THE BIOLOGY OF RUMEN FLUKES (TREMATODA: PARAMPHISTOMATIDAE) IN MOOSE, *Alces alces L.*, IN NORTHWESTERN ONTARIO

BY

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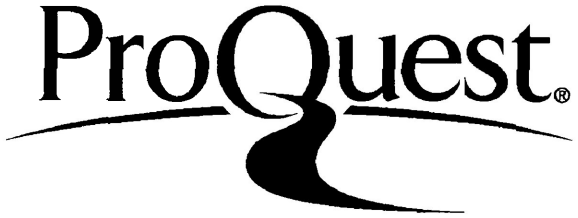

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ABSTRACT

Paramphistomes (*Paramphistomum sp.*) were found in 86% of 160 moose rumens collected from northwestern Ontario at all seasons of the year. All moose older than 2.4 years were infected. The number of flukes per moose ranged from 16 to 28,262 (median = 1,135). Their distribution in moose was overdispersed and approximated a negative binomial. The intensity of infection did not vary with age with the exception of calves (0.5 - 1.4 years) which had fewer flukes than older moose. Small, newly acquired flukes were first seen in the rumen of calves and older moose in October.

Few gravid flukes were found in moose over winter. The proportion of worms with eggs increased in March and April and 100% were gravid from May to July. Thereafter, the proportion gravid declined, reaching 0.5% in November. Similarly, few eggs were found in feces of wild moose during winter but the numbers began to rise in March and exceeded 100 eggs/g feces in July. By September, few eggs could be found. This annual fluctuation in the production of eggs is not related to seasonal transmission and life span of the flukes. Experimentally infected moose which had no opportunity for reinfection began to pass eggs in spring and stopped in the fall for up to 3 consecutive years following a single infection. This adaptation, which enables the parasite in a northern host to recognize favourable seasons, may compensate in part for the inability of paramphistome eggs to survive freezing.

Three captive moose given 3,000 to 11,750 paramphistome metacercariae in September began to pass eggs the following April (185-225 days). Another, not given metacercariae (9,000) until December, came patent in 169 days (May). One experimental animal given non-gravid flukes from a wild moose by rumenotomy in late February also began to pass eggs in April. No signs of disease due to

rumen fluke infections were seen in experimental animals.

A total of 7,910 aquatic snails representing 15 species were examined from 32 lakes and rivers thought to be used by moose. Only *Helisoma trivolvis* and *H. campanulatum* were shedding paramphistome cercariae (0.9% and 1.1% respectively). Paramphistome metacercariae were found attached to the ventral side of floating vegetation in 4 of 12 lakes investigated. The black, pin-head-sized metacercariae were present on vegetation from late June until late August and September when aquatic plants began to die. Attempts to infect *H. trivolvis* and *H. campanulatum* in the laboratory were unsuccessful.

Paramphistome eggs held at 11°C did not develop. When the temperature was raised to 19°C, eggs began to hatch after 30 days. Since lakes in northwestern Ontario do not reach 11°C before mid-May it is most likely that metacercariae appearing on aquatic vegetation in late June originate from snails infected in previous summers. Transmission to moose is probably greatest when they feed most intensively on aquatics from mid-June to mid-July.

ACKNOWLEDGEMENTS

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INTRODUCTION

Rumen flukes of the genus *Paramphistomum* Fischoeder, 1901 have been reported from moose (*Alces alces L.*) throughout much of the range of this host in North America (Anderson and Lankester 1974, Lankester *et al.* 1979). The identity of the species involved, however, has not been resolved. Reports in the literature generally refer specimens from moose to *P. cervi* (Zeder 1790) and those from white-tailed deer (*Odocoileus virginianus*) to *P. liorchis* Fischoeder, 1901. This practice implies strict host specificity but reports of both species in other wild and in domestic ruminants suggest otherwise (Price and McIntosh 1944, Sey 1980). During the course of the present study, specimens from moose in northwestern Ontario were sent to 3 experienced taxonomists and 3 different determinations resulted. A more detailed morphological study presently underway may help to clarify the problem. For the purpose of this work, however, it was necessary to assume that only one species of rumen fluke was involved and it is herein referred to as *Paramphistomum sp.*

A preliminary study by Lankester *et al.* 1979 revealed that most rumen flukes collected from moose in winter (November - February) were small and contained no eggs. Specimens from moose during summer (July and August) were larger and all were gravid. Intensity of infection did not appear to increase with the age of moose although accurate estimates of total numbers were not attempted. At least one calf became infected in its first summer of life. This evidence suggested that rumen flukes were picked up by moose during summer, possibly while feeding on aquatic vegetation. Worms did not mature until the following spring and died by September or October after a relatively short life span of about 16 months.

The commencement of egg production in March by flukes acquired the previous summer represents an unusually long prepatent period. Eggs of *P. cervi* were

passed by roe deer (*Capreolus capreolus*) 82-96 days after experimental infection and by sheep and cattle after 103-115 days (Kraneburg and Boch 1978). If moose became infected from mid-June to mid-July when aquatic feeding is most intensive (Cobus 1972, Fraser *et al.* 1984), gravid rumen flukes might be expected by November. This apparent delay in the maturation of flukes suggested to Lankester *et al.* (1979) that some environmental cue such as change in diet may signal favourable conditions and stimulate egg production in spring. Gupta *et al.* (1984) similarly reported that the maturation of *P. cervi* is delayed in sheep and apparently synchronized with appropriate meteorologic factors and the availability of the snail intermediate host in India.

The purpose of this study was to confirm observations made by Lankester *et al.* (1979) and to collect additional data which would test their hypotheses regarding the life-cycle of rumen flukes in moose. Specifically, the following questions were asked. Do rumen flukes only live about 16 months and does their life span and a restricted period of transmission explain the absence of gravid worms in moose over winter? Alternatively, are flukes long-lived? Does intensity increase with age of moose and can flukes in their host detect changes in season that cause egg production to stop in the fall and resume again when favourable conditions return the following spring? Efforts were also made to determine the species of aquatic gastropod important as intermediate hosts of rumen flukes in northwestern Ontario and how and when moose become infected. Finally, observations of experimentally infected moose and necropsy of wild moose killed by hunters or vehicles allowed an assessment of the parasite's pathogenicity.

METHODS AND MATERIALS

Examination of wild moose

The rumens from 160 moose were examined for flukes during the period 1976-1981. Most samples were obtained from moose killed by hunters or by vehicles. All moose came from an area of Ontario along the north shore of Lake Superior extending west to Turtle Lake (48°50'N., 92°40'W.), east to Lake Superior Provincial Park and north to Stevens (49°32'N., 85°49'W.).

Whenever possible, the sex of each animal was recorded and its age determined. Calves and yearlings were aged by the extent of tooth eruption and wear (Passmore *et al.* 1955). Calves were sometimes identified by their much smaller hoof size. Older animals were aged by staff of the North Central Region's ageing unit of the Ontario Ministry of Natural Resources. Age was determined by counting annual layers in the cementum of the first incisor tooth (Sergeant and Pimlott 1959).

Rumens were examined either fresh or after being frozen and thawed. They were opened mid-dorsally and the inner surface and contents inspected for flukes. Specimens were stored in 10% formalin or in a 10% solution of glycerin in 70% alcohol. In 1980 and 1981, the lining and contents of the omasum and abomasum were also searched for flukes. When present, the proximal 20 cm of the duodenum was opened and searched for immature flukes, sometimes by pressing between glass plates and holding to the light. Flukes found in these latter locations were stored separately.

Estimates were made of the total number of flukes present in the rumens of 60 moose in 1980 and 1981. In 1980 the volume of the contents of the rumen and reticulum were measured in a graduated polyethylene container. The contents were

then placed in a 200-L drum. Water was added to achieve a total volume 3 times that of the rumen and reticulum contents to facilitate thorough mixing. The material was stirred with a paddle and while stirring, 4, 1-L samples were taken by dipping a 1-L beaker. Each sample was washed in a sieve and stored in 70% alcohol. Flukes still attached to the rumen lining were washed off with a strong current of water along with the adhering vegetation. This material was brought to 15 L with water. While stirring vigorously, 4, 0.5-L samples were removed, washed in a sieve and stored in alcohol. In 1981, flukes and vegetation adhering to the rumen lining were washed off with a strong current of water. This was then sieved and the material (generally about 1 L or less) added to the extracted rumen and reticulum contents. The rumen and reticulum contents were then diluted and sampled as in 1980.

In both 1980 and 1981, samples of flukes were sometimes removed prior to sampling or sometimes a few flukes were found on the rumen wall after washing with the strong current of water. Flukes removed in this way were counted and added to the estimated count.

The subsamples of rumen material were later examined for flukes. Portions of the material were spread on gridded white enamel trays, immersed in water and carefully searched. Flukes recovered were counted and stored in 70% alcohol.

To determine whether the 4, 1-L rumen samples and 4, 0.5-L rumen lining samples were randomly selected, chi-square tests were run on each set of 4 samples. The chi-square tests indicated that 4 of the 59 sets of rumen samples and 2 of the 31 sets of rumen lining samples were non-randomly selected ($P = 0.05$). However, it is to be expected that if enough random sets of samples are drawn that eventually a set will be drawn that will test as non-random. The number expected can be calculated by the product of the probability level and sample size. In the

case of rumen samples, the expected number is 3.9 (0.05 x 59). The 3.9 is not significantly different from the observed 4 (X^2 = 0.186, df = 1, 0.50< P< 0.75). Similarly the observed 2 rumen lining samples that tested as being non-random are not significantly different from the expected 1.6 (0.05 x 31) (X^2 = 0.046, df = 1, 0.75< P< 0.90). It was concluded that the sampling methods did sample in a random manner.

Total number of flukes (N) in a rumen was estimated in 1980 by:

$$N = \left(\frac{\Sigma \text{ Rum SS}}{4} \times 3 \text{ Rum Vol}\right) + \left(\frac{\Sigma \text{ RumLin SS}}{4} \times 30\right) + \text{Others}$$

and in 1981 by:

$$N = \left(\frac{\Sigma \text{ Rum SS}}{4} \times 3 \text{ Rum Vol}\right) + \text{Others}$$

Where Rum SS is rumen subsample counts

Rum Vol is rumen-reticulum volume

RumLin is rumen lining subsample counts

Others is flukes collected prior to sampling

To determine the ratio of gravid to non-gravid flukes, up to 70 worms were arbitrarily selected from counted rumen subsamples. When 70 worms were not available, additional specimens were selected from the rumen lining subsample. In rumens that were not subsampled for population estimates, 70 worms were selected. Samples of less than 10 flukes were not used in analysis.

Flukes were dehydrated and cleared in graded alcohol and xylene series respectively and examined with a Bausch and Lomb dissecting microscope at 20 X magnification. If a fluke had one or more eggs in its uterus it was considered gravid.

To determine if the method of collecting flukes from the rumen influenced

the proportion gravid, flukes from 20 rumen subsamples were compared to flukes from the rumen lining subsamples from the same 20 moose. All 20 comparisons had a similar proportion gravid to non-gravid as tested by chi-square tests. These results justified the comparison of samples collected from rumen counts to those samples collected directly from the rumen.

Gravid and non-gravid flukes were rehydrated in an alcohol series and measured in 70% alcohol. Flukes to be measured were placed in a Petri dish, just covered with 70% alcohol, and placed on an overhead projector. Projected images were traced on a screen. A vinylite rule was placed in each projection to determine the degree of magnification. The projector was checked for distortion by placing the vinylite rule in different locations and no distortion was found. The curved length of rumen flukes was measured directly from the tracings using an electronic planimeter. A random sample of 53 flukes was measured for length while in a 70% alcohol solution prior to clearing in xylene, and were measured again after being returned to a 70% alcohol solution. The worms averaged 0.96 mm less in length, a significant difference ($t = 5.13$, $df = 102$, $P < 0.001$). The F ratio for the length measurements was 1.46, indicating the variances were not significantly different ($0.10 < P < 0.20$). Although the xylene did shrink the flukes, the low F ratios indicated that all worms were shortened a similar amount and therefore measurements would have value when comparing length of worms obtained at different times of the year.

Examination of moose feces for rumen fluke eggs

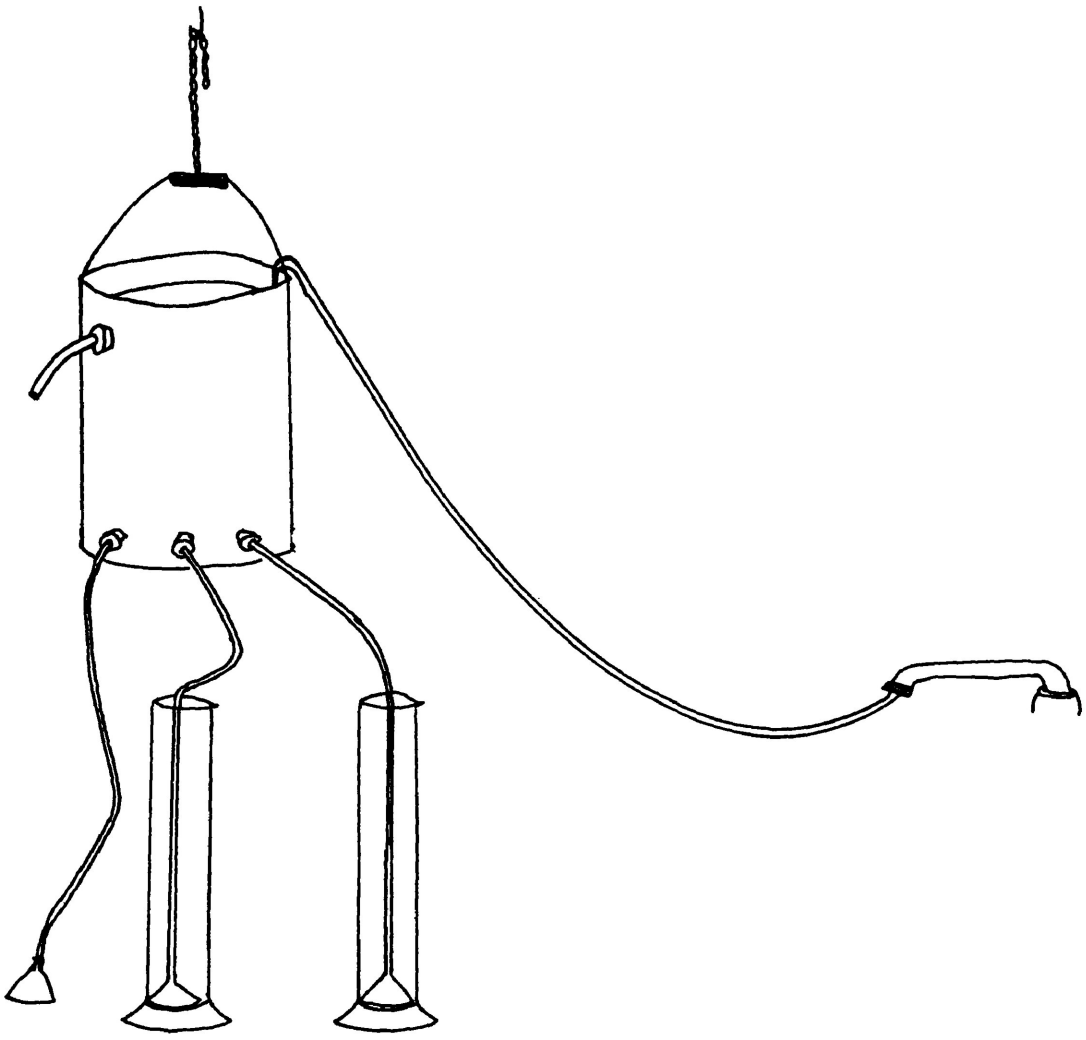
Fecal material deposited by free-ranging or experimentally infected moose and fecal material from the rectum of vehicle-killed and hunter-killed moose were examined for rumen fluke eggs using a sedimentation technique. Feces were examined while fresh or after being frozen and thawed. Twenty grams of feces

were macerated in 0.5 L of water and poured through a No. 80 Endicott sieve to remove larger particles. More water was added to the sieve and the material stirred until a 2-L graduated cylinder was filled. The paramphistome eggs, which easily passed through the sieve, were allowed to settle to the bottom of the cylinder. A tube with an opening of 1 mm diameter at its end was inserted into the stem of a plastic funnel. The diameter of the large end of the funnel was slightly smaller than the inside diameter of the cylinder. The funnel and attached tube were lowered to the bottom of the cylinder (Fig. 1). Water flowed through the tubing from an elevated header tank that could be adjusted in height to provide a flow of about 100 ml per minute. Light material floated up and out of the cylinder but the heavier eggs did not. Each sample was gently washed in this way for 4 to 8 hours before allowing eggs to settle and decanting the cylinder to a volume of 200 ml. The 200 ml sample was agitated and 3, 2 ml subsamples were removed with a pipette and examined in a gridded syracuse glass using a dissecting microscope at 20 X magnification. The number of eggs per gram of feces was estimated by:

$$\text{Eggs/g} = \frac{\text{Eggs in three, 2 ml subsamples}}{3} \times \frac{200 \text{ ml}}{20 \text{ g} \times 2 \text{ ml}}$$

The accuracy of the sedimentation technique was assessed by dividing a single fecal sample into 9, 20 g subsamples and 3, 1 g subsamples. The 9, 20 g subsamples were put through the sedimentation process. The estimated mean of the 9 subsamples was 372 eggs/g with a range of 232 to 470 and a standard deviation of 76.2 eggs/g. The 3, 1 g subsamples were each mixed in enough water to make it possible to count the eggs on a gridded syracuse glass. The counts obtained were 429, 519 and 402 eggs. There was no significant difference between the 3 direct counts and the 9 sedimentation estimates ($t = 1.59$, $df = 10$, $0.10 < P < 0.20$). Eggs were measured using a Wild microscope and drawing tube.

Fig. 1. Apparatus for sedimentation technique used to separate *Paramphistomum sp*. eggs from feces.



Examination of other ruminants for paramphistomes

Eleven (11) adult cattle (*Bos taurus*) and 4 adult goats (*Capra hircus*) killed at a local abattoir were examined for rumen flukes. Only animals pastured near forested areas were examined. The rumens of 5 adult white-tailed deer (*Odocoileus virginianus*) from areas also occupied by moose were examined for rumen flukes. Two samples of deer feces collected during July from Sibley Provincial Park were examined for fluke eggs.

Experimental infection of captive moose

Five moose calves (3 males and 2 females) were captured May 14th, June 13th, July 19th, 22nd and 23rd when they were from about 1-10 weeks old. Calves caught in May and June were given a formula of condensed milk diluted by half with water and with egg yolks added. Bottle feeding was supplemented with freshly-cut browse. By mid-August calves were weaned onto a solid diet of commercial, calf starter plus good quality alfalfa hay. Calves caught in July were placed on the above solid diet supplemented initially by freshly-cut browse. All moose were housed in large pens free of standing water and aquatic snails. In the third year, the moose were given access to a larger penned area with a stream running through one corner. No planorbid snails were seen in the stream.

One calf caught in July (No. 5) was given 10 tablets of Yomasum 20 (Niclosamide) to eliminate any migrating flukes on December 5, 1981. At this dosage the moose was given 142 mg of Niclosamide per kilogram of body weight. Horak (1971) found Niclosamide at 50-150 mg/kg body weight very effective against immature flukes not yet in the rumen. Flukes already in the rumen were not affected.

Two calves (No.'s 1 and 3) and 1 yearling (No. 4) were infected *per os* in

September and another calf (No. 5) in December with various numbers of rumen fluke metacercariae. Metacercariae for one infection (No. 1) came from naturally infected snails (*Helisoma trivolvis*) held in aquaria with the accumulated metacercariae being scraped off the aquaria walls. Metacercariae for moose infections No.'s 3, 4 and 5 came from Pickerel Lake where the metacercariae were scraped from the underside of water lily (*Nuphar variegatum*) leaves. Moose No. 2 was not infected until February 26th when it was given non-gravid flukes removed from the rumen of a freshly killed, wild, yearling moose. The worms were placed into the rumen by means of a rumenotomy. One holstein heifer was given metacercariae from naturally infected *Helisoma trivolvis* held in aquaria with the accumulated metacercariae being scraped off the aquaria walls.

Blood for hematological study was collected from the jugular vein of 2 infected moose and from another moose used as a control prior to its infection. Blood was also taken post-infection from the holstein heifer. Feces were collected regularly from all 5 moose and examined for paramphistome eggs using the sedimentation technique.

Examination of wild gastropods

Gastropods were examined from 32 lakes that were either known to be used by moose for aquatic feeding or were considered likely to be used for aquatic feeding. Emphasis was placed on the lakes in Sibley Provincial Park because of their frequent use by moose (Fraser and Hristienko 1983). Gastropods were collected by hand on wading through the shallow water from early June to mid-August. A small number of snails was collected from Pickerel Lake by skin diving in deeper water. Snails were placed in the dark for at least 8 hours before being examined for paramphistome infections following the method of Swart and Reinecke (1962). Most snails were tested in a growth chamber at 20°C. Snails

were placed in 250 ml jars in deionized water under 35 watt, cool, white, fluorescent tubes (40 cm above the jars) for 4 hours. Viewing the jars with a lighted background, the darkly pigmented cercariae or metacercariae could be seen easily with the naked eye. A few snails were tested at the collection site by placing them in direct sunlight in a cool location for 4 hours and examining as described above. Infected snails were kept as a source of metacercariae for other experiments.

Naturally infected snails that died in the laboratory were dissected and the different life stages of the paramphistomes drawn and measured using a Wild compound microscope and drawing tube. Paramphistome cercariae from naturally infected snails were killed in one of two ways before being measured. Cercariae from *H. trivolvis* were killed by the addition, dropwise, of 1N HCl until they ceased moving, and cercariae from *H. campanulatum* were killed by gentle heating on a microscope slide. Encysted paramphistome metacercariae for measuring were collected on a glass slide taped to the inside of an aquarium containing naturally infected snails or pried from the underside of water lily leaves from Pickerel Lake and placed on a glass slide.

Floating vegetation was examined for metacercariae in 12 lakes in July and August as an indicator of the presence of infected snails.

Ten floating styrofoam discs (7 cm diameter) covered with green cellophane were anchored in position in Pickerel Lake on June 9th and replaced with new discs at 2 week intervals throughout the summer until September 8th. Metacercariae encysted on the discs were counted using a dissecting microscope at 20 X magnification.

An experiment was conducted to determine whether cercariae encysted selectively on floating versus submerged vegetation. *Sparganium angustifolium*, which

grows vertically to the water surface with the upper portion floating on the water surface, was collected from Pickerel Lake. Each blade of the grasslike *Sparganium angustifolium* was divided into the submerged vertical portion and floating portion. The number of metacercariae was counted on both the submerged and floating portions. The lengths of each floating and each submerged portion were measured.

Experimental infection of snails

Paramphistome eggs from feces of an experimentally infected moose were collected using the sedimentation technique and incubated in finger bowls with distilled water at room temperature under a light regime of 12 hours light and 12 hours dark. When hatching of the eggs was first observed, the bowls were placed in the dark to prevent further hatching until snails were ready to infect. *Helisoma trivolvis* (1-5 mm diameter), hatched from eggs in the laboratory, were suspended in gauze cloth in the finger bowls for 1 to 5 hours. Snails were dissected at irregular intervals (usually about 1 per week) after being exposed to miracidia using a Bausch and Lomb dissecting microscope at 3 X. The viscera of the snails was pressed under a cover slip and examined for sporocysts using a compound microscope at 200 X.

Young *H. campanulatum* (2 weeks old and 1-2 mm in diameter), raised from eggs, were exposed to miracidia for 30 minutes. The snails were then removed with light forceps, placed in an aquarium and examined at 10 days and 41 days post-infection. Young *H. campanulatum* (9 weeks old and 5-6 mm in diameter), raised from eggs, were exposed to miracidia from eggs collected from a wild moose. These snails were examined 134 days post-infection.

A separate sample of *H. campanulatum* was kept in an aquarium for a 2 year period during which time paramphistome eggs were added to the aquarium from time to time. The aquarium walls were examined periodically for metacercariae.

Effect of temperature on hatching of paramphistome eggs

Feces were collected from an experimentally infected moose and eggs separated using the sedimentation technique. Eggs were divided into 9 finger bowls (15 cm diameter), each bowl containing several hundred eggs. Three finger bowls were kept at each of three temperatures (11°C, 19°C and 27°C) under a light regime of 12 hours of light and 12 hours dark. The finger bowls were checked regularly with a Bausch and Lomb stereo microscope at 20 X magnification and kept filled to a depth of 4 cm with distilled water. A sample of 20 to 30 eggs were examined in each bowl for development and the proportion of hatched eggs in the sample was recorded. Examination was completed quickly and the bowls returned to their respective locations to minimize temperature changes. One of the 3 finger bowls held at 11°C was transferred to 19°C on the 57th day of the experiment.

On August 18, 1981, a large sample of paramphistome eggs from an experimentally infected moose was placed in the dark at 11°C until May 19, 1982, and thereafter at room temperature (18°C) on a natural light regime. Eggs were examined periodically for development and hatching.

The effect of desiccation on the viability of paramphistome eggs

A fecal sample was collected from an experimentally infected moose and divided into 4, 20 g portions. Eggs were immediately separated from one portion using the sedimentation technique and kept in distilled water in a finger bowl. The eggs from this portion were placed in the laboratory at ambient room temperature (22°C) and a light regime of approximately 14 hours of light, 10 hours of darkness. The 3 experimental portions of fecal material were allowed to desiccate in open Petri dishes in a growth chamber at 19°C under 12 hours light, 12 hours dark and were removed after 8, 13 and 22 days. Eggs were collected by sedimentation and held in distilled water at room conditions. A Bausch and Lomb stereo microscope at 20 X

was used to check the development of miracidiae within the eggs and the proportion of eggs hatched.

The effect of freezing on paramphistome egg viability

Paramphistome eggs were removed from the feces of an experimentally infected moose using the sedimentation technique. The eggs, in distilled water, were divided into 6 equal samples. The control was placed in a finger bowl and kept in the laboratory at approximately 22°C. Five experimental samples were put in plastic bottles and frozen at -4 to -5°C. Samples were removed after 24 hours, 2, 4, 5 and 8 days, thawed at room temperature, and placed in finger bowls at control conditions. All samples were checked regularly for hatching and miracidia development.

All statistical analyses were made according to Zar (1974) and statistical significance was considered at $P \leq 0.05$.

RESULTS

Prevalence and intensity of paramphistomes in wild moose

The rumens of a total of 160 moose were examined from 1976 to 1981; 137 (86%) contained rumen flukes (Appendix 1). Ages were determined for 140 moose. Of this sample, none of 8 newborns (<2.5 months) was infected (Table 1). Seventy-two percent of calves (defined herein as animals 0.5 - 1.4 years) and 86% of yearlings (1.5 - 2.4 years) and all moose older than 2.4 years were infected. There was no significant difference in the prevalence of infection between calves and yearlings ($X^2 = 2.27$, $0.10 < P < 0.25$) but moose older than 2.4 years had a higher prevalence of infection than yearlings ($X^2 = 8.60$, $0.001 < P < 0.005$). The prevalence of infection in males and females was not significantly different ($X^2 = 0.11$, $0.50 < P < 0.75$).

Estimates of rumen fluke numbers were made on 60 wild moose rumens older than 0.4 years. There was a mean of 3,435 flukes in the 56 infected moose with a very large standard deviation of 5,650. The number of flukes was not normally distributed about the mean but was extremely overdispersed as shown in Fig. 2. A large proportion of the moose had low fluke numbers while 2 individuals had over 20,000 flukes each. The strong departure from a normal distribution made the use of parametric statistics invalid and consequently non-parametric statistics were used in analyses.

The median number of flukes was 1,135 (16 - 28,262, n = 56) (range followed by sample size). The use of a Kruskal-Wallis test indicated that there were differences in rumen fluke numbers (Table 2) among the various ages of moose ($H = 21.41$, $df = 4$, P 0.001). Calves had significantly less flukes than yearlings ($U = 173$, $P < 0.001$) and less than moose older than 1.5 years ($Z = 3.51$, $P < 0.001$) (Table 2). Older moose (4.5+) had significantly fewer flukes ($U = 44$, $P =$

TABLE 1. Prevalence of rumen flukes (*Paramphistomum sp.*) in wild moose of northwestern Ontario

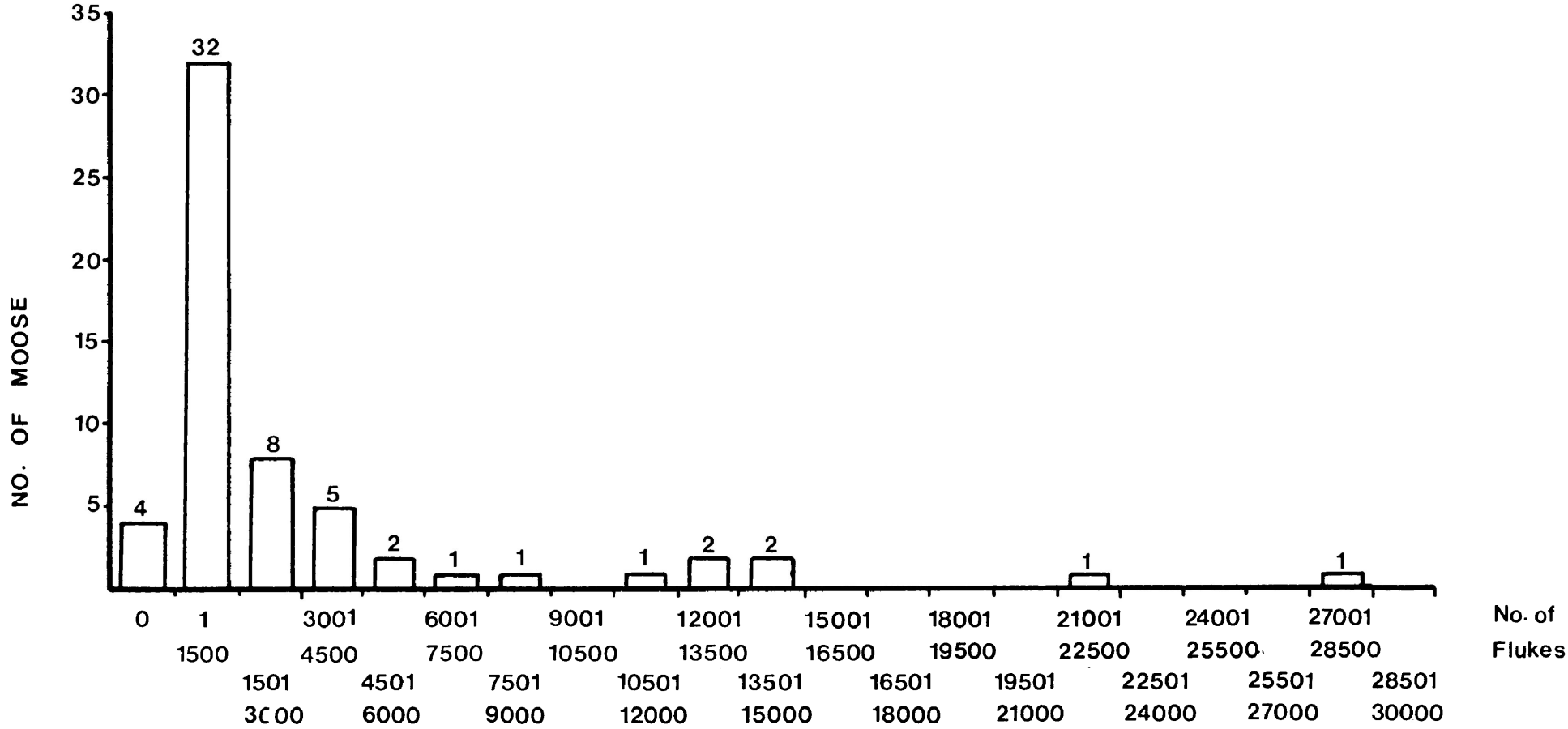
	Age of moose (yrs.)[1]				
	2.5 mths.	0.5-1.4 yrs.	1.5-2.4 yrs.	2.5 yrs.	Total no.
Males	0%(3)[2]	84%(19)	81%(21)	100%(25)	68
Females	0%(3)	56%(16)	93%(15)	100%(34)	68
Males and females combined[3]	0%(8)	72%(36)	86%(37)	100%(59)	140

[1] The birth date of all moose is assumed to be June 1st. Animals 2.5 mths. are referred to as newborns, age 0.5-1.4 yrs. as calves and age 1.5-2.4 yrs. as yearlings.

[2] Percent of the moose infected followed by the number of moose examined.

[3] The sex was not recorded for 4 moose which are included in this category. In addition, several moose not aged are omitted from this table.

Fig. 2. Number of rumen flukes from wild moose rumens showing dispersed distribution.


NO. OF MOOSE
35
30
25
20
15
10
5
32
8
5
4
2
1
1
1
2
2
1
1
0
1
1500
1501
3000
3001
4500
4501
6000
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25500
25501
27000
27001
28500
28501
30000
No. of
Flukes

TABLE 2. Estimates of intensity of rumen flukes (*Paramphistomum sp.*) in wild moose of northwestern Ontario

Age of moose	No. examined[1]	No. infected	Median no. of rumen flukes[2]	Range in no. of rumen flukes		
0.5-1.4	15	14	202	16-2,297	U = 167	P < 0.001
1.5-2.4	17	15	2,127	166-13,553	U = 95	ns
2.5-3.4	9	8	2,881	262-14,987	U = 26	ns
3.5-4.4	5	5	4,102	480-21,227	U = 44	P = 0.002
4.5	9	9	812	73-28,262		

[1] Five moose that were not aged are omitted from this table.

[2] A Kruskal-Wallis test indicated a significant difference in fluke numbers among the different ages ($H = 21.4$, $P < 0.001$). Mann-Whitney U tests were subsequently used on pairs of data and ages 0.5-1.4 and 1.5-2.4 were found to have significantly different numbers of flukes.

0.001) than moose aged 3.5 - 4.4 years (Table 2).

There was no significant difference between male calves with a median of 88 (n = 9) flukes and females with a median of 315 (n = 5) (U = 38, $P > 0.20$). Nor was there a significant difference between male moose aged 1.5 years and older with a median of 628 (n = 20) flukes and females with 470 (n = 20) (U = 155, $P > 0.20$).

Differences in intensity of infection between sampling years were not significant. Calves had a median of 37 (n = 5) flukes in 1980 and 402 (n = 9) in 1981. There was no difference in the intensity of infection between years (U = 35, $0.20 < P < 0.10$). Moose older than 1.5 years had a median of 2,133 (n = 26) in 1980 and 814 (n = 16) in 1981. There was no difference in intensity of infection between years (Z = 1.61, $0.20 < P < 0.10$).

Within the study area there were 7 localities corresponding to the different O.M.N.R. districts or management areas. No significant differences (H = 1.9, $0.90 < P < 0.75$) were found in the number of flukes among the 5 localities which contributed to counts for moose aged 0.5 to 1.4 years. There were also no significant differences in numbers of flukes among the 7 localities for moose aged ≥ 1.5 years (H = 1.8, $0.95 < P < 0.90$).

The effect of time of year on fluke numbers was examined by dividing the year into 3 seasons from January 1st to April 30th, May 1st to September 30th, and October 1st to December 31st. Calves had median counts of 1,981 (n = 2), 88 (n = 11) and 37 (n = 1) in these 3 seasons respectively. There were no significant differences among the 3 seasons (H = 5.2, $0.10 < P < 0.05$). Moose ≥ 1.5 years had median counts of 745 (n = 4), 1,196 (n = 28), and 2,133 (n = 10). There were no significant differences among the counts from the 3 seasons for moose aged ≥ 1.5 (H = 2.57, $0.50 < P < 0.25$).

Migration of paramphistomes in wild moose

None of 8 newborns examined between June 1st and August 1st had flukes in the rumen. Nor were migrating flukes seen in the duodenum, abomasum or omasum of the 3 newborns in which these organs were available for examination. Four of 8 calves collected in October had flukes in the rumen. Two of 4 specimens from the rumen of one of these calves were gravid and were a mean of 4.6 mm long and 1.2 mm wide; non-gravid specimens were 3.9 x 1.2 mm. The duodenum, abomasum and omasum were examined only in the 4 infected calves; 2 small migrating paramphistomes were found in the omasum of one.

In older moose, no migrating flukes were found in the duodenum, abomasum or omasum during summer (Table 3). A 9.5-year-old, female moose (No. 24, Appendix 1) collected October 18th had 20 small, thin, pink flukes in its omasum. A 3.5-year-old, male moose (No. 13) that was shot October 18th had 2 migrating flukes in its omasum. Small, thin flukes ($\leq$ 3.5 mm in length) resembling those found in the omasum were found in the rumen of some moose. These small individuals are presumed to have been acquired the previous summer and to have migrated recently from the duodenum. They were first seen in the rumen of 19 of 28 moose collected in October and were found in two calves as late in the year as April (Table 3). The small, thin flukes were absent from all moose examined May through September.

Small, thin flukes were present in 3 of 5 moose aged 0.5 to 1.4 years, 3 of 8 moose aged 1.5 to 2.4 years, 6 of 9 moose aged 2.5 to 3.4 years, and 10 of 19 moose older than 3.5 years. Different age moose did not have significantly different proportions of small, thin flukes ($X^2 = 1.55$, $0.50 < P < 0.75$).

Seasonal maturation of rumen flukes

Paramphistome eggs were absent in feces of wild moose in November and

TABLE 3. Numbers of wild moose with recently acquired[1] rumen flukes (*Paramphistomum sp.*) at different times of the year

Month	Locations in gastro-intestinal tract			
	Duodenum	Abomasum	Omasum	Rumen
January	----	----	----	1(5)
February	0(1)[2]	0(1)	0(1)	3(5)
March	----	----	----	0(5)
April	0(1)	0(1)	0(1)	2(4)
May	0(6)	0(8)	0(8)	0(18)
June	0(15)	0(20)	0(20)	0(26)
July	0(8)	0(9)	0(9)	0(11)
August	0(2)	0(1)	0(1)	0(4)
September	----	----	----	0(4)
October	1(8)	0(10)	3(9)	17(24)
November	0(2)	0(2)	0(2)	1(3)
December	----	----	----	1(1)

[1] Recently acquired flukes were short (< 3.5 mm) and thin.

[2] Number of a particular organ examined in parenthesis.

remained at or near 0 through December, January and February (Fig. 3). Egg counts rose in March and continued to increase exponentially, reaching a mean of over 100 eggs/g in July. By September and October, egg counts had dropped to low levels.

From 1976 to 1981, samples of flukes were obtained from moose rumens throughout the year and the proportion gravid determined. A mean of 69 specimens (range 10-178) were examined from each of 106 moose. The proportion gravid rose from 8% in March to 47% in April (Fig. 4). Almost 100% were gravid from May to July. Thereafter, the proportion of gravid flukes declined reaching 0.5% gravid in November.

Examining the sharp increase in the proportion gravid from March to May more closely (Fig. 5) it can be seen that of a group of 4 moose killed March 29, 1979 (killed as a group in a train collision), 3 had no gravid worms while 1 animal had 14% gravid. A cow and her calf killed April 4, 1979 had no gravid flukes. Ninety-nine percent of flukes obtained April 25, 1978 from an adult moose were gravid. Flukes from a calf moose killed April 21, 1980 were 87% gravid. Nearly all flukes from moose killed during May were gravid except for a yearling killed May 13, 1980 when only 51% were gravid.

Size of rumen flukes collected from wild moose

All flukes in moose 0.5 to 1.4 years will have been picked up during the animals' first summer of life whereas older moose may harbour flukes acquired over several summers. Examination of the size of the flukes from these 2 age groups might be expected to show that older moose have flukes of 2 or more sizes. For the purpose of analysis the year was divided into 4 seasons; November 1st to February 28th, March 1st to May 31st, June 1st to August 31st, and September 1st to October 31st. The few gravid flukes collected November to February were not significantly different in length from the non-gravid flukes (Table 4). Gravid

Fig. 3. Mean number (± 1 S.E.) of paramphistome eggs in feces of wild adult moose showing seasonal variation. Numerals represent sample size.

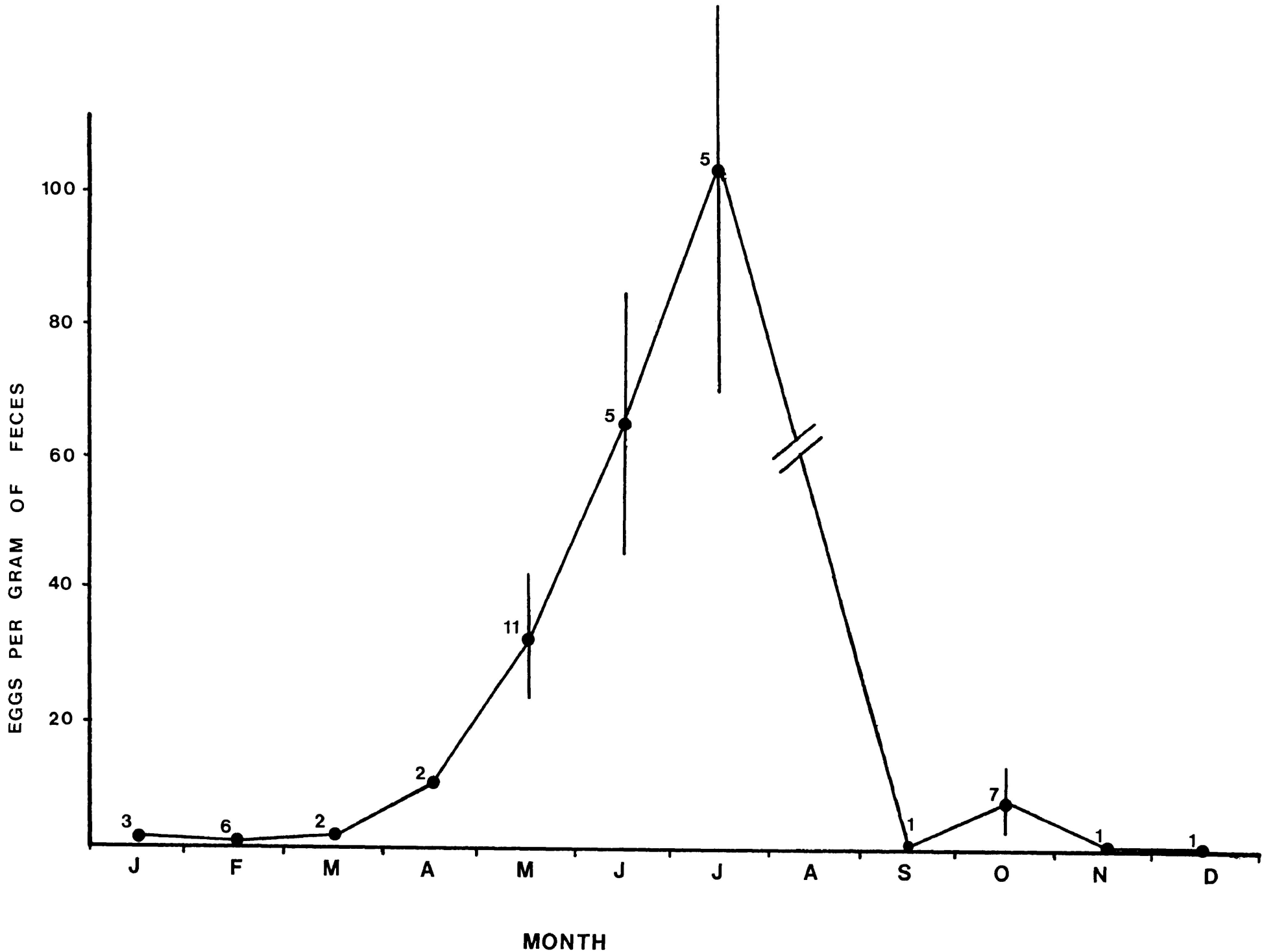

EGGS PER GRAM OF FECES
100
80
60
40
20
3
6
2
2
11
5
5
1
7
1
1
J
F
M
A
M
J
J
A
S
O
N
D
MONTH

Fig. 4. Mean proportion of gravid rumen flukes (± 1 S.E.) taken from wild moose showing seasonal variation. Numerals represent sample size.

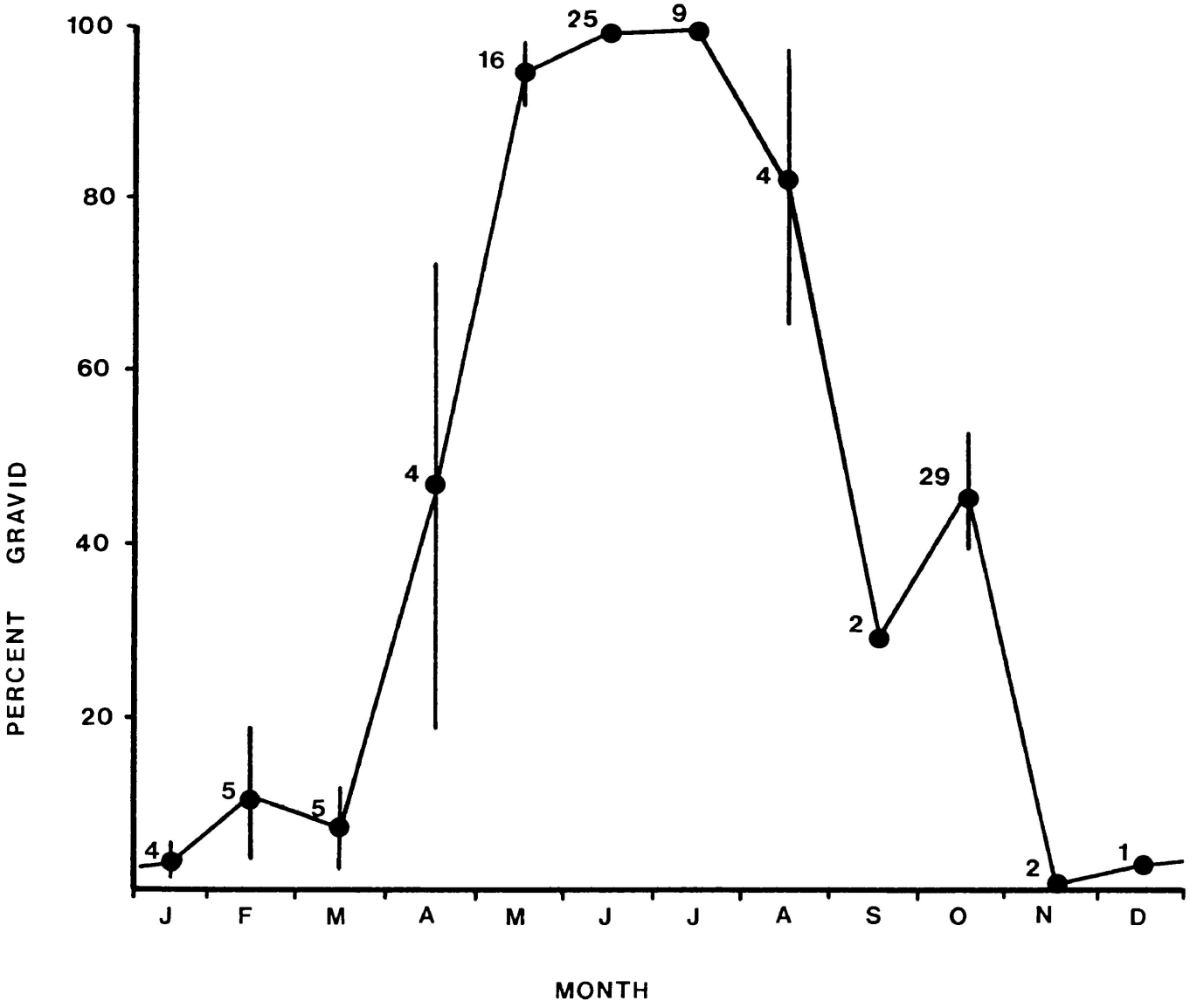

100
80
60
40
20
PERCENT GRAVID
4
5
5
4
16
25
9
4
2
29
2
1
J
F
M
A
M
J
J
A
S
O
N
D
MONTH

Fig. 5. Proportion of gravid rumen flukes taken from wild moose during the spring showing the spring maturation.

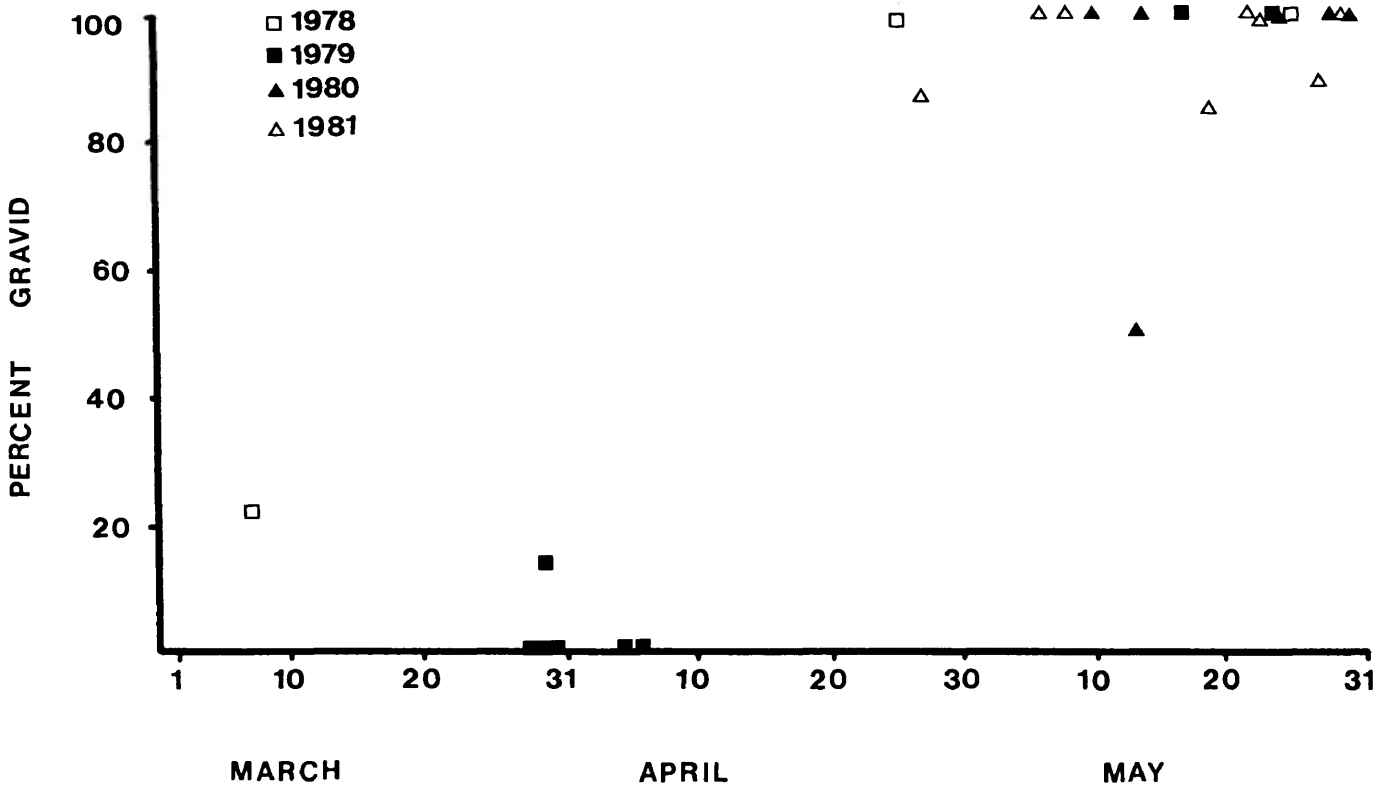

□ 1978
■ 1979
▲ 1980
△ 1981
PERCENT GRAVID
100
80
60
40
20
1
10
20
31
10
20
30
10
20
31
MARCH
APRIL
MAY

TABLE 4. Mean lengths (mm) of rumen flukes obtained from calf and adult moose at various seasons of the year

Month		Calves (0.5-1.4 years)		Adults (>1.4 years)	
Nov. - Feb.	gravid	3.80 ± 0.8[1] (n = 3)	$t^2 = 0.95$ ns	4.36 ± 1.0 (n = 9)	t = 0.21 ns
	non-gravid	4.53 ± 1.3 (n = 36)		4.44 ± 1.1 (n = 200)	
Mar. - May	gravid	5.21 ± 1.1 (n = 33)	t = 4.32 P < 0.001	5.40 ± 1.3 (n = 331)	t = 1.08 ns
	non-gravid	4.22 ± 0.7 (n = 33)		5.26 ± 0.8 (n = 111)	
June - Aug.	gravid	5.49 ± 1.0 (n = 106)		5.86 ± 1.3 (n = 616)	t = 3.81 P < 0.001
	non-gravid	------		6.84 ± 1.0 (n = 27)	
Sept. - Oct.	gravid	------		5.29 ± 1.6 (n = 264)	t = 9.91 P < 0.001
	non-gravid	4.3 (n = 2)		4.02 ± 1.3 (n = 248)	

[1] ± 1 standard deviation

[2] students t test

and non-gravid flukes from older moose examined March to May were also similar in length. The few non-gravid flukes found in older moose from June to August were significantly longer than gravid flukes (Table 4).

Flukes from calves sampled from November to February had a mean length of 4.47 ± 1.29 mm (n = 36) (± 1 standard deviation) and were not significantly longer ($t = 0.17$, $0.5 < P$) than flukes from adult moose (4.43 ± 1.05 mm, $n = 209$). Gravid flukes from calves (0.5 to 1.4 years) killed June to August were significantly shorter (5.49 ± 1.0 mm, n = 106) than those from older moose (5.86 ± 1.3 mm, n = 264) killed at the same time ($t = 2.82$, $0.002 < P < 0.005$). Most flukes obtained from November to February were non-gravid (96% from adults and 92% from calves) and flukes obtained from June to August were mostly gravid (96% from adults and 100% from young moose). Any differences between the length of worms in calves and older moose in these 2 periods are the least influenced by differences in size associated with the state of sexual maturation. The distributions of fluke lengths from calves and older moose in November to February were very similar (Fig. 6) with only one mode evident. The length distributions of gravid flukes collected from June to August also form a single mode for flukes from calves and from older moose (Fig. 7).

The mean length of 17 rumen flukes from an experimentally infected moose (No. 4) November 27, 1983 was 7.1 ± 1.1 mm which is significantly larger ($t = 9.85$, $P < 0.001$) than flukes from wild adult moose at this time of the year.

Experimental infection of captive moose

Four (4) moose approximately 4, 4, 6.5, and 16 months old were infected with 3,000 to 11,750 paramphistome metacercariae (Table 5). The viability of the metacercariae administered was not determined. The 4 moose infected in the fall or early winter (No's 1, 3, 4 and 5) all had rumen fluke eggs in their feces the

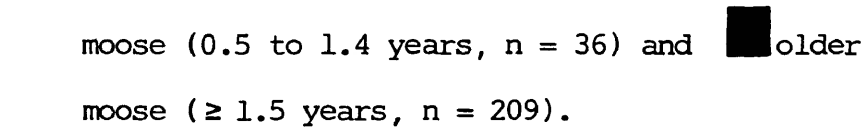
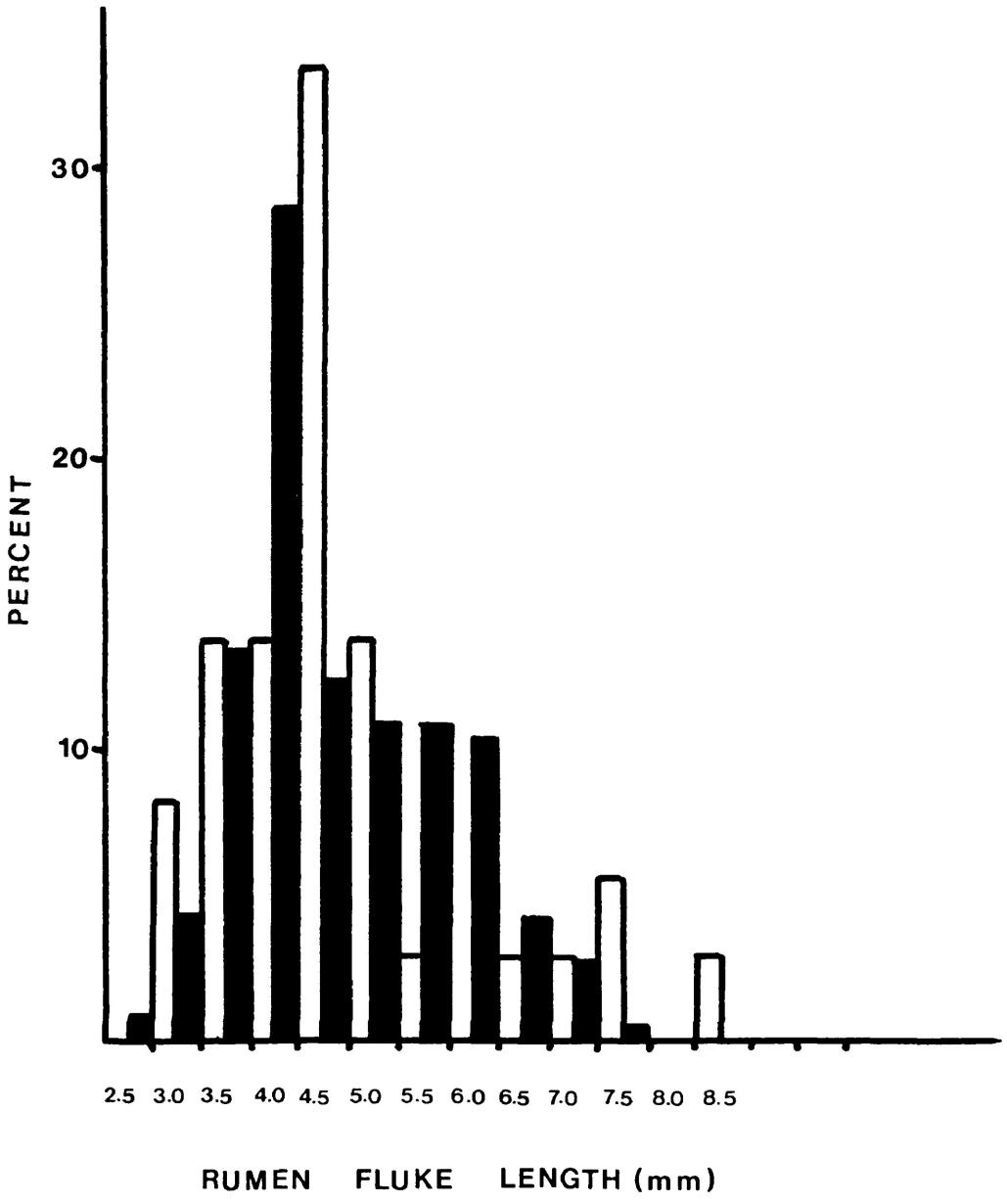


Fig. 6. Distribution of lengths of *Paramphistomum sp.* taken from the rumens of young and older moose obtained from November to February. ⊓ young moose (0.5 to 1.4 years, n = 36) and ■ older moose (≥ 1.5 years, n = 209).


30
20
10
PERCENT
2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0 8.5
RUMEN FLUKE LENGTH (mm)

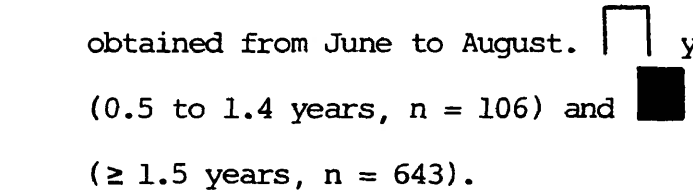


Fig. 7. Distribution of lengths of *Paramphistomum sp.* taken from the rumens of young and older moose obtained from June to August. ⌐¬ young moose (0.5 to 1.4 years, n = 106) and ■ older moose (≥ 1.5 years, n = 643).

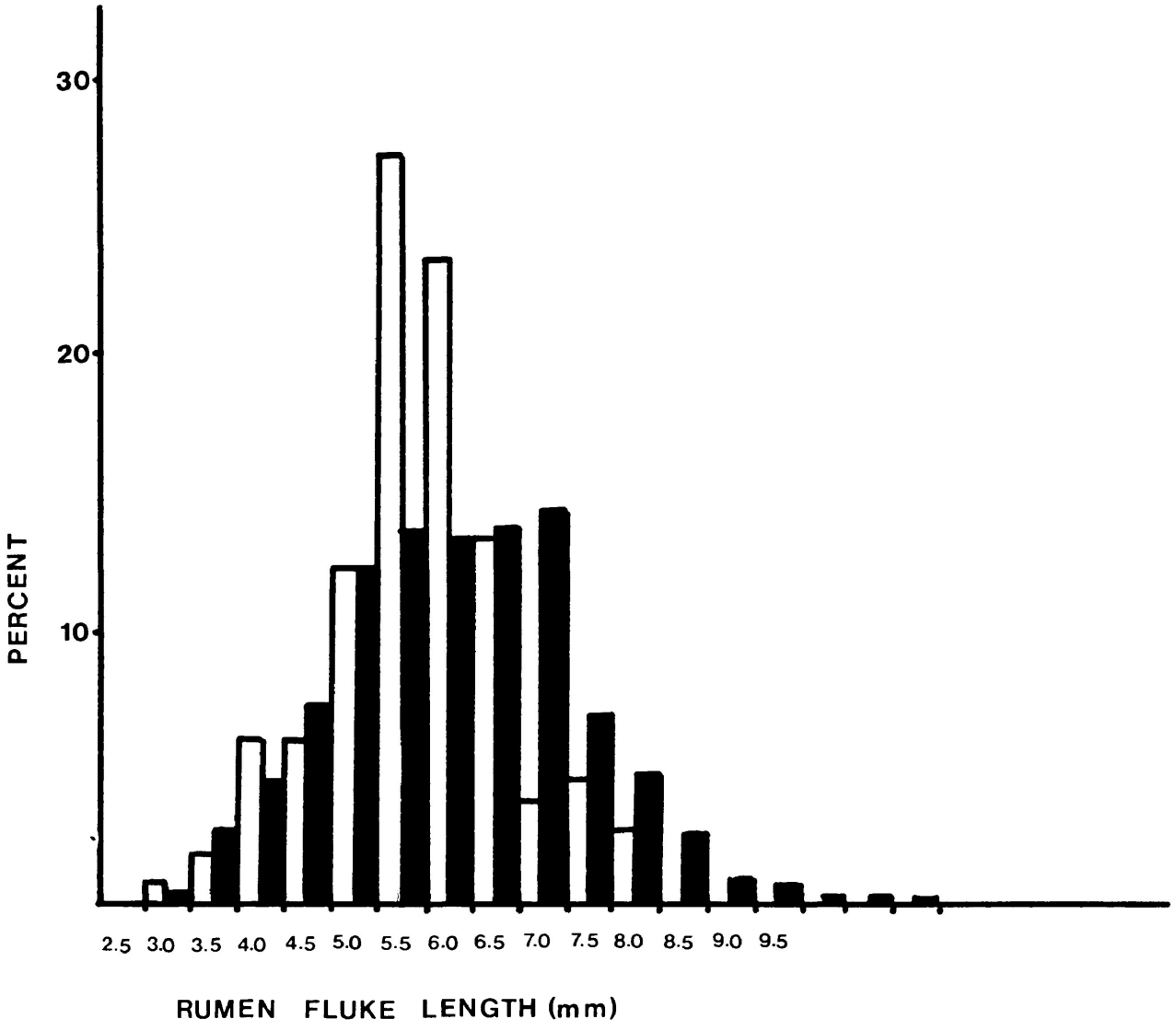

30
20
10
PERCENT
2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0 8.5 9.0 9.5
RUMEN FLUKE LENGTH (mm)

TABLE 5. Details of experimentally produced paramphistome infections in captive moose

Moose no.	Date of capture	Date of infection	Age of moose at infection	No. of metacercariae given	Animal terminated
1	23.07.80	29.09.80	4 months	11,750[2]	08.05.82
2	22.07.80	26.02.81	8 months	300[1]	12.06.82
3	14.05.81	11.09.81	4 months	3,000[3]	02.04.84
4	13.06.80	16.09.81	16 months	8,000[3]	27.11.83
5	19.07.81	12.12.81	6.5 months	9,000[3]	

[1] Approximately 300 rumen flukes (*Paramphistomum sp.*) taken live from a rumen of a wild moose and transplanted by rumenotomy to the rumen of experimental moose.

[2] Metacercariae from naturally infected *Helisoma trivolvis* collected from Slab Lake and maintained in aquaria.

[3] Metacercariae collected from vegetation on Pickerel Lake which contained infected *H. campanulatum*.

following spring. The prepatent periods varied from 169 days to 225 days (Fig. 8) and averaged 194 days. Although the moose infected December 12th came patent later in the spring than the others, it had the shortest prepatent period of 169 days. Moose No. 2, infected with non-gravid rumen flukes via rumenotomy in February, started to pass paramphistome eggs in April. Although highly unlikely, the possibility that 3 of the moose were infected prior to capture in July cannot be excluded. If this did occur in any of these 3 moose (No's 1, 2 and 5) the prepatent data would be affected but not the data pertaining to the longevity of the worm and also the seasonal fluctuation from gravid to non-gravid states.

Changes in the numbers of eggs passed in feces of all 5 experimentally infected moose (Fig.'s 9 and 10) followed the same seasonal pattern as seen in wild moose (Fig. 3). Egg levels rose in the summer and dropped to 0 over winter in 4 moose and to nearly 0 in the remaining moose. This pattern was repeated over 3 years for 2 moose, over 2 years for 2 others, and over 1 year for 1 moose. During this time there were no opportunities for reinfection.

Egg levels were compared for the months of May and June in year 1 and year 2 of the experimental infections. Four comparisons were possible involving 4 different moose. Egg counts were variable with the average number of eggs being slightly lower in year 2 than year 1. A paired t-test indicated no significant difference between years ($t = 0.36$, $0.50 < P$).

Moose No. 1 was terminated May 8, 1982. A population estimate of approximately 600 flukes was obtained. The proportion of gravid flukes was not determined for this moose. Moose No. 2 died June 12, 1982 of causes unrelated to the fluke infection. Approximately 12 flukes were obtained from this moose. The proportion gravid was not obtained. Experimental moose No. 4 was killed on November 27, 1983 by which time the fecal egg count had declined to 0. Seventeen (17) flukes were

Fig. 8. Prepatent period of paramphistome infections in moose experimentally infected with metacercariae.

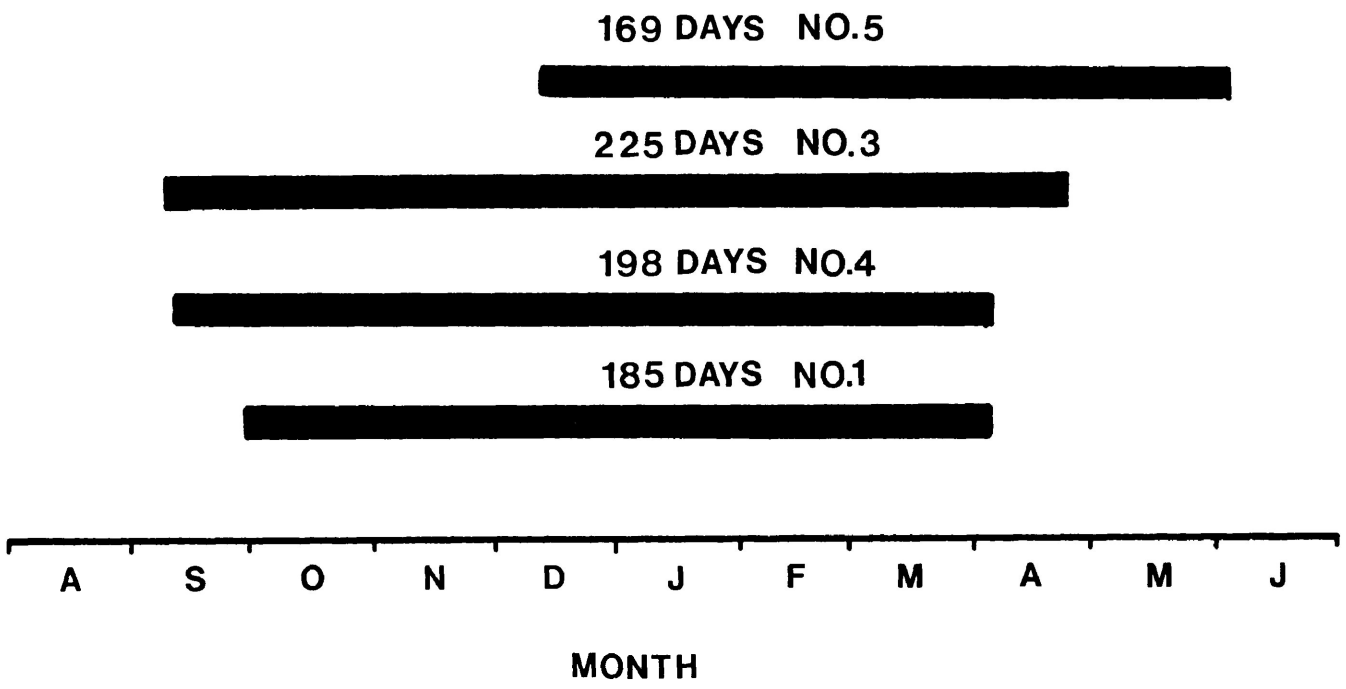

169 DAYS NO.5
225 DAYS NO.3
198 DAYS NO.4
185 DAYS NO.1
A
S
O
N
D
J
F
M
A
M
J
MONTH

Fig. 9. Number of *Paramphistomum sp*. eggs in feces of experimentally infected moose showing seasonal variation.

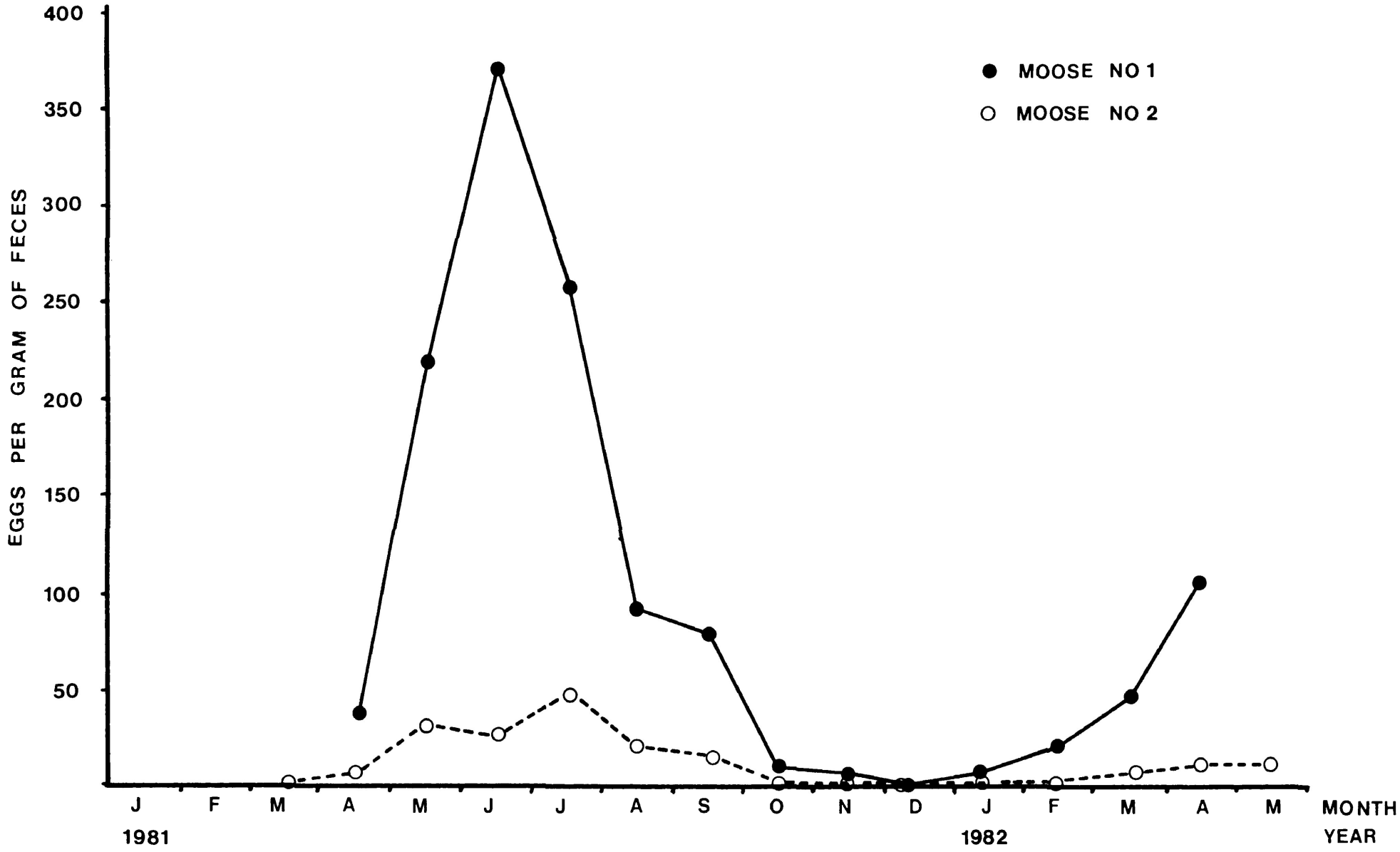
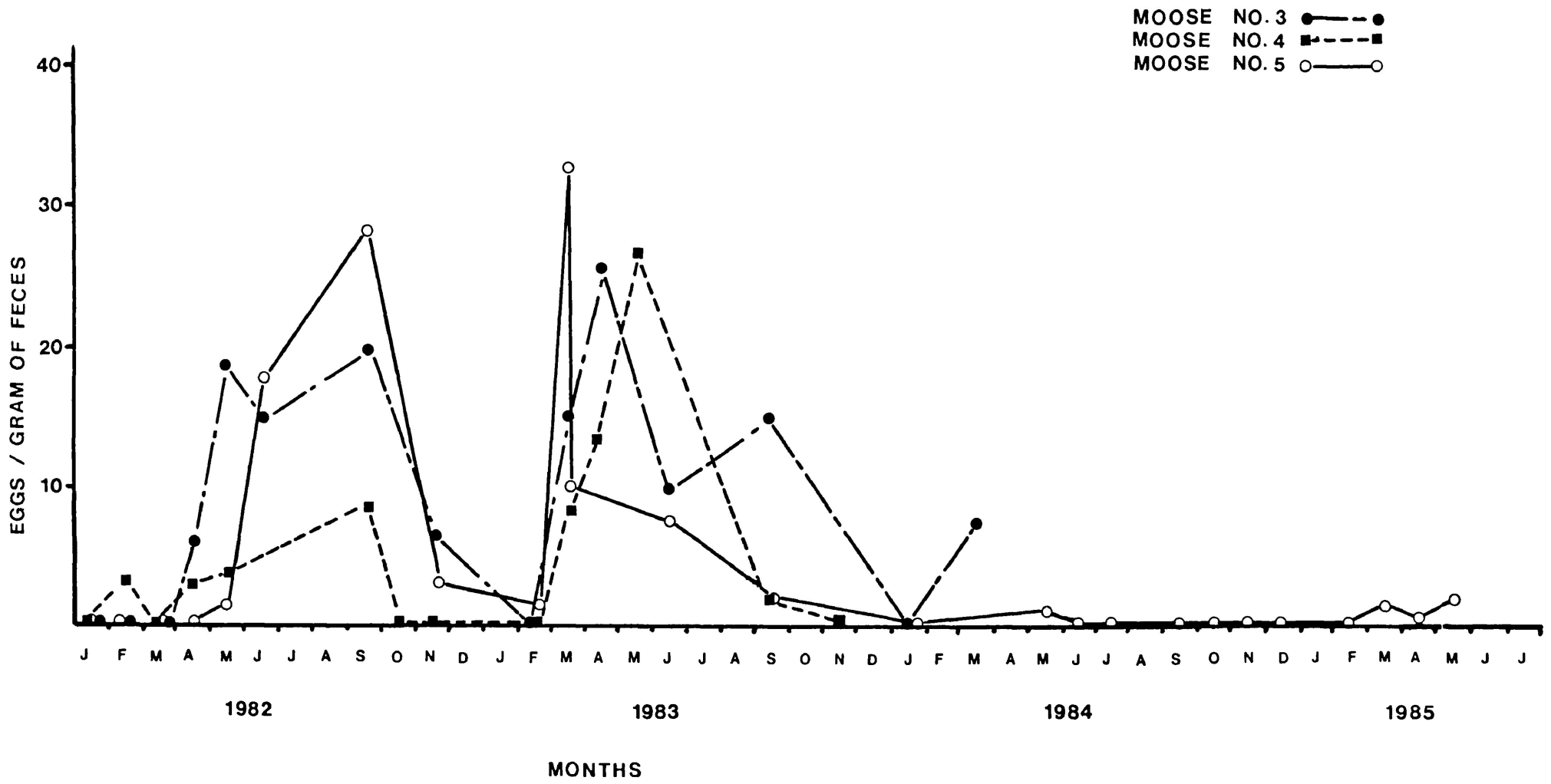

400
350
300
250
200
150
100
50
EGGS PER GRAM OF FECES
MOOSE NO 1
MOOSE NO 2
J
F
M
A
M
J
J
A
S
O
N
D
J
F
M
A
M
MONTH
1981
1982
YEAR

Fig. 10. Numbers of rumen fluke eggs in feces of 3 experimentally infected moose showing variation and longevity of the parasite. Samples taken at monthly or longer intervals.


MOOSE NO. 3
MOOSE NO. 4
MOOSE NO. 5
40
30
20
10
EGGS / GRAM OF FECES
J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J J
1982
1983
1984
1985
MONTHS

collected from this animal which probably represents the majority of flukes present. All 17 flukes were non-gravid. Experimental moose No.'s 3 and 5 were maintained through the winter. Neither moose passed eggs in January. Moose No. 3 had a fecal egg count of 3.3 eggs/g on March 20, 1984 and a count of 10.0 eggs/g on March 29, 1984. A total count of 161 flukes was obtained when this moose was terminated on April 2, 1984 and 91% of a subsample of 110 flukes were gravid.

As of May, 1985, moose No. 5 had been maintained for 3.5 years post-infection (Fig. 10). Eggs were produced through the summers of 1982 and 1983. In May of 1984 eggs were again present in the feces of this animal, however, there were no eggs in June through to February, 1985. Low numbers of eggs were again present in March, April, and May of 1985 (Fig. 10).

Examination of other ruminants and an attempted experimental infection of a heifer

During the study, the rumens of 5 white-tailed deer (*Odocoileus virginianus*) were examined for flukes and summer feces from 2 deer were also examined for rumen fluke eggs. Deer were collected off range occupied by infected moose. No evidence of rumen flukes was found in any of the deer samples. Eleven (11) adult cattle (*Bos taurus*) and 4 adult goats (*Capra hircus*) pastured in the Thunder Bay area were examined at a local abattoir; no rumen flukes were found.

A holstein heifer (4 months old, 100 kg) was given 11,750 metacercariae *per os* on September 29, 1980. Metacercariae were collected from naturally infected *H. trivolvis* obtained from Slab Lake and maintained in aquaria. No clinical signs of infection were observed and no flukes were found at necropsy 30 days later. Blood samples were taken on September 30th and October 3rd and 30th. Eosinophils increased from 3% September 30th to 12% October 3rd, and 11% on October 30th (Table 6). Total protein was 7.2 g/100 ml on all 3 dates while albumin levels were 2.7 g/100 ml September 30th and 2.9 g/100 ml both October

TABLE 6. Blood parameters of a moose and a holstein heifer each infected with 11,750 paramphistome metacercariae on September 29th

Date sampled	Total protein (g/100 ml)		Albumin (g/100 ml)		% Eosinophil	
	Moose No. 1	Holstein	Moose No. 1	Holstein	Moose No. 1	Holstein
September 30	6.4	7.2	3.9	2.7	0	3
October 3	6.0	7.2	3.6	2.9	2	12
October 10	---	7.2	---	2.9	-	11
November 5	6.3	---	3.9	---	2	--
November 19	6.6	---	3.7	---	1	--
November 27	5.5	---	3.2	---	1	--
December 11	6.0	---	---	---	1	--
December 19	6.1	---	4.6	---	0	--

3rd and 30th (Table 6).

Responses of moose to infection with paramphistome metacercariae

None of the 4 experimentally infected moose showed clinical signs of infection. Moose No. 1 infected with 11,750 metacercariae September 29th had albumin and total blood protein measured on 8 occasions between September 3rd and December 19th. Albumin levels ranged from 3.2 to 4.6 g/100 ml (Table 6) and total proteins ranged from 5.5 to 6.6 g/100 ml. Eosinophils measured as a percent of the number of white blood cells ranged from 0 to 2% (Table 6). Eosinophil counts were done on 2 other infected moose (No.'s 3 and 4) and 1 control moose (No. 5) (Table 7). Eosinophil counts, done as direct counts only, were obtained on 8 occasions on experimental moose No. 3 and ranged from 9 to 237 per mm^3 and the 1 count on experimental moose No. 4 was 131 per mm^3. The control moose (No. 5) was unusual in having eosinophil counts ranging from 25 to 1,128 per mm^3.

Paramphistomes attached to the wall of the rumen by drawing the base of a rumen papilla into the acetabulum. The distal two-thirds of the rumen papilla was lost leaving the basal stub to which worms were attached. The worms occurred in patches on the rumen wall and these areas were distinguished by the damaged papillae (Fig. 11). Most worms, however, would detach themselves (Fig. 12) from the rumen wall soon after the death of the moose. Attached rumen flukes or the characteristic papillae damage was only found in the rumenus atrium portion of the rumen (Fig. 13). When infections were heavy in wild moose the portion of the rumenus atrium denuded of papillae was greatest.

No other lesions of the rumenus atrium were noted in any of the 95 wild moose examined in 1980 and 1981 or in the experimental moose. No lesions attributable to immature paramphistomes were found in the duodenum of 10 moose killed in July and August or in 33 moose killed at other periods of the year.

TABLE 7. Numbers of eosinophils in 2 moose experimentally infected with paramphistome metacercariae

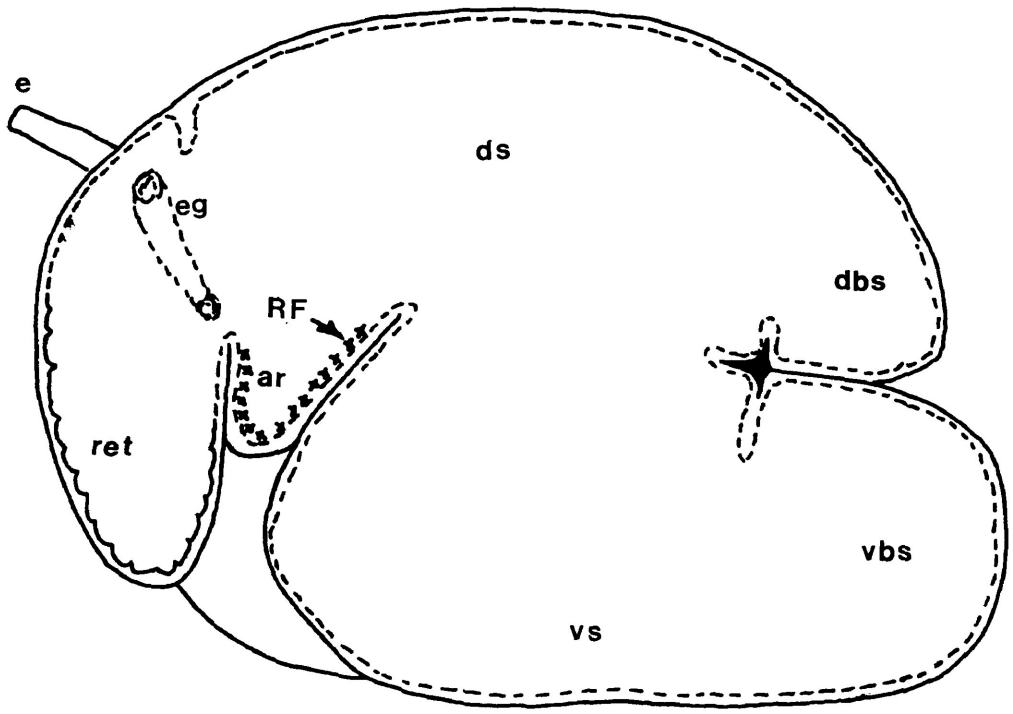
Date	Infected experimental moose		Not infected
	No. 3	No. 4	No. 5
September 18, 1981	150 mm^{-3}	---	300
September 25, 1981	237	---	37
October 2, 1981	12	---	25
October 16, 1981	9	---	312
October 23, 1981	220	---	920
October 30, 1981	72	---	1,128
November 11, 1981	140	---	---
November 27, 1981	140	131	106

Moose No. 3 infected with 3,000 metacercariae on September 11, 1981, moose No. 4 infected with 9,000 metacercariae on September 16, 1981.

Fig. 11. Damage to papillae of moose rumen by *Paramphistomum sp.*

Fig. 12. Most *Paramphistomum sp.* become detached from the rumen wall soon after the death of the moose.

Fig. 13. Location of rumen flukes shown in left lateral view of a wild moose rumen. RF = rumen flukes, ret = reticulum, ar = atrium rumenus, e = esophagus, eg = esophageal groove, ds = dorsal sac, dbs = dorsal blind sac, vs = ventral sac, vbs = ventral blind sac.


e
ds
eg
RF
dbs
ar
ret
vbs
vs

Prevalence of paramphistomes in wild gastropods

A total of 7,910 aquatic snails representing 15 species from 6 families were collected from 32 locations (Table 8) and tested for paramphistomes. *Helisoma trivolvis* and *Helisoma campanulatum* of the family Planorbidae were the only species found to be infected with paramphistome metacercariae (Table 8). The prevalence of paramphistome infections in *H. trivolvis* was 0.94% (n = 3,954) and in *H. campanulatum* 1.12% (n = 1,689). These rates of infection were not significantly different ($\chi^2 = 0.43$, $0.50 < P < 0.75$).

Helisoma trivolvis and *H. campanulatum* were both widely distributed in the study area (Table 8) with *H. trivolvis* occurring in 50% and *H. campanulatum* in 22% of the 32 lakes studied. Fifty-nine percent of the lakes contained either *H. trivolvis* or *H. campanulatum*. Infected *H. trivolvis* were found only at Slab Lake (Table 9) and infected *H. campanulatum* were found only at Pickerel Lake. Generally, samples of these 2 species were not large enough from the other lakes to exclude their being infected at rates statistically different from *H. trivolvis* in Slab Lake or *H. campanulatum* in Pickerel Lake. *Helisoma trivolvis* from Lake 24A and Joe Boy Lake were exceptions. A total of 505 *H. trivolvis* from Lake 24A were negative, which is significantly different ($\chi^2 = 6.94$, $0.01 < P < 0.025$) from the infection rate observed in Slab Lake. Similarly, all 285 *H. trivolvis* from Joe Boy Lake tested negative which is significantly different from the infection rate observed in Slab Lake ($\chi^2 = 3.91$, $0.01 < P < 0.05$). Insufficient numbers of *H. campanulatum* were collected to be able to state that there was a significant difference ($\chi^2 = 1.82$, $0.10 < P < 0.25$) in infection rate between *H. campanulatum* collected in Pickerel Lake and the combined total collected in the other lakes.

Paramphistome metacercariae were found encysted on floating vegetation in Pickerel Lake, Rita Lake, Grassy Lake and Bear Trap Lake. All 4 lakes contained

TABLE 8. Snail species in 32 lakes of northwestern Ontario and the prevalence of natural paramphistome infections

	No. examined	No. infected	% infected	No. of lakes with snail present
Planorbidae				
Helisoma trivolvis	3,954	37	0.94	16
Helisoma anceps	341	0	0.00	12
Helisoma campanulatum	1,689	19	1.12	7
Helisoma corpulentum	21	0	0.00	1
Planorbula armigera	139	0	0.00	1
Gyraulus deflectus	2	0	0.00	2
Gyraulus parvus	55	0	0.00	3
Promenetus exacuous	10	0	0.00	3
Lymnaeidae				
Lymnaea stagnalis	731	0	0.00	18
Bulimnea megasoma	129	0	0.00	4
Physidae				
Physa gyrina	459	0	0.00	20
Physa jennessi	289	0	0.00	7
Hydrobiidae				
Amnicola limosa	53	0	0.00	4
Valvatidae				
Valvata sincera	4	0	0.00	1
Succineidae				
Succinea ovalis	34	0	0.00	3
Total	7,910	56		

TABLE 9. Prevalence of paramphistome infections in *Helisoma trivolvis* and *H. campanulatum* from lakes in northwestern Ontario, 1980 and 1981

Lake	*H. trivolvis*		*H. campanulatum*		All other snail spp.	
	No. examined	No. infected	No. examined	No. infected	No. examined	No. infected
Slab Lake	2,727	37	-----	--	318 (9)[1]	0
Pickerel Lake*	195	0	1,543	19	414 (3)	0
Joe Boy Lake	285	0	-----	--	390 (3)	0
Pounsford Lake	-----	--	-----	--	2 (2)	0
Rita Lake*	28	0	33	0	3 (1)	0
Lake 24A	505	0	5	0	90 (4)	0
Lake 7B	-----	--	-----	--	262 (3)	0
Kay Lake	1	0	-----	--	1 (1)	0
Gardiner Lake	4	0	-----	--	11 (4)	0
Ravine Lake	-----	--	-----	--	32 (2)	0
Surprise Lake	-----	--	-----	--	57 (3)	0
Sibley Creek	105	0	-----	--	158 (4)	0
Lizard Lake	20	0	-----	--	35 (2)	0
Grassy Lake*	22	0	34	0	62 (4)	0
Lake 15D	-----	--	-----	--	80 (1)	0
Lake 15A	7	0	-----	--	43 (3)	0
Sawbill Lake	-----	--	-----	--	3 (1)	0
Marie Louise Lake	39	0	-----	--	10 (3)	0
Addison Lake	-----	--	-----	--	43 (5)	0
Beaver Pond #1	8	0	-----	--	24 (2)	0
Beaver Pond #2	6	0	-----	--	--- (0)	-

TABLE 9. (Cont'd)

	H. trivolvis		*H. campanulatum*		All other snail spp.	
	No. examined	No. infected	No. examined	No. infected	No. examined	No. infected
Blend Lake	-----	--	-----	--	54 (3)	0
Moose Pond Lake	1	0	-----	--	14 (2)	0
Bear Trap Lake*	-----	--	17	0	15 (2)	0
Elbow Lake	-----	--	-----	--	22 (2)	0
Henderson Lake	-----	--	-----	--	21 (1)	0
Savanne Lake	-----	--	-----	--	49 (2)	0
Dexter Lake	-----	--	25	0	8 (1)	0
Location 'A'	-----	--	32	0	9 (1)	0
East Dog River	-----	--	-----	--	21 (1)	0
Matawin River	1	0	-----	--	--- (0)	-
Pasture Stream	-----	--	-----	--	16 (2)	0
TOTAL	3,954	37	1,689	19	2,267	0

[1] Number of different species examined.

* Indicates lakes in which paramphistome metacercariae found on vegetation.

H. campanulatum and 3 of them contained *H. trivolvis*. Only 12 of the 32 lakes were examined for metacercariae. No metacercariae were found in Joe Boy Lake, Addison Lake, Lake 24A or Lake 15A. Floating discs were placed in good *H. campanulatum* habitat in Pickerel Lake and checked (Table 10) at regular intervals for metacercariae in order to determine seasonal development patterns. The first metacercariae appeared on the discs between June 24 and July 8, 1983. The first snail found to be producing cercariae was collected from Pickerel Lake on June 27, 1981. No metacercariae were found on the floating discs between August 6th and 16th. Between August 16th and September 8th metacercariae were found on the discs. Water temperatures were very high in August of 1983 and *H. campanulatum* were difficult to find at this time. In 1981, infected *H. campanulatum* that were shedding cercariae were collected from Pickerel Lake as late as August 25th.

The leaves of *Sparganium angustifolium* grow vertically to the water's surface and then float horizontally. This characteristic allowed a check of the distribution of metacercariae on the submerged portion of the leaf versus the floating portion. The underside of the floating portions of *Sparganium angustifolium* collected from Pickerel Lake on August 25, 1981 had a mean of 0.60 ± 0.722 (s.d.) metacercariae per cm^2 of leaf area as measured on 21 stems equalling 338 cm^2 in area. Submerged vertical portions of *Sparganium angustifolium* had a mean of 0.02 ± 0.034 metacercariae per cm^2 of leaf area as measured on 21 stems equalling 806 cm^2 in area. The number of metacercariae per cm^2 between the floating and submerged portions of the plant was significantly different ($t = 3.49$, $0.001 < P < 0.002$).

From examination of water lily leaves and floating discs it was apparent that metacercariae were not uniformly spaced across the bottom of these floating objects but were concentrated on the periphery.

TABLE 10. Seasonal occurrence of paramphistome metacercariae in Pickerel Lake in 1983

Time period	No. of metacercariae on floating discs	Presence of metacercariae on floating vegetation	Surface water temperature[1]
June 9	----	Absent	15°C
June 9 to June 24	0.0 (n = 10)	Absent	26°C
June 24 to July 8	3.9 ± 4.6 (n = 10)[2]	Present	24.5°C
July 8 to July 22	4.6 ± 4.1 (n = 10)	Present	25.5°C
July 22 to August 6	0.3 ± 0.6 (n = 3)	Present	27°C
August 6 to August 16	0.0 (n = 8)	Present	23°C
August 16 to September 8	7.7 ± 13.1 (n = 10)	Present	21.5°C

[1]Water temperatures were higher than average in 1983.

[2]Mean number of metacercariae on each 0.7 cm diameter disc followed by ± 1 S.E. with number of discs in parenthesis. Discs were collected and replaced with new discs on each check date. On 2 dates several discs were partially submerged and were eliminated from the sample.

Paramphistome morphometrics

Naturally infected *H. campanulatum* that died in the laboratory were examined. In each specimen the digestive gland tissue had been largely replaced by rediae and immature cercariae. In some of the rediae, daughter rediae were observed (Fig. 14). Developing cercariae, some with eye spots, could be observed inside the rediae. The mean curved length of rediae was 966µm (range 475µm to 1,550µm, n = 22). Many cercariae had emerged from rediae and were free in the digestive gland.

Cercariae emerging from *H. trivolvis* were killed by the addition, dropwise, of 1N HCl while cercariae from *H. campanulatum* were killed by gentle heating. Cercariae from *H. trivolvis* and *H. campanulatum* were similar in length ($t = 2.04$, $0.05 < P < 0.1$), being a mean of 731.5µm (n = 8) and 667.5µm (n = 12) long respectively (Table 11). Cercariae from *H. trivolvis* and *H. campanulatum* were also similar in width ($t = 0.22$, $0.50 < P$) being 354.4µm (n = 8) and 352.1µm (n = 12) respectively (Table 11). Cercariae from *H. trivolvis* had a shorter tail (613.4 n = 8) than those from *H. campanulatum* (927.7µm, n = 12) ($t = 9.33$, $P < 0.001$). However, the two methods of killing cercariae may differentially affect their morphometrics and comparisons of this sort may not be valid.

The metacercaria cyst is dome-shaped, transparent and is expanded at the base where it attaches to the substrate. Metacercariae curled inside retain their dark brown colour but have lost their tails. The cysts which appear as small, black dots to the naked eye are firmly attached to the substrate and can be felt with the fingertip. Metacercariae from naturally infected *H. trivolvis* and *H. campanulatum* were observed in the laboratory. Metacercariae from *H. campanulatum* in the laboratory were not measured but those on vegetation in Pickerel Lake and assumed to have come from *H. campanulatum* were measured. The diameter of the expanded base of the metacercaria cyst from *H. trivolvis* (442.1µm) was signifi-

Fig. 14. Redia of *Paramphistomum sp.* taken from the digestive gland of a naturally infected *Helisoma trivolvis* showing daughter redia. dr = daughter redia, c = immature cercaria.

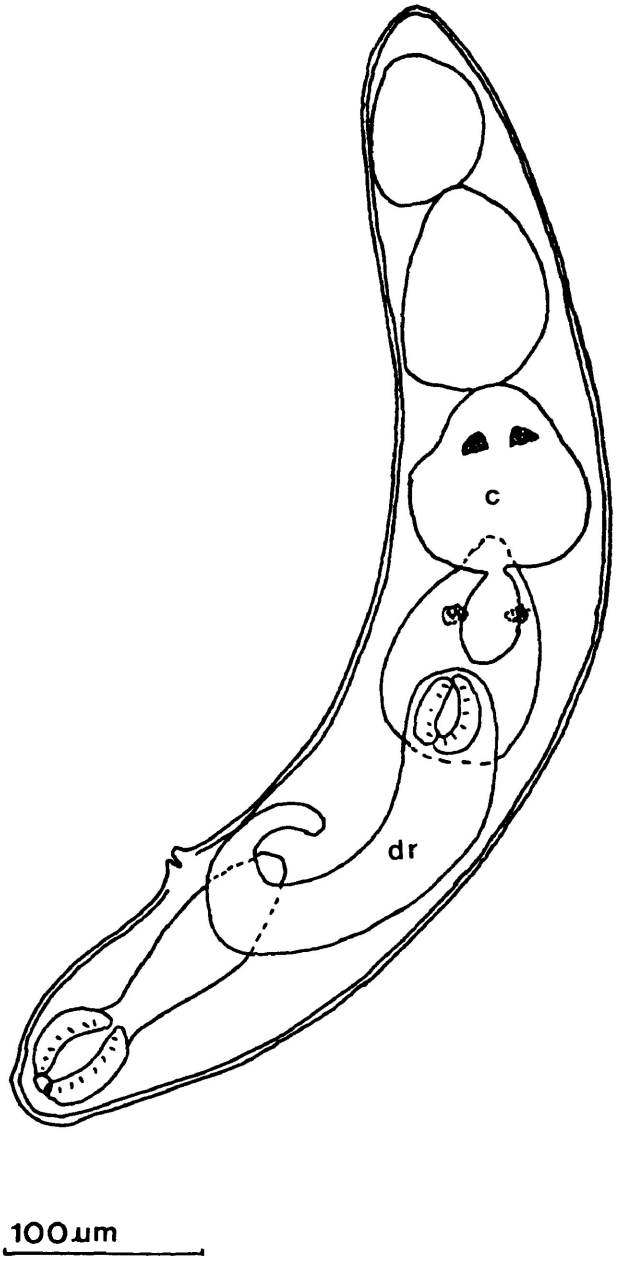

c
dr
100 µm

TABLE 11. Dimensions (µm) of paramphistome cercariae and metacercariae from naturally infected *Helisoma trivolvis* and *H. campanulatum*

	Snail host		
	H. trivolvis	*H. campanulatum*[2]	Statistics
Cercariae[1]			
body length	731.5 ± 16.4(8)[3] (670-790)	667.5 ± 26.8(12) (510-780)	F = 4.03 ns t = 2.04 ns
body width	354.4 ± 6.2(8) (330-390)	352.1 ± 8.4(12) (310-400)	F = 2.82 ns t = 0.22 ns
tail length	613.4 ± 4.8(8) (595-640)	927.7 ± 28.3(11) (700-1025)	F = 48.28 P < 0.001 t = 9.33 P < 0.001
Metacercariae			
maximum diameter of cyst	442.1 ± 4.1(30) (400-490)	308.0 ± 4.6(15) (280-340)	F = 1.58 ns t = 20.18 P < 0.001
maximum diameter of metacercariae	295.6 ± 3.6(30) (250-320)	264.4 ± 1.5(25) (250-280)	F = 6.61 P < 0.001 t = 7.41 P < 0.001

[1] Cercariae from *H. trivolvis* were killed by the addition, dropwise, of 1N HCl prior to measuring while cercariae from *H. campanulatum* were killed by gentle heating.

[2] Metacercariae collected from aquatic vegetation in Pickerel Lake and assumed to come from *H. campanulatum*.

[3] Mean ± S.E. sample size in parenthesis and range in parenthesis.

cantly larger ($t = 20.18$, $P < 0.001$) than that of metacercariae cysts from Pickerel Lake (308.0µm) (Table 11). Similarly the diameter of the larval metacercaria within the cyst was significantly greater for those from *H. trivolvis* (295.6µm) ($t = 7.42$, $P < 0.001$) than for those from Pickerel Lake (264.4µm).

Paramphistome eggs from experimentally infected moose and wild moose were oval in shape, transparent, and had a visible operculum. Eggs were dense and quickly sank when placed in water. Eggs from wild moose were 156.8 ± 3.1µm (±1 SE, n = 5) long, similar in length to eggs collected from experimentally infected moose (151.7 ± 2.0µm, n = 7) ($t = 1.24$, $0.20 < P < 0.50$). The width of eggs from wild moose were 85.4 ± 1.9µm (n = 5), similar in size to those eggs from experimentally infected moose which measured 85.0 ± 1.2µm (n = 7) ($t = 0.2$, $P < 0.50$).

Three miracidia which hatched from eggs of paramphistomes taken from a wild moose measured 190 by 55µm, 165 by 60µm, and 180 by 62µm.

Experimental infection of snails with paramphistomes

Young *H. trivolvis*, raised from eggs, were placed in finger bowls with miracidia hatched from eggs collected from a moose experimentally infected with paramphistomes. When miracidia approached a snail they would move in tight circles. If a miracidium touched the mantle of the snail, the snail would withdraw quickly into its shell and twist 90°. Miracidia were observed to enter the mantle cavity of the young *H. trivolvis* and not seen to leave. Dissection of *H. trivolvis* at intervals after exposure to miracidia did not reveal any paramphistome infections.

Young *H. campanulatum* (2 weeks old and 1-2 mm in diameter), raised from eggs, were exposed for 30 minutes to miracidia hatched from eggs collected from a moose experimentally infected with paramphistomes. After 24 days, 10 of these snails

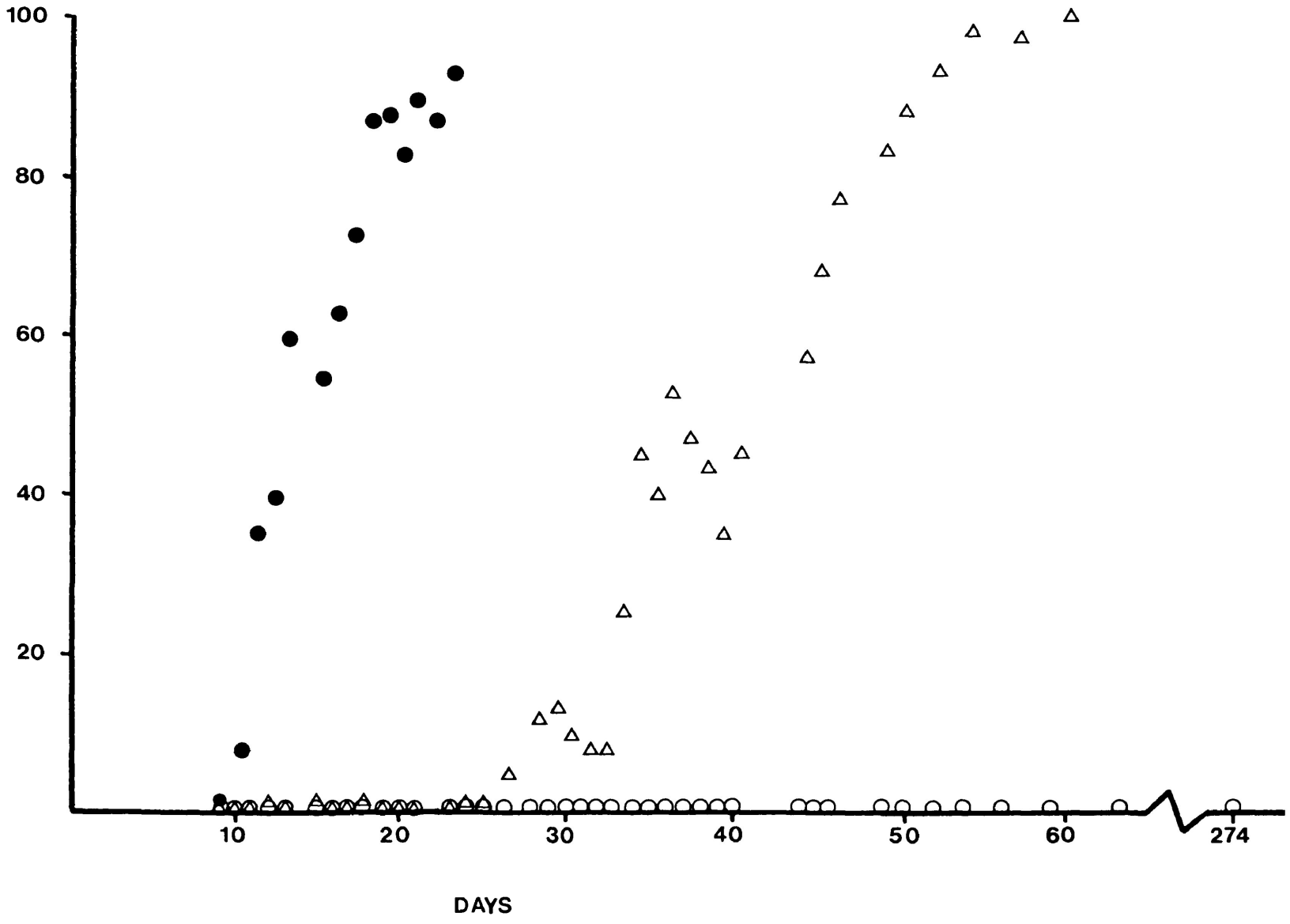
(then 4-5 mm in diameter) were examined but no sporocysts or rediae were seen. After 41 days, 5 more snails (then 4-6 mm in diameter) were examined and again no sporocysts or rediae were seen. Another group of young *H. campanulatum* (9 weeks old and 5-6 mm in diameter) were exposed to miracidia hatched from eggs collected from wild moose feces. After 140 days, 23 of these snails were examined (then 6-12 mm in diameter) but no sporocysts or rediae were found. A separate sample of *H. campanulatum* was kept in an aquarium for 2 years. Eggs collected from feces of experimentally infected moose were added to the aquarium several times over the 2 year period but no metacercariae were observed attached to the aquarium walls. Oligochaetes in high numbers were observed in the mantle cavity of both *H. trivolvis* and *H. campanulatum* that were used in the paramphistome infection experiments.

Factors affecting the incubation period and viability of paramphistome eggs

Eggs kept at 27°C in a light regime of 12 hours of illumination and 12 hours of darkness began to hatch after 10 days, and by 19 days 80% of the eggs had hatched (Fig. 15). Eggs kept at 19°C began to hatch after 26 days, and by 49 days 80% had hatched. No eggs kept at 11°C had hatched after 57 days nor could any development within the eggs be detected at this time. After 57 days the eggs were placed at approximately 22°C. After 21 days, 100% of these eggs had hatched. A sample of eggs in water at 11°C, and in total darkness, had not hatched nor developed when checked after 274 days. These eggs were then placed in water at 20°C with approximately 12 hours of illumination and 12 hours of darkness. When checked after 43 days, 87% of the eggs had hatched.

To test the resistance of eggs to desiccation, a fecal sample from an experimentally infected moose was divided into 4, 20 g subsamples. Eggs were removed from 1 subsample using the sedimentation technique and placed in water at approxi-

Fig. 15. Effect of temperature on hatching of *Paramphistomum sp*. eggs.


EGGS AT 27°C
EGGS AT 19°C
EGGS AT 11°C
100
80
60
40
20
10
20
30
40
50
60
274
DAYS

mately 22°C. The other 3 fecal subsamples were placed in open Petri dishes and put in a growth chamber at 19°C. After varying periods of desiccation the feces were broken up and the eggs removed using the sedimentation technique and placed in water at approximately 22°C. Only 3% of the eggs subjected to 8 days of desiccation hatched (Table 12), while no eggs desiccated for 13 and 21 days hatched. Eighty-three (83) percent of the eggs in the undesiccated control sample hatched.

To test their resistance to freezing, eggs were removed from feces using the sedimentation technique, divided into subsamples, placed in water and frozen at -4 to -5°C for varying lengths of time up to 8 days. A control sample was not frozen. Freezing eggs for 24 hours reduced the proportion that hatched to 10% (Table 13). The subsample frozen for 8 days still had 5% of the eggs hatch. The unfrozen control sample had a 93% hatch.

TABLE 12. Proportion *Paramphistomum sp.* eggs hatching after varying periods of desiccation

Period of desiccation at 19°C	Proportion of eggs hatching[1]
8 days	1/30
13 days	0/10
22 days	0/30
No desiccation	25/30

[1] Number hatching within 2 months of desiccation treatment at room temperature (19-22°C).

TABLE 13. Proportion of *Paramphistomum sp.* eggs hatching after varying periods of freezing

Period of freezing at -4 to -5°C	Proportion of eggs hatching[1]
24 hours	4/40
46 hours	3/50
90 hours	6/30
114 hours	1/20
186 hours	1/20
No freezing	56/60

[1] Number hatching within 2 months of freezing treatment at room temperature (19-22°C).

DISCUSSION

In this study, 86% of 160 moose had rumen flukes. Olsen and Fenstermacher (1942) found 30% (n = 30) of moose in Minnesota were infected with *P. cervi* while Threlfall (1967) reported 5% (n = 109) of moose in Newfoundland had *P. cervi*. Peterson (1955) reported that 12% (n = 25) of moose examined from northwestern Ontario were infected with *P. cervi*. The low prevalence reported in these studies may have resulted from incomplete searches. For example, most examinations of moose in Newfoundland were made on only a 225 cm^2 sample of rumen wall (Threlfall 1967).

Paramphistomum spp. have been reported from other cervids in North America and Europe. In Newfoundland, 69% of 26 caribou were infected with *Paramphistomum sp.* (Bergerud 1971). *Paramphistomum liorchis* was found in 7.3% (n = 788) of the white-tailed deer examined from the southeastern United States, with an estimated mean intensity of 402 (n = 39) flukes (Prestwood *et al.* 1970). In Germany, *P. cervi* was found in 11% of 73 red deer (*Cervus elaphus*) with numbers ranging from 2 to 1,562, and a mean of 246 (n = 8) (Graubmann *et al.* 1978). In the same study, Graubmann *et al.* (1978) found 2 of 15 roe deer infected with 15 and 206 *P. cervi*.

In results reported here the youngest infected animals were calves that died in October. Eight newborns that died in June, July and on August 1st were not infected. However, there were no newborns examined between August 2nd and September 30th. Seventy-two (72) percent of 36 moose aged 0.5 to 1.4 years were infected, 86% of 37 moose aged 1.5 to 2.4 years were infected, and 100% of 59 moose older than 2.5 years were infected. The high prevalence of infection in all ages of moose indicates that even newborns are feeding in aquatic areas at a time suitable for transmission of this parasite and that by their third summer all moose have ingested metacercariae. The 100% infection rate in moose older

than 2.5 years indicates that all areas of the study were suitable for transmission.

Counts of the number of flukes in the rumen of 56 infected moose ranged from 16 to 28,262 with a mean of 3,435 and a median of 1,135 flukes. The only counts of rumen flukes reported in the literature for moose were from the USSR where one heavily infected moose had 40,000 *P. cervi* (Aleksandrova 1962). Moose in the present study had both a high prevalence and intensity of rumen flukes compared to other reports of paramphistomes in cervids.

The number of flukes in each rumen was not normally distributed but were overdispersed and resembled a negative binomial distribution. The distribution of many parasites in their definitive hosts approximates a negative binomial (Anderson 1974). In this type of distribution most host individuals contain only a few parasites while a large proportion of the parasite's suprapopulation (in sense of Kennedy 1977) is concentrated in a few hosts. These few heavily infected hosts are of particular value to the parasite suprapopulation because they contain a large portion of the species' reproductive capacity. In this study the 6 most heavily infected moose contained 54% of the flukes present in the 56 infected moose sampled.

This overdispersion may have resulted from differences in acquiring the infective metacercariae. Anderson (1974) attributed the overdispersed distribution of the tapeworm *Caryophyllaeus laticeps* in European bream (*Abramis brama*) to the non-random distribution of the infected intermediate host, a tuberficid. The tuberficids were clumped with some clumps having many infected members. When a bream encountered a group of tuberficids it would search the area and consume a large proportion of that group. In this way some bream would pick up heavy infections while many others would have low numbers of tapeworms. Although 59%

of the 32 lakes investigated in this study had a suitable snail host, it was apparent in collections that a few of the lakes had large numbers of either *H. trivolvis* or *H. campanulatum* and presumably the populations of these 2 species were overdispersed in relation to the lakes in which they were found. If both the chances of transmission from egg to a snail and feeding intensity by moose are similar in 2 different lakes, one with a high density of snail intermediate host and the other with a low density, then the high density lake would be expected to contain a higher number of infected snails and, consequently, more metacercariae than the lake with the low snail density. Moose in Ontario have relatively static home ranges (Peterson 1955, Goddard 1970, Snider 1978). Those with heavily infected lakes within their home range would acquire heavy infections while moose living in areas with less heavily infected lakes would acquire low numbers of rumen flukes. In this way an overdispersed distribution could result.

The overdispersed distribution may also result from differences in host immunity from one moose to another. Horak (1971) found that if the initial infection of *P. microbothrium* in sheep, goats or cattle was large enough, a strong partial immunity to suprainfections developed. If infections were slowly acquired in some moose, immunity might not develop and large populations of rumen flukes could result in those individuals. All age groups of moose had similar occurrences of newly acquired flukes and this would suggest that most individuals continue to collect at least some rumen flukes each year throughout their life.

Calves had a median of 202 flukes which was significantly lower than the median of 1,427 from all older moose. This is probably the result of limited aquatic feeding by calves. Fraser *et al.* (1982) reported that only 15 of 195 moose observed in Sibley Park lakes through the summer were calves. In 29 of 30 cow-calf groups observed by Cobus (1972) at Joe Boy Lake the calf or calves were left bedded down on the shore. Cobus (1972) saw only 1 calf feeding on aquatics

during his study and that was on August 13th.

It was determined in this study from experimentally infected moose that the rumen flukes are long-lived. With a long-lived parasite it would be expected that each successive age group of moose would have more flukes. However, in wild moose the number of rumen flukes was similar for most age groups. Moose older than 4.5 years had significantly fewer flukes than 3.5 to 4.4 year-old animals. If a partial immunity against suprainfections was established in moose early in life as Horak (1971) found for *P. microbothrium* in domestic ruminants, then similar numbers of flukes might be expected in younger age classes. A large proportion of the rumen flukes may die after 3 years in wild moose as was found in experimentally infected moose. If recruitment of new flukes is regulated by a partial immunity then little change might be expected in the number of flukes in older moose as flukes acquired 3 years earlier die out and are replaced by new ones. Klesov and Mereminski (1973) reported that *P. cervi* lives for 4 years in cattle in the USSR. It was found that older moose were just as likely to have newly acquired flukes in their rumens as younger moose, however, no estimate of the number of newly acquired flukes in each moose was made. If immunity against suprainfection was incomplete then a few flukes could be acquired each year in older moose but the number acquired could be greatly reduced.

Male and female moose had similar numbers of rumen flukes. Both Cobus (1972) and Fraser *et al.* (1982) observed that bulls and cows participated equally in the intensive aquatic feeding seen from mid-June to mid-July. Rumen fluke metacercariae were being shed from snails and were encysting on aquatic vegetation during this period. After mid-July metacercariae continued to accumulate on vegetation but moose feed little on aquatics after this time (Cobus 1972, Fraser *et al.* 1982). There were no significant differences in the numbers of flukes obtained in 1980 and 1981 either in moose 0.5 to 1.4 years old or in moose 1.5

years. This would imply that the extent of aquatic feeding and acquisition of metacercariae was similar in both years.

There was no difference in fluke numbers in moose rumens in relation to 7 administrative and geographical localities making up the study area. This probably reflects the widespread use of aquatic vegetation by moose and wide distribution of suitable intermediate hosts. There were no differences in fluke numbers among the 3 defined seasons either for calves (0.5 to 1.4 years) or older moose. Flukes were present in the rumen throughout the year and in approximately similar numbers. As already mentioned, moose born in May were not found to have flukes in their rumens until October. Small, thin, migrating flukes were found in the duodenum and omasum only in October. Similar small, thin flukes were found in the rumen of moose of all ages from October to April.

Flukes obtained from calf moose during the time period of November to February were the same size as flukes from older moose ($\geq$ 1.5 years) examined in winter. The flukes from the calf moose could only have been acquired during the previous summer. Those from older moose presumably would include those from several preceding summers. Flukes obtained from an experimentally infected moose killed on November 27th, 2 years and 2 months post-infection, were significantly larger than flukes from wild moose during the November to February period. All of the flukes from the experimental moose were non-gravid. It may be that the quality of the diet given to the captive moose resulted in larger flukes. The size distribution of flukes from older, wild moose killed in the November to February period showed no evidence of more than one size group. Flukes from calf moose were smaller than flukes from older moose ($\geq$ 1.5 years) in the June to August period. It may be that flukes acquired more than one year previously can put more energy into reproductive tissues, resulting in a larger size. The size distribution of flukes taken from June to August from older moose, although not

showing 2 peaks, did show a truncated curve that could easily have included smaller flukes from the summer before. The size distribution of flukes from calf moose for this same time period showed a much sharper peak, presumably a result of only one age of fluke being present. Calf moose had a prevalence of infection of 72%, moose age 1.5 to 2.4 years had a prevalence of infection of 86%, and 59 moose older than 2.5 years had a prevalence of infection of 100%. Presumably, the increase in prevalence with age is evidence of a parasite that lives for more than 1 year, although different age animals may have different aquatic feeding habits.

Paramphistomiasis is characterized by outbreaks of acute gastroenteritis with high morbidity and mortality, particularly in young animals (Horak 1971). Several species of paramphistomes are known to cause the disease (Horak 1971). *Paramphistomum cervi* has been reported to cause paramphistomiasis in domestic ruminants of Europe by many authors (eg. Visnjakov and Ivanov 1964, Mereminski and Gluzman 1967). Zadura (1960) reported paramphistomiasis as causing death in 2 captive red deer (*Cervus elaphus*) in Poland. In this disease the excysting worms in the duodenum attach themselves by drawing a plug of mucosal tissue into their acetabulum, causing necrosis of the tissue involved (Horak 1971). The lesions caused by this activity result in intestinal discomfort leading to anorexia. Plasma albumin drops very low, presumably due to losses through intestinal lesions (Horak 1971), resulting in generalized edema. Death occurs generally from pulmonary edema with exhaustion and starvation (Horak 1971). Eosinophil levels rise in domestic ruminants infected with *P. microbothrium* in the first week post-infection, peak the fourth week and decrease gradually to normal by the eleventh week (Horak 1971). An elevated eosinophil level was also reported by Tsretzeva (1959) for cattle infected with *Paramphistomum sp.* and by Lengy (1962) for sheep infected with *P. microbothrium*.

Intestinal lesions as described by Horak (1971) in domestic ruminants were

not observed in wild moose nor were clinical signs of paramphistomiasis seen in any of the 4 experimentally infected moose. Haematologic values did not change in relation to infections. The high level of eosinophils found in the control moose may have been due to an infection of Trichostrongyles picked up within the moose enclosure.

Paramphistomes can, on occasion, cause pathology after they reach the rumen. Horak (1967), on summarizing the findings of many authors, stated that heavy infections in the rumen can cause edema of the epithelial layer with lymphocyte infiltration in the lamina propria and sometimes in the submucosal layer. Acute inflammation of the mucous membrane of the rumen and reticulum of 2 red deer that died from paramphistomiasis was noted by Zadura (1960). No evidence of such inflammatory changes was seen at the site of attachment of the rumen flukes in wild moose or in experimentally infected moose.

In observations reported here, rumen flukes occurred in dense patches in the rumenus atrium portion of the rumen of moose. Attachment of the worms to the base of the papillae caused the epithelial papillae to slough off leaving only stubs. This was also reported for *P. cervi* infecting moose in the USSR (Aleksandrova 1962). When infections were heavy, large patches of papillae were lost. Graubmann *et al*. (1978) also found patches of papillae sloughed in roe and red deer. In domestic ruminants, much of the rumen is covered by keratinized epithelium with the main site of volatile fatty acid absorption occurring in the rumenus atrium (Hofmann 1973). The reduction of absorptive papillae in this region could cause nutritional impairment in domestic ruminants. In moose, however, the entire rumen has a dense covering of absorptive papillae and significant impairment of volatile fatty acid absorption would therefore be unlikely.

Paramphistomes in the rumen of sheep were found to feed on rumen ciliates

thereby impairing carbohydrate digestion (Mikhailova *et al*. 1973). Ciliates probably make up a minor component of the moose rumen's microfauna. The microfauna of browsing cervids is dominated by bacteria (Giesecke 1970).

It would appear that moose and their rumen flukes are co-adapted with few pathologic changes being observed in the wild or experimental animals. *Paramphistomum microbothrium* did not cause serious disease in sheep when 20,000 metacercariae were ingested (Horak 1971) and the chance of serious disease in moose being brought on by much higher infection rates should not be ruled out.

In this study, 2 species of planorbid snails, *H. trivolvis* and *H. campanulatum*, were found naturally infected with paramphistomes as confirmed by experimental infection in captive moose. In Europe, *P. cervi* has been found to naturally infect *Planorbis planorbis* and *Anisus vortex* (Kraneburg 1977). Kraneburg (1978) successfully infected *Planorbis planorbis*, *A. vortex*, *A. leucostomus*, *Bathyomphalus contortus*, *Hipperitis complanatus* and *Armiger crista*, all of which are planorbid snails. Of these, only *A. crista* (= *Gyraulus crista*) occurs in North America but it was not found in this study. Krull (1933) reported *P. cervi* in the southern United States in naturally infected *Stagnicola bulimoides* and experimentally infected *Pseudosuccinea columella*; both are lymnaeid snails. Sey (1982) considered that Krull (1933) must have been working with another species of paramphistome. *Paramphistomum cervi* is quite rare in the southern United States (Price 1953). The common species of that area is *P. microbothroides* whose intermediate hosts are lymnaeid snails (Sey 1980).

Each paramphistome species infects only snail species of a single family. The common *P. microbothrium* of Africa and the Middle East infects only species of bulinids (Dinnik and Dinnik 1954, Lengy 1960). *Paramphistomum daubneyi*, which occurs in Europe, has been found to infect the lymnaeid *Lymnaea truncatula* (see

Sey 1980). *Paramphistomum ichikawai* has been identified in Hungary and its intermediate hosts are the planorbid species *Planorbis planorbis* and *Segmentina nitida* (Sey 1978).

In this study, the infection rates of both *H. trivolvis* and *H. campanulatum* were near 1%. This is similar to the infection rates for *Liorchis scotia* and *Liorchis hibernae* in planorbid snails taken from areas pastured by infected cattle in the USSR, which varied from 0.45 to 2.2% (Katkov 1970). Both *L. scotia* and *L. hibernae* are considered synonyms of *P. cervi* (Odening *et al.* 1978, Sey 1980). In an examination of over 9,000 snails in the USSR, paramphistomes were found in 4.5% of *Planorbis planorbis*, 1% of *Anisus vortex*, and 0.4% of *A. contortus* (Orlovskii and Zharikov 1970).

Even though in this study only about 1% of *H. trivolvis* and *H. campanulatum* were infected, metacercariae were abundant on vegetation and experimental discs. The metacercariae, as observations demonstrated, occurred almost entirely on the underside of floating portions of vegetation. Experiments conducted with naturally infected *H. trivolvis* in conjunction with this study demonstrated that the paramphistome cercariae were positively phototropic and encysted on dark objects at the water's surface (Hoeve 1982). Positive phototropism has been reported for *Cotylophoron cotylophorum* (Srivastava 1938), *P. microbothrium* (Dinnik and Dinnik 1954, Swarte and Reinecke 1962), and *P. cervi* (see Olsen 1974). Positive phototropism in *P. microbothrium* cercariae was considered by Horak (1967) to be an adaptation to get the metacercariae onto vegetation where grazing ruminants could ingest them once water levels had subsided. Fraser and Hristienko (1983) found that moose in Sibley Provincial Park fed on the floating leaves of water lilies (*Nuphar variegatum*) to such an extent that the plant was eliminated from some lakes. Positive phototropism in paramphistome cercariae would thus appear to be an adaptive strategy, well suited to the aquatic feeding behaviour

of moose.

Helisoma trivolvis occurred in half of the 32 lakes investigated, but infected individuals were found only in Slab Lake. *Helisoma campanulatum* was found in 7 of the lakes with infected individuals being collected only from Pickerel Lake. However, the collections of either *H. trivolvis* or *H. campanulatum* from other lakes were too small to exclude their being infected at a rate similar to that found in Slab Lake or Pickerel Lake, with the exception of *H. trivolvis* in Lake 24A and Joe Boy Lake. With the exception of these 2 lakes, it would appear that infected snails were found in Slab Lake and Pickerel Lake only because of the availability of large numbers of snails for testing. The presence of infected snails in a lake was most easily detected by searching for metacercariae on the underside of floating vegetation. Four lakes that were found to have paramphistome metacercariae on vegetation also had *H. campanulatum*. *Helisoma trivolvis* was present in 3 of these 4 lakes. No paramphistome metacercariae were found on floating vegetation in Joe Boy Lake or Lake 24A despite the occurrence of suitable snail hosts.

Clark (1973) conducted an extensive survey of freshwater molluscs in Canada. He found *H. trivolvis* common in lakes of various sizes and in rivers and streams with little or no current. Vegetation was moderate to thick at most locations and mud was the most frequent bottom type. *Helisoma trivolvis* was found distributed throughout the boreal forest region of Canada but not in Newfoundland. *Helisoma campanulatum* was found in lakes and in slow moving portions of rivers. It was most often associated with abundant aquatic vegetation. It was found in the boreal forest region from Newfoundland west to Saskatchewan. It thus appears that the 2 intermediate hosts identified in this study are suited in both habitat requirements and in geographical distribution for efficient transmission of this parasite.

In this study it was not possible to experimentally infect *H. trivolvis* or *H. campanulatum*. Miracidia moved in tight circles when close to *H. trivolvis* and upon contacting a snail caused it to quickly pull into its shell and twist 90°C. Similar behaviour has been described prior to successful infection of snails by paramphistomes (Olsen 1974). Odening *et al.* (1978) experimentally infected the following planorbid snails with *P. cervi*: *Anisus vortex*, *Armiga crista*, *Bathyomphalus contortus*, *Planorbis planorbis*, and *Segmentina nitida*. Other workers have experimentally infected snails with various paramphistome species: *P. microbothrium* (Dinnik and Dinnik 1954, Swarte and Reinecke 1962), *Cotylophorus cotylophorum* (Srivastava 1938), *P. daubneyi* (Sey 1980). Nikitin (1967) found that oligochaetes present on *Planorbis planorbis* consume *Liorchis hibernae* (= *P. cervi*) miracidia, preventing infection of the snails. Gluzman (1972) found that the oligochaete *Chaetogaster limnaei*, present on *Planorbis planorbis* snails, reduce to 1/3 the expected success of experimental infections with *Liorchis hibernae* (= *P. cervi*) and Patzig and Schmid (1981) also found that *Chaetogaster limnaei* present on *Planorbis planorbis* protect the snails from *P. cervi* infection. In the present study, oligochaetes were seen attached to both *H. trivolvis* and *H. campanulatum* in the laboratory on a number of occasions. It is quite possible that snails could become more heavily infected with oligochaetes in an aquarium environment. Heavy infections of oligochaetes could have prevented the successful infection of the experimental snails. Oligochaetes should be destroyed by heating the snails in water to a temperature of 24°C (Sankurathri and Holmes 1976) prior to attempting future experimental infections.

It is quite possible that the infection rates of paramphistomes reported here and the differences in infection rates among lakes could be influenced by *Chaetogaster limnaei* on *H. trivolvis* and *H. campanulatum*. Sankurathri and Holmes (1976) found that the loss of *Chaetogaster limnaei* from the gastropod *Physa gyrina*

resulted in a dramatic increase in the natural infection of *P. gyrina* with the larval trematode *Echinoparyhium recurvatum*. Infection rates in *P. gyrina* vary inversely with the number of *Chaetogaster limnaei* present on each snail.

Cercariae from *H. trivolvis* were similar in body size but had longer tails than those from *H. campanulatum*. Cercariae from *H. trivolvis* were killed by addition of 1N HCl while cercariae from *H. campanulatum* were killed by gentle heating. These 2 methods may have resulted in different tail lengths. Metacercariae from *H. trivolvis* were significantly larger than metacercariae on vegetation in Pickerel Lake and considered to have come from *H. campanulatum*. However the range in size of the metacercariae from *H. trivolvis* collected from Slab Lake completely overlapped the metacercariae collected from Pickerel Lake and assumed to have come from *H. campanulatum*. Egg size and miracidia size were similar to that stated for *P. cervi* (Sey 1982). Sey (1982) reported that mature rediae were 700-1,100 while in this study rediae from *H. campanulatum* ranged from 475 to 1,550 . However, all of these rediae may not have been mature. The presence of daughter rediae were noted in this study and are characteristic of several trematodes (Noble and Noble 1976) including paramphistomes (Dinnik and Dinnik 1954).

With no opportunities for reinfection in the experimental moose, it was demonstrated in the one moose maintained for 3.5 years post-infection that the rumen flukes live for at least 3.5 years. Rumen flukes were obtained from all of the other 4 moose when terminated 2 to 2.5 years after infection. Egg levels were similar in May and June in year 1 and year 2, indicating that there was probably no major mortality during the first 2 years of infection. Dinnik and Dinnik (1962) reported that there was no major mortality of *P. microbothrium* in 2 Kenyan cattle 5 and 7 years after infection. In contrast, *P. cervi* infecting cattle in the Soviet Union were found to live 4 years (Klesov and Mereminski

1973).

All 5 experimentally infected moose started to pass paramphistome eggs in their feces in the spring following infection. Eggs were present through the summer, dropped to 0 in the winter, and appeared again in feces the following spring. Similar seasonal changes in egg numbers were seen in wild moose. It was shown experimentally that paramphistomes go into a non-gravid state each winter, becoming gravid each spring. Rumen flukes in the experimentally infected moose appeared to become gravid somewhat earlier in their second year of infection as determined by fecal egg counts. This was reinforced by the 91% gravid rate found in flukes from a moose terminated April 2nd (2.5 years post-infection). The reason for this departure from observations on wild moose is not known.

Several studies report no such seasonal variation in egg output. The fecal egg levels for *P. microbothrium* in cattle in South Africa reached a plateau in 7 to 13 months post-infection and remained constant thereafter (Horak 1967). The level of *P. microbothroides* eggs in feces from cattle in Quebec did not exhibit seasonal variation (Bouvry and Rau 1983). Many immature paramphistomes were found in the rumens of sheep from the Volga River delta in October (Karaboev and Amangelieu 1964) while through November and subsequent winter months numerous mature worms were found, suggesting that the maturation process was not synchronized with season. In contrast, Rodonaya (1960) found that *P. skryabini* infecting cattle in the USSR had low fecal counts in November and peak egg counts in June. In India, Gupta *et al.* (1984) found that *P. cervi* infecting sheep were gravid from April to August and immature from September to March. The peak egg production was during the monsoon season of July and August, coinciding with the availability of the intermediate host, *Indoplanorbis exustus*.

Gupta *et al.* (1984), in their study of *P. cervi*, worked in the tropical

regions of India at 31°N. latitude. At this latitude, seasonal photoperiod fluctuations would be considerably reduced. Mean monthly temperatures did fluctuate in their study area but much less than in northern Ontario. Assuming that the sheep were free ranging, their diets would be expected to change from dry season vegetation to more succulent wet season vegetation. It is interesting to note that seasonal maturation has been observed both in tropical and temperate regions while evidence for the lack of seasonal maturation in different paramphistome species has also been reported from tropical and temperate regions.

A seasonal pattern of maturation occurs in some nematode species, eg. *Haemonchus contortus* in sheep, *Ostertagia ostertagia* in cattle and *Dictyocaulus viviparous* in cattle (Schad 1977). The increased production of eggs in spring is commonly referred to as spring rise. In these nematodes the larval stages in the definitive hosts are arrested from further development during the winter. Generally, the arrested nematodes are those acquired late in the season after having experienced low temperatures or reduced photoperiod while in their free-living stage. In the spring there is a synchronized maturation of the arrested larval nematodes, due to what is generally considered to be the result of a relaxation of the hosts' immune response. In this study no reservoir of recently acquired immature flukes was found in the lower stomachs or intestines that would make this paramphistome species analogous to the nematode situation.

Individual paramphistomes alternate seasonally from a gravid to a non-gravid state similar to that described for the cestode *Davainea tetraoensis* infecting ruffed grouse (*Bonasa umbellus*) (see Dick and Burt 1971). Mature forms of the cestode are present in the gut of ruffed grouse only during the summer and only immature forms are present in the winter. A decline in environmental temperature below 0°C was considered to cause the cestode to change to an immature form and that a change to a mature form was thought to be caused by gonadal development in

the host or factors responsible for this gonadal development.

The mean prepatent period in the 4 moose experimentally infected with metacercariae was 195 days. This is much longer than most reports for paramphistomes in the literature. *Paramphistomum cervi* have been found to have a prepatent period of 96-130 days in cattle (Sey 1982), 96-107 days in sheep (Sey 1982), and 82-96 days in roe deer (Kraneburg and Boch 1978). Prepatent periods for other paramphistome species are somewhat shorter; *P. microbothrium* has a prepatent period of 56 days in cattle, 69 days in goats and 71 days in sheep (Horak 1971) and *P. ichikawai* has a prepatent period of 42-51 days in both sheep and cattle (Kisileve 1967 in Horak 1971). It is interesting to note that in the study by Gupta *et al.* (1984), of the seasonal maturation of *P. cervi* of sheep in India, the authors believed their sheep were getting infected in June to August coinciding with the period of peak snail activity and the occurrence of *P. cervi* metacercariae. Gravid flukes were not observed by Gupta *et al* (1984) until April, giving a prepatent period of approximately 250+ days.

The 3 moose experimentally infected in September became patent in April with prepatency periods of 225, 198 and 185 days. It is unlikely that wild moose become infected as late as September. Aquatic vegetation has largely disappeared by September. Aquatic feeding by moose occurs predominately in the period from mid-June to late July (Fraser *et al.* 1982, Cobus 1972). The first snail shedding cercariae was found on June 27, 1982 and in 1983 the first metacercariae occurred in Pickerel Lake between June 24th and July 8th. It is therefore likely that moose become infected while feeding on aquatic plants in July. Wild calf moose, as well as older moose, have rumen flukes that become patent in April. A mid-July (to mid-April) infection gives a very long prepatent period of 273 days. Since the moose experimentally infected in September started to pass eggs in April, this would imply that rumen flukes enter a dormancy period until some stimulus in late

winter synchronizes the production of eggs. It should be noted that 2 small flukes obtained from a calf killed in October already had a few eggs in their uteri. It may be that development of the worm starts as soon as they reach the rumen in October and that some stimulus is required to put them into a dormant or non-developing state. If this seasonal stimulus is not properly received the flukes may start to develop eggs in a time period similar to that reported in most paramphistome studies.

Three environmental factors have been considered as ultimate pacemakers for the observed cyclic maturation pattern. These are seasonal changes in diet, temperature, and photoperiod. Moose have a major shift in diet from leafy vegetation in the summer to a diet of woody twigs after leaf-fall in the autumn, and back to leafy plants after leaf-out in the spring (Stewart *et al*. 1977). However, leaf-fall generally occurs between October 7th and October 18th (unpublished data) after fecal egg counts and the proportion of worms gravid have started to decline. Similarly, average leaf-out is May 21st (n = 9) (Snider, unpublished data), and occurs after eggs are already present in moose feces and nearly 100% of the flukes are gravid. The experimental moose were maintained on mixed grain diets and had only limited amounts of natural foods available. Fecal egg levels in the experimental moose showed the same seasonal fluctuation as in wild moose in spite of a fairly constant diet throughout the year.

Photoperiod and environmental temperature are 2 parameters that change seasonally but whose effects are often difficult to separate. An experiment to test the effects of photoperiod on fluke maturation could be performed by placing an infected moose inside a windowless, unheated barn in mid-winter. The moose would be maintained on a constant diet and exposed to light for 8 hours each day, simulating a constant mid-winter light regime. Disproof of the photoperiod hypothesis would result if eggs were passed in April.

There is evidence that metacercariae appearing on aquatic vegetation in June and July come from snails infected in the summer of the previous year. In this study it was reported that paramphistome eggs would not hatch or develop at 11°C. Similarly, Kraneburg (1978) working in Germany reported that *P. cervi* eggs would not develop at 13°C. At 19°C rumen fluke eggs would not start to hatch for 30 days and 50% of the eggs would not be hatched until 40 days. Kraneburg (1978) found that *P. cervi* took 20 days to hatch at 20°C. The time required for the development of paramphistomes in snails was not determined in this study. However, Kraneburg (1978) found cercariae of *P. cervi* were shed 50 days after infections of snails at 20°C.

Even shallow water lakes in northwestern Ontario do not warm up soon enough in the spring for paramphistome eggs to hatch and for cercariae to develop in infected snails by the end of June. Shallow water lakes (10-15 meter mean depth) in the study area are ice covered for an average of 185 days of the year (Shuter *et al.* 1983) with surface temperatures not reaching 11°C before mid-May (Bacante pers. comm.) and with summer maximum temperatures of about 20°C (Shuter *et al.* 1983). Water temperatures decline to 11°C by late September (Bacante pers. comm.). Under such a temperature regime paramphistome eggs could not start to develop until the end of May with hatching occurring in late June or early July. If development in snails occurred at a similar rate to that reported by Kraneburg (1978) for *P. cervi* (at 20°C) then 50 days would be required for production of cercariae. Metacercariae would not be expected on vegetation before mid-August. The peak of aquatic feeding is well past by this time (Fraser *et al.* 1982). Metacercariae and shedding snails were first found in late June, implying that paramphistomes survive through the winter in the snail host. Paramphistomes surviving through a temperate winter in the intermediate snail host have been reported by Kraneburg (1978) for *P. cervi* in planorbid snails. The overwinter

survival of paramphistomes in *H. trivolvis* and *H. campanulatum* would appear to be an adaptation to ensure that metacercariae are present when moose feed most intensively on aquatic vegetation in June and July.

The most distinctive adaptation of the rumen fluke seen in this study is its seasonal maturation cycle. Newly acquired worms have a prolonged prepatent period and become gravid in the spring with egg production peaking in June and July. The worms enter a non-gravid period through the fall and winter only to repeat the cycle in the spring. The parasite husbands its reproductive potential to coincide with the most opportune time to get eggs into the aquatic environment and to avoid the 6 month-long winters when freezing would kill any eggs produced.

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APPENDIX 1. Information on infection rates and development of *Paramphistomum sp.* in wild moose from northwestern Ontario

Moose specimen no.	Date of death			Age[2] of moose	Sex of moose	Infected with flukes	Flukes from rumen samples		Flukes from other than rumen samples		No. of newly acquired flukes[1]				Fecal egg levels
	Year	Month	Day				Gravid	Non-gravid	Gravid	Non-Gravid	Duodenum	Abomasum	Omasum	Rumen	
005	1980	10	15	02	M	yes	2	29	7	62	0	0	0	5/31	3.3
006	1980	10	15	00	F	yes	0	1	2	1	0	0	2	0/3	0
007	1980	10	17	02	M	yes	0	80	--	--	0	0	0	80/80	0
008	1980	09	09	03	M	yes	63	40	42	32	--	--	--	0/32	--
009	1980	10	17	02	F	yes	6	51	15	17	0	0	0	3/17	4.9
011	1980	10	16	08	M	yes	--	--	0	107	--	--	--	1/75	--
012	1980	10	16	04	F	yes	--	--	36	68	--	--	--		--
013	1980	10	18	03	M	yes	3	82	--	--	0	0	2	0/19	1.7
014	1980	10	09	98	F	yes	12	4	65	9	--	0	--	0/16	--
015	1980	08	01	00	F	no	--	--	--	--	0	0	0		--
016	1980	07	30	98	F	yes	2	0	8	0	0	0	0	0/10	--
017	1980	10	18	01	M	yes	14	90	--	--	--	0	0	3/32	--
018	1980	10	18	02	M	yes	7	76	9	68	--	--	--	4/18	0
021	1980	10	20	01	F	yes	0	62	--	--	0	0	0	5/16	6.5
022	1980	07	26	03	F	yes	--	--	--	--	0	0	0		--
023	1980	06	27	00	F	no	--	--	--	--	0	0	0		--
024	1980	10	18	09	F	yes	24	46	--	--	20	0	2	0/32	--
025	1980	10	02	00	M	yes	--	--	--	--	--	--	--		--
027	1980	06	24	04	F	yes	103	0	--	--	0	0	0	0/72	--
028	1980	06	12	03	M	yes	--	--	20	0	0	0	0	0/20	--
029	1980	06	03	01	M	yes	11	0	--	--	0	0	0	0/14	16.2
030	1980	05	28	02	M	yes	102	1	--	--	--	0	0	0/18	--
031	1980	06	12	08	F	yes	--	--	104	0	--	0	0	0/72	--
032	1980	06	19	02	M	yes	105	0	--	--	--	0	0	0/32	--
033	1980	05	14	01	M	yes	98	0	68	0	--	--	--	0/33	--
034	1980	06	08	05	M	yes	103	0	--	--	--	--	--	0/34	--
035	1980	06	06	02	M	yes	62	0	30	0	--	0	0	0/35	--

APPENDIX 1. (Cont'd)

Moose specimen no.	Date of death			Age of moose[2]	Sex of moose	Infected with flukes	Flukes from rumen samples		Flukes from other than rumen samples		No. of newly acquired flukes[1]				Fecal egg levels
	Year	Month	Day				Gravid	Non-gravid	Gravid	Non-Gravid	Duodenum	Abomasum	Omasum	Rumen	
036	1980	06	06	05	F	yes	65	0	--	--	0	0	0	0/70	38.5
037	1980	06	13	06	F	yes	38	0	25	0	0	0	0	0/38	--
038	1980	11	14	02	M	yes	1	99	--	--	0	0	0	1/31	--
039	1980	05	29	00	M	yes	--	--	24	0	--	0	0	0/25	--
040	1980	05	24	00	M	yes	55	1	--	--	0	0	0	0/32	--
041	1980	06	--	02	-	yes	38	0	--	--	--	0	0		--
042	1980	05	18	00	M	yes	--	--	--	--	--	--	--		--
043	1980	06	12	03	F	yes	105	0	--	--	0	0	0	0/32	--
044	1980	06	14	04	M	yes	64	0	--	--	0	0	0	0/58	--
045	1980	05	11	05	F	yes	18	0	4	0	0	0	0	0/18	--
046	1980	07	03	01	M	Yes	2	0	2	0	0	0	0	0/4	--
047	1980	07	01	01	M	yes	32	0	50	0	0	0	0	0/32	--
048	1980	07	09	02	F	yes	103	0	75	0	0	0	0	0/32	--
049	1980	05	17	00	M	yes	1	0	--	--	--	0	0	0/1	--
051	1980	05	01	10	M	yes	--	--	--	--	--	--	--	0/3	--
052	1980	05	29	00	M	yes	--	--	--	--	--	--	--	0/5	2.2
053	1980	05	13	01	F	yes	49	47	--	--	0	--	--	0/32	122.2
054	1980	04	29	00	F	no	--	--	--	--	0	--	--		0
055	1980	01	29	02	F	yes	--	--	--	--	--	--	--	0/1	--
056	1980	01	16	98	-	yes	--	--	--	--	--	--	--		--
057	1980	05	31	00	F	yes	--	--	--	--	--	--	--	0/16	--
058	1980	06	11	98	F	yes	--	--	--	--	--	--	--	0/8	--
059	1980	07	11	07	F	yes	--	--	70	0	0	--	--	0/32	162.7
060	1980	08	08	01	F	yes	--	--	--	--	0	--	--		--
061	1980	08	22	98	F	yes	--	--	12	24	--	--	--	0/63	--
064	1981	05	08	01	F	yes	10	0	--	--	--	--	--	0/4	3.5
065	1981	05	19	01	F	yes	23	4	--	--	--	--	--	0/4	16.3
066	1981	05	06	01	M	yes	100	0	--	--	--	--	--	0/30	--
067	1981	05	11	01	M	no	--	--	--	--	--	--	--		--

APPENDIX 1. (Cont'd)

Moose specimen no.	Date of death			Age[2] of moose	Sex of moose	Infected with flukes	Flukes from rumen samples		Flukes from other than rumen samples		No. of newly acquired flukes[1]				Fecal egg levels
	Year	Month	Day				Gravid	Non-gravid	Gravid	Non-Gravid	Duodenum	Abomasum	Omasum	Rumen	
068	1981	05	21	98	F	unk	--	--	--	--	--	--	--		18.2
069	1981	05	22	03	M	yes	--	--	47	0	--	--	--		74.6
070	1981	05	23	03	M	yes	103	2	--	--	0	0	0	0/32	61.5
071	1980	06	27	00	F	no	--	--	--	--	--	--	--		--
072	1981	05	29	00	M	yes	65	0	--	--	0	0	0		44.1
073	1981	05	26	09	F	yes	27	5	52	4	0	0	0	0/32	14.6
075	1981	06	02	03	M	yes	--	--	100	4	--	--	--	0/1	220.0
076	1981	05	27	00	F	unk	--	--	--	--	--	--	--		8.1
077	1981	04	27	00	F	yes	89	13	--	--	--	0	0	1/30	--
078	1981	06	08	04	M	yes	62	0	18	0	--	0	0	0/18	16.2
079	1981	06	01	06	M	yes	4	0	10	0	0	0	0	0/14	--
080	1981	06	04	02	M	no	--	--	--	--	0	0	0		--
081	1981	02	13	04	M	yes	--	--	0	8	--	--	--	0/8	--
082	1981	06	04	02	M	yes	103	1	--	--	--	0	0	0/32	--
083	1981	06	03	01	M	yes	104	0	19	0	0	0	0	0/72	--
084	1981	01	12	00	M	yes	--	--	0	4	--	--	--	1/4	--
085	1981	06	24	01	M	yes	1	0	--	--	--	--	--	0/1	--
086	1981	02	26	01	F	yes	32	53	--	--	0	0	0		--
087	1981	01	21	98	-	yes	0	102	--	--	--	--	--	0/32	--
088	1981	01	21	98	M	yes	3	20	--	--	--	--	--		1.6
089	1981	01	21	98	F	yes	0	24	--	--	--	--	--	0/24	--
090	1981	01	06	00	M	yes	3	64	--	--	--	--	--	0/26	--
091	1981	06	20	00	-	no	--	--	--	--	0	--	--		--
092	1981	07	02	01	M	yes	8	0	--	--	--	0	0	0/8	--
093	1981	07	07	03	F	unk	--	--	--	--	--	--	--		3.3
094	1981	07	02	02	M	yes	--	--	101	0	--	0	0	0/31	--
095	1981	06	21	02	F	yes	29	0	--	--	0	0	0	0/29	--
096	1981	06	17	02	F	yes	20	1	--	--	--	--	--		--
097	1981	07	13	02	M	no	--	--	--	--	0	0	0		--

APPENDIX 1. (Cont'd)

Moose specimen no.	Date of death			Age[2] of moose	Sex of moose	Infected with flukes	Flukes from rumen samples		Flukes from other than rumen samples		No. of newly acquired flukes[1]				Fecal egg levels
	Year	Month	Day				Gravid	Non-gravid	Gravid	Non-Gravid	Duodenum	Abomasum	Omasum	Rumen	
098	1981	06	--	00	-	no	--	--	--	--	0	0	0		--
099	1981	07	28	06	F	yes	102	1	--	--	0	0	0	0/31	145.0
101	1981	07	07	01	F	yes	30	0	--	--	--	--	--	0/30	--
102	1981	09	03	01	F	yes	--	--	--	--	--	--	--	0/4	0.0
103	1981	10	21	00	F	yes	--	--	--	--	0	0	0		
104	1981	11	16	98	F	yes	--	--	--	--	0	0	0		0.0
105	1981	10	22	00	M	no	--	--	--	--	--	--	--		--
026	1980	06	--	01	-	yes	--	--	--	--	0	0	0		--
106	1981	10	22	00	F	no	--	--	--	--	--	--	--		--
107	1980	10	08	00	F	yes	--	--	0	1	--	--	--		--
200	1979	05	17	03	F	yes			86	0				0/32	
201	1979	10	--	03	M	yes			91	19				1/27	38.3
202	1979	10	15	99	-	yes			39	81				13/19	
203	1979	04	06	00	F	yes			0	24				1/24	
204	1979	03	29	01	F	yes			0	32				0/32	
205	1979	06	11	98	M	yes			40	1				0/41	
206	1979	06	25	08	F	yes			32	0					
207	1979	04	06	98	F	yes			0	102				0/32	
208	1979	10	14	99	-	yes			16	16				15/30	
209	1979	10	--	03	M	yes			39	88				12/33	
210	1979	10	--	01	M	yes			30	52					
211	1978	06	08	99	M	yes			1	0					
212	1978	10	17	02	F	yes			1	7				1/8	
213	1978	10	17	02	F	yes			1	0				0/1	
214	1979	05	24	01	M	yes			64	0				0/33	
215	1978	05	25	03	M	yes			17	0				0/17	
216	1978	06	17	98	F	yes			21	0				0/21	
217	1978	10	17	10	F	yes			53	29				7/79	
218	1978	09	26	15	M	yes			0	104				0/32	

APPENDIX 1. (Cont'd)

Moose specimen no.	Date of death			Age of moose[2]	Sex of moose	Infected with flukes	Flukes from rumen samples		Flukes from other than rumen samples		No. of newly acquired flukes[1]				Fecal egg levels
	Year	Month	Day				Gravid	Non-gravid	Gravid	Non-Gravid	Duodenum	Abomasum	Omasum	Rumen	
219	1978	10	17	02	M	yes			13	2				0/15	
220	1977	06	12	02	M	yes			11	0				0/12	
221	1977	08	30	18	M	yes			18	1				0/19	
222	1978	02	24	05	F	yes			21	83				0/70	
223	1977	08	11	02	F	yes			29	0				0/29	
224	1979	03	29	01	F	yes			0	19				0/19	
225	1979	06	05	98	M	yes			9	0				0/9	
226	1977	06	10	01	M	yes			0	1				0/1	
227	1978	04	25	08	F	yes			103	1				0/32	
228	1979	06	05	02	M	yes			170	0				0/70	
229	1977	07	19	03	F	yes			33	1				0/34	
230	1978	03	07	07	F	yes			22	80				0/32	
232	1978	10	14	01	F	yes			28	12					
233	1976	10	04	03	M	yes			0	12				1/12	
234	1976	10	04	13	F	yes			21	4				0/26	
235	1976	10	05	10	F	yes			134	0				7/134	
236	1976	10	06	03	F	yes			11	0					
237	1976	10	07	08	F	yes			68	0					
238	1976	10	05	06	F	yes			14	0				0/14	
239	1976	10	14	01	F	yes			22	1				0/23	
240	1976	10	17	02	F	yes			8	112				5/81	
241	1976	11	13	01	M	yes			0	1				0/1	
242	1976	11	20	17	F	yes			0	25				0/30	
243	1976	06	18	09	F	yes			98	2				0/34	
244	1976	07	05	02	M	yes			22	0				0/22	
245	1976	08	14	02	M	yes			25	0				0/24	
246	1976	12	09	03	F	yes			2	62				1/52	
248	1976	09	13	03	M	yes			5	0				0/5	
249	1977	02	07	99	M	yes			1	100				5/32	

APPENDIX 1. (Cont'd)

Moose specimen no.	Date of death			Age[2] of moose	Sex of moose	Infected with flukes	Flukes from rumen samples		Flukes from other than rumen samples		No. of newly acquired flukes[1]				Fecal egg levels
	Year	Month	Day				Gravid	Non-gravid	Gravid	Non-Gravid	Duodenum	Abomasum	Omasum	Rumen	
250	1977	02	17	06	F	yes			0	32				5/32	
251	1976	02	10	12	F	yes			0	55				4/55	
252	1979	10	07	00	M	no			--	--					0.0
253	1976	10	07	00	F	no			--	--					
254	1976	10	04	01	F	no									
255	1976	10	04	01	M	no									
256	1977	09	06	01	F	no									
257	1977	04	27	00	F	no									
258	1977	06	22	01	F	no									
259	1978	07	28	00	M	no									
260	1979	06	21	00	M	no									
261	1979	06	12	01	F	no									
262	1976	02	--	00	M	no									
263	1979	06	27	00	M	no									
264	1979	03	29	98	F	yes			5	25				0/30	
265	1979	03	29	01	M	yes			0	32				0/32	
266	1976	10	14	01	F	yes			1	0					
267	1979	10	--	99	M	yes			1	1					
268	1979	10	15	01	M	yes			16	14				3/12	
269	1979	10	--	99	-	yes			16	14					

[1] Full counts were not possible in any of these organs. In the rumen newly acquired flukes are presented as a proportion of the flukes examined.

[2] When the age is listed as 98 it is an animal 1.5 years or older in age, a 99 is a moose whose age is unknown.

APPENDIX 2. Information on population estimates of *Paramphistomum sp.* obtained from rumens of wild moose from northwestern Ontario

Moose specimen no.	Total volume of rumen and reticulum	Worm counts from 1 litre samples of diluted rumen contents				Subtotal from rumen samples	Worm counts from 0.5 litre rumen wall wash samples				Subtotal from rumen wash samples	Other worms counted	Total[1] population estimates
		No. 1	No. 2	No. 3	No. 4		No. 1	No. 2	No. 3	No. 4			
005	29.7	4	7	8	12	691	18	8	16	29	533	0	1,224
006	18.8	1	0	0	0	14.1	2	0	1	0	22.5	0	37
007	33.0	3	2	0	5	247.5	40	55	54	61	1,575		2,139
008	31.5	75	107	87	80	8,250	187	172	160	176	5,213	0	13,462
009	28.3	19	14	10	14	1,210	17	16	12	11	420		1,630
013	45.5	16	17	27	29	3,044	34	37	38	33	1,065		4,109
014	51.0	3	6	5	2	612	19	17	21	19	570		1,182
015	9.0	0	0	0	0	0	0	0	0	0	0		0
016	10.0	1	0	0	1	15	1	2	3	2	60		75
017	28.0	5	14	13	10	882	41	42	44	39	1,245		2,127
018	30.0	23	20	23	28	2,115	45	53	52	51	1,509		3,624
021	38.0	7	4	3	2	456	14	14	10	12	375		831
022	26.0	0	0	0	0	0	0	0	0	0	0		0
023	2.8	0	0	0	0	0	0	0	0	0	0		0
024	36.8	254	277	244	249	28,262						0	28,262
025	22.8	0	0	0	0	0	0	0	0	0	0	0	0
027	36.8	138	156	132	132	15,401	199	199	208	166	5,790	37	21,131
028	30.7	1	2	0	1	92	4	5	4	6	144	27	263
029	10.7	1	0	7	0	64	1	0	1	1	23	0	87
030	16.9	44	44	46	44	2,256	30	37	32	36	1,014	0	3,270
032	5.2	26	26	23	27	398	7	12	10	4	249	0	647
033	26.5	147	140	164	146	11,869	52	53	58	62	1,689	0	13,558
034	10.7	269	260	276	288	8,773	70	85	86	91	2,490	0	11,263
035	17.5	10	20	17	15	814	7	11	9	11	285	0	1,099
036	28.2	17	16	4	13	1,058	4	7	3	1	114	0	1,172
037	10.7	10	12	8	8	305	4	2	10	11	204	0	509
038	38.1	88	93	81	100	10,344	151	169	155	144	4,635	0	14,979
040	14.4	8	16	10	12	497	0	2	4	5	83	0	580

APPENDIX 2. (Cont'd)

Moose specimen no.	Total volume of rumen and reticulum	Worm counts from 1 litre samples of diluted rumen contents				Subtotal from rumen samples	Worm counts from 0.5 litre rumen wall wash samples				Subtotal from rumen wash samples	Other worms counted	Total[1] population estimates
		No. 1	No. 2	No. 3	No. 4		No. 1	No. 2	No. 3	No. 4			
041	21.4	6	3	1	6	257	3	5	9	5	165		422
043	15.1	107	94	105	100	4,598	27	35	36	30	960		5,558
044	10.1	3	1	1	1	45	10	16	20	12	435		480
045	20.8	2	6	5	5	281	1	1	2	0	30	0	311
046	13.8	0	0	0	2	21	1	0	0	0	8	0	28
047	3.6	5	6	10	11	86	11	8	17	16	390	0	476
048	24.3	140	137	152	140	10,374	34	70	61	68	1,749	0	12,123
049[3]	13.8	1	0	0	0	10	0	0	1	0	8	0	18
053[2]												2,666	2,666
060	17.5	1	6	8	8	403						0	403
064	35.3	6	3	2	3	371						27	398
065	19.6	12	3	5	7	397						36	433
066	30.9	27	35	50	42	3,569						16	3,585
070	32.6	42	53	75	63	5,701						4	5,705
072	15.6	17	11	10	17	646						33	679
073	22.4	12	18	10	5	756						56	812
077	24.3	22	27	27	15	1,662						11	1,672
078	25.9	17	13	11	21	1,204						18	1,222
079	21.0	1	0	1	2	63						10	73
080	15.0	0	0	0	0	0						0	0
081	46.2	1	5	2	3	388						8	396
082	15.7	176	198	165	184	8,516						20	8,536
083	15.7	33	32	39	34	1,625						19	1,644
085	21.0	1	0	0	0	16						0	16
086	34.7	27	25	22	15	2,321						370	2,691
087	42.2	53	46	55	68	7,026						1	7,027
088	38.4	8	5	7	5	720						6	726
089	45.0	4	11	5	4	810						6	816
090	32.3	23	20	28	23	2,277						15	2,292

APPENDIX 2. (Cont'd)

Moose specimen no.	Total volume of rumen and reticulum	Worm counts from 1 litre samples of diluted rumen contents				Subtotal from rumen samples	Worm counts from 0.5 litre rumen wall wash samples				Subtotal from rumen wash samples	Other worms counted	Total[1] population estimates
		No. 1	No. 2	No. 3	No. 4		No. 1	No. 2	No. 3	No. 4			
092	19.4	2	1	1	1	73						15	88
095	13.8	9	8	4	8	300						4	304
096	16.2	5	3	2	3	158						8	166
097	32.4	0	0	0	0	0						0	0
099	20.4	66	67	63	64	3,978						71	4,049
101	13.2	5	9	5	11	297						18	315
102	20.0	0	2	2	0	60						0	60

[1] No. 15 and 23 not used in analysis of population estimates because they were young calves and the counts from 16 and 47 not used because rumen material had been lost.

[2] No. 60 volume of rumen and reticulum contents increased by 4X rather than 3X.

[3] No. 53 counted directly with a total count being done.