and measured relative humidities was small for the intermediate humidity values, but greater for the highest and lowest relative humidities. Since this method of humidity control relies on a diffusion process, it was assumed that the discrepancies at the high and low humidities were due mainly to insufficient equilibration time for the chambers. In all subsequent experimentation the chambers were allowed to equilibrate for a minimum of one week, and at cooler temperatures for two weeks, prior to the introduction of samples.

Since saturated salt solutions regulate humidity up or down towards the theoretical values as long as precipitate remains in the solution, no further measurements were made. Salt solutions were frequently checked, more water or salts added if necessary, and the solutions stirred to prevent the formation of an unsaturated water layer at their surface.

Table I. Theoretical and measured values of percentage relative humidity (% RH) in humidity control chambers. Measurements were made only at the temperatures indicated below. Theoretical values are taken from Table II. Discrepancy is the deviation of the measured value from the theoretical value.

Solution	Temperature (^oC)	Theoretical % RH	Measured % RH	Discrepancy (% RH)
H_2O	45	100	94.0	- 6.0
KCl	45	81.0	82.5	+ 1.5
	35	83.0	82.0	- 1.0
NaCl	35	75.5	77.0	+ 1.5
K_2CO_3	48	40.0	48.0	+ 8.0
	35	41.5	42.0	+ 0.5
	25	43.0	50.0	+ 7.0
LiCl	35	11.5	22.0	+10.5

D. Preparation of Larvae for Experimentation

Feces containing larvae were wrapped in a double layer of cheesecloth and placed overnight in a Baermann apparatus. Approximately 100 ml of fluid was drawn off and refrigerated ($8^{o}C$). Larvae in this fluid were repeatedly washed in tap water at $8^{o}C$ to remove as much fecal debris as possible from the solution. Before samples were prepared, larvae were allowed to reach room temperature, and were given a final wash in room-temperature distilled water. Washing procedure allowed larvae to sediment out by gravity before drawing off the supernatant by vacuum.

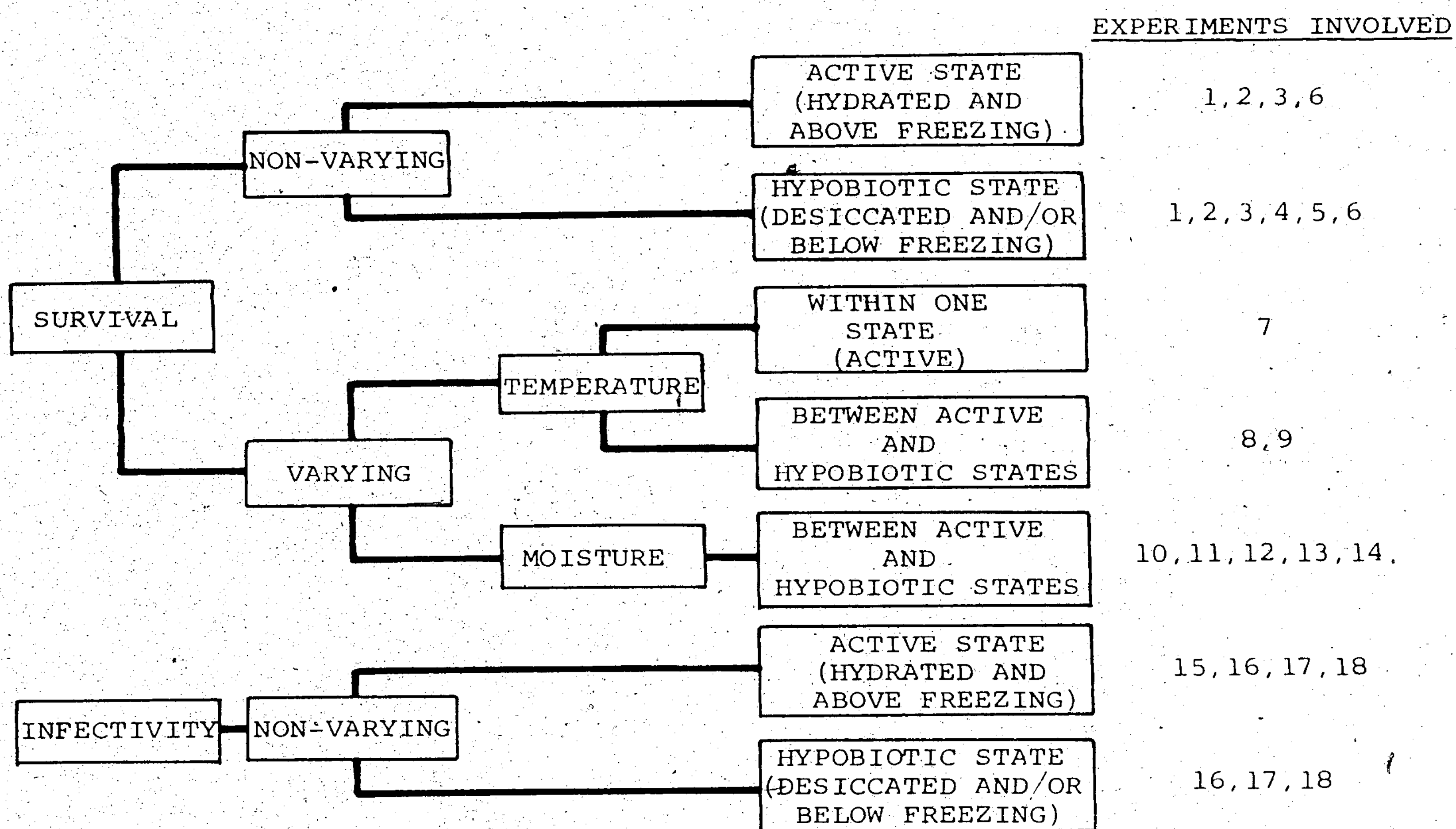
Fluid containing an estimated 100 larvae was pipetted into 60-mm Petri dishes. The water level in all dishes was equilibrated so that evaporation in all samples that were to be desiccated would proceed similarly.

Control samples (i.e. those remaining hydrated) were covered to prevent evaporation. In those to be desiccated, the water was allowed to evaporate under ambient conditions until only a thin film remained. At that time, experimental treatment of all samples, controls included, was started.

E. Experimental Design

The experiments were designed to determine the effects of several types of environmental factors on the first-stage larvae of Parelaphostrongylus. The outline of the experimental program is given in Figure 5. Two criteria, survival and infectivity, were

Figure 5. Flow diagram outlining the organization of the experimental program. Experiment numbers correspond to those used in the text.


EXPERIMENTS INVOLVED
SURVIVAL
NON-VARYING
ACTIVE STATE (HYDRATED AND ABOVE FREEZING)
1,2,3,6
HYPOBIOTIC STATE (DESICCATED AND/OR BELOW FREEZING)
1,2,3,4,5,6
VARYING
TEMPERATURE
WITHIN ONE STATE (ACTIVE)
7
BETWEEN ACTIVE AND HYPOBIOTIC STATES
8,9
MOISTURE
BETWEEN ACTIVE AND HYPOBIOTIC STATES
10,11,12,13,14
INFECTIVITY
NON-VARYING
ACTIVE STATE (HYDRATED AND ABOVE FREEZING)
15,16,17,18
HYPOBIOTIC STATE (DESICCATED AND/OR BELOW FREEZING)
16,17,18

used to assess the influence of experimental treatments on first-stage larvae.

The experimental conditions were of two types: one in which the physical conditions were maintained non-varying, the other in which they were varied. Two types of non-varying conditions were used. In one type the larvae were hydrated and stored at temperatures above freezing, so that they remained in an active state. In the other type the larvae were desiccated and/or frozen, so that their response while in a hypobiotic state (sensu Keilin 1959) could be determined.

Varying conditions comprised either temperature fluctuations with non-varying moisture conditions, or moisture-level fluctuations with non-varying temperature. Temperature fluctuations were of two types: one in which the larvae remained in the active state, the other in which they were repeatedly cycled between the active state and cryobiotic state (hypobiosis induced by low temperature [Keilin 1959]). Moisture fluctuations were only of the type where larvae were cycled between the active state and the anhydrobiotic state (hypobiosis induced by water deficiency [Keilin 1959]).

A total of 18 experiments was performed to determine the survival or infectivity of first-stage larvae of P. odocoilei and P. tenuis. The following sections describe basic procedures for each type of experiment along with variations on the basic procedure that were employed in specific experiments. The experiments were numbered, and the numbers correspond to those used in Appendix I and in Figure 5.

1. Survival Following Non-varying Treatment

The survival of larvae of *P. odocoilei* and *P. tenuis* was determined at several combinations of temperature and moisture. Desiccated samples of larvae were stored at a variety of relative humidities in the humidity control chambers which each contained an appropriate saturated salt solution. Hydrated samples were also stored in the chambers, to control for the effects of possible contaminants in the chambers. Those chambers containing hydrated samples had relative humidities maintained near 100 percent with distilled water; this prevented desiccation of the hydrated samples with their covers removed. Temperature was controlled by placing the humidity control chambers in either coolers or incubators set to desired temperatures.

Where larvae from two sources were being compared, each chamber contained samples from both sources. At various intervals a number of samples was removed to monitor survival. These samples were not returned to the experiment following monitoring. If larvae from two sources were involved, survival of those in the same chamber was monitored simultaneously.

The relative humidities maintained by saturated salt solutions vary slightly with temperature. For the solutions used in these experiments, the relative humidities reported in the literature are given in Table II for a range of temperatures from 2 to 50°C. Further reference to the humidities maintained by each of these

Table II. Percentage relative humidity (% RH) over saturated salt solutions and water. Top rows are values from Winston and Bates (1960); bottom rows are from O'Brien (1948).

	Temperature (°C)											Range in
	2	5	10	15	20	25	30	35	40	45	50	% RH
LiCl	14.5	14.0	13.5	13.0	12.5	12.0	11.5	11.5	11.0	11.0	11.0	16.0- 11.0
	16.0	--	--	15.0	15.0	13.0	13.0	--	--	--	--	
K_2CO_3	47.0	--	47.0	44.0	44.0	43.0	43.5	--	40.0	--	--	50.0- 40.0
	50.0	--	47.0*	44.0*	44.0	43.0	--	--	40.0*	--	--	
NaCl	75.0	75.0	76.5	76.0	76.0	75.5	75.5	75.5	75.0	75.0	74.5	77.5- 74.5
	75.0	--	76.5	77.5	77.0	76.0	75.5	--	75.5	--	74.5	
KCl	88.0	--	88.0	86.5	85.0	85.0	84.5	83.0	82.0	81.0	80.5	88.0- 80.4
	88.0	--	87.5	86.7	86.5	87.0	84.8	--	82.5	--	80.4	
H_2O	--	--	--	--	--	--	--	--	--	--	--	100
	100	--	100	100	100	100	100	--	100	--	100	

* Determination was made at a temperature slightly different from the column head.

solutions were expressed as a single percentage relative humidity (% RH), with an implied range of ± 5% RH. For K_2CO_3, NaCl, and KCl, the values were 45, 75, and 85% RH, respectively. The values of H_2O and LiCl were 95 and 20% RH, respectively, with allowance for the difficulty of maintaining very high or very low humidities. Given the implied ranges of these values, all five solutions provided a gradient from high to low relative humidity, with no overlap of relative humidity regardless of temperature.

a. Experiment Number 1

This experiment, in conjunction with the next one, was designed to determine the survival of P. odocoilei stored under a variety of moisture conditions, at temperatures above freezing. In this experiment, desiccated samples at 20, 45, 75, 85, or 95% RH, and hydrated samples, were stored at 5, 36, or 48°C. Experimentation at the combination of 20% RH and 5°C was not performed, due to the inefficiency of humidity control by LiCl at this low temperature (O'Brien 1948). The survival of larvae was monitored after up to eight time periods in each condition. In most cases, four replicates were examined during monitoring.

b. Experiment Number 2

This experiment was similar in design to Experiment Number 1 (Exp't 1), except that intermediate temperatures of storage (14 and 26°C) were used. The conditions tested comprised hydrated samples, and desiccated samples at 20, 45, 75, or 95% RH.

c. Experiment Number 3

To determine if larvae of the same species but with different sources of origin had similar survival, the following experiment was performed. Samples of Jasper-source and Vancouver Island-source larvae of P. odocoilei were placed in a variety of moisture conditions at 25°C. These were desiccated at 45, 75, or 95% RH, and hydrated. A pair of samples from each source was placed on each shelf in the humidity control chambers, giving a total of six samples per moisture treatment per source of larvae. The survival of larvae from all six samples of each source was monitored after 5 days at 95% RH, 7 days at 75% RH, 19 days at 45% RH, and 1[illegible]ated. These times were chosen to allow for an interm[illegible]l of mortality to occur at each moisture condition, the[illegible]ilitating comparison of survival between sources.

d. [illegible]iment Number 4

[illegible] next two experiments were designed to determine the survival of [illegible]. odocoilei while frozen. The purpose of this experiment was t[illegible]fold; to determine the effect of desiccation prior to freezing on survival while frozen, and again to compare the survival of P. odocoilei larvae from the two sources (Jasper and Vancouver Island), this time following freezing.

Six hydrated and six desiccated (at ambient = 35% RH) samples from each source were frozen at -25°C. Survival was monitored after 100, 190, and 280 days. Between one and three samples per source was monitored at each time period.

e. Experiment Number 5

The long-term survival of P. odocoilei while frozen on feces was estimated in this experiment. Platt (1978) determined larval output per gram of host feces (LPG) from experimentally infected mule deer, using the Baermann technique on subsamples of fecal pellet groups. He then placed the remainders of those pellet groups in a freezer at -25°C. For this experiment, those remainders were thawed, and LPG were determined by the same method that Platt used.

The duration of freezing was 32 months for the pellet groups from one deer (n=4), and 34 months for those from a second deer (n=4). Since the Baermann technique tends to result in recovery of live larvae only, before-and-after LPG could be used to estimate survival of larvae while frozen.

f. Experiment Number 6

This experiment, similar in design to Exp't 3, was to determine if larvae of the two species, P. odocoilei and P. tenuis, had similar survival following storage under non-varying conditions at a temperature above freezing. Larvae of each species were placed in three moisture conditions at 30°C. They comprised hydrated, and desiccated at 45 or 95% RH. Survival of larvae in each moisture condition was monitored after up to four time periods. Four replicates per species and moisture condition were monitored after each time period.

2. Survival Following Varying Temperature Treatment

To determine the effect of repeated temperature changes, such as those that might occur on a daily basis in natural conditions, on larvae of P. odocoilei and P. tenuis, hydrated larvae were repeatedly moved between two temperature levels a number of times.

a. Experiment Number 7

This experiment examined the effects of repeated changes in temperature, during which the larvae remained in the active state. Three hydrated samples of 200 larvae of P. odocoilei were repeatedly moved between 8 and 37°C, for a total of 16 complete cycles of temperature over a four day period. To control for the effects of high temperature per se on survival, a control group of three hydrated samples (200 larvae each) was placed non-varying at 37°C for a similar timespan (89 hours) to that spent by larvae in the experimental samples at the high temperature of the cycle (86 hours at 37°C). If survival in the experimental groups was lower than that observed in the control group, then the excess mortality would be a result of the change of temperature, and not the lethal action of the high temperature alone.

b. Experiment Number 8

The next two experiments involved repeatedly changing the temperature of samples between above-freezing and below-freezing levels, so larvae were repeatedly cycled between the active state and the cryobiotic state.

This experiment was of a preliminary nature, to see if repeated freezing would reduce survival of the larvae. Two hydrated samples were repeatedly frozen at -25°C and thawed to +14°C, for a total of 13 cycles over a four-day period. Following the final thawing, survival of larvae was monitored.

c. Experiment Number 9

This experiment was designed to determine whether or not survival following repeated freezing differed between _P. odocoilei_ and _P. tenuis_. Twelve hydrated samples of each species were subdivided into four groups; two controls and two experimentals (Fig. 6). The non-frozen control remained at +14°C. the frozen control was placed at -25°C for the duration of the experiment, 11 complete days, when the samples were thawed and survival monitored. The two experimental groups were repeatedly forzen and thawed, 10 and 20 times, respectively. Survival was monitored after the final thawing.

3. Survival Following Varying Moisture Treatment

As with temperature, moisture conditions experienced by larvae may change on a daily or other basis. Five experiments were performed to determine the effects of repeated desiccation on larvae of _P. odocoilei_ or _P. tenuis_. A generalization of the experimental design is presented schematically in Figure 7. All experiments involved varying the moisture conditions between hydration and desiccation, so that larvae were repeatedly moved between the active, hydrated state, and the anhydrobiotic state.

Several hydrated samples were divided into control and experimental groups. There were two control groups in each experiment. The samples in the hydrated control were covered

Figure 6. Schematic representation of the design of Experiment Number 9 (Exp't 9) showing temperatures of samples in each of the four groups over time. "S" indicates the time when survival of larvae was monitored.

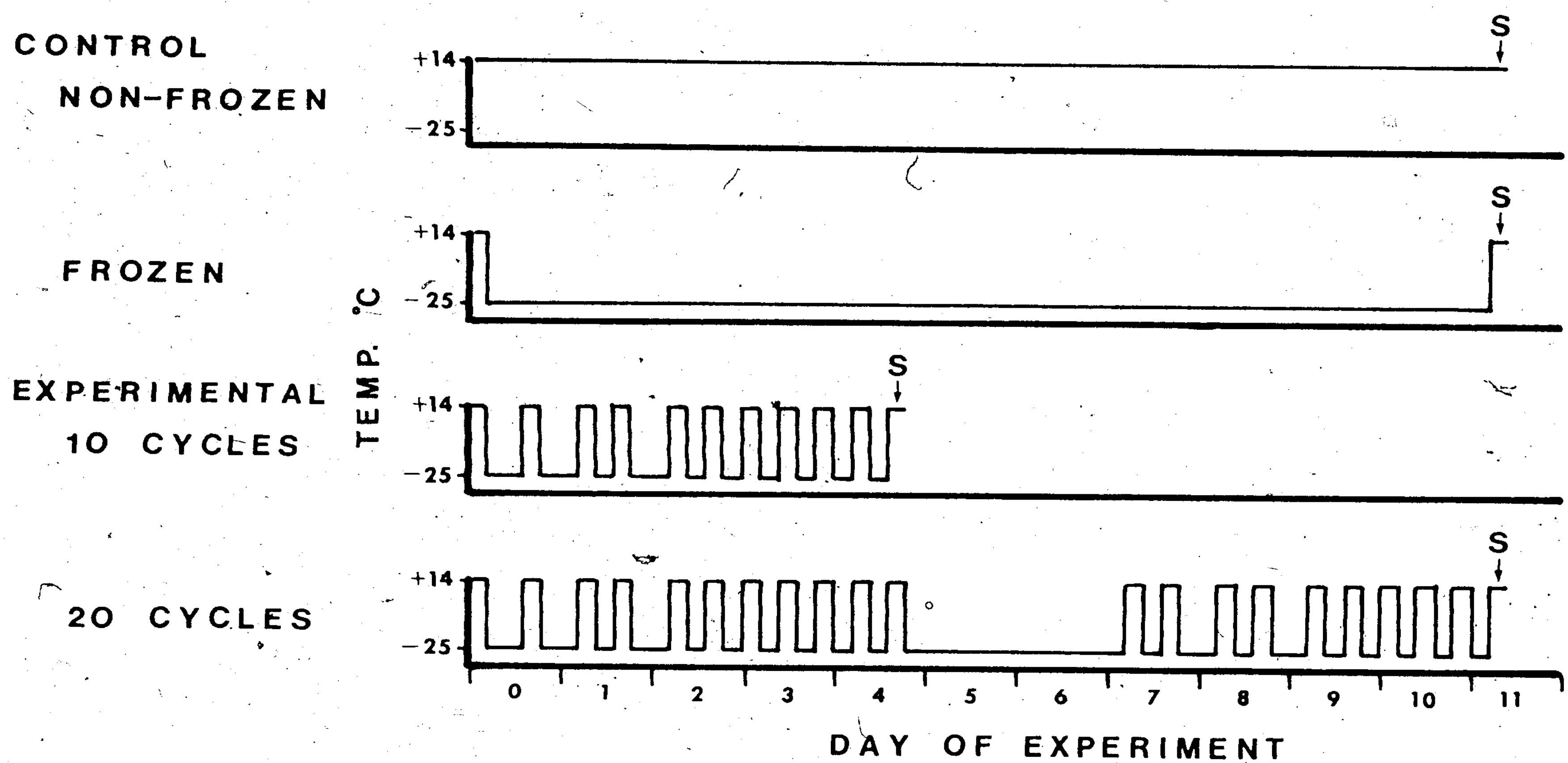
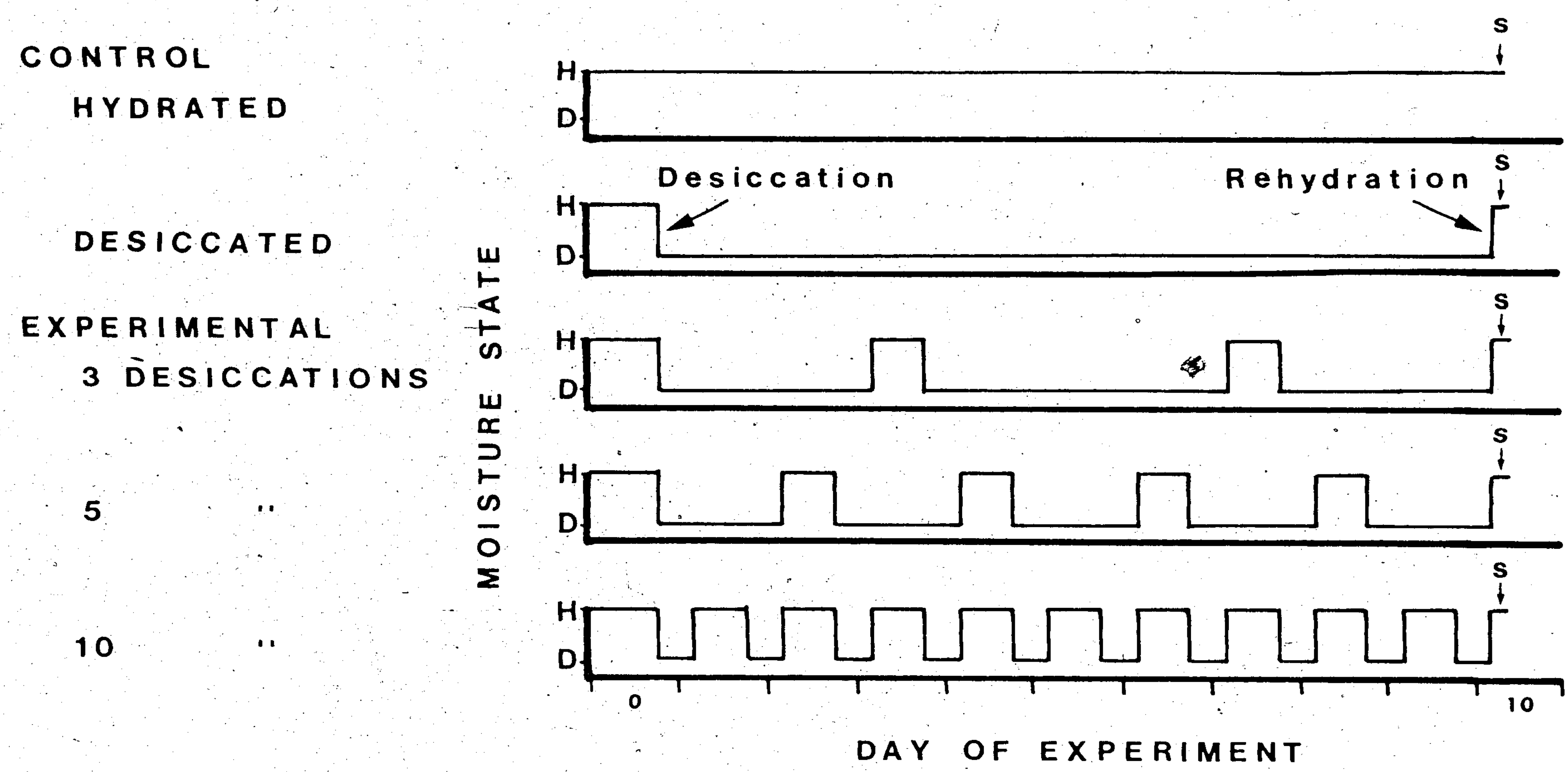

CONTROL
NON-FROZEN
FROZEN
EXPERIMENTAL
10 CYCLES
20 CYCLES
TEMP. °C
+14
-25
S
0
1
2
3
4
5
6
7
8
9
10
11
DAY OF EXPERIMENT

Figure 7. Schematic representation of the basic design of all varying-moisture experiments (Exp'ts 10-14). Moisture states of samples in each group over time are shown (H= hydrated; D= desiccated). "S" indicates time when survival of larvae was monitored.


CONTROL
HYDRATED
DESICCATED
EXPERIMENTAL
3 DESICCATIONS
5 ''
10 ''
MOISTURE STATE
H
D
S
Desiccation
Rehydration
0
10
DAY OF EXPERIMENT

to prevent evaporation of the water (Fig. 7). The samples in the desiccated control, were allowed to desiccate and remain that way for the duration of the experiment. These samples were rehydrated prior to survival monitoring (Fig. 7). The two control groups served to account for the effects of continuous hydration or desiccation on the larvae. The samples in the experimental groups were repeatedly desiccated, with a small amount of distilled water added at various intervals to rehydrate the larvae. The example of experimental design (Fig. 7) has three experimental groups, receiving 3, 5, or 10 desiccations.

All experiments were conducted at ambient relative humidity (30-40% RH). Evaporation of the water in the samples occurred within 24 hours at room temperature (18^oC) or 48 hours when refrigerated (8^oC). All groups, control and experimental, were prepared at the same time, and survival monitored on the same days. The number of replicates in each group varied from one to four.

a. Experiment Number 10

Larvae of P. odocoilei were divided into two control groups and one experimental group, which received five desiccations. The experiment was run over eight days, at room temperature.

b. Experiment Number 11

Larvae of P. odocoilei were divided into two control and three experimental groups. The experimental groups received 3, 5, or 10 desiccations. The experiment was carried out at room temperature over a nine day period.

c. Experiment Number 12

This experiment was identical in design to Exp't 11, but was carried out at a lower temperature (8°C), and over a longer time period (22 days).

d. Experiment Number 13

Larvae of P. odocoilei and P. tenuis were each divided into two control and three experimental groups. Five, six, or nine desiccations were administered to the experimental groups over 15 days, at room temperature.

e. Experiment Number 14

This experiment was similar to Exp't 13, except that the experimental groups received three, six, or nine desiccations, over 12 days.

4. Infectivity Trials

Infectivity of first-stage larvae was determined by exposing a known number of surviving larvae to the snail Triodopsis multilineata. After a period of one month, larvae were recovered from the snails by artificial pepsin digestion (0.6 g pepsin powder, 0.7 ml HCl per 100 ml distilled water; incubated at 37°C) of the snail tissues.

Larvae were exposed to snails in 10-cm diameter glass dishes with two discs of filter paper lining the bottom. A suspension of larvae in water, with numbers of live larvae estimated by standard survival monitoring on a subsample of the suspension, was pipetted onto the filter paper. A group of snails was then placed on the filter paper and the dish covered. The snails were allowed to crawl on the filter paper for several hours. At intervals of about one-half hour, any snails on the sides or cover of the dish were placed back on the filter paper.

In Exp't 15, numbers of larvae and snails varied between exposure dishes, since this experiment was designed to determine the influence of different density conditions of exposure on final larval recoveries. In Exp'ts 16-18 the same number of larvae and snails was used in all dishes; the variable here was the prior treatment of the larvae. Prior to being exposed to snails, larvae were allowed to revive, if necessary, from the treatments they were administered. For example, if the experimental treatment had involved desiccation of the larvae, they would be placed in water for several hours to revive.

a. Experiment Number 15

To determine the effect of exposure conditions on subsequent recovery of second- and third-stage larvae, P. odocoilei was exposed to snails under four different densities of snails and first-stage larvae: 1) many larvae/many snails, 2) many larvae/few snails, 3) few larvae/many snails, and 4) few larvae/few snails. "Many" and "few" larvae were 4710 and 1570 total, or 60 and 20 larvae per cm^2 on the filter paper, respectively. For snails, "many" and "few" were 15 and 5 per dish, or 0.192 and 0.064 snails per cm^2 on the filter paper, respectively. Larvae and snails were chosen at random from common sources prior to allocation. Exposure was for nine hours, and digestion of the snails was after four weeks.

b. Experiment Number 16

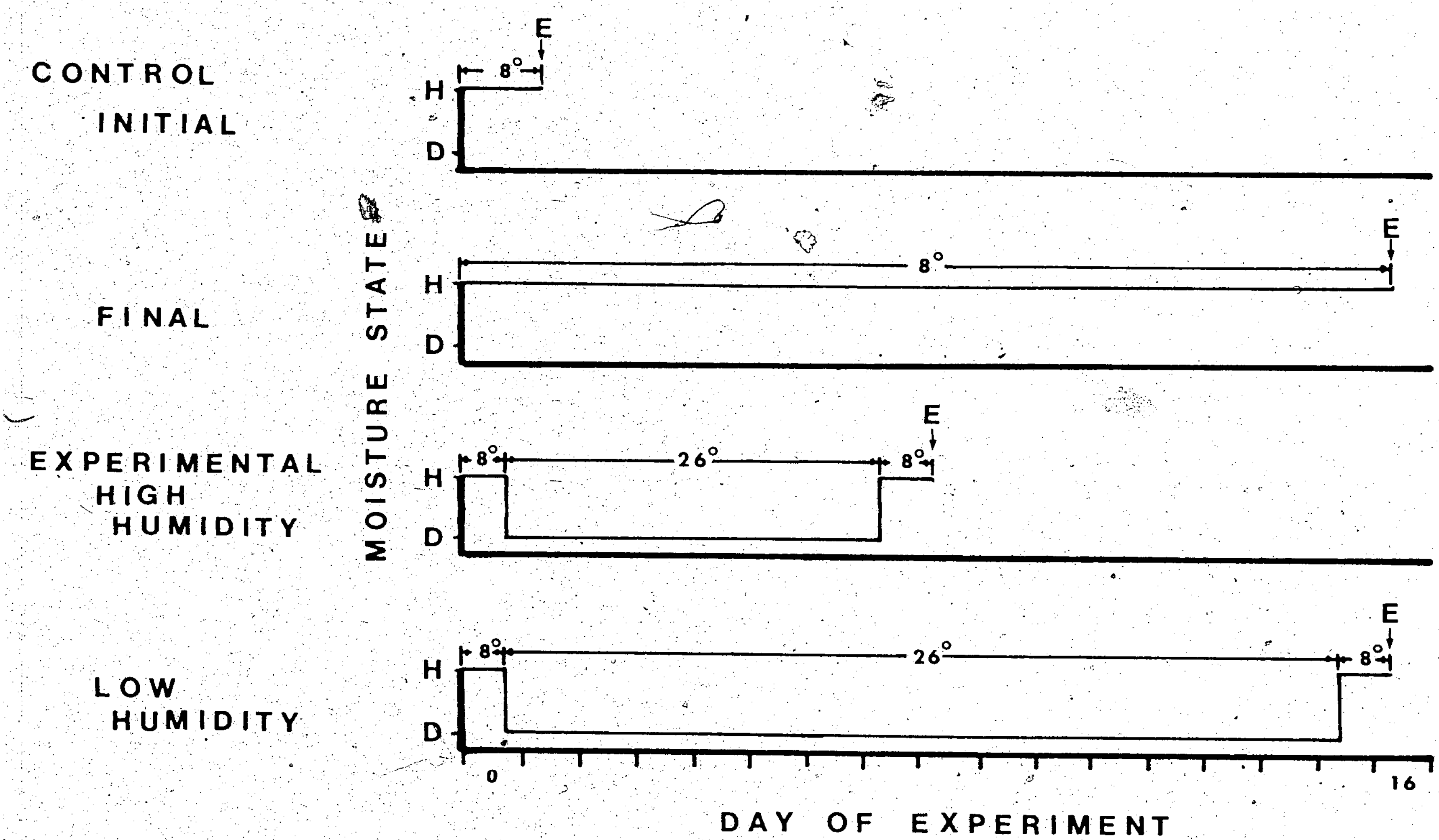
This was a preliminary experiment to determine the influence of temperature and moisture stress on infectivity of first-stage larvae. A large quantity of P. odocoilei was washed repeatedly in water at 8°C, and subdivided into four groups. The summarization of experimental design (Fig. 8) illustrates the sequence of events for these four groups regarding: changes in moisture state of the larvae between hydrated and desiccated, changes in temperatures, and times of exposure of the surviving larvae to snails.

One group of larvae was designated as initial control, and was exposed to snails at the start of the experiment to determine the initial infectivity of the larvae.

Two of the remaining groups were designated as experimental groups. One was allowed to desiccate and was then placed at 95% RH and 26°C for six days. This was the high-humidity experimental group. The second group, a low-humidity experimental, was allowed to desiccate, and was then placed at 45% RH and 26°C for 14 days. Following desiccation, the larvae were rehydrated and placed over-night at 8°C to revive. They were then exposed to snails. A shorter treatment time was chosen at the high humidity based on other experimental results which indicated that few or no larvae would survive 95% RH and 26°C for 14 days.

The fourth group of larvae was designated as final control, and was stored in water at 8°C for the duration of the experiment. It was designed to account for any effects that passage of time since the start of the experiment would have on infectivity of the

Figure 8. Schematic representation of the design of Exp't 16. Moisture states of samples in each group over time are shown (H= hydrated; D= desiccated). Temperature of samples (degrees C) is shown above each graph. "E" indicates time when larvae were exposed to snails.


CONTROL
INITIAL
FINAL
EXPERIMENTAL
HIGH
HUMIDITY
LOW
HUMIDITY
MOISTURE STATE
H
D
E
8°
26°
0
16
DAY OF EXPERIMENT

original [illegible] larvae. Any reduction in infectivity of the experimental groups below that of the final control would be due to [illegible] experimental treatment, and not loss of infectivity over time.

The exposure of larvae from each of the four groups was as follows: two units of 10 000 surviving larvae were selected from each group, and each unit was exposed to 10 snails for 6.5 hours. Snails were digested four weeks after exposure to the first-stage larvae.

c. Experiment Number 17

This experiment was designed to determine the influence of moisture conditions, temperature, and length of treatment on the infectivity of first-stage larvae of P. odocoilei. A single stock of larvae was subdivided into 12 groups, each to receive a different treatment. Four groups of larvae received one of three moisture treatments: hydrated, desiccated at 45% RH, or desiccated at 75% RH. Within each moisture condition two groups were treated at 20°C, two at 26°C. At each of these six temperature-moisture combinations, one group was treated for 2 days, the other for 10 days. The choice of conditions was such that even under the harshest of them (95% RH at 26°C for 10 days) there would be sufficient numbers of surviving larvae to expose to snails.

Following treatment, 10 000 surviving larvae from each group were exposed to 10 snails for six hours. Snails were digested five weeks post-exposure. For logistic reasons, all treatments were not started simultaneously, but were staggered so that exposure took place in three-group units for each of four successive weeks.

d. Experiment Number 18

The objectives of this experiment were twofold: first, to examine the effects of freezing on infectivity of first-stage larvae of P. odocoilei and P. tenuis; second, to determine whether infectivity of the two species differed following freezing.

Two sources of larvae of each species were used: one fresh on feces; the other collected from the same animals one month previously, and stored on the feces since then at -25°C. All fecal samples, fresh or frozen, were brought to room temperature on the same day, and larvae recovered from them in a Baermann apparatus. The recovered larvae were washed five times over the next week; their temperature during this period was kept at 8°C.

The exposure of larvae from each of the four groups was as follows. Two units of 5000 surviving larvae were selected from each group, and each unit exposed to five snails for 4 hours. At 28 days post-exposure, the snails exposed to one unit of larvae from each of the four groups were digested. The remaining snails were digested 38 days post-exposure.

F. Data Analysis

Data were analyzed statistically using procedures outlined by Snedecor and Cochran (1967) and Sokal and Rohlf (1969). Regression analyses were performed on an Amdahl 470 computer using APL, with programs obtained from the public library of the University of Alberta Computing Center.

Pairs of means were compared using t-tests. Where critical values of t had to be calculated, these are given along with their probability values. Groups of means were compared by analysis of variance (anova). Heteroscedasticity was reduced to within acceptable limits by appropriate transformations. Angular transformation was used in the case of percentage data, while logarithmic transformation was used for count data. Reduction of heteroscedasticity was confirmed using either the F-max test, or Bartlett's test. Departures from normality were tested for using the Kolgomorov-Smirnov test.

Where overall statistical analysis of a given set of data could not be done due to violation of necessary assumptions, but where analyses on subsets of the data could be validly performed, the technique of combining probability values was used to test the hypothesis (Sokal and Rohlf 1969). The proviso of this test was that the separate tests, from which probability values were obtained, all tested the same scientific, though not necessarily statistical, hypothesis.

Means, standard errors, and confidence limits on all percentage data were calculated following angular transformation, but are reported here following conversion back to the percentage scale. The same information on count data was calculated, and is reported, in the original scale.

Any departure from the procedures outlined here will be noted when they occur.

III. RESULTS

A. Survival Under Non-varying Conditions

The survival of first-stage larvae of Parelaphostrongylus odocoilei under non-varying conditions at temperatures above freezing (Exp'ts 1,2) is shown in Figure 9 (a more complete presentation of the data, including confidence limits for the mean percentages, is in Appendix II). Generally, survival declined in a linear manner.

The maximum length of survival could only be accurately determined by direct inspection of the data in a few cases, such as for the samples stored at 36 or 48°C. In other cases, either the time period between observations was too great to determine when the last larvae died (e.g. 75% RH at 14°C), or insufficient numbers of samples were available to monitor survival for the entire lifespan of all the larvae (e.g. 45% RH at 5°C). In the latter two cases maximum length of survival was estimated by regression analysis. Linear regression was used to estimate the x- intercept (maximum days survival), unless a significant x^2 component was detected, in which case polynomial regression was used. The maximum survival times of the larvae in all conditions, as determined by the appropriate one of the above methods, are given in Table III.

At all moisture conditions survival was inversely related to temperature of storage. Survival of desiccated larvae was inversely related to the relative humidity of storage, regardless

Figure 9. Survival of first-stage larvae of P. odocoilei at various temperature and moisture conditions (Exp'ts 1,2).

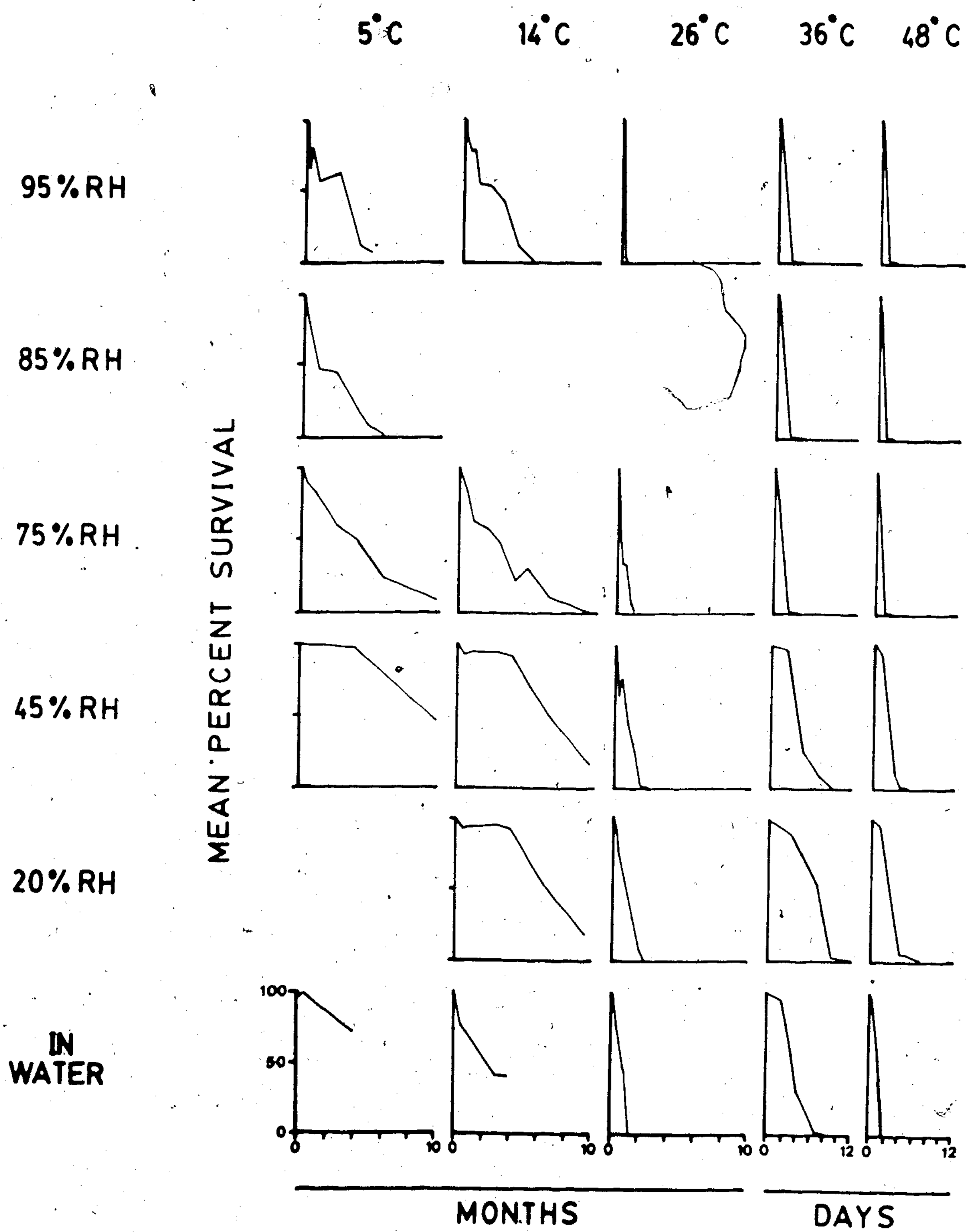

5°C
14°C
26°C
36°C
48°C
95% RH
85% RH
75% RH
45% RH
20% RH
IN WATER
MEAN PERCENT SURVIVAL
100
50
0
0
10
12
MONTHS
DAYS

Table III. Maximum days survival of first-stage larvae of *Parelaphostrongylus odocoilei* under various temperature and moisture conditions in Experiment Numbers 1 and 2 (Exp'ts 1,2). Where maximum survival could not be determined directly from the data, it was estimated by either linear or polynomial regression.

Moisture Conditions	Temperature (^{o}C)				
	5	14	26	36	48
Desiccated:					
95% RH	198	175	13	2.0	1.5
85% RH	196	--	--	2.5	1.5
75% RH	380	277	44	2.5	1.0
45% RH	406	332	68	8.0	4.5
20% RH	--	347	68	11.0	7.5
Hydrated	413	269	51	7.0	1.3

of temperature. The survival of larvae desiccated at the lower relative humidities was similar to that of hydrated larvae when at low temperatures, but was lengthier than hydrated larvae when at high temperatures.

Thus P. odocoilei can survive at high temperatures for up to one week, and at low temperatures from six months to over one year, depending on moisture conditions.

The survival of P. odocoilei originating from Vancouver Island did not differ from survival of P. odocoilei originating from Jasper, either at above-freezing temperatures (Exp't 3; Fig. 10) or below-freezing temperatures (Exp't 4; Fig.11). When survival of the larvae was correlated with source of larvae, shelf position in the humidity control chamber, and moisture treatment above freezing (Exp't 3) by anova, there was no significant effect of shelf position or larval source (Table IV). However, the different moisture conditions did have a significant effect on survival of the larvae. When mean survival of P. odocoilei larvae from the two sources was correlated with source of larvae, moisture condition prior to freezing, and length of freezing (Exp't 4) by anova, again there was no significant difference of survival between sources of larvae. However, there was a significant decrease in survival over time, dependent upon the moisture state of the larvae prior to freezing, as indicated by the significant interaction between moisture and time (Table V).

First-stage larvae of P. odocoilei can withstand freezing on feces for much longer than the 32 and 34 month periods used

Figure 10. Survival of first-stage larvae of *P. odocoilei* from two sources, at 25^{o}C and various moisture conditions (Exp't 3). Bars represent mean $\pm$ SE.

a. Hydrated for 12 days.

b. Desiccated for 19 days at 45% RH.

c. Desiccated for 7 days at 75% RH.

d. Desiccated for 5 days at 95% RH.

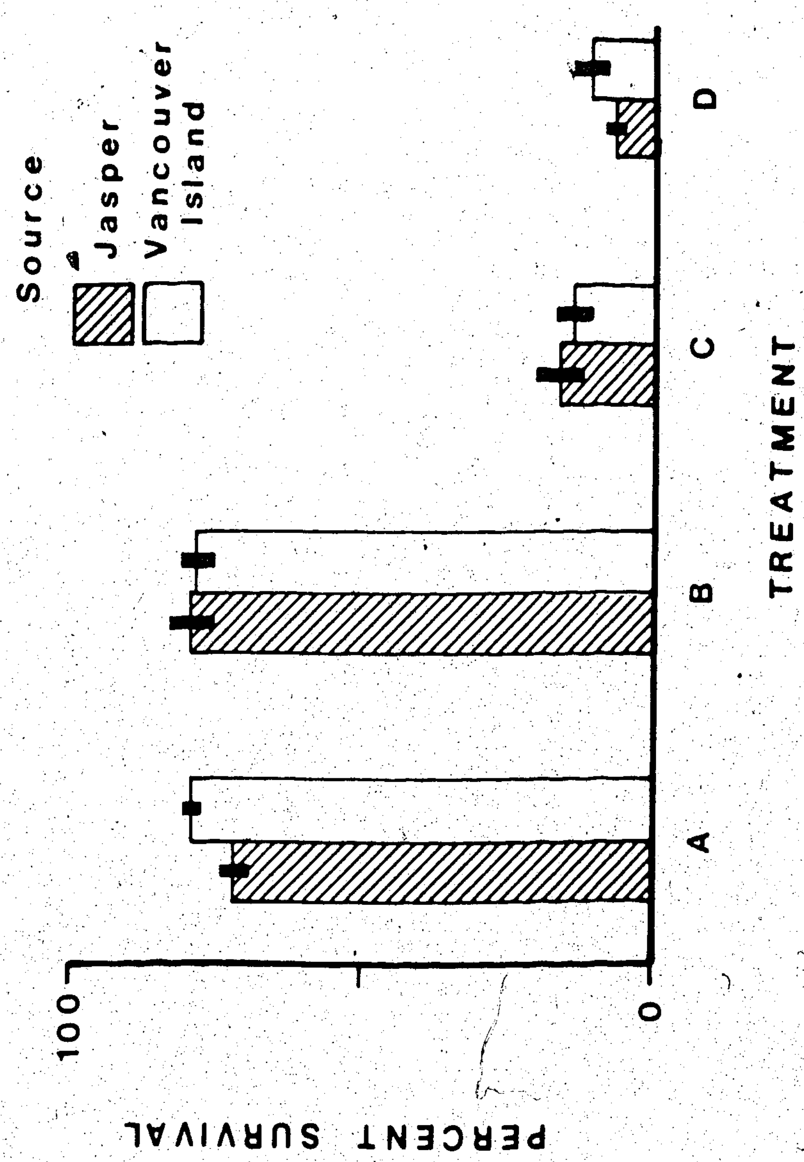

Source
Jasper
Vancouver Island
PERCENT SURVIVAL
100
0
A
B
C
D
TREATMENT

Figure 11. Mean percent survival of first-stage larvae of P. odocoilei from two sources, following freezing (Exp't 4). Open circles and dashed lines represent Jasper source larvae; closed circles and solid lines represent Vancouver Island source. Group "H" samples were frozen while hydrated; group "D" were frozen while desiccated.

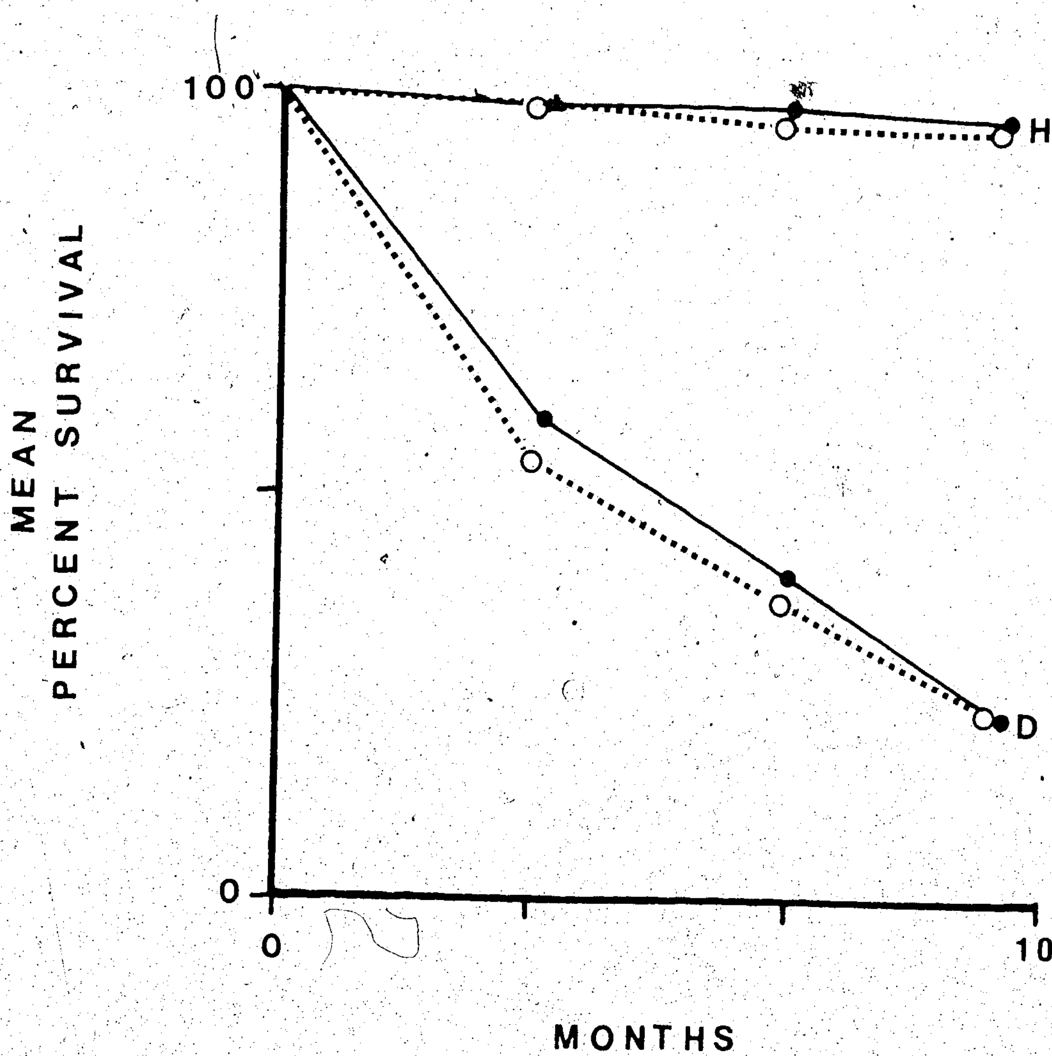

100
0
MEAN
PERCENT SURVIVAL
H
D
0
10
MONTHS

Table IV. Anova table for the survival of first-stage larvae of P. odocoilei at 25°C, by source of larvae, position in humidity control chamber, and treatment (Exp't 3). Data were transformed by $\arcsin\sqrt{p}$.

Source of Variation	DF	Sum of Squares	Mean Square	F	P
Main Effects					
Source	1	33.5	33.5	1.01	ns
Position	2	38.1	19.0	<1.00	ns
Treatment	3	20556.9	6852.3	199.56	<0.001
First-order Interactions					
Source x Position	2	61.5	30.8	<1.00	ns
Source x Treatment	3	108.0	36.0	1.09	ns
Position x Treatment	6	130.5	21.8	<1.00	ns
Second-order Interaction					
Source x Position x Treatment	6	95.5	15.9	<1.00	ns
Error	24	791.6	33.0		
Total	47	21815.6			

Table V. Anova table for the survival of first-stage larvae of P. *odocoilei* at -25°C, by source of larvae, moisture condition of freezing, and length of freezing (Exp't 4). Data were transformed by arcsin $\sqrt{p}$.

Source of Variation	DF	Sum of Squares	Mean Square	F	P
Main Effects					
Source	1	5.9	5.9	2.08	ns
Moisture	1	5104.5	5104.5	1797.10	<0.001
Length	2	270.2	135.1	47.56	<0.05
First-order Interaction					
Source x Moisture	1	0.1	0.1	<1.00	ns
Source x Length	2	1.5	0.8	<1.00	ns
Moisture x Length	2	164.4	82.2	28.93	<0.05
Second-order Interaction (Error)					
Source x Moisture x Length	2	5.7	2.8		
Total	11	5552.1			

in Exp't 5. Survival after that time period was still close to 75 percent (Table VI). Median, rather than mean, survival was used due to the asymmetric distribution of survival values from the four samples from each deer. The survival estimate of 75 percent for survival on feces is only slightly less than the 86 percent which is predicted from the data of Exp't 4 ($\hat{Y}$= 98.8 - 0.01319X), for survival of Jasper-source P. odocoilei which were frozen while hydrated.

The survival of first-stage larvae of the two species, P. odocoilei and Parelaphostrongylus tenuis, under non-varying conditions at a temperature above freezing (Exp't 6) is shown in Figure 12. Survival could not be analyzed by anova as was done for the results of the similarly designed Exp't 3 (using P. odocoilei from two different sources) due to overall heteroscedasticity which could not be reduced to an acceptable level. However, t- tests on the difference in survival between species could be performed for the individual treatment groups. There was a significant difference between species in two cases: Class "B" ($t=4.920$, $df=6$, $p<0.01$), and "D" ($t=3.506$, $df=6$, $p<0.05$). Probability values were suggestively low in several other classes (Class "A", $t=2.057$, $df=2$, $p<0.20$; "E", $t=2.287$, $df=6$, $p<0.10$; and "H", $t=2.263$, $df=6$, $p<0.10$).

Th[illegible]lity values from the t- tests on the six treatment g[illegible]ot significantly different were combined, the [illegible] that survival of P. odocoilei = P. tenuis [illegible]ing statistic, $-2\Sigma \ln P$ (distributed as X^2)

Table VI. Survival of first-stage larvae of *P. odocoilei*, frozen on fecal pellets at -25^{o}C (Exp't 5). Initial and final larvae-per-gram (LPG) determinations are from subsamples of the same pellet group.

Source animal	Date feces collected (Day-Month-Year)	Initial LPG	Time frozen (months)	Final LPG	Percent survival
Mule Deer No. 1	4-II-76	112	32.5	99	88
	6-II-76	533	32.5	369	69
	16-II-76	7075	32.0	3462	48
	19-II-76	10790	32.0	8990	83
			32.2*		76*
Mule Deer No. 3	12-XII-75	4271	34.5	7143	167
	13-XII-75	8725	34.5	5925	68
	14-XII-75	9079	34.5	7050	78
	19-XII-75	7217	34.5	4385	61
			34.5*		73*

* median

Figure 12. Survival of first-stage larvae of _P. odocoilei_ (P.o.) and _P. tenuis_ (P.t.) at 30°C and various moisture conditions (Exp't 6). Bars represent mean ± SE.

a. Hydrated for 20 days.

b. Desiccated for 3 days at 45% RH.

c. Desiccated for 9 days at 45% RH.

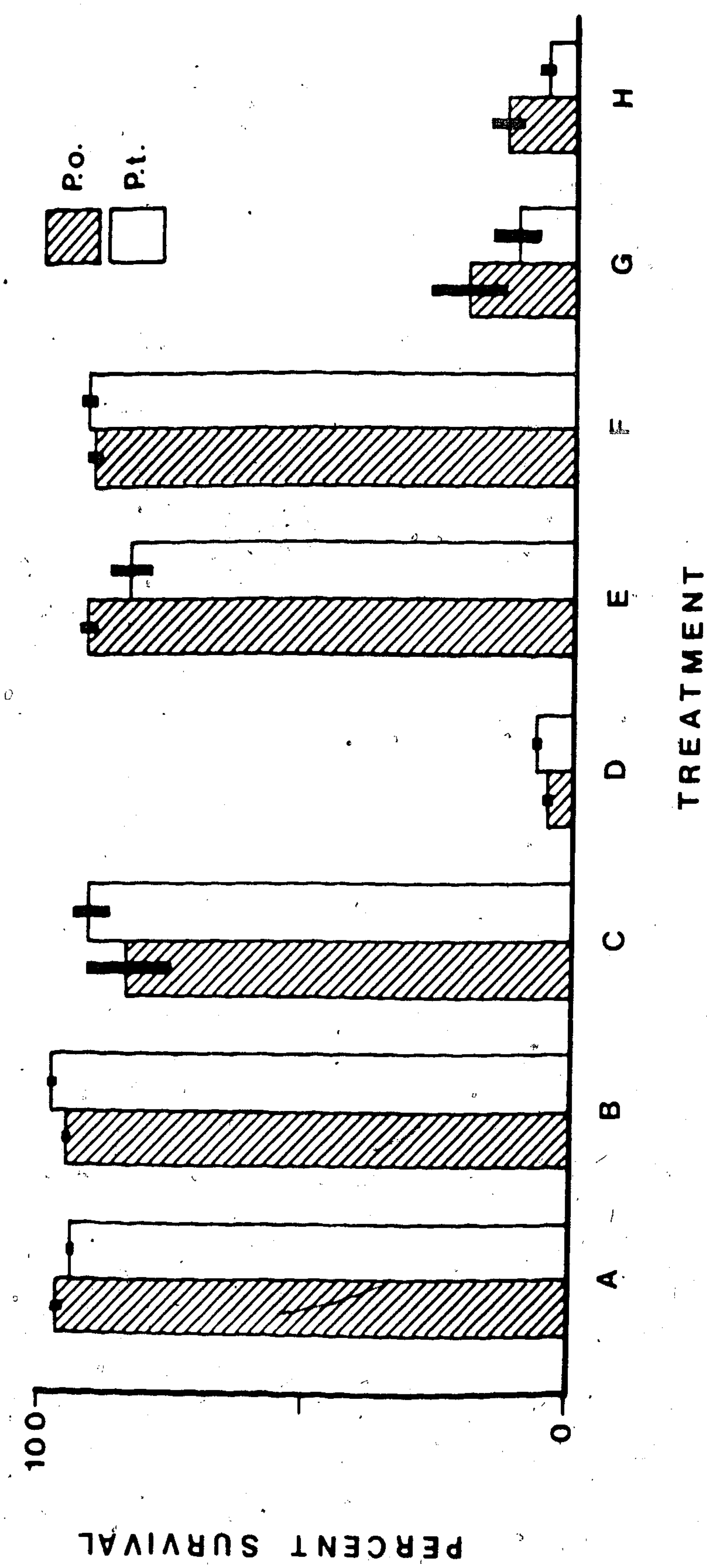
d. Desiccated for 20 days at 45% RH.

e. Desiccated for 2 days at 95% RH.

f. Desiccated for 4 days at 95% RH.

g. Desiccated for 6 days at 95% RH.

h. Desiccated for 8 days at 95% RH.



P.o.
P.t.
100
0
PERCENT SURVIVAL
A
B
C
D
E
F
G
H
TREATMENT

was 20.12, df=12, $p<0.05$. Of the two species in this experiment, P. tenuis generally had higher survival at low relative humidity (Fig. 12b-d), while P. odocoilei generally had higher survival at high relative humidity (Fig. 12e-h).

Three experiments in this section (Exp'ts 3,4,6) compared the survival of larvae of different types following storage under non-varying conditions. In two of them (Exp'ts 3,4) the different types of larvae used were of the same species, but from different geographical regions, and the null hypothesis that their survival under non-varying conditions is equal was accepted. In the other (Exp't 6) the different types of larvae were of different species, and the null hypothesis that their survival is equal was rejected.

B. Survival Under Varying Temperature Conditions

The survival of first-stage larvae of P. odocoilei was not affected by the repeated cycling of temperature between 8 and 37°C (Exp't 7). Mean percent survival with 95% confidence limits was 90 (88- 93) after 16 complete cycles between the two temperatures, compared with 87 (74-97) in the control. The control had spent almost as much total time at 37°C as the experimental group but was not cycled. The difference in survival between control and experimental groups was not significant ($t=1.11$, df=4, $p<0.40$).

The cycling of larvae of P. odocoilei between above- and below-freezing temperatures did reduce their survival. In the preliminary experiment (Exp't 8), mean percent survival was reduced to 82 percent (95% confidence limits: 45 - 93% survival) after 13 complete freeze-thaw cycles.

The reduction in survival following repeated cycles of freezing was confirmed for P. odocoilei, and also observed for P. tenuis, in Exp't 9 (Fig. 13). Survival of both species decreased in relation to the number of cycles. There was no significant difference in survival between the non-frozen controls of the two species ($t=1.027$, $df=4$, $p<0.40$). The survival of the three groups which underwent freezing (the control a single time, and the two experimental groups 10 or 20 times) was correlated with species and number of freezings by anova (Table VII). The effects of both species and number of freezings were significant, with no interaction. P. tenuis not only had lower survival than P. odocoilei in the two experimental groups (10 freezings, $t=2.979$, $df=4$, $p<0.05$; 20 freezings, $t=5.560$, $df=4$, $p<0.01$), but also in the frozen control, which had just a single freezing ($t=3.463$, $df=4$, $p<0.05$).

In summary, temperature variation not involving a change of state of the hydrated first-stage larvae of P. odocoilei did not affect their survival. However, when the temperature variations involved a change of state for the larvae (from active to cryobiotic), survival of both P. odocoilei and P. tenuis was reduced in relation to the number of times the change occurred. P. tenuis was slightly more susceptible than P. odocoilei to repeated freezing.

Figure 13. Survival of first-stage larvae of _P. odocoilei_ (P.o.) and _P. tenuis_ (P.t.) following repeated freezing (Exp't 9). Bars represent mean ± SE.

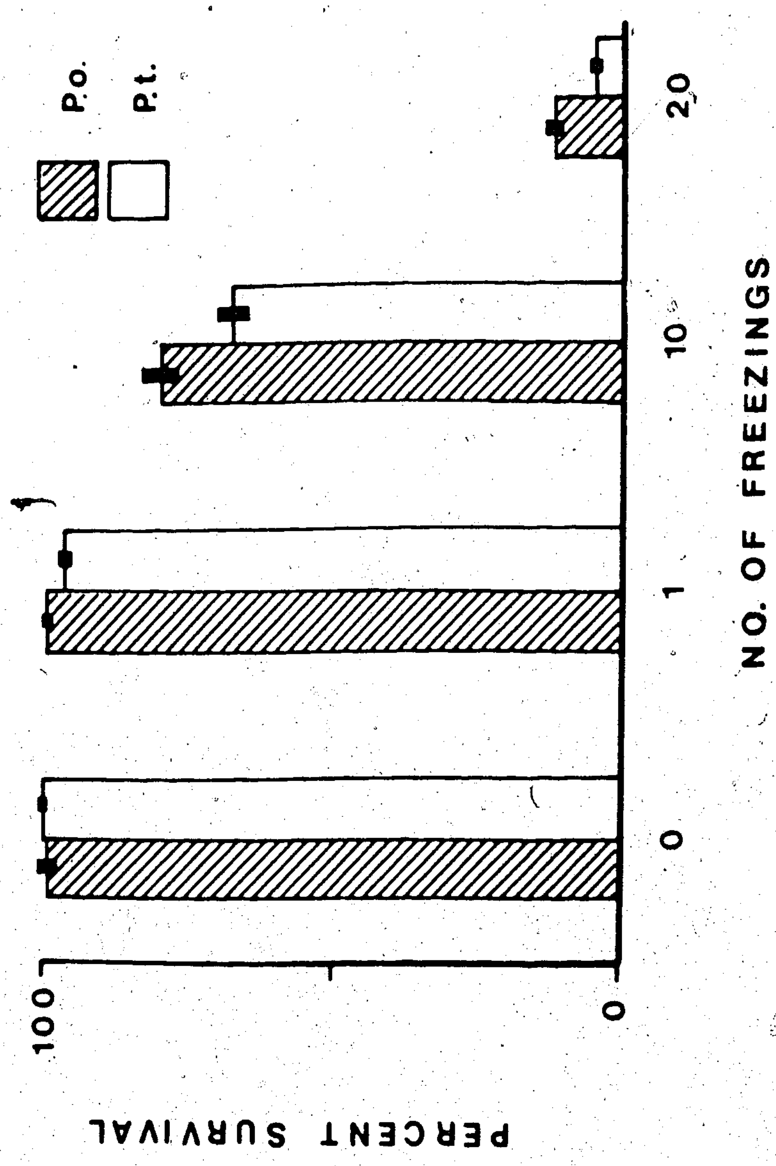

P.o.
P.t.
PERCENT SURVIVAL
100
0
NO. OF FREEZINGS
0
1
10
20

Table VII. Anova table for the survival of first-stage larvae of _P. odocoilei_ and _Parelaphostrongylus tenuis_ following repeated freezing, by species and number of freezings (Exp't 9). Data were transformed by arcsin $\sqrt{p}$.

Source of Variation	DF	Sum of Squares	Mean Square	F	P
Main Effects					
Species	1	221.9	221.9	46.72	<0.001
Number	2	12966.3	6483.2	1364.90	<0.001
First-order Interaction					
Species x Number	2	12.9	6.4	1.37	ns
Error	12	57.0	4.8		
Total	17	13258.1			

C. Survival Under Varying Moisture Conditions

The results obtained in the four repeated-desiccation experiments which involved P. odocoilei at 18°C (Exp'ts 10,11,13,14) were in close agreement (Fig. 14). In only one desiccation class ("3-5") was there a significant difference in survival between experiments ($F=54.8$, $df=3,11$, $p<0.01$). The results from the two experiments involving repeated desiccation of P. tenuis at 18°C (Exp'ts 13,14) were also in close agreement (Fig. 15), with only one desiccation class ("9-10") having a difference in survival between experiments ($t=3.613$, $df=6$, $p<0.05$). Because of the general similarity of results from the different experiments, they were pooled to allow comparison between species (Fig. 16).

For each species, survival of the two control groups (hydrated and desiccated) was high, although survival in the desiccated controls, which had undergone one desiccation, was about 3 percent lower than that of the corresponding hydrated controls. The survival in all experimental groups was lower than in the corresponding controls, and decreased in relation to the number of desiccations. P. tenuis had significantly higher survival than P. odocoilei in the hydrated control ($t=2.697$, $df=19$, $p<0.05$) and in the three experimental groups (Class "3-5", $t=2.891$, $df=20$, $t_{[01]}$ crit.$= 2.702$; "6-8", $t=7.614$, $df=14$, $p<0.01$; "9-10", $t=2.996$, $df=18$, $p<0.01$).

A lowered temperature (Exp't 12) reduced, but did not eliminate, the effect of repeated desiccation on survival of

Figure 14. Survival of first-stage larvae of P. odocoilei following repeated desiccations at 18°C (Exp'ts 10, 11,13,14). Bars represent mean ± SE.

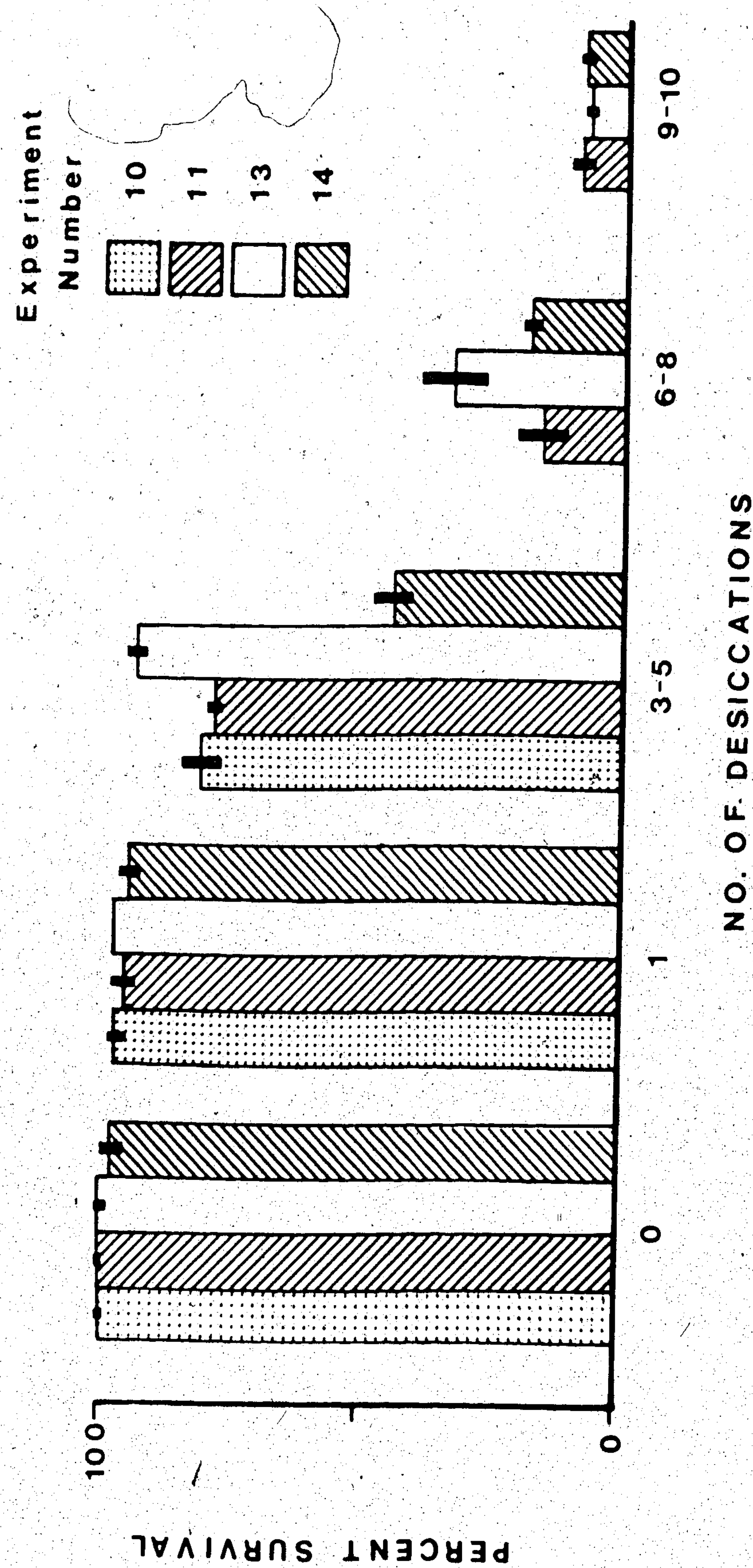

Experiment Number
10
11
13
14
100
0
PERCENT SURVIVAL
0
1
3-5
6-8
9-10
NO. OF DESICCATIONS

Figure 15. Survival of first-stage larvae of _P. tenuis_ following repeated desiccations at 18°C (Exp'ts 13,14). Bars represent mean ± SE.

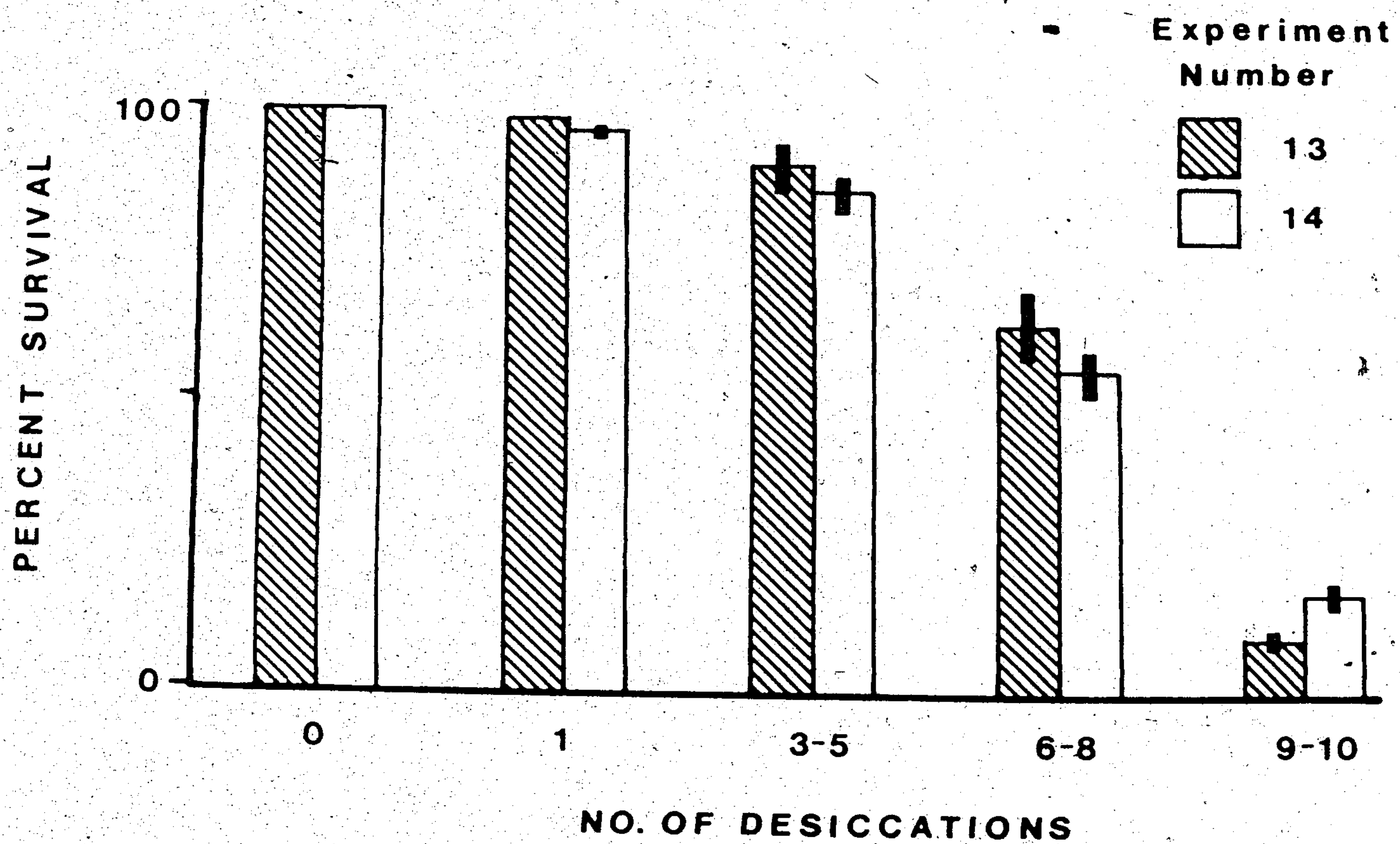
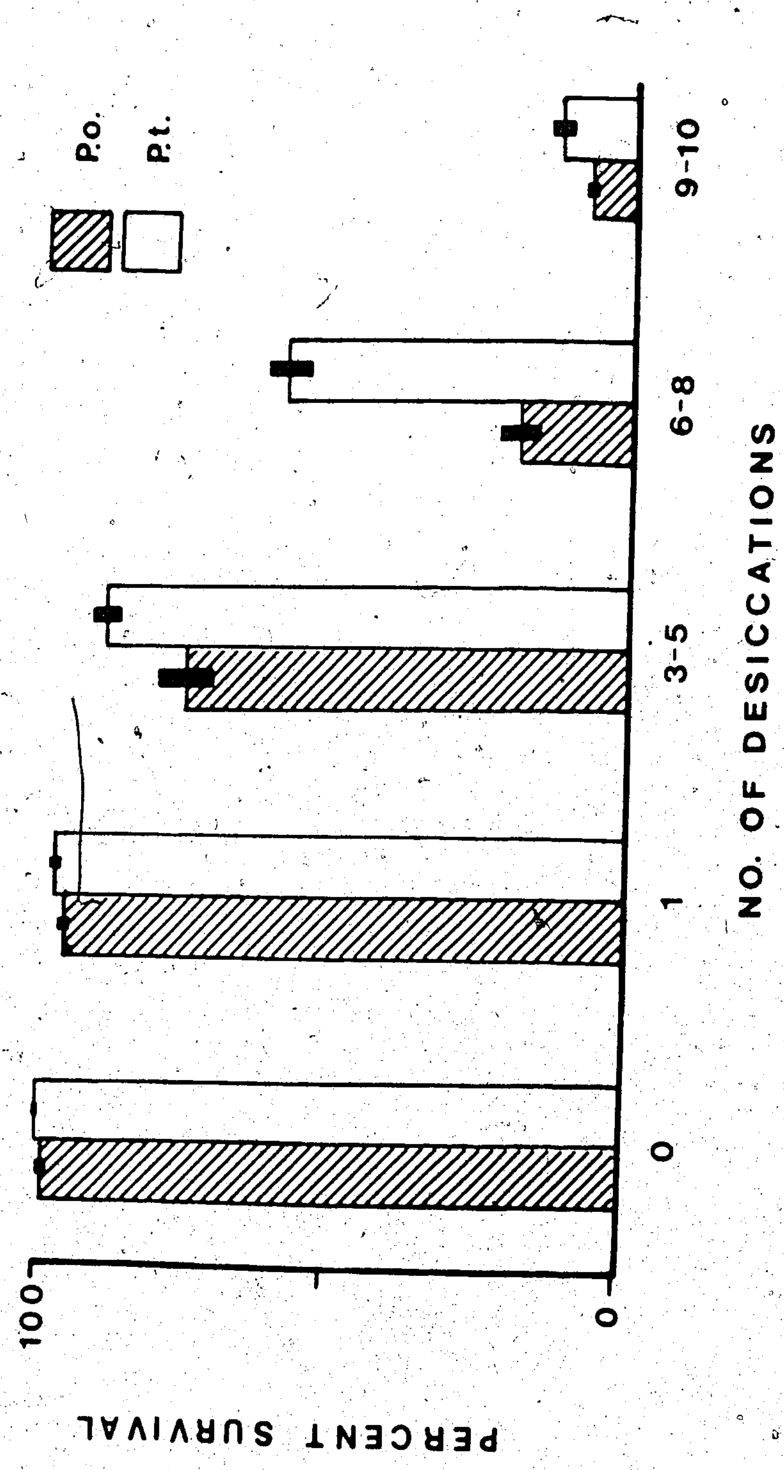

Experiment Number
13
14
100
0
PERCENT SURVIVAL
0
1
3-5
6-8
9-10
NO. OF DESICCATIONS

Figure 16. Survival of first-stage larvae of P. odocoilei (P.o.) and P. tenuis (P.t.) following repeated desiccations at $18^{o}C$ (pooled data from Exp'ts 10,11,13,14). Bars represent mean $\pm$ SE.



P.o.
P.t.
PERCENT SURVIVAL
100
0
0
1
3-5
6-8
9-10
NO. OF DESICCATIONS

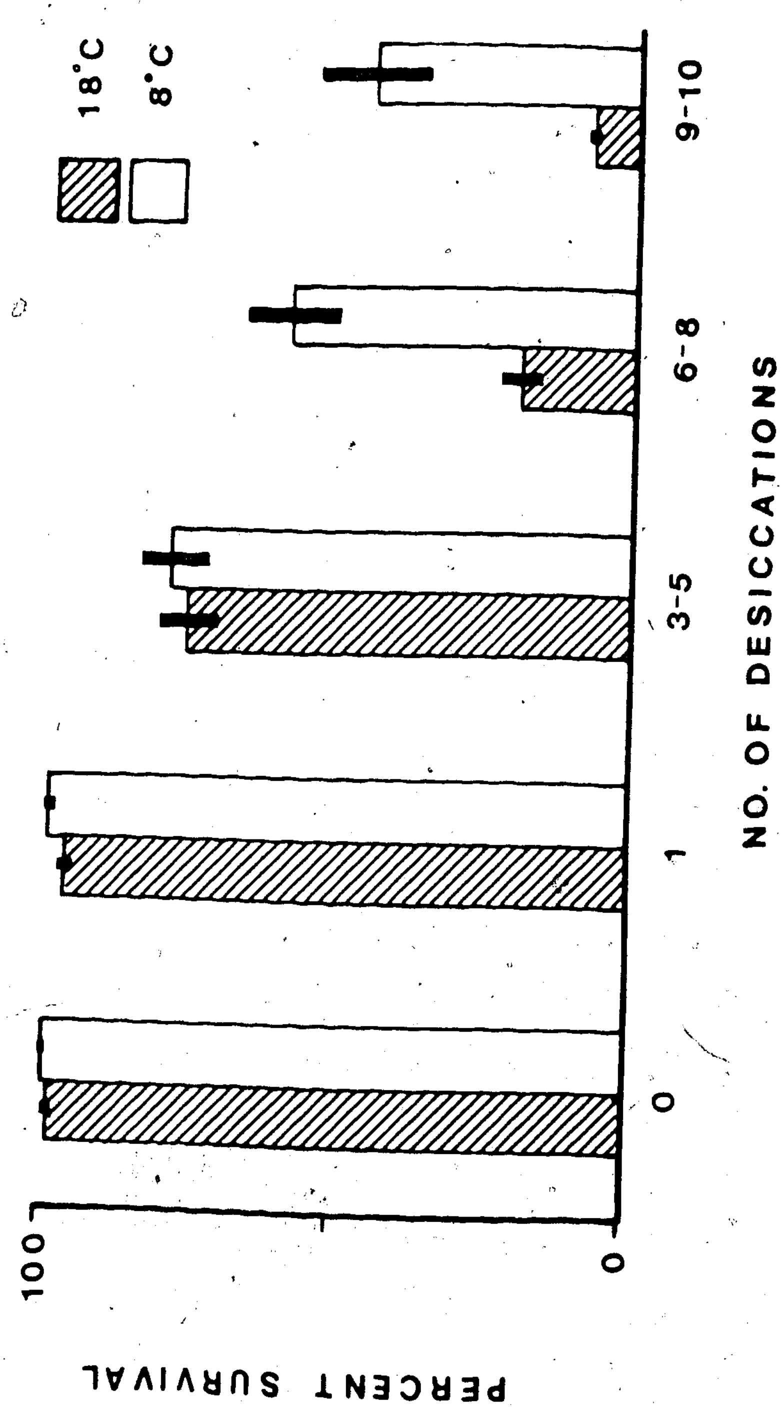
P. odocoilei larvae (Fig. 17). At both temperatures survival decreased in relation to the number of desiccations. At five or fewer desiccations, survival at 8°C was only a few percentage points higher than at 18°C, but after 6 to 10 desiccations the margin increased to nearly 40 percent.

The results from this section are similar to the observations on larval survival following repeated freezing. Moisture fluctuations which elicited a change of state of the larvae (in this case between active and anhydrobiotic) reduced their survival in relation to the number of fluctuations. In contrast to their survival following repeated freezing, larvae of P. odocoilei were slightly more susceptible than P. tenuis to the effects of repeated desiccation on survival.

D. Infectivity Trials

The conditions under which experimental exposure of P. odocoilei to Triodopsis multilineata occurs appeared to markedly affect the number of larvae which successfully entered the snails and developed (Table VIII). Using product-moment correlations, the total number of larvae recovered from all snails in a given exposure (Exp't 15) was significantly correlated with the overall density of the exposure conditions ($r=0.996$, $p<0.01$), but not with either snail density ($r=0.702$, ns) or larval density ($r=0.602$, ns), the two components of overall density. Further evidence for the importance of density of both snails and larvae during exposure comes from the lack of correlation between numbers of first-stage

Figure 17. Survival of first-stage larvae of _P. odocoilei_ following repeated desiccations at 8 and 18°C (Exp'ts 10-14). Bars represent mean $\pm$ SE.



18°C
8°C
PERCENT SURVIVAL
100
0
0
1
3-5
6-8
9-10
NO. OF DESICCATIONS

Table VIII. Recovery of second- and third-stage larvae from *Triodopsis multilineata* exposed to first-stage larvae of *P. odocoilei* under various exposure conditions (Exp't 15).

Conditions of exposure				Recovery of larvae	
Number of snails per dish	Number of first-stage larvae per dish	Number of first-stage larvae per snail	Exposure density*	Total number	Number per snail
5 (= 0.064 per cm^2)	1570 (= 20 per cm^2)	314	1.28	8	1.6
	4710 (= 60 per cm^2)	942	3.84	28	5.6
15 (= 0.192 per cm^2)	1570	105	3.84	37	2.5
	4710	314	11.52	125	8.3

* Number of snails per cm^2 x number of larvae per cm^2

larvae available per snail and the recovery of second- and third-stage larvae from those snails (r=0.333, ns). In both the few snails/few larvae, and many snails/many larvae conditions of Exp't 15 there were 314 larvae per snail in the exposure dish, but in the denser conditions of the latter group, five times as many second- and third-stage larvae were recovered per snail.

In the first experiment to examine the effect of desiccation and high temperature on the infectivity of first-stage larvae of P. odocoilei (Exp't 16), there was much lower recovery of larvae from the snails in the two experimental groups than from either of the control groups (Table IX). There was no loss of infectivity of the larvae as a result of the two-week timespan over which the experiment was run, since recovery of larvae from the final control was not significantly different from the initial control ($t=2.753$, $df=2$, $p<0.20$).

The conditions under which first-stage larvae of P. odocoilei were stored prior to exposure to snails (Exp't 17) had great influence on their infectivity (Table X). Desiccation of the first-stage larvae, treatment at higher temperatures or for longer periods of time, all resulted in some loss of their infectivity. Those larvae desiccated at lower (45%) relative humidity retained their infectivity more than those stored at higher (95%) relative humidity, even though both desiccated groups had lower infectivity than the corresponding groups of hydrated larvae. The correlation of total numbers of larvae recovered with treatment conditions of the first-stage larvae by anova (Table XI) showed that the

Table IX. Recovery of second- and third-stage larvae from T. multilineata exposed to treated first-stage larvae of P. odøcoilei (Exp't 16).

Group designation	Treatment conditions			Number recovered (mean ± SE)
	Moisture	Temperature (°C)	Length (days)	
Initial control	Hydrated	8	1	2035 ± 145
Final control	Hydrated	8	14	2768 ± 241
High-humidity experimenatl	Desiccated (95% RH)	26	6	374 ± 1.0
Low-humidity experimental	Desiccated (45% RH)	26	14	237 ± 9.2

Table X. Recovery of second- and third-stage larvae from T. multilineata exposed to treated first-stage larvae of P. odocoilei (Exp't 17).

Treatment conditions			Number recovered (% of total)		
Moisture	Temperature (^{o}C)	Duration (days)	Second-stage	Third stage	Total
Hydrated	20	2	42 (6)	691 (94)	733
		10	3 (5)	59 (95)	62
	26	2	9 (4)	236 (96)	245
		10	0 (-)	0 (-)	0
Desiccated: 45% RH	20	2	3 (3)	90 (97)	93
		10	0 (-)	0 (-)	0
	26	2	3 (12)	23 (88)	26
		10	0 (-)	0 (-)	0
Desiccated: 75% RH	20	2	0 (0)	1 (100)	1
		10	0 (-)	0 (-)	0
	26	2	0 (-)	0 (-)	0
		10	0 (-)	0 (-)	0

Table XI. Anova table for numbers of larvae recovered from *T. multilineata* exposed to treated first-stage larvae of *P. odocoilei*, by moisture condition, temperature, and length of treatment (Exp't 17). Data were transformed by $\log_{10}(x+1)$.

Source of Variation	DF	Sum of Squares	Mean Square	F	P
Main Effects					
Moisture	2	2.126	1.063	23.62	<0.05
Temperature	1	0.301	0.301	6.69	ns
Length	1	1.353	1.353	30.06	<0.05
First-order Interactions					
Moisture x Temperature	2	0.209	0.104	2.31	ns
Moisture x Length	2	0.726	0.363	8.07	ns
Temperature x Length	1	0.001	0.001	<1.00	ns
Second-order Interaction (Error)					
Moisture x Temperature x Length	2	0.090	0.045		
Total	11	4.806			

effects of moisture condition and duration of treatment were significant, while the $6^{o}C$ difference between the two temperature treatments did not have a significant effect. In all groups of larvae recovered from snails, the majority had reached the third stage.

Several differences between the infectivity of P. odocoilei and P. tenuis (Exp't 18) were observed (Table XII). The mean number of P. odocoilei recovered from T. multilineata that were exposed to previously-frozen first-stage larvae was not significantly different from those exposed to fresh ones ($t= 0$, $df=2$, ns). However, significantly fewer (about one-twentieth the number) P. tenuis were recovered from snails exposed to previously-frozen first-stage larvae than from those exposed to fresh ones ($t= 6.212$, $df=2$, $p<0.05$). Total numbers of larvae recovered from T. multilineata was correlated with species and prior treatment of first-stage larvae by anova (Table XIII). The significant interaction indicates that significantly fewer larvae of P. tenuis retained their infectivity following freezing, compared to first-stage larvae of P. odocoilei. Under the exposure conditions used in this experiment, the first-stage larvae of P. tenuis were generally much more infective to T. multilineata than those of P. odocoilei exposed in a similar manner.

In all four groups in Exp't 18 the proportion of third-stage larvae recovered from snails was greater in the second replicate, which was digested 10 days after the first. Twenty-eight days after exposure to T. multilineata, the proportion of third-stage

Table XII. Recovery of second- and third-stage larvae from _T. multilineata_ exposed to treated first-stage larvae of _P. odocoilei_ and _P. tenuis_ (Exp't 18). Replicate 1 was digested 28 days post-exposure; replicate 2, after 38 days.

Treatment	Replicate	Number recovered (% of total)					
		P. odocoilei			_P. tenuis_		
		Second-stage	Third-stage	Total	Second-stage	Third-stage	Total
Fresh	1	6 (100)	0 (0)	6	322 (81)	77 (19)	399
	2	0 (0)	2 (100)	2	152 (15)	846 (85)	998
	Mean			4.0			698
Frozen	1	3 (100)	0 (0)	3	29 (88)	4 (12)	33
	2	1 (25)	3 (75)	4	17 (45)	21 (55)	38
	Mean			3.5			36

Table XIII. Anova table for numbers of larvae recovered from _T. multilineata_ exposed to treated first-stage larvae of _P. odocoilei_ and _P. tenuis_, by species and temperature of treatment (Exp't 18). Data were transformed by $\log_{10}(x)$.

Source of Variation	DF	Sum of Squares	Mean Square	F	P
Main Effects					
Species	1	5.3467	5.3467	105.46	<0.01
Temperature	1	0.7824	0.7824	15.432	<0.05
First-order Interaction					
Species x Temperature	1	0.7822	0.7822	15.428	<0.05
Error	4	0.2028	0.0507		
Total	7	7.1141			

larvae of P. tenuis recovered did not differ between the fresh and previously-frozen groups ($X^2 = 0.613$, df=1, $p<0.50$). However, after 38 days there was a significantly lower proportion of third-stage to second-stage larvae recovered in the frozen group compared to the fresh group ($X^2 = 21.23$, df=1, $p<0.01$).

IV. DISCUSSION

A. Survival of P. odocoilei

First-stage larvae of Parelaphostrongylus odocoilei appear extremely resistant to both high and low temperatures: the maximum temperature they can tolerate is at least 48°C; at temperatures just above freezing they can survive in the neighborhood of one year, depending on moisture conditions; while frozen they can survive several years. Their infectivity to snails does not appear excessively reduced by any particular temperature condition.

While resistant to a wide range of temperatures, moisture conditions are critical to the first-stage larvae. When hydrated or desiccated their survival was lengthy. However, while survival was not necessarily reduced by desiccation, infectivity of the surviving larvae always was. Thus, first-stage larvae of P. odocoilei appear susceptible to desiccation, in terms of its apparent potential for reducing their transmission.

Survival of larvae was modified by several factors. Both repeated freezing and repeated desiccation reduced survival, although repeated temperature changes above freezing did not. Desiccation of the larvae, while increasing their resistance to high temperatures, reduced their resistance to freezing.

These features of larval survival of P. odocoilei are interesting from two points of view. First, they indicate that the ability of the first-stage larvae to resist extremes of environmental moisture and temperature conditions (at least in terms of survival) is exceptional, although the mechanisms by which they do so appear unusual for a nematode (see later). Second, they provide a groundwork for discussing the epizootiology of P. odocoilei. While much of their life in the intermediate and definitive hosts has been documented, prior to this study little information other than on morphology or prevalence was available for the free-living, first-stage larvae of any species of Parelaphostrongylus. However, before examining the epizootiological implications of this study, the relationship of P. odocoilei survival characteristics to those of the free-living stages of other nematodes will be examined.

Survival of the free-living stages of parasitic nematodes depends not only upon whether they are in water or desiccated, but also if desiccated upon the relative humidity of the environment (Rose 1957; Prasad 1959; Hansson 1974; Nath 1978). It is therefore important, in comparing survival of different groups of nematodes, that moisture as well as temperature conditions for which survival was determined be precisely specified. Unfortunately, most of the earlier studies reported the moisture conditions to which larvae were subjected with such ill-defined terms as "humid" and "moist", in addition to the more accurate descriptors, "wet", and "dry" or "desiccated".

The following paragraphs discuss the survival of first-stage larvae of P. odocoilei in relation to that of the free-living stages of other parasitic nematodes. The purpose will be to point out both that great variation in temperature and moisture tolerance exists between species, and that the tolerance of P. odocoilei to the range of conditions tested in this study is similar or superior to that reported for other nematode species. The greatest use will be made of literature in which experimental conditions are accurately described. A large number of studies, though vague in description of some aspects of experimental conditions, contain valid qualitative information, and will be referred to where appropriate.

The high-temperature tolerance of *P. odocoilei* larvae is similar to that of other metastrongyles. The larvae survived a temperature of 48°C for one day in water; one week when desiccated at 20 or 45% relative humidity (% RH). The larvae of *Cystocaulus ocreatus* and *Protostrongylus* sp. tolerated temperatures of 45-50°C for one-half hour (Morev 1966). The upper lethal temperature for *Elaphostrongylus cervi* in water was near 50°C (Mitskevich 1964), but a temperature of 40°C could be tolerated for one week (Lorentzen and Halvorsen 1976) under unspecified moisture conditions. Some larvae of *Protostrongylus stilesi* could survive at least one day on dried fecal pellets at 72°C (Forrester and Senger 1963); this was the only metastrongyle studied at such a high temperature. These studies indicate that species in this group have similar resistance to high temperatures, being able to survive exposure to temperatures of 45-50°C at least for a short while.

Lower survival of hydrated than desiccated larvae at high temperatures has been reported for many species. In studies with larvae on feces (Rose 1957; Forrester and Senger 1963) this might be interpreted as due to the presence of contaminants such as bacteria, rotifers and fungi on the decaying, moist pellets.

Susceptibility of first-stage metastrongyle larvae to such conditions has been reported (Hobmaier and Hobmaier 1930; O'Roke 1936; Pillmore 1956). In this study however, isolated, clean, desiccated larvae of P. odocoilei exhibited superior survival over similarly treated but hydrated larvae at high temperature. Third-stage larvae of trichostrongyle nematodes also reacted similarly (Poole 1956, on Nematodirus filicollis; Andersen and Levine 1968, on Trichostrongylus colubriformis; Todd et al. 1976, on Haemonchus contortus). This suggests that desiccation of larvae promotes their high-temperature survival, not by reducing decay of their surroundings, but by action on the larvae themselves.

While resistance of first-stage larvae to high temperature is similar among the metastrongyles, their long-term survival at cooler, more favorable temperatures differs markedly. In water, Protostrongylus kochi lived only 4 months at 2- 4°C (Davtian 1949, cited by Morev 1966) as did P. stilesi at an unspecified temperature (Pillmore 1956), yet larvae of Protostrongylus rufescens lived in water over a year at an unspecified temperature (Hobmaier and Hobmaier 1930). P. odocoilei, also able to survive a year in water at 5°C, lived even longer (18 months) when desiccated at 45% RH. Protostrongylus (=Synthetocaulus) hobmaieri was able to survive 19 months at 10- 20°C, while desiccated at 35- 50% RH (Matekin et al. 1954, cited by Forrester and Senger 1963). Under similar conditions, survival of P. odocoilei was only slightly shorter (14 months).

In studies on other strongylids (Belle 1959; Gupta 1961; Herlich 1966; Andersen and Levine 1968; Todd et al. 1976) a range in maximum lifespan (from 4 to over 18 months) similar to that existing among the metastrongyles has been reported. In contrast to the response of P. odocoilei, studies on trichostrongyles have shown a lower survival at cool temperatures when desiccated than when hydrated (Andersen and Levine 1968; Todd et al. 1976).

Thus while desiccation appears to promote high-temperature survival of strongylid larvae, regardless of species, the benefits at cooler temperatures vary between species. Too few studies have been done to permit generalization of particular types of survival responses as characteristic of any of the major taxa within the Strongylida.

There is as much variation in the resistance of various species of strongylid larvae to freezing as was demonstrated in their ability to survive at above-freezing temperatures. Studies on strongyles (Belle 1959; Balasingam 1964) indicated a range in survival from two hours to over 30 days when frozen at -20°C; on trichostrongyles (Andersen and Levine 1968; Todd et al. 1976), from a few days to two months at -28°C; and on metastrongyles (Pillmore 1956; Rose 1957; Lankester and Anderson 1968; Hansson 1974), from nearly two weeks to 10 months at -20°C. The ability of larvae of P. odocoilei to survive freezing for several years thus appears exceptional for this group of nematodes.

Two of the factors that can influence the survival of larvae while frozen are: whether the larvae are on or off feces, and whether they are hydrated or desiccated prior to freezing. For example, Skrjabingylus nasicola survived freezing at -20°C when on pellets, but not when off (Hansson 1974); H. contortus survived freezing better when off fecal pellets (Todd et al. 1976). Survival estimates for P. odocoilei frozen on feces or in water were similar.

In summary, larvae of P. odocoilei generally have higher survival, under comparable conditions, than most other Strongylida studied. However, the extremes of high and low temperature and desiccation that have been examined in some other studies were not part of this study, and the response of P. odocoilei to those conditions was not determined. The range of conditions chosen for this study was similar to what might be expected in nature; the data obtained would aid in understanding the epizootiology of Parelaphostrongylus. The use of temperature and moisture extremes would not serve that purpose, but rather would be useful for studying the mechanisms of resistance to high temperature, freezing, and desiccation; such was not the purpose of this study. However, while the elucidation of survival mechanisms was not the purpose of this study, some observations on P. odocoilei warrant special attention in that regard.

The consistent observation in studies on other nematodes has been that while desiccated, survival was lengthiest at the highest relative humidities, and shortest at the lowest. To illustrate this point, survival data for several species whose desiccation tolerance was studied over a range of relative humidities is shown in Figure 18. Because absolute lengths of survival varied between species, survival at the different relative humidities in each study was converted to a proportion of the maximum survival noted for each species. Thus the differences between species in their sensitivities to the level of relative humidity are emphasized, while differences in the length of survival at a given relative humidity are not.

Strongyloides papillosus (Fig. 18a) was most sensitive to the level of relative humidity, surviving only if it was above 90% RH. Ditylenchus myceliophagus, S. nasicola, and Bunostomum trigonocephalum (Fig. 18b-d) were somewhat less sensitive, surviving desiccation at relative humidities as low as 20 percent. Heterodera rostochiensis and Heterodera schachtii (Fig. 18e), although demonstrating rapid decrease in survival at relative humidities below 100 percent, still had some survival at almost 0% RH. The survival of Ditylenchus dipsaci, Muellerius capillaris, and Trichostrongylus retortaeformis (Fig. 18f-h) did not fall as rapidly with decreasing relative humidity as the previous species, and in the case of T. retortaeformis and M. capillaris was quite substantial, even at the lowest relative humidities.

These nine species, while showing a continuum from extreme to mild sensitivity to reduced relative humidity, all responded in the same qualitative manner. The survival of P. odocoilei, when displayed in the same manner (Fig 18i) had the opposite qualitative

Figure 18. Survival of desiccated free-living larvae of a variety of plant- and animal-parasitic nematodes at a variety of relative humidities.

a. Infective larvae of *Strongyloides papillosus* (after Nath 1978).

b. Fourth-stage larvae of *Ditylenchus myceliophagus* (after Perry 1977).

c. First-stage larvae of *Skrjabingylus nasicola* (after Hansson 1974).

d. Infective larvae of *Bunostomum trigonocephalum* (after Belle 1959).

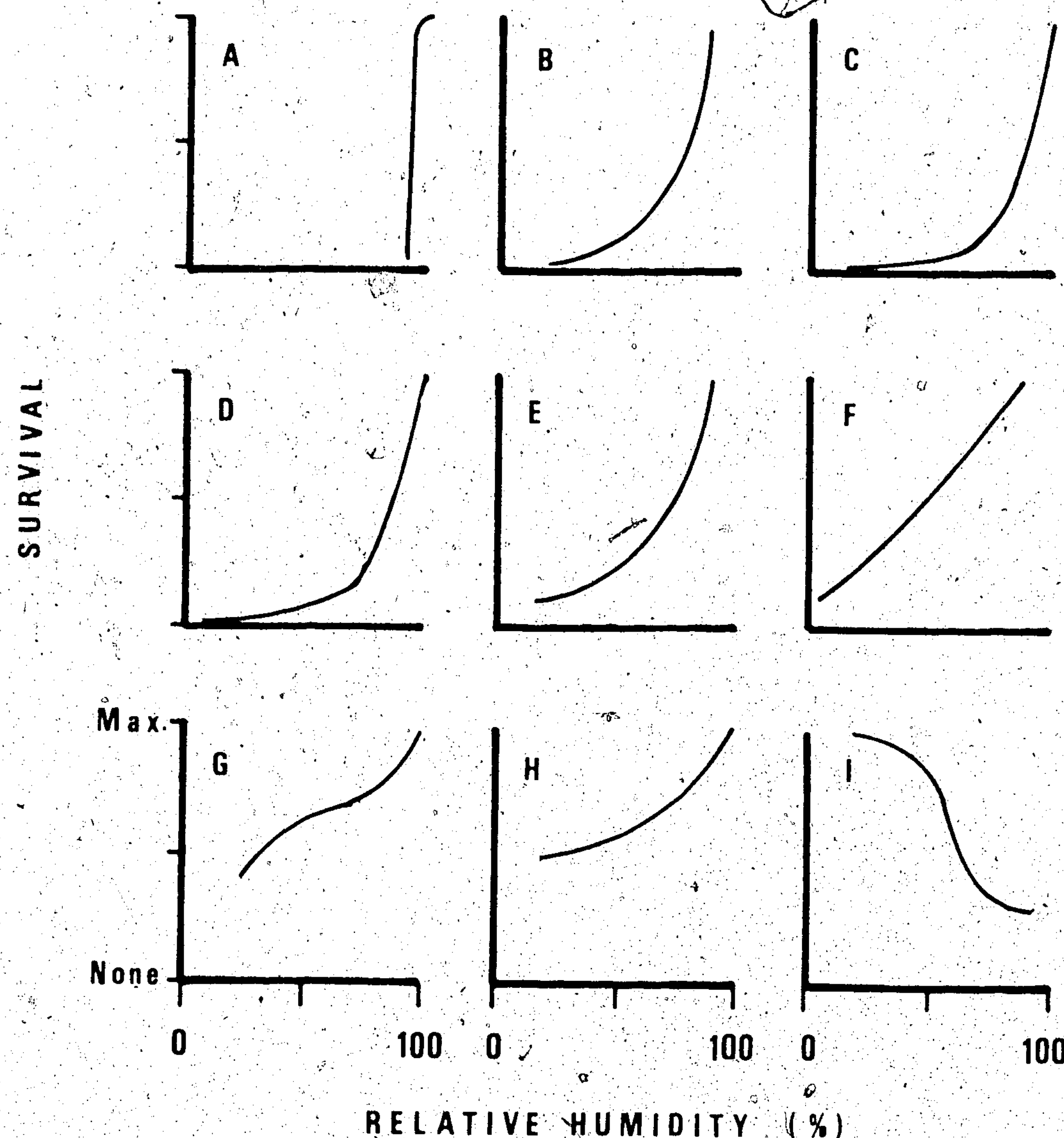
e. Second-stage larvae of *Heterodera schachtii* and *Heterodera rostochiensis* (after Ellenby 1968b).

f. Fourth-stage larvae of *Ditylenchus dipsaci* (after Perry 1977).

g. First-stage larvae of *Muellerius capillaris* (after Rose 1957).

h. Infective larvae of *Trichostrongylus retortaeformis* (after Prasad 1959).

i. First-stage larvae of *P. odocoilei*.


A
B
C
D
E
F
G
H
I
SURVIVAL
Max.
None
0
100
0
100
0
100
RELATIVE HUMIDITY (%)

response to the level of relative humidity: greater survival with decreasing relative humidity.

This suggests three possibilities. First, the observed survival pattern of P. odocoilei may merely be an extension of the continuum of increasing resistance to low relative humidity, with the same mechanism for survival while anhydrobiotic (see p. 21) operating as in the nine other species. A second possibility is that a different mechanism for survival operates in larvae of P. odocoilei than in the other nine species studied. The third possibility is that the results obtained in this or the other studies were artifactual, arising only from the techniques employed. The last possibility will now be addressed in detail.

The experimental protocol in this study comprised five parts. First, larval survival in this study was examined using larvae that had been removed from feces, cleaned, and placed in clear water prior to desiccation. Of the eight other studies used to compile Figure 18, all but Hansson (1974) used larvae prepared similarly. Second, in order to desiccate the larvae, evaporation of the water in which they were contained was done in a Petri dish under ambient room conditions. All the other studies but those of Belle (1959), Prasad (1959), and Nath (1978) also used a smooth substrate for evaporation of the water. Rose (1957) and Nath (1978) allowed the samples to desiccate under ambient conditions, as in the present study. Third, humidity control in this study was by saturated salt solutions. Only Rose (1957), Ellenby (1968b), and Perry (1977a) used other methods. Fourth, survival in this and all other studies was monitored after rehydrating larvae, without a period of "pre-hydration" (see later). Finally, survival of larvae in this study, as in all the others, was monitored using motion of the larvae as a criterion. In summary, the methods of this study were similar to those of the other eight studies, and likely did not contribute to the different qualitative results observed.

From this study there are two other pieces of supporting evidence that the unusual desiccation survival of P. odocoilei is a real phenomenon. First, the congener Parelaphostrongylus tenuis had a similar response; survival after nine days at low relative humidity (Fig. 12c) was greater than after a shorter time at a higher relative humidity (Fig. 12g). Second, the infectivity of larvae surviving storage at high relative humidity was lower than infectivity after the same time spent at lower relative humidity (Exp't 17).

If the evidence that survival of first-stage larvae of P. odocoilei (and P. tenuis) is indeed better when desiccated at lower than high humidity is accepted, the question is raised: what mechanisms could be responsible?

Although the ability of many organisms to survive lengthy periods of desiccation has been recognized for over two centuries (Keilin 1959), the study of anhydrobiosis is unfortunately still largely in the descriptive stage (as in this study). Several factors are known to enhance desiccation resistance of nematodes, although the mechanisms by which they do so are still poorly understood.

Evaporative water loss, for example, must be slow to result in maximum survival while desiccated (Ellenby 1968a,b; Crowe and Madin 1975). This can be accomplished by several means: coiling of the body, aggregations into groups ("eel-worm wool"), retention of cuticles from previous molts, or water loss occurring at high relative humidities (Ellenby 1968a, 1969; Bird and Buttrose 1974; Crowe and Madin 1975; Rössner and Perry 1975; Perry 1977a). Similarly, slow water uptake during rehydration also promotes revival of larvae. With *Aphylenchus avenae* stored at 0% RH, a period of "pre-hydration" at 95% RH before the addition of water resulted in 95% revival of the larvae and adults; without the "pre-hydration" treatment, revival following the addition of water was only 60 percent (Crowe and Madin 1975).

These studies however, have not provided answers as to how larvae survive desiccation, apart from suggestions that "preparatory" biochemical events must occur (Crowe and Madin 1975), or that slow dehydration might permit an orderly packing of tissues during shrinking (Bird and Buttrose 1974). No one has addressed the question of why survival at different relative humidities differs, let alone why some species should favor low relative humidities and others high.

Pigoń and Weglarska (1955) and Bhatt and Rohde (1964) have suggested that metabolism in anhydrobiotic organisms, which is almost non-existent at low relative humidities, increases sharply at relative humidities of 90 percent and above. Burns (1964) suggested that a water content just sufficient to support oxygen consumption, but not other biochemical reactions (20- 60%) may be lethal. This may be of little consequence to the nematode, either hydrated or desiccated. Studies on water content during dehydration (Ellenby 1968a; Rössner and Perry 1975; Perry 1977b) have resulted in estimates of the initial water content of several species at about 75 percent, exceeding the lethal value of 60 percent estimated by Burns (1964). As well, upon drying the water content is reduced to below the 20 percent lethal value suggested by Burns in a very short time. For example, even when dried slowly at ambient relative humidity of 95-99 percent, water content of Rotylenchus robustus dropped to below 5 percent after only 5 minutes (Rössner and Perry 1975). Only a brief time was spent in the critical zone, even when dried slowly. Thus, factors other than a lethal water content must be operating during mortality of larvae experiencing desiccation. No solution appears at hand to explain the effect of relative humidity on survival. Subsequent discussion of the phenomenon will deal only with its epizootiological consequences.

The previous sections have dealt with survival of P. odocoilei under a variety of constant conditions. The information gained from controlled studies of that nature can be applied to field situations only if it is assumed that the influence of different conditions in combination is additive; that under changing conditions the changes themselves do not have any influence beyond what the summation of their individual components would have. The results from several experiments in this study indicate that this assumption is valid only under certain circumstances.

The factor which appears to determine whether added influence on larval survival beyond the summation of the components occurs, is whether a change of the physical state of the larvae is involved. In the case where repeated temperature changes occurred, but larvae remained in the active state (Exp't 7), no added mortality in the temperature-cycled group of larvae beyond that in the control group was detected. However, when repeated temperature changes resulted in the larvae repeatedly moving between the active and cryobiotic (see p. 21) states (Exp'ts 8,9), additional mortality of larvae was observed, above the level expected when they were constantly in either of the two states. This phenomenon was also observed when moisture changes involved larvae repeatedly moving between the active and anhydrobiotic (see p. 21) states (Exp'ts 10-14).

Changing states acted on the larvae by killing or weakening a certain proportion of the individuals each time a change of state occurred. There was slightly reduced survival observed after even one freezing (or desiccation), and the increased mortality after each succeeding freezing (or desiccation) cycle. The processes involved in freezing larvae appeared to be less demanding than those involved in desiccating larvae. Survival of larvae of <u>P. odocoilei</u> after 20 freezings was similar to the survival resulting from only 10 desiccations.

Susceptibility of nematode larvae to repeated desiccations has been reported frequently (Poole 1954, in Todd et al. 1970; Keilin 1959; Schmidt et al. 1974; Evans and Perry 1976; Todd et al. 1977). This susceptibility has been attributed to an increase of solutes with each desiccation, resulting from impurities in the water added for each rehydration (Todd et al. 1970). The susceptibility to repeated desiccation varies between species. Third-stage larvae of H. contortus were able to survive 70 days of daily desiccation in triple-distilled water (Todd et al. 1970). They survived at least 64 days while constantly desiccated under similar ambient conditions (Todd et al. 1976), indicating no added mortality due to the repeated desiccations. On the other hand, larval T. colubriformis could survive only 30 days of repeated daily desiccation in triple-distilled water (Schmidt et al. 1974), but survived constant desiccation under similar ambient conditions for at least 128 days (Andersen and Levine 1968). Resistance of Cooperia punctata to repeated desiccation was found to be intermediate between that of H. contortus and T. colubriformis (Todd et al. 1977). The survival of P. odocoilei, determined in this study using distilled water as the medium, was similar to that of T. colubriformis in distilled water (Schmidt et al. 1974).

The effects of repeated freezing have been examined only infrequently. Species of Nematodirus have been shown to resist at least a dozen cycles of freezing and thawing with little reduction in survival (Turner 1953; Poole 1956). Survival of P. odocoilei was slightly poorer after a similar number of freezing cycles.

In summary, it appears that survival data derived under constant temperature and moisture conditions can be interpreted in relation to the varying conditions of nature only with caution. If ambient conditions are such that freezing or desiccation of larvae occurs on a repeated basis, then their survival will probably be less than that predicted using data derived under constant experimental conditions.

B. Infectivity of P. odocoilei

Forrester and Senger (1963), studying the survival of larvae of P. stilesi under temperature and moisture stress, concluded that, "... it seems unlikely that temperature and humidity can influence the survival of first stage protostrongylid larvae on fecal material to a significant degree." They felt that due to the remarkable survival capabilities exhibited by the larvae of P. stilesi, they would survive environmental stresses and probably still be available in sufficient quantities to allow infection of the intermediate host. They acknowledged that they had not examined the viability (= infectivity) of the surviving larvae, but that the surviving larvae could have been affected physiologically, and may have been unable to complete their life cycle. Their statement now appears prophetic in light of recent studies dealing with the infectivity of surviving larvae.

The infectivity of nematode larvae which survived a variety of different storage conditions has been shown to be reduced gradually over time. First-stage larvae of Angiostrongylus (= Parastrongylus) costaricensis had a period of infectivity that was shorter than their period of survival under both hydrated and desiccated storage conditions (Arroyo and Morera 1978; Bullick and Ubelaker 1978). Third-stage larvae of T. *colubriformis* gradually lost infectivity after 12 months storage on moist filter paper at 4°C (Herlich 1966). First-stage larvae of P. *tenuis* also lost infectivity after desiccation or freezing (Lankester and Anderson 1968; this study).

The results of this study indicate that P. *odocoilei* also loses infectivity before death occurs. Those conditions which resulted in the poorest survival also resulted in the lowest infectivity among the survivors. Two conditions which resulted in lengthy survival of the larvae, storage in water at temperatures just above freezing, and freezing, resulted in little loss of infectivity among the surviving larvae. On the other hand, desiccation of larvae resulted in drastic reduction of the survivor's infectivity. Reduction in infectivity, just as in survival, was a function of the degree of desiccation. At 45% RH, survival was better and the loss of infectivity less than for larvae at 75% RH (Table XI). It is critical to note that while first-stage larvae of P. *odocoilei* survived as long when desiccated at low relative humidities as when hydrated, the infectivity of larvae surviving desiccation was never found to be more than 15 percent that of larvae which had not been desiccated.

C. Comparative Studies

The experiments just discussed pertain to larvae of P. *odocoilei* originating from mule deer of Jasper National Park. The remaining discussion along those lines will deal with the survival of Jasper-source larvae of P. *odocoilei* in relation to P. *odocoilei* larvae of Vancouver Island source, and to larvae of P. tenuis originating from Pennsylvania.

No difference in survival between the Vancouver Island and Jasper source larvae was detected, though a broad range of conditions was tested: hydration and desiccation; above-freezing and below-freezing temperatures. Climatic conditions in the coastal areas of Vancouver Island are certainly more moderate than those of Jasper. Based on mean values for the period 1941-1970, daily minimum temperatures reported from Vancouver Island meteorological stations were generally only a few degrees below freezing in January, while those for Jasper averaged -17°C (Anonymous 1973). Greater total annual precipitation and annual days with measurable rain were much greater on the Island (Anonymous 1973). Snowfall was much less frequent on the coastal areas of Vancouver Island than in Jasper (Anonymous 1973). This would result in more direct exposure to ambient conditions for larvae shed on Vancouver Island sites, while those larvae shed in Jasper would have a higher probability of being covered with snow, thereby receiving a degree of protection from ambient conditions. The sites for transmission of P. odocoilei on Vancouver Island are not known, but the results of this study suggest that not only would transmission be possible in the coastal areas, but that cooler temperatures in the upland regions would not likely be limiting.

Differences in survival between P. odocoilei and its congener P. tenuis were observed. The differences, slight when under constant conditions, were more prominent under fluctuating temperature and moisture conditions. These differences comprised a lower survival of P. tenuis than P. odocoilei following repeated freezing, but superior resistance of P. tenuis over P. odocoilei to repeated desiccation. Many other species also exhibited differences from close relatives in their survival under various storage conditions. Several examples follow.

Studies on congeneric species pairs of plant-parasitic nematodes (Ellenby 1968b; Perry 1977a) have demonstrated that one member of the pair had superior desiccation resistance to the other. Ellenby (1968b) suggested that in the case of Heterodera schachtii and Heterodera rostochiensis that the difference in resistance was related to differences in the characteristics of the host plants on which they evolved.

Balasingam (1964), studying Uncinaria (= Dochmoides) stenocephala, Arthrocephalus (= Placoconus) lotoris, and Ancylostoma caninum, demonstrated that the first species, which has a northerly distribution, had greater freezing resistance than larvae of A. caninum, a parasite southern in distribution. A. lotoris, which has a distribution somewhat intermediate to the other two species, had characteristics which were intermediate.

Differences in survival capabilities also exist between species of Trichostrongylus (Gupta 1961; Herlich 1966; Andersen and Levine 1968; Rojo-Vazquez 1976). It is of interest that although larvae of T. colubriformis exhibited greater resistance to continuous desiccation than did H. contortus (Todd et al. 1976;

Hsu and Levine 1977), H. contortus was the more resistant of the two to repeated desiccation (Todd et al. 1970).

Todd et al. (1970) suggested that the resistance to repeated desiccation exhibited by H. contortus and T. colubriformis may be of adaptive value in places such as Urbana, Illinois, where daily dew formation and evaporation occur during the summer months. The difference in resistance between the two was suggested as a contributing cause of the difference in distribution of the two species (Schmidt et al. 1974).

While the differences in survival between P. odocoilei and P. tenuis were slight, the difference in the infectivity of the surviving larvae was not. Following freezing, the infectivity of P. tenuis was reduced to only 5 percent that of larvae which had not been frozen, while larvae of P. odocoilei had only slight loss of infectivity. Following desiccation though, the infectivity of P. odocoilei was reduced to only about 15 percent of its normal levels, as discussed previously. Infectivity of P. tenuis larvae following desiccation was not determined in this study, but similar information can be derived from the study of Lankester and Anderson (1968). They reported a recovery of P. tenuis larvae from snails (Mesodon thyroidus) exposed to first-stage larvae in dried and remoistened soil that was 50% that of the recovery from snails exposed to larvae in moist soil that had not been dried. The recovery from dried and remoistened feces was about 25% that from fresh feces containing P. tenuis larvae. The 25 and 50% infectivity retention figures for P. tenuis following desiccation are not strictly comparable with the infectivity figures derived in this study. They do not take into account the number of live larvae available to infect the snails, as this study does; therefore if mortality of larvae of P. tenuis in soil or feces occurred, those estimates are conservative. Compared to the maximum infectivity retention for P. odocoilei following desiccation, those figures lend credence to the argument that P. tenuis can better resist desiccation.

D. Epizootiological Considerations

It has been alluded to in the previous discussion that slight differences in survival of the free-living stages of different species of nematodes can serve to segregate them geographically. However, conditions in the environment which we might perceive as being potentially limiting to free-living larvae may or may not have any bearing on the conditions actually experienced by the nematode larva in the soil. Temperature and moisture conditions occurring in the vegetation mat or in the soil may be markedly different from the conditions measured less than two meters above ground in a standard weather shelter (Levine and Todd 1975).

Crofton (1963) reported that relative humidities in the vegetation mat were still above 90 percent even after three weeks of drought. Collis-George (1959) outlined several factors regarding moisture conditions within the soil which would be of importance to soil-inhabiting nematodes. Even at water levels low enough to elicit wilting in plants, the soil atmosphere is still above 98.5% RH. The relative humidity in soil near the surface could, at midday with a drying wind, be as low as 50 percent. To keep from desiccating the nematode must either expend energy sufficient to retain water, which becomes more expensive as the soil dries, or migrate deeper into the soil; failing these two routes it must become anhydrobiotic (Collis-George 1959).

Changes in temperature result in temporary modifications of soil relative humidity: increases in temperature result in a drop in relative humidity; decreases in temperature result in an increase through temporary saturation of the air. Despite occasional drops in relative humidity, soil nematodes spend the majority of the time in a water film sufficient to keep them hydrated (Collis-George 1959), in a soil atmosphere of high relative humidity. Movement of nematodes through the soil is restricted to within relatively narrow ranges of water suction, which is in part related to soil structure. In shrinking-soil systems, pore spaces might be only one-tenth the size they would be in a rigid-soil system with the same water suction (Collis-George 1959). Once the pores empty of water, movement of nematodes becomes difficult, for although a film of water remains on the walls of the pores, even with relatively abundant water it may only be a fraction of a micron thick.

It is unfortunate that measurements of soil water suction are made only infrequently, for they would provide much information of use in understanding local microclimates; the climate the parasite experiences. As a generalization, the soil offers a degree of protection for the larvae, compared with conditions above its surface.

The majority of first-stage larvae of *P. tenuis* is located near the periphery of the fecal pellet (Lankester and Anderson 1968), and although the mucus coat on a fecal pellet may reduce the rate of drying by up to 30 percent in the case of sheep pellets (Crofton 1963), it is conceivable that larvae on the exposed portions of the pellets would desiccate rather quickly, suffering any associated loss of infectivity. It is not known whether first-stage larvae of *Parelaphostrongylus* spp. migrate readily off the fecal pellets soon after they are shed, thus reaching the protection of the soil or vegetation; another metastrongyle, *M. capillaris*, does not (Rose 1957), even though it survives better in soil (Nickel 1960). Pellets containing first-stage larvae could be protected if dropped in areas of lush vegetation as opposed to open areas, and frequent rains would aid survival by washing larvae into the protective environment of the soil.

The extent to which transmission of *Parelaphostrongylus* spp. would be impaired or aided by larvae leaving the fecal pellets and entering the soil has not been carefully examined, but Lankester and Anderson (1968) observed that *M. thyroidus* acquired substantial numbers of *P. tenuis* larvae after crawling on infected pellets or infected soil. However, previous drying of the soil did not reduce the numbers of larvae acquired by *M. thyroidus* as much as previous drying of fecal material containing first-stage larvae. This indicates that first-stage larvae of *P. tenuis* may have been more protected from desiccation by the soil than by fecal material. While the results of the present study indicate that transmission of *P. odocoilei* to *Triodopsis multilineata* is enhanced by crowded conditions of both snails and first-stage larvae, the movement of larvae off the fecal material into the surrounding soil may not have any deleterious consequences for transmission as long as the larvae are not dispersed too far.

The first-stage larva is the age class in the life cycle of *Parelaphostrongylus* most susceptible to climatic factors. Regardless of the availability of suitable intermediate and definitive hosts, if this stage succumbs to environmental pressure, transmission will not occur. Environmental pressure on the first-stage larvae is very likely involved in the current known distribution of *Parelaphostrongylus* spp.

There is an apparent segregation of *P. tenuis* and *Parelaphostrongylus andersoni* in the southeastern United States. White-tailed deer were examined for both parasite species in 24 counties in the southeast (Prestwood et al. 1974). *P. andersoni* was present alone in 12 counties, *P. tenuis* in 10, but in only two counties were the two species found together. A particularly striking example of this segregation occurred in South Carolina, where 11/30 white-tailed deer from five counties were infected with *P. andersoni*. None had *P. tenuis* even though a large proportion of the deer to the north and south harbored *P. tenuis* (Prestwood et al. 1974). As well, in a previous study (Prestwood and Smith 1969) none of 87 white-tailed deer from nine other counties in South Carolina was infected with *P. tenuis*.

Arguments were put forth by Platt (1978) that the elaphostrongylines are relatively non-specific at the intermediate host level. While such appears to be the case for _P. tenuis_ (Lankester and Anderson 1968) and _P. odocoilei_ (Platt 1978), little is known of the intermediate-host specificity of _P. andersoni_. Barring any extreme idiosyncracies in intermediate host suitability for _P. andersoni_, it is unlikely that intermediate hosts serve as the means for segregating these two parasite species in this region. The white-tailed deer is the host for both parasite species, and concomitant infections of the two parasites have been reported (Prestwood et al. 1974), so it is unlikely the definitive host plays a role in the segregation.

This leaves, of course, the first-stage larvae of the two species as the point where a differential influence on the transmission of the two species is occurring. It was observed that _P. tenuis_ occurred primarily in the oak-hickory-pine subclimax and the climax deciduous forest habitats, but tended to be absent from the southern floodplain and southern mixedwood vegetation habitats (Prestwood and Smith 1969; Prestwood et al. 1974). In contrast, _P. andersoni_ tended to be found in the southern floodplain and southern mixed vegetation habitats as well as in the oak-hickory-pine subclimax (Prestwood et al. 1974). The distributional pattern related to vegetation types in this case is strongly suggestive of microclimatological conditions having a differential influence on the survival or subsequent infectivity of the larvae of these two species.

A second oddity in the distribution of *Parelaphostrongylus* spp. is the apparent lack of any representatives in the North American grassland biome, as defined by Carpenter (1940). The reported distribution of *P. tenuis* extends westward only to the boundaries of the grassland. The only place where the confirmed distribution of *P. tenuis* has not reached the grassland is in Illinois, but Schaeffer and Levine (unpub., cited by Levine 1968, p. 287) found dorsal-spined larvae, similar to those of *P. tenuis*, in white-tailed deer feces from Illinois.

Unfortunately, there are few reports of searches for elaphostrongyline nematodes from the grassland; parasite surveys of deer from that region (Boddicker and Hugghins 1969; Worley and Eustace 1972) have not included examination for them. Samuel and Holmes (1974) reported finding dorsal-spined larvae in deer feces only from the forested regions of Alberta. Pellet groups from parkland (n= 43) and grassland (n= 13) were negative, while feces from adjacent forested regions were infected.

Bindernagel and Anderson (1972) found elaphostrongyline-like larvae in the feces of white-tailed deer from eastcentral Saskatchewan. Positive samples were found in areas of forest or parkland, not in grassland areas. Prevalence of dorsal-spined larvae decreased from a high in the mixedwood forest to complete absence in the grassland (Table XIII) during a followup study (1976-1978) in Saskatchewan (Shostak, unpub.). These pieces of evidence, admittedly scanty, suggest that deer in the grassland do not harbor elaphostrongyline nematodes.

Table XIV. Recovery of dorsal-spined larvae from the feces of white-tailed deer from different regions of southern Saskatchewan (Shostak, unpub.).

Region	Number of samples examined	Number of samples positive	Percent of samples positive
Prairie	35	0	0
Prairie/Parkland transition	61	2	3
Parkland	57	8	14
Parkland/Mixedwood transition	25	4	16
Mixedwood	386	123	32
Total	564	137	27

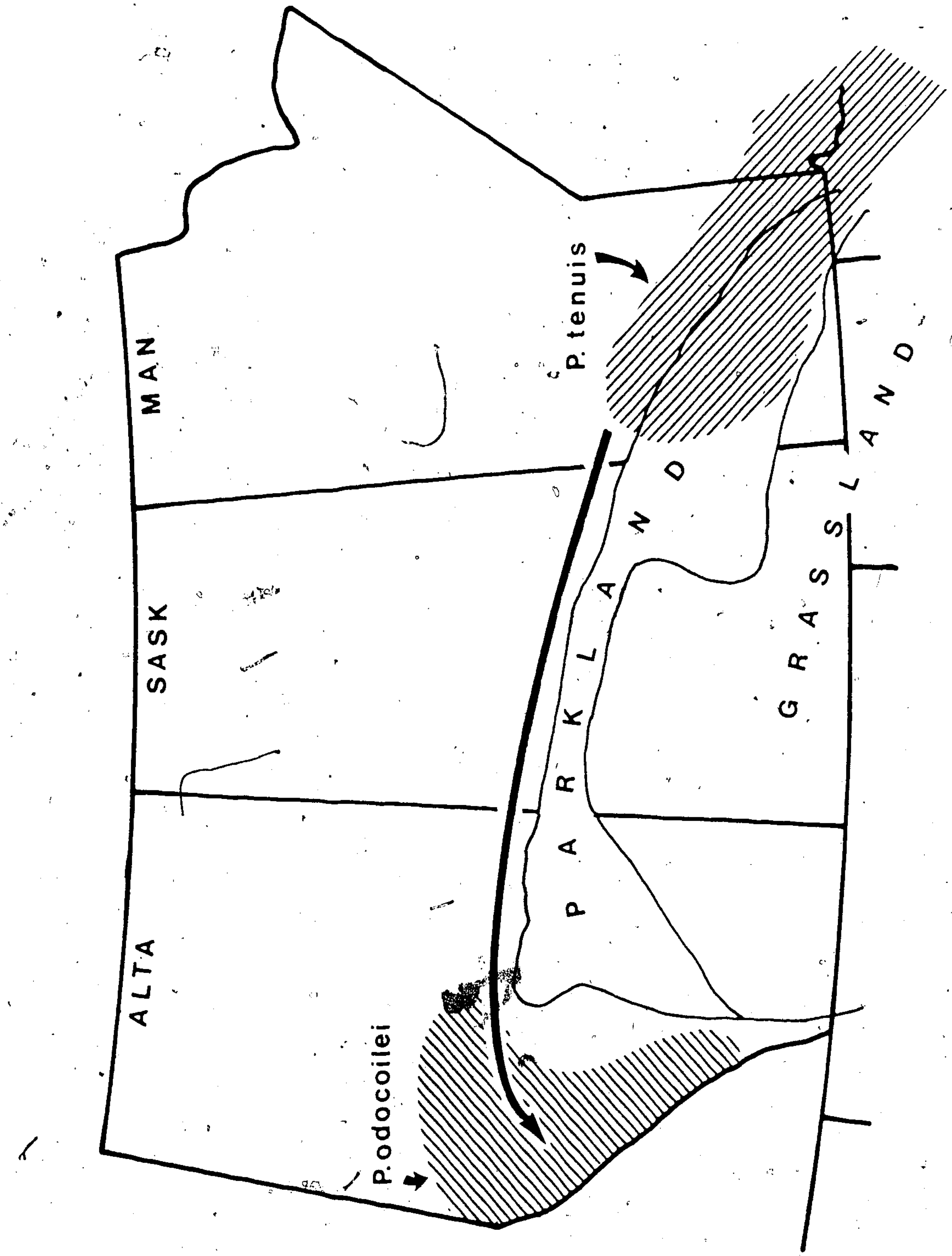
The grassland has populations of white-tailed and mule deer, providing a definitive host for elaphostrongylines. Gastropods that are known intermediate hosts for Parelaphostrongylus (see Lankester and Anderson 1968; Platt 1978) range throughout the grassland (Carpenter 1940; Burch 1962). The availability of suitable hosts for the parasitic phases of the life cycle would not appear to be limiting for transmission of Parelaphostrongylus in the grassland. It is possible that transmission of elaphostrongylines in the grassland is being at least partially impaired at the point involving the first-stage larvae, as was suggested already as partial explanation for the disjoint distribution of P. tenuis and P. andersoni in the southeastern United States.

The seasonal precipitation pattern in the grassland has been implicated in limiting the activity of small animals and plants (Carpenter 1940). Conditions of desiccation (on the soil surface at least) are easily conceivable given the characteristic drought at the end of the hot season. Prior desiccation has been shown to reduce the infectivity of P. odocoilei (this study) and P. tenuis (Lankester and Anderson 1968). Exposure to ultraviolet light, either from sunlight or atificial sources, has also been found to result in mortality of trichostrongylid (Senger 1964; Conder 1978) and metastrongylid (Rose 1957) nematode larvae. In contrast, Mitskevich (1964) reported that first-stage larvae of E. cervi were resistant to direct sunlight. Desiccation and exposure to high levels of solar radiation, working in conjunction, may result in sufficient larval mortality or loss of infectivity to seriously impair transmission of *Parelaphostrongylus* in the grassland. Reduced gastropod activity under dry conditions might also act to reduce the efficiency of parasite transmission. It seems probable that, even if conditions in the majority of the grassland are severe enough to impair elaphostrongyline transmission, these parasites may be present there in a discontinuous distribution, associated with protected habitats such as river valleys.

It was mentioned previously that the westward distribution of *P. tenuis* in North America appears limited by the grassland biome. In 1972, Bindernagel and Anderson hypothesized that *P. tenuis* could reach the foothills of the Rocky Mountains in western Alberta by spreading westwards from Manitoba along the aspen parkland in central Saskatchewan and Alberta (Fig. 19). Thus, *P. tenuis* could circumvent the grassland barrier. The arrival of *P. tenuis* in Alberta could have disastrous consequences for a variety of wild ruminants there. Bindernagel and Anderson's hypothesis was met with an alternative hypothesis (Samuel and Holmes 1974) which stated that some ecological feature, possibly associated with drier western conditions, limited *P. tenuis* populations in the west. They cited the apparent absence of *P. tenuis* from the grassland, and from the sandy-soiled pine forests of the southeastern United States, as supporting evidence.

The results from the present study may be used to test the hypothesis of Samuel and Holmes (1974) as it might apply to the first-stage larvae of *P. tenuis*. Their hypothesis can be restated

Figure 19. Potential route of expansion of P. tenuis range into the foothills of the Rocky Mountains, as suggested by Bindernagel and Anderson (1972). Vegetation regions are from Rowe (1972).


MAN
SASK
ALTA
P. tenuis
PARKLAND
GRASSLAND
P. odocoilei

as follows: "The first-stage larvae of *P. tenuis* have insufficient tolerance of climatic conditions in the Canadian west for the parasite to become established there".

A conclusive test of this hypothesis would involve the seeding of an area in the west with white-tailed deer feces containing larvae of *P. tenuis*, and the subsequent documentation of any spread of the parasite into non-infected deer in the area. Unfortunately, such an exercise would have grave consequences if the hypothesis were disproved. The hypothesis can be tested in a more indirect manner.

The life cycle of *P. tenuis* is almost identical to the life cycle of *P. odocoilei*, apart from the definitive hosts. Both parasites even share several species of intermediate hosts (see Lankester and Anderson 1968; Platt 1978). Since *P. odocoilei* is native to western Alberta (Fig. 19) it is obviously adapted to the climatic conditions found there. If the larvae of *P. tenuis* have environmental tolerances equal to or superior to those possessed by the native *P. odocoilei*, the hypothesis that they cannot tolerate western climatic conditions should be rejected. This method uses the environmental tolerances of *P. odocoilei* as a biological measurement of the stress imposed by western Alberta microclimates.

This study examined the effect of two climatic factors, temperature and moisture, on the two parasite species. In terms of moisture stress, the difference between survival of the two species under a variety of constant conditions (Fig. 12) was never more than a few percentage points. In the case of repeated desiccation (Fig. 16), *P. tenuis* survival was always slightly greater. The similar or superior survival of *P. tenuis* compared to *P. odocoilei*, coupled with greater retention of infectivity by *P. tenuis* larvae following desiccation (see previous discussion), leads to rejection of the hypothesis that moisture conditions limit distribution of *P. tenuis* in the west.

In terms of temperature stress, *P. odocoilei* had consistently greater survival than *P. tenuis* following repeated freezing (Fig. 13), although the difference in survival between the two species was slight. Length of survival while continuously frozen was considerably longer for *P. odocoilei* (at least 34 months) than *P. tenuis* (10 months; Lankester and Anderson 1968). The infectivity of *P. tenuis* was also considerably reduced by freezing (Table XII). Not only did just 5 percent of the *P. tenuis* larvae retain their infectivity, compared with over 85 percent for *P. odocoilei*, but those larvae of *P. tenuis* which had been frozen appeared delayed in their development from second-stage larvae to third-stage larvae. The data suggest that a susceptibility to freezing might be a means by which *P. tenuis* is prevented from expanding its range westwards.

To support the hypothesis that susceptibility to freezing may bar *P. tenuis* from the west, it must be assumed that winter is an important time for transmission of the parasite. It is obvious that infection of the intermediate host cannot occur in winter, but must await spring. An advantage accruing

Parelaphostrongylus in having larvae highly resistant to freezing would be that a winter's accumulation of first-stage larvae would survive until spring, and following snow-melt would flood the environment with massive numbers of larvae, increasing the chances for larval contact with molluscs. The results from this study (Table VIII) suggest that larval acquisition by molluscs might be facilitated under conditions of high larval density.

The necessity for infection of the intermediate host to occur in the spring has not been established. However, the strategy of releasing greatest numbers of first-stage larvae to the environment during the winter months appears to have been adopted by at least two species of metastrongylid nematodes. An increase in larval shedding by infected definitive hosts has been reported during the late winter and early spring months for Protostrongylus spp. (Forrester and Senger 1964; Uhazy et al. 1973) and P. odocoilei (Platt 1978; Samuel, unpub.). Platt (1978) discussed this phenomenon in relation to the epizootiology of P. odocoilei in Jasper National Park. He suggested that dispersal of mule deer during the summer months would tend to drastically reduce contact between larvae and molluscs, and infected molluscs and deer; additionally, larvae shed during the summer months would run serious risk of desiccation due to only sporadic rainfall at that time. The winter's accumulation of larvae, on the other hand, would be swept to the protection of the soil, surviving there to infect molluscs throughout the summer months. Lengthy survival of larvae in the soil would make it unnecessary that infection of the intermediate host occur only in the spring. Deer, returning to the wintering grounds in the autumn, would thus face densities of infected molluscs sufficient to result in acquisition of _P. odocoilei_ by many of the returning deer. There is evidence suggesting that fawns in Jasper do not acquire _P. odocoilei_ until they return to the wintering grounds (Samuel, unpub.).

Seasonal changes in larval output might be unimportant for the transmission of parasites possessing that characteristic. It may represent only a host-regulated phenomenon. The scheme suggested by Platt (1978) for the transmission of _P. odocoilei_ might be applicable to _P. tenuis_ only if the latter species has a seasonal fluctuation in larval output. Such information is lacking for _P. tenuis_. Until more information is available on the extent to which winter is important in the transmission of _P. tenuis_, the potential of its reduced freezing resistance to serve as a means for preventing its spread westwards can only be speculated on.

The present study can go no further than to suggest that at least two mechanisms exist by which climatic factors might influence the transmission of _P. odocoilei_ and _P. tenuis_ in a differential manner. One, a greater susceptibility to repeated desiccation, may serve to prevent the establishment of _P. odocoilei_ in areas which _P. tenuis_ could occupy. The other, a greater susceptibility of _P. tenuis_ to freezing (both continuous and repeated), might also serve to segregate the two species under certain circumstances.

LITERATURE CITED

Alibasoglu, M., D.C. Kradel and H.W. Dunne. 1961. Cerebral nematodiasis in Pennsylvania deer (Odocoileus virgineanus). Cornell Vet. 51:431-441.

Andersen, F.L. and N.D. Levine. 1968. Effect of desiccation on survival of the free-living stages of Trichostrongylus colubriformis. J. Parasitol. 54:117-128

Anderson, R.C. 1956. Elaphostrongylus odocoilei Hobmaier and Hobmaier, 1934 in the cranial case of Odocoileus virginianus borealis Miller. Can. J. Zool. 34:167-173.

_______. 1963. The incidence, development, and experimental transmission of Pneumostrongylus tenuis Dougherty (Metastrongyloidea:Protostrongylidae) of the meninges of the white-tailed deer (Odocoileus virginianus borealis) in Ontario. Can. J. Zool. 41:775-792.

_______. 1971. Metastrongyloid lungworms. In Davis, J.W. and R.C. Anderson (Eds.). Parasitic Diseases of Wild Mammals. The Iowa State University Press, Ames, Iowa. pp. 81-126.

Anonymous. 1973. Environment Canada (Atmospheric Environment): Canadian Normals. Vol. 1, Temperature (1941-1970); Vol. 2, Precipitation (1941-1970). Downsview, Ontario.

Arroyo, R. and P. Morera. 1978. Viability of the first stage larvae of Angiostrongylus costaricensis in rat feces. J. Parasitol. 64:146.

Balasingam, E. 1964. Comparative studies on the effects of temperature on free-living stages of Placoconus lotoris, Dochmoides stenocephala, and Ancylostoma caninum. Can. J. Zool. 42:907-918.

Behrend, D.F. and J.F. Witter. 1968. Pneumostrongylus tenuis in white-tailed deer in Maine. J. Wildl. Manage. 32:963-966.

Belle, E.A. 1959. The effect of microenvironment on the free-living stages of Bunostomum trigonocephalum. Can. J. Zool. 37:289-298.

Bhatt, B.D. and R.A. Rohde. 1970. The influence of environmental factors on the respiration of plant-parasitic nematodes. J. Nematol. 2:277-285.

Bindernagel, J.A. and R.C. Anderson. 1972. Distribution of the meningeal worm in white-tailed deer in Canada. J. Wildl. Manage. 36:1349-1353.

Bird, A.F. and M.S. Buttrose. 1974. Ultrastructural changes in the nematode Anguina tritici associated with anhydrobiosis. J. Ultrastruct. Res. 48:177-189.

Boddicker, M.L. and E.J. Hugghins. 1969. Helminths of big game mammals in South Dakota. J. Parasitol. 55:1067-1074.

Brown, T.T., Jr., H.E. Jordan and C.N. Demorest. 1978. Cerebrospinal parelaphostrongylosis in llamas. J. Wildl. Dis. 14:441-444.

Brunetti, O.A. 1969. Redescription of Parelaphostrongylus (Boev and Schuls, 1950) in California deer, with studies on its life history and pathology. Calif. Fish and Game 55:307-316.

Bullick, G.R. and J.E. Ubelaker. 1978. Ecological factors involved in survival of Parastrongylus costaricensis free-living larvae. Abstract No. 247, Annual Meeting of American Society of Parasitologists. Chicago Illinois, 5-10 November 1978.

Burch, J.B. 1962. How to Know the Eastern Land Snails. Wm. C. Brown Publishers, Dubuque, Iowa. 214 p.

Burns, M.E. 1964. Cryobiology as viewed by the microbiologist. Cryobiology 1:18-39.

Carpenter, J.R. 1940. The grassland biome. Ecol. Monogr. 10:617-684.

Carpenter, J.W., H.E. Jordan and J.A. Morrison. 1972. Meningeal worm (Parelaphostrongylus tenuis) infection in white-tailed deer in Oklahoma. J. Wildl. Dis. 8:381-383.

Collis-George, N. 1959. The physical environment of soil animals. Ecology 40:550-557.

Conder, G.A. 1978. The effect of UV radiation on survival of the free-living stages of Haemonchus contortus. Proc. helminthol. Soc. Wash. 45:230-232.

Crofton, H.D. 1963. Nematode parasite population in sheep and on pasture. Commonwealth Agric. Bur. Technical Comm. No. 35. 104 p.

Crowe, J.H. and K.A.C. Madin. 1975. Anhydrobiosis in nematodes: evaporative water loss and survival. J. exp. Zool. 193:323-333.

Davtian, E.A. 1949. [Larval development of lung nematodes of sheep and goats in Armenia]. Zool. Sbornik AN Arm. SSR Vol. 6. (cited by Morev, Yu. B. 1966) (In Russian).

DeGiusti, D.L. 1955. The occurence of a protostrongylid nematode in the meninges of Michigan deer. J. Parasitol. (Suppl.) 41:42.

Dougherty, E.C. 1945. The nematode lungworms (Suborder Strongylina) of North American deer of the genus Odocoileus. Parasitology 36:199-208.

Ellenby, C. 1968a. Desiccation survival of the infective larva of Haemonchus contortus. J. Exp. Biol. 49:469-475.

______. 1968b. The survival of desiccated larvae of Heterodera rostochiensis and H. schachtii. Nematologica 14:544-548.

______. 1969. Dormancy and survival in nematodes. Soc. Exp. Biol. Symposium No. 23. pp. 83-97.

Evans, A.A.F. and R.N. Perry. 1976. Survival strategies in nematodes. In Croll, N.A. (Ed.). The Organisation of Nematodes. Academic Press, London. pp. 383-424.

Forrester, D.J. and C.M. Senger. 1963. Effect of temperature and humidity on survival of first stage Protostrongylus stilesi larvae. Exp. Parasitol. 13:83-89.

______ and ______. 1964. A survey of lungworm infection in bighorn sheep of Montana. J. Wildl. Manage. 28:481-491.

Gibbs, H.C. 1973. Transmission of parasites with reference to the strongyles of domestic sheep and cattle. Can. J. Zool. 51:281-289.

Gordon, H. McL. 1948. The epidemiology of parasitic diseases, with special reference to studies with nematode parasites of sheep. Aust. vet. J. 24:17-45.

Gupta, S.P. 1961. The effects of temperature on the survival and development of the free-living stages of Trichostrongylus retortaeformis Zeder (Nematoda). Can. J. Zool. 39:47-53.

Hansson, I. 1974. Seasonal and environmental conditions affecting the invasion of mustelids by larvae of the nematode Skrjabingylus nasicola. Oikos 25:61-70.

Herlich, H. 1966. Effects of cold storage on the infectivity of third-stage larvae of the intestinal worm, Trichostrongylus colubriformis (Giles, 1892) Ransom, 1911. Proc. helminthol. Soc. Wash. 33:101-102.

Hobmaier, A. and M. Hobmaier. 1930. Life history of Protostrongylus (Synthetocaulus) rufescens. Proc. Soc. exp. Biol. Med. 28:156-158.

______ and ______. 1934. Elaphstrongylus odocoilei n. sp., a new lungworm in black tail deer (Odocoileus columbianus). Description and life history. Proc. Soc. exp. Biol. Med. 31:509-514.

Hsu, C.-K. and N.D. Levine. 1977. Degree-day concept in development of infective larvae of Haemonchus contortus and Trichostrongylus colubriformis under constant and cyclic conditions. Am. J. vet. Res. 38:1115-1119.

Karns, P.D. 1967. Pneumostrongylus tenuis in deer in Minnesota and implications for moose. J. Wildl. Manage. 31:299-303.

Kates, K.C. 1965. Ecological aspects of helminth transmission in domesticated animals. Am. Zoologist 5:95-130.

Kearney, S.R. and F.F. Gilbert. 1978. Terrestrial gastropods from the Himsworth Game Preserve, Ontario, and their significance in Parelaphostrongylus tenuis transmission. Can. J. Zool. 56:688-694.

Keilin, D. 1959. The problem of anabiosis or latent life: history and current concept. Proc. R. Soc. Lond. Ser. B 150:149-191.

Lankester, M.W. and R.C. Anderson. 1968. Gastropods as intermediate hosts of Pneumostrongylus tenuis Dougherty of white-tailed deer. Can. J. Zool. 46:373-383.

Levine, N.D. 1963. Weather, climate, and the bionomics of ruminant nematode larvae. Adv. vet. Sci. 8:215-261.

______. 1968. Nematode Parasites of Domestic Animals and of Man. Burgess Publishing Company, Minneapolis, Minn. 600 p.

______ and K.S. Todd, Jr. 1975. Micrometeorological factors involved in development and survival of free-living stages of the sheep nematodes Haemonchus contortus and Trichostrongylus colubriformis. A review. Int. J. Biometeor. 19:174-183.

Lorentzen, G. and O. Halvorsen. 1976. The biology of the free-living first stage larva of Elaphostrongylus rangiferi (Nematoda, Metastrongyloidea). Proc. Scand. Soc. Parasitology, 20-22 August, 1976. p. 461.

Matekin, P.V., E.S. Turlygina and N.M. Shalaeva. 1954. [Contribution to the biology of the larvae of protostrongylids of sheep and goats and epizootiology of protostrongylosis in Central Asia]. Zoologichesky Zhurnal 33:373-394. (cited by Forrester, D.J. and C.M. Senger 1963) (In Russian).

Mitskevich, V.Y. 1964. [Cycle of development of Elaphostrongylus rangiferi Miz. 1958]. In Boev, S.N. (Ed.). Parazity [illegible]el'skokh. Zhiv. Kazakh. Izdatel. Akad. Nauk. Kazakh. SSSR. [illegible]:49-60. (In Russian).

[illegible] S. 1978. Effect of relative humidity on the free-living stages of Strongyloides papillosus (Rhabdiasoidea:Nematoda). Experientia 34:580-582.

[illegible]ttles, V.F. and A.K. Prestwood. 1976. Experimental Parelaphostrongylus andersoni infections in white-tailed deer. Vet. Pathol. 13:381-393.

Nickel, S. 1960. Das Verhalten der freilebenden Erstlarve des kleinen Lungenwurmes Muellerius capillaris in Freiland und Labor. Angew. Parasit. I:88-92. (Abstract).

O'Brien, F.E.M. 1948. The control of humidity by saturated salt solutions. J. Sci. Instr. 25(3):73-76.

O'Roke, E.C. 1936. The lungworm situation in the white-tailed deer (Odocoileus virginianus borealis) in Michigan. Proc. North Am. Wildl. Conf. 3-7 February, 1936. pp. 473-478.

Perry, R.N. 1977a. Desiccation survival of larval and adult stages of the plant parasitic nematodes, Ditylenchus dipsaci and D. myceliophagus. Parasitology 74:139-148.

_______. 1977b. The water dynamics of stages of Ditylenchus dipsaci and D. myceliophagus during desiccation and rehydration. Parasitology 75:45-70.

Pigoń, A. and B. Weglarska. 1955. Rate of metabolism in tardigrades during active life and anabiosis. Nature 176:121-122.

Pillmore, R.E. 1956. Investigations of the life history and ecology of the lungworm, Protostrongylus stilesi. Federal Aid Div. Quart. Rep. Colorado Department of Game and Fish 47-70.

Platt, T.R. 1978. The life cycle and systematics of Parelaphostrongylus odocoilei (Nematoda:Metastrongyloidea), a parasite of mule deer (Odocoileus hemionus hemionus), with special reference to the molluscan intermediate host. Ph. D. Thesis. University of Alberta. 233 p.

_______ and W.M. Samuel. 1978a. A redescription and neotype designation for Parelaphostrongylus odocoilei (Nematoda: Metastrongyloidea). J. Parasitol. 64:226-232.

______ and ______. 1978b. Parelaphostrongylus odocoilei: life cycle in experimentally infected cervids including the mule deer, Odocoileus h. hemionus. Exp. Parasitol. 46:330-338.

Poole, J.B. 1954. Studies on the microenvironment of the free-living stages of nematodes. Ph. D. Thesis. McGill University. 115 p. (cited by Todd, K.S., Jr., N.D. Levine and C.C. Whiteside 1970).

______. 1956. Reaction to temperature by infective larvae of Nematodirus filicollis, Trichostrongylidae (Nematoda). Can. J. comp. Med. 20:169-172.

Prasad, D. 1959. The effects of temperature and humidity on the free-living stages of Trichostrongylus retortaeformis. Can. J. Zool. 37:305-316.

Prestwood, A.K. 1972. Parelaphostrongylus andersoni sp. n. (Metastrongyloidea:Protostrongylidae) from the musculature of white-tailed deer (Odocoileus virginianus). J. Parasitol. 58:897-902.

______ and V.F. Nettles. 1977. Repeated low-level infection of white-tailed deer with Parelaphostrongylus andersoni. J. Parasitol. 63:974-978.

______, ______ and F.E. Kellogg. 1974. Distribution of muscleworm, Parelaphostrongylus andersoni, among deer of the southeastern United States. J. Wildl. Dis. 10:404-409.

______ and J.F. Smith. 1969. Distribution of meningeal worm (Pneumostrongylus tenuis) in deer in the southeastern United States. J. Parasitol. 55:720-725.

Pursglove, S.R., Jr. 1977. Helminth parasites of white-tailed deer (*Odocoileus virginianus*) from New Jersey and Oklahoma. Proc. helminthol. Soc. Wash. 44:107-108.

Rogers, W.P. and R.I. Sommerville. 1963. The infective stages of nematode parasites and its significance in parasitism. Adv. Parasitol. 1:109-177.

Rojo-Vazquez, F.A. 1976. Effect of temperature on the development of pre-parasitic stages of *Trichostrongylus axei* and *T. colubriformis*. Rev. Iber. Parasitol. 36:249-255

Rose, J.H. 1957. Observations on the bionomics of the free-living first stage larvae of the sheep lungworm, *Muellerius capillaris*. J. Helminthol. 31:17-28.

Rössner, J. and R.N. Perry. 1975. Water loss and associated surface changes after desiccation in *Rotylenchus robustus*. Nematologica 21:438-442.

Rowe, J.S. 1972. Forest Regions of Canada. Department of Fisheries and the Environment, Canadian Forest Service. Publ. No. 1300. 172 p.

Samuel, W.M. and J.C. Holmes. 1974. Search for elaphostrongyline parasites in cervids from Alberta. Can. J. Zool. 52:401-403.

______ and D.O. Trainer. 1969. A technique for survey of some helminth and protozoan infections of white-tailed deer. J. Wildl. Manage. 33:888-894.

Schmidt, J.M., K.S. Todd Jr. and N.D. Levine. 1974. Moisture stress effects on survival of infective *Trichostrongylus colubriformis* larvae. J. Nematol. 6:27-29.

Senger, C.M. 1964. Lethal effect of ultraviolet radiation on free-living nematodirid larvae. J. Parasitol. 50 (3, Sec. 2):56.

Smith, H.J. and R. McG. Archibald. 1967. Moose sickness, a neurological disease of moose infected with the common cervine parasite, Elaphostrongylus tenuis. Can. Vet. Jour. 8:173-177.

Snedecor, G.W. and W.G. Cochran. 1967. Statistical Methods. The Iowa State University Press, Ames, Iowa. 593 p.

Sokal, R.R. and F.J. Rohlf. 1969. Biometry. W.H. Freeman and Company, San Francisco. 776 p.

Stock, T.M. 1978. Gastro-intestinal helminths in white-tailed deer (Odocoileus virginianus) and mule deer (Odocoileus hemionus) of Alberta: a community approach. M. Sc. Thesis. The University of Alberta. 111 p.

Thurston, D.R. and R.G. Strout. 1978. Prevalence of meningeal worm (Parelaphostrongylus tenuis) in white-tailed deer from New Hampshire. J. Wildl. Dis. 14:89-96.

Todd, K.S., Jr., N.D. Levine and P.A. Boatman. 1976. Effect of desiccation on the survival of infective Haemonchus contortus larvae under laboratory conditions. J. Parasitol. 62:247-249.

_______, _______ and B.N. Wagher. 1977. Effects of repeated desiccation on survival of infective Cooperia punctata larvae. J. Parasitol. 63:956-957.

_______, _______ and C.C. Whiteside. 1970. Moisture stress effects on survival of infective Haemonchus contortus larvae. J. Nematol. 2:330-333.

Turner, J.H. 1953. Viability studies on eggs and infective larvae of Nematodirus spathiger (Railliet, 1896), a trichostrongylid nematode of ruminants. Parasitology 39:589-598.

Uhazy, L.S., J.C. Holmes and J.G. Stelfox. 1973. Lungworms in the Rocky Mountain bighorn sheep of western Canada. Can. J. Zool. 51:817-824.

Winston, P.W. and D.H. Bates. 1960. Saturated salt solutions for the control of humidity in biological research. Ecology 41:232-237.

Worley, D.E. and C.D. Eustace. 1972. Prevalence of helminth parasites in mule deer from eastern Montana. Proc. helminthol. Soc. Wash. 39:135-138.

Appendix I. History of first-stage larvae used in this study. Information is arranged in the following order: 1) experiment number; 2) species of _Parelaphostrongylus_ and source; 3) host animal from which larvae on feces were obtained; 4) date feces were collected from the host animal; 5) the temperature at which the feces were stored; 6) the date larvae were recovered from the feces; and 7) the date the experiment was initiated.

Experiment #	Species	Host animal	Collected from deer	Temperature stored (^{o}C)	Recovered from feces	Exp't started
1	P.o.(J)	MD-7	29-Nov-77	8	29-Nov-77	30-Nov-77
2	P.o.(J)	MD-6,8	14-Dec-77	8	15-Dec-77	16-Dec-77
3	P.o.(J)	MD-?	?	8	22-Feb-78	1-Mar-78
	P.o.(V)	BTD-?	?	8	22-Feb-78	1-Mar-78
4	P.o.(J)	MD-?	?	-25	10-Mar-78	15-Mar-78
	P.o.(V)	BTD-?	?	-25	10-Mar-78	15-Mar-78
5	P.o.(J)	MD-1	Feb-76	-25	25-Oct-78	25-Oct-78
		MD-3	Dec-75	-25	26-Oct-78	26-Oct-78
6	P.o.(J)	MD-4	Mar-77	-25	25-Apr-79	1-May-79
	P.t.	WTD	early-78	-25	25-Apr-79	1-May-79
7	P.o.(J)	MD-?	?	-25	?	10-Feb-78
8	P.o.(J)	MD-?	?	-25	?	22-Mar-78
9	P.o.(J)	MD-1	Feb-76	-25	26-Apr-78	1-May-78
	P.t.	WTD	early-78	-25	26-Apr-78	1-May-78
10	P.o.(J)	MD-?	?	-25	?	21-Feb-78
11	P.o.(J)	MD-?	?	-25	?	1-Mar-78
12	P.o.(J)	MD-?	?	-25	?	23-Mar-78
13	P.o.(J)	MD-1	Feb-76	-25	26-Apr-78	3-May-78
	P.t.	WTD	early-78	-25	26-Apr-78	3-May-78
14	P.o.(J)	MD-1	Feb-76	-25	26-Jun-78	14-Aug-78
	P.t.	WTD	early-78	-25	26-Jun-78	14-Aug-78
15	P.o.(J)	MD-?	?	-25	?	16-Mar-78

Experiment #	Species	Host animal	Collected from deer	Temperature stored (°C)	Recovered from feces	Exp't started
16	P.o.(J)	MD-1	Feb-76	-25	26-Apr-78	2-May-78
17	P.o.(J)	MD-1	Feb-76	-25	26-Jun-78	14-Aug-78
18	P.o.(J)	MD-20	12-Dec-78	8	13-Dec-78	22-Dec-78
		MD-20	14-Nov-78	-25	13-Dec-78	22-Dec-78
	P.t.	WTD-25	12-Dec-78	8	13-Dec-78	22-Dec-78
		WTD-25	14-Nov-78	-25	13-Dec-78	22-Dec-78

Abbreviations used:

P.o.(J) - P. odocoilei of Jasper Park origin

P.o.(V) - P. odocoilei of Vancouver Island origin

P.t. - P. tenuis

MD - captive mule deer; number follows

BTD - captive blacktailed deer; number follows

WT - captive white-tailed deer; number follows

WTD - wild white-tailed deer

? - information not noted

Appendix II. Mean percent survival with 95% confidence limits for first-stage larvae of *P. odocoilei* at a variety of temperature and moisture combinations (Exp'ts 1,2).

Percent RH	Temperature (°C)	Days	Number of samples	Mean percent survival	95% confidence limits Lower	Upper
In water	5	6	4	96.8	93.2	99.1
		12	4	97.7	95.6	99.1
		124	2	71.1	0	100
	14	16	8	75.9	64.5	85.7
		32	4	70.5	33.9	96.0
		56	4	57.7	29.1	83.8
		87	4	41.5	6.7	82.3
		121	2	41.1	0	100
	26	4	8	91.7	83.4	97.3
		12	8	75.8	53.5	92.4
		31	4	48.4	22.7	74.7
		38	3	0.9	0	22.3
	36	2	4	96.3	90.7	99.4
		4	12	30.2	8.0	59.1
		7	4	0.2	0	4.2
		9	4	0	0	0
	48	1	4	57.4	0.3	100
		1.5	4	0	0	0
95	5	6	4	65.9	62.4	69.3
		14	8	80.7	71.8	88.3
		32	8	56.3	38.3	73.5
		72	4	62.0	34.7	85.7
		124	4	11.2	2.3	25.6
		142	4	7.9	3.7	12.9
	14	4	4	87.2	78.6	93.8
		14	4	77.5	70.6	84.0
		24	4	79.1	67.1	89.0
		33	4	54.9	35.2	73.8
		56	4	53.7	39.8	67.3
		87	4	41.9	33.9	50.2
		121	4	11.9	0	44.7
		154	4	1.2	0	10.9
	26	4	8	51.5	32.7	70.0
		10	4	4.1	3.4	4.8
		13	4	0	0	0
	36	2	4	0.2	0	2.8
		4	4	0	0	0

Percent RH	Temperature (^oC)	Days	Number of samples	Mean percent survival	95% confidence limits	
					Lower	Upper
95 (cont'd)	48	1	5	0.7	0	5.7
		2	4	0	0	0
85	5	6	4	89.0	71.3	98.8
		12	4	79.5	71.5	86.4
		32	8	47.1	31.5	62.9
		72	4	45.0	26.0	64.9
		124	4	18.7	9.4	30.2
		142	4	8.2	4.9	12.3
		175	4	1.5	0	6.7
	36	2	4	1.1	0	4.8
		4	4	0	0	0
	48	1	5	1.0	0	4.9
		2	4	0	0	0
75	5	9	8	88.2	59.2	100
		28	4	83.2	67.3	94.5
		75	4	59.5	36.4	80.6
		124	4	49.6	11.7	87.7
		175	4	24.7	1.5	63.5
		306	4	9.2	3.2	17.8
	14	14	4	84.0	75.6	90.9
		32	4	62.4	50.4	73.7
		59	4	57.8	43.1	71.8
		87	4	47.9	36.5	59.5
		121	4	20.2	0	69.7
		154	4	31.1	8.4	60.3
		194	4	12.1	6.9	18.4
		289	4	0	0	0
	26	4	8	67.2	58.5	78.0
		12	8	33.3	18.4	50.2
		18	4	34.6	1.8	81.3
		24	4	18.7	0	63.6
		31	4	7.1	0.7	19.3
		38	4	1.4	0	10.7
	36	2	4	1.0	0	4.5
		4	4	0	0	0
	48	1	5	0.1	0	1.0
		3	4	0	0	0

Percent RH	Temperature (°C)	Days	Number of samples	Mean percent survival	95% confidence limits Lower	95% confidence limits Upper
45	5	6	4	98.8	82.8	100
		12	4	98.5	97.6	99.2
		47	4	99.0	95.5	100
		124	4	96.5	91.4	99.3
		306	3	45.2	12.1	80.9
	14	14	4	91.5	87.4	94.9
		32	4	93.5	86.0	98.3
		87	4	94.2	89.9	97.4
		125	4	91.1	78.9	98.4
		194	4	50.9	45.3	56.4
		289	4	16.6	3.5	36.8
	26	3	4	85.2	74.1	93.6
		10	4	63.9	26.8	97.2
		14	8	73.1	55.1	87.8
		31	4	45.1	28.4	62.5
		46	4	26.8	9.3	49.4
		58	4	3.9	0	27.0
		68	4	0	0	0
	36	2	4	95.7	89.6	99.2
		4	8	24.7	11.6	40.8
		7	4	9.0	0.8	24.8
		9	4	0	0	0
	48	1	4	93.8	84.4	99.1
		3	4	11.4	1.8	27.8
		4	4	0.1	0	2.2
		5	4	0	0	0
20	14	14	4	90.7	85.3	94.9
		32	4	93.7	90.7	96.1
		87	4	95.6	88.7	99.4
		125	4	92.9	85.9	97.6
		194	4	52.2	44.9	59.5
		289	4	19.1	12.7	26.5
	26	3	4	94.4	87.7	98.5
		10	4	90.4	79.9	97.3
		14	8	74.2	58.8	87.1
		31	4	52.6	33.0	71.8
		46	4	30.7	20.2	42.5
		58	4	8.7	0	37.4
		68	4	0	0	0

Percent RH	Temperature (^{o}C)	Days	Number of samples	Mean percent survival	95% confidence limits	
					Lower	Upper
20 (cont'd)	36	3	4	89.4	84.3	93.6
		5	4	71.1	46.4	90.4
		7	4	52.7	5.1	97.1
		9	4	2.6	0	15.4
		12	4	0	0	0
	48	1	4	96.5	93.8	98.5
		3	4	32.2	13.5	54.5
		4	4	5.8	0	21.6
		5	4	5.3	0.8	13.4
		7	4	0.5	0	4.2