THE UNIVERSITY OF MANITOBA

HELMINTHS IN *Rana pipiens* SCHREBER, AND *Bufo hemiophrys* COPE, FROM THE DELTA MARSHES, MANITOBA

by

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ABSTRACT

Nine species of helminths were found in specimens of Rana pipiens (5 of trematodes, 3 of nematodes, and 1 of cestodes), and nine in specimens of Bufo hemiophrys (5 of trematodes, 3 of nematodes, and 1 of acanthocephalans) from Delta. Four helminth species were specific for a particular host. Seven helminth species occurred in both hosts. Evidence suggested that the separation of Rhabdias bufonis and R. ranae is doubtful. Nine new host records and one new Canadian record were established. Anurans living mainly in a semi-aquatic environment were parasitized by higher numbers and more kinds of helminths than anurans living mainly in a terrestrial environment. Larger anurans had more kinds of helminths than smaller anurans. No significant difference was found in the intensity of helminthic infections between males and females of a particular host species. A statistical comparison of the expected and observed cases of juvenile and adult helminths in lungs suggested an antagonism between trematodes and nematodes. Changes in the extensity and intensity of infections were related to the habits of the definitive hosts and the life-cycles of the helminths.

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INTRODUCTION

Studies of anuran helminths are scarce in recent parasitological literature. Older American reports surveyed the kind and distribution of helminths in anurans in various regions, but few investigated the ecological factors determining helminthic infections. The scarcity of either surveys or ecological studies on these parasites in published Canadian literature is a curious gap in the otherwise intense and wide-ranging scope of Canadian parasitology. This study surveys the helminths of _Rana pipiens_ and _Bufo hemiophrys_ in the Delta Marsh of Manitoba, and attempts to relate their infection to ecological factors.

A survey in the summer of 1968 revealed a total of 10 helminth species in both hosts. Plots of the helminth numbers per host against time revealed that a high helminthic infection occurred in spring and early summer with a gradual decline towards autumn in both hosts. To explain these data, a broad objective was chosen; namely an examination of the factors influencing the incidence of helminthic infection in _Rana pipiens_ and _Bufo hemiophrys_. To facilitate this, eight questions will be examined. These are as follows:

1. Do the leopard frog and the Canadian toad have the same or different helminth fauna?
2. Are certain helminths specific to one host and not to the other?
3. Does the helminth fauna of a frog or toad depend on its environmental preference?

4. Is parasitism related to the size or sex of the host?
5. Do changes occur in the intensity or extensity of helminthic infections with the progression of seasons?
6. Are seasonal changes in parasitism correlated with the habits of the host and/or life cycles of the helminths?
7. To what extent does multiple species parasitism occur?
8. When two kinds of helminths occupy the same organ in a host is there evidence of antagonism, synergism, or no interaction?

LITERATURE REVIEW

DISTRIBUTION OF *Rana pipiens* AND *Bufo hemiophrys*

The systematics of *Rana pipiens* or leopard frog is in a state of flux. The species includes various subspecies or races in its distribution over North America (Wright and Wright, 1949; Stebbins, 1954). The following distribution is given for the *R. pipiens* complex, and no attempt will be made to deal separately with the controversial subspecies and races.

The leopard frog is the most widely distributed amphibian in North America. Its range extends from Great Slave Lake in the District of Mackenzie, Canada, south to Panama; and from the Atlantic Coast to the western edge of the Great Basin (Wright and Wright, 1949; Stebbins, 1954).

Bufo hemiophrys or Canadian toad is generally restricted to the Canadian prairies in its distribution. Its range extends from the District of Mackenzie to eastern South Dakota; and from south-eastern British Columbia to eastern Manitoba (Stebbins, 1966).

ECOLOGICAL RELATIONS OF *Rana pipiens* AND OF *Bufo hemiophrys*

Table I gives the ecological relations of the leopard frog and Canadian toad. The major ecological distinction between the two species stems from the greater aquatic affinity of *R. pipiens*

TABLE I.

Ecological relations of *R. pipiens* and *B. hemiophrys*

Descriptions	*Rana pipiens*	*Bufo hemiophrys*
habitat during active phase	frequents springs, creeks, rivers, ponds, pools, canals, marshes, and resevoirs where there is permanent water and aquatic or emergent aquatic vegetation(Wright and Wright,1949; Stebbins, 1954).	found close to water; frequents lakes, ponds, streams, marshes and roadside ditches. Chiefly diurnal during the breeding season, retiring to sandy banks to bury itself at night(Stebbins, 1966).
feeding habitat	in water basins or near their margins but may forage far from water in damp meadows(Noble,1931; Moore and Strickland,1954; Stebbins,1966).	generally terrestrial; in or near, pond margins (Moore and Strickland, 1954;Breckenridge and Tester,1961).
breeding	in Canada and Northern U.S.A.,breeds from April 1 to May 15(Wright and Wright,1949).	breeds from May onward (Wright and Wright,1949).
habitat during dormant phase (hibernation)	aquatic; spend the winter in pools or marshes (Wright and Wright,1949).	terrestrial; spend the winter buried 75 to 115 feet from pond margins (Breckenridge and Tester 1961).

and the greater terrestrial affinity of *B. hemiophrys*. This distinction is most apparent during their period of hibernation.

Moore and Strickland (1954) in Alberta compared the food ingested by *R. pipiens* and *B. hemiophrys*. They found that toads fed mainly on ground-dwelling insects such as ground beetles, carrion beetles, and ants. Frogs, take a greater proportion of flying insects, and feed on ground-dwelling insects with the exception of such common types as ants. Furthermore, frogs because of their more aquatic habits, feed on aquatic insects more than do toads.

SURVEYS AND LIFE HISTORY STUDIES OF ANURAN HELMINTHS

World literature of anuran helminths is extensive. Most of the recent contributions, chiefly morphological, are from the Oriental biogeographic region and were not considered in detail in this review.

A review of Nearctic literature prior to 1936 revealed that Brandt's summary in 1936 was reasonably accurate. I was able to make 22 additions to his list (Table III) for the period from 1936 to 1969.

Some omissions in Brandt's summary (1936) were early Canadian records. This is not surprising for it was with considerable diligence that I was able to find 26 Canadian host/parasite records (Table II). Nine papers were my sources; namely, Stafford (1900, 1902a, b, c, d, and 1905); Simon (1922); Fantham and Porter (1948); and Anderson (1960, 1964).

Most literature on helminths of anurans is devoted to descriptions of new species, host records, or morphological studies.

TABLE II.

Helminths from Canadian anurans

Helminths	Host	Local	Author
trematodes			
Polystoma integerrimum (Froelich,1791) Rud.,1808	Bufo americanus	Quebec	Fantham and Porter(1948)
Manodistomum occultum Stafford,1905	Rana virescens	Canada *	Stafford(1905)
Loxogenes arcanum (Nickerson,1900) Stafford,1905	Rana catesbeiana,R.clamitans	Nova Scotia,Ont.	Stafford(1905); Simon(1922)
Glypthelmins quieta (Stafford,1900) Stafford,1905	R.catesbeiana,R.virescens Hyla pickeringii	Canada	Stafford(1905)
Glypthelmins subtropica Harwood,1932	R.clamitans	Quebec	Fantham and Porter(1948)
Cephalogonimus americanus Stafford,1902	R.virescens,R.clamitans	Quebec	Stafford(1902); Fantham and Porter (1948)
Haematoloechus longiplexus Stafford,1902	R.catesbeiana	Canada	Stafford(1902)
H. breviplexus Stafford,1902	R.catesbeiana,R.clamitans,R.pipiens R.virescens,Bufo americanus	Quebec	Stafford(1902); Fantham and Porter (1948)
H. varioplexus Stafford,1902	Rana spp.,B.americanus	Canada	Stafford(1902)
H. similiplexus Stafford,1902	R.virescens,R.pipiens,R.clamitans B.lentiginosus,B.americanus	Canada	Stafford(1902)
H. medioplexus Stafford,1902	B.lentiginosus,R.catesbeiana R.pipiens,R.virescens	Quebec	Stafford(1902); Fantham and Porter (1948)
Gorgoderina amplicava Looss,1899	R.catesbeiana,R.clamitans	Canada	Stafford(1905)
G. attenuata(Stafford,1902)Stafford,1905	R.clamitans,R.pipiens,R.catesbeiana	Quebec	Stafford(1905); Fantham and Porter (1948)
G. simplex (Looss,1899) Looss,1902	B.americanus	Quebec	Stafford(1905); Fantham and Porter (1948)
G. opaca (Stafford,1902) Stafford,1905	toads and frogs	Canada	Stafford(1905)
G. translucida (Stafford,1902) Stafford,1905	toads and frogs	Canada	Stafford(1905)

* Stafford gives no specific Canadian local, however eastern Canada(Ontario & Quebec) are likely locals.

TABLE II. continued

Helminths	Host	Local	Author
Halipegus occidualis Stafford,1905	R.clamitans,R.catesbeiana	Canada	Stafford(1905)
Diplodiscus temperatus Stafford,1905	R.virescens,R.catesbeiana	Canada	Stafford(1905)
Brachycoelium salamandrae (Froelich,1789) Stafford,1905	R.catesbeiana	Quebec	Stafford(1905); Fantham and Porter (1948)
cestodes			
Diphyllobothrium ranarum Meggitt,1925	R.catesbeiana,B.americanus	Quebec	Fantham and Porter(1948)
Ophiotaenia saphena Osler,1931	R.clamitans	Quebec	Fantham and Porter(1948)
nematodes			
Rhabdias bufonis (Schrank,1788)	R.catesbeiana,R.pipiens	Quebec	Fantham and Porter(1948)
R. ranae Walton,1929	R. pipiens	Manitoba	Walton(1929)
Aplectana americana (*)	B.americanus	Quebec	Fantham and Porter(1948)
Cosmocercoides dukae Holl,1928	R.pipiens,R.catesbeiana,R.clamitans R.septentrionalis,B.americanus	Ontario	Anderson(1960)
Oxysomatium inglisi Anderson,1964	R.catesbeiana	Ontario	Anderson(1964)

* authority could not be found in the available literature

TABLE III.

Additions to Brandt's 1936 list of helminths infecting N.American anurans north of Mexico

Helminths:	Host	Location	Author	Country
trematodes				
Halipegus aspina	Rana boyli	stomach	Ingles,1936	U.S.A.
Megalodiscus microphagus	Bufo boreas	rectum	Ingles,1936	U.S.A.
Gorgoderina aurora	R.aurora	bladder	Ingles,1936	U.S.A.
Haematoloechus buttensis	R.boyli	lungs	Ingles,1936	U.S.A.
Glypthelmins shastai	B.boreas	intestine	Ingles,1936	U.S.A.
Brachycoelium lynchi	R.aurora	intestine	Ingles,1936	U.S.A.
Haplometrana utahensis	R.pretiosa	intestine	Olsen,1937a	U.S.A.
Gorgoderina tanneri	R.pretiosa	bladder	Olsen,1937b	U.S.A.
Halipegus eccentricus	R.clamitans R.pipiens R.catesbeiana	ear ear ear	Thomas,1939 " "	U.S.A. " "
Langeronia provitellaria	R.pipiens	intestine	Sacks,1952	U.S.A.
Phyllodistomum bufonis	B.boreas	intestine	Frandsen,1957	U.S.A.
Glypthelmins pennsylvaniensis	Hyla crucifer	intestine	Cheng,1961	U.S.A.
Bunoderella netteri	Ascaphus truei	intestine	Schell,1964	U.S.A.
cestodes				
Crepidobothrium olor	R.aurora	intestine	Ingles,1936	U.S.A.
Cylindrotaenia quadrijugosa	R.pipiens	intestine	Lowler,1939	U.S.A.
Ophiotaenia gracilis	R.catesbeiana	intestine	Jones,1957	U.S.A.
nematodes				
Rhabdias joaquinensis	R.aurora	lungs	Ingles,1936	U.S.A.
Oswaldocruzia waltoni	B.boreas R.aurora	intestine "	Ingles,1936 "	U.S.A. "
Spironura pretiosa	R.pretiosa	intestine	Ingles,1936	U.S.A.
Spironura rankini	R.clamitans	intestine	Walton,1941	U.S.A.
Oxysomatium inglisi	R. catesbeiana	rectum	Anderson,1964	Canada
Strongyloides physali	B.valiceps	intestine	Little,1966	U.S.A.

Helminth surveys of anurans (Table IV) are generally of two types: one deals with a particular class of helminth and its occurrence in one or several host species, while the other deals with all classes of helminths in one or more host species.

One hundred and four genera of helminths are known from world amphibians. Fifty-four of these genera are known from North American amphibians (26 nematode genera, 20 trematode genera, 6 cestode genera, and 2 acanthocephalan genera), while 4 nematode, 16 trematode, and 2 cestode genera are known from Canadian amphibians.

Known life-cycles of helminths are chiefly those of the Digenean Trematodes (Table V). The lack of literature on nematode life-cycles may be due to the fact that many are thought to lack an intermediate host and to enter the definitive host directly. Acanthocephalans and cestodes are infrequent parasites of anurans and consequently are rarely reported in the literature.

Polystoma integerrimum (Froelich, 1791) Rud., 1808 is the only Monogenea species reported in North America. It is apparently limited to two locales, northeastern North America (Quebec) and Minnesota (Yamaguti, 1963), even though widespread in Europe.

A REVIEW OF SOME PARTICULAR HELMINTH GENERA PARASITIC IN ANURANS

The following review will deal with nine genera, that pertain to the helminth species found in this study. Discussions are based mainly on the work of Hyman (1951), and Yamaguti (1958, 1959, 1961 and 1963).

TABLE IV.

Helminth surveys

Authors		Number of anuran species surveyed	Country	Helminth classes *
Ingles	1936	7	U.S.A.	T N C
Brandt	1936	6	U.S.A.	T N C A
Rankin	1945	7	U.S.A.	T N C
Bouchard	1951	6	U.S.A.	T
Grossman and Sandner	1953	2	Poland	T A
Markov and Rogoza	1953	1	U.S.S.R.	T N A
Najarian	1955	9	U.S.A.	T
Mazurmovich	1957	5	U.S.S.R.	T N C A
Lehmann	1960	3	U.S.A.	T N
Waitz	1961	10	U.S.A.	T N
Lees	1962	1	Britain	T N A

* A - acanthocephalans
T - trematodes
N - nematodes
C - cestodes

TABLE V.

Additions to Brandt's 1936 list of life history studies of helminths infecting some North American anurans north of Mexico.

Author	Helminth species	First intermediate host	Second intermediate host	Infection of definitive host
Krull (1936) Odlaug (1937) Goodchild (1948)	Gorgodera amplicava (Trematoda)	Musculium partumetium	Helisoma antrosa	ingestion of infected second intermediate host
Olsen (1937)	Haplometrana utahensis (Trematoda)	Physella spp.	tadpoles, Rana spp. epidermis	ingestion of tadpoles or shed skins bearing cysts
Leigh (1937)	Glypthelmins quieta (Trematoda)	Physa spp.	anuran epidermis	ingestion of shed skin bearing cysts
Thomas (1939)	Halipegus eccentricus (Trematoda)	Physa spp., Helisoma spp.	Cyclops spp.	ingestion of infected Cyclops
Herber (1939)	Diplodiscus temperatus (Trematoda)	Helisoma trivolvis	tadpole and frog epidermis	ingestion of tadpoles or shed skins bearing cysts
Rankin (1939)	Gorgoderina attenuata (Trematoda)	Sphaerium spp.	tadpoles	ingestion of tadpoles harboring metacercaria
Ogren (1953, 1959) Anderson (1960)	Cosmocercoides dukae (Nematoda)	terrestrial gastropods		ingestion of infected gastropods
Lang (1968, 1969)	Cephalogonimus americanus (Trematoda)	Helisoma spp.	tadpole or frog epidermis	ingestion of tadpole or shed skins bearing cysts

TREMATODA

Family: Paramphistomatidae

Diplodiscus Diesing, 1836

Adults are parasitic in the endgut of amphibians and rarely in fishes. The five species listed in the literature have a world-wide distribution. Cercariae encyst on both bottom debris and the epidermis of tadpoles and frogs. Infection ensues when either debris or shed skin bearing the cysts is eaten by tadpoles or frogs.

Family: Gorgoderidae

Gorgoderina Looss, 1902

Members of the genus are commonly found in the kidney, ureters, and bladder of amphibians. Twenty-four species are listed in the literature, twelve of which occur in North America. The genus is well distributed in the Western hemisphere with additional records in Europe and Asia. Miracidia penetrate a bivalve of the family Sphaeriidae. The cercariae are ingested by a damsel fly nymph or other aquatic insect larvae. The metacercariae, after hatching in the definitive host, seem to inhabit the kidneys first, passing into the bladder as sexual maturity is attained.

Family: Plagiorchiidae

Haematoloechus Looss, 1899

Members are parasitic in lungs of amphibians. Forty-four species are known to have a world-wide distribution while nineteen have been reported from North America. Cercariae develop in snails of the genera Planorbula and Gyraulus, and encyst in dragonfly nymphs of the genus Sympetrum. Infection results when the definitive host ingests infected dragonflies (Krull, 1930).

Family: Cephalogonimidae

Cephalogonimus Poirier, 1886

Species of this genus are parasitic in the intestine of amphibians and reptiles. Five species known to parasitize amphibians are recorded from North American hosts with one species reported also from a European frog. Cercariae develop in snails of the genera Lymnaea and Planorbis, and then encyst on the epidermis of tadpoles or frogs. Infection occurs when a tadpole or shed skin bearing the cysts is ingested by frogs (Lang, 1969).

CESTODA

Family: Proteocephalidae

Ophiotaenia La Rue, 1911

These worms are parasites in the intestine of amphibians and reptiles. Sixteen species are known from amphibians, nine of them from North America. Other records are from South and Central America, Australia, Japan and South Africa. The oncosphere is ingested by copepods of the genus Cyclops and develops into a procercoid in its body cavity. The definitive host can be infected directly by eating parasitized Cyclops or by eating tadpoles that have fed on such *Cyclops* (Osler, 1931).

NEMATODA

Family: Rhabdiasidae

Rhabdias Stiles et Hassall, 1905

Adults and juveniles of this genus are commonly found in lungs of amphibians and reptiles. Twenty-three species are known from amphibians, four of them from North American amphibians. The distribution of the genus is world-wide. The life cycle includes parasitic and free-living adults. The parasitic form is either a protandrous hermaphrodite or a parthenogenetic female. The eggs from this hatch to free-living young which may develop directly into the parasitic form, in which case the cycle is said to be direct or homogonic; or they may develop into free-living males and females, the offspring of which proceed to the parasitic phase so that the cycle is then indirect or heterogonic.

Family: Trichostrongylidae

Oswaldocruzia Travassos, 1917

Adults and juveniles of the genus are parasitic in the intestine of amphibians and reptiles. The twenty-five species reported from amphibians give the genus a world-wide distribution. The definitive host may be infected by skin penetration, or more likely by ingestion of the infective juvenile with food. Eggs are embryonated when deposited.

Family: Oxyuridae

Cosmocercoides Wilkie, 1930

These worms are parasitic in the endgut of amphibians and reptiles. The five species of this genus are known from North America, Europe, Japan and India. The only North American species in amphibians and reptiles is C. dukae. Amphibians and reptiles become infected by ingesting gastropods harboring the infective larvae. According to the Chitwoods (1937) and Anderson (1960), several species occur in the intestine of slugs and other terrestrial snails.

ACANTHOCEPHALA

Family: Pomphorhynchidae

Pomphorhynchus Monticelli, 1905

Representatives of this genus are usually parasitic in fishes. Eight species are known, of which one was described from an anuran. Species of this genus are distributed in temperate North America, Northern Europe, and Japan. Acanthors of this genus occur in amphipods, Gammarus. The definitive host is infected by ingestion of an amphipod harboring the acanthellas.

ECOLOGY OF HELMINTHIC INFECTIONS

GENERAL

Ecological studies (eg. Brandt, 1936; Rankin, 1937, 1945) are less frequent in Neartic than in Palearctic literature (eg. Mazurmovich, 1951; Markov and Rogoza, 1953; Grossman and Sandner, 1953; Lees, 1962). Although few authors investigated the ecology of anuran parasitism, most made observations and valuable suggestions on the ecological factors that influence parasitism. Thus ecological information was obtained from three kinds of surveys. The first was primarily concerned with helminth systematics. The second was surveys investigating the helminth fauna of anuran hosts with coincidental ecological considerations. The third, and in my opinion most valuable kind of survey, was that which relates the helminth fauna to the ecology of the host. According to Brandt (1936) the third type of survey gave a systematic and comprehensive study of the relations of parasitic infections to the ages, habitats, and habits of hosts.

Mazurmovich (1951) used the terms 'intensity' and 'extensity' to describe the host-parasite relationship. 'Intensity' was defined as the average number of helminths per host and 'extensity' referred to the per cent of the hosts infected.

HELMINTHS AND ENVIRONMENT

The most aquatic host species generally harbored a greater number of helminths and greater number of species than did the more terrestrial host (Brandt, 1936; Rankin, 1937 and 1945; Bouchard, 1951; Markov and Rogoza, 1953; Mazurmovich, 1957).

Brandt's data suggested a high correlation between aquatic environment preference and degree of metazoan parasitism (Table VI). A comparatively higher platyhelminth and acanthocephalan infection and per cent total infection were found in the more aquatic hosts.

TABLE VI.

Helminthic infections and the environment of the host.

(from Brandt, 1936)

Host species	Environment	No. hosts examined	% hosts infected	Mean trematode infection	Mean cestode infection	Mean nematode infection	Mean acanthocephalan infection	Mean helminthic infection
Rana catesbeiana Shaw, 1802	aquatic	71	98.6	27.6	17.1	77.9	5.93	128.9
R. sphenocephala Cope, 1886	semi-aquatic	60	100.0	44.8	10.0	19.3	2.62	76.7
Bufo fowleri Hinckley, 1882	semi-terrestrial	62	96.8	0.73	3.58	19.6	0.016	23.9
Scaphiopus holbrookii Harlan, 1835	terrestrial	60	83.3	0.0	1.25	17.6	0.0	18.9

Rankin (1945) and Bouchard (1951) attributed this to the dependence of these helminths on aquatic media and aquatic intermediate hosts for their transmission. Thus aquatic hosts are more apt to become infected directly in the water or indirectly by ingestion of aquatic intermediate hosts. The complete absence of flukes in the least aquatic amphibian species, Scaphiopus holbrookii, is noteworthy. Brandt suggested that a subterranean environment with an almost complete reduction of the time spent in an aquatic habitat at the breeding period (estimated minimum of one day) effectively minimized the success of trematode parasitism.

Nematode infections were found to be erratic in their distribution between aquatic and terrestrial environments. Oswaldocruzia pipiens Walton, (1929), is widespread in North America and occurs in all host species. On the basis of his data (Table VII) Brandt suggested that O. pipiens showed an adaptation to a terrestrial environment. Palearctic literature (Mazurmovich, 1951; Markov and Rogoza, 1955; Lees, 1962), supports Brandt's observation - that frogs become infected by nematode parasites during the terrestrial phase of their existence. Rankin (1945) found O. pipiens to be the commonest nematode infecting the amphibians and reptiles in western Massachusetts. He concluded that O. pipiens was commonly found in aquatic hosts, rarely in terrestrial hosts. This contradictory conclusion may be explained by the fact that Rankin oversimplified his definition of 'aquatic' environment. Thus amphibian hosts associated with an aquatic environment were aquatic and reptile hosts associated with a terrestrial environment were terrestrial.

TABLE VII.

Oswaldocruzia pipiens distribution and environment of the host
(from Brandt, 1936)

Host species	Environment description	No. hosts examined	Mean *O. pipiens* per frog	% hosts infected by *O. pipiens*
R. catesbeiana	aquatic	71	0.231	13.7
R. sphenocephala	semi-aquatic	60	2.18	48.3
B. fowleri	semi-terrestrial	62	3.5	61.3
S. holbrookii	terrestrial	60	1.52	38.3

His failure to subdivide the amphibian habitat as did Brandt, probably resulted in this contradictory conclusion.

SEASONAL PERIODICITY OF HELMINTHIC INFECTIONS

Seasonal periodicity of helminths in amphibians is difficult to summarize from the literature. The progression of seasons is thought to be the limiting factor for variation in helminthic infections. Most variation in life-cycles of the parasite and host can be correlated with seasonal and physiographic changes (Brandt, 1936; Rankin, 1937). Due to many variants within seasonal progression and because the literature refer to a variety of geographical areas, this review will be concerned with only some general variations of helminthic infections.

Brandt (1936) found no significant seasonal periodicity in the intensity of helminthic infections when he considered each group of trematodes, nematodes, cestodes and acanthocephalans. Small sample sizes for each of the six hosts and the lack of uniformity in sampling intervals, may have contributed to his negative results.

Rankin (1937), working on salamanders of North Carolina, suggested that the intensity of trematode infections was higher from May to September due to higher temperatures and greater host exposure to aquatic intermediate hosts. Lees (1962) found that nematodes were abundant in Rana temporaria Linné, at all times of the year, except summer. He suggested that May and June were dry months in north-eastern England and thus were detrimental to the survival of the infective stages of nematodes in, or on the soil.

Cestodes are rare parasites of anurans (Brandt, 1936; Rankin, 1945). Brandt and also Rankin (1937) found cestode infections more prevalent from June to November.

Some agreement exists on the seasonal incidence of acanthocephalans, though few species have been found in amphibians. Most authors of the publications reviewed found greatest infections with acanthocephalans during the hibernation or in the spring. Rankin (1945) found *Pomphorhynchus bulbocolli* (Linkins 1919) Van Cleave, 1919, in *Triturus v. viridescens* in the autumn and suggested that these worms survive best at low temperatures.

Considering all helminths, the literature suggests that the highest infection occurs in the spring when the amphibians are in aquatic breeding habitats. The fact that few species of helminths show significant seasonal periodicity does not prove the absence of such variation. As Van Cleave (1916) said, "the mere fact that a parasite is present in a definitive host for the entire year is no proof that there is no periodicity."

AGE AND SIZE OF HOST AND THE HELMINTHIC INFECTIONS

The effect of host size on helminths was investigated by Krull (1931). He suggested that the size of mature lung flukes of the genus *Haematoloechus* is independent of the size of the host and the number of flukes present, but depends upon the physiological condition of the host. Krull observed that the longevity of *Haematoloechus spp.* was, on the average, one year. Brandt (1936) found that larger and presumably older bullfrogs harbored greater numbers of parasites than smaller individuals. A study of salamander parasites by Rankin (1937) bears out the observation of Brandt, that an increase in age and size of the host is associated with an increase in infection.

HOST BREEDING AND THE HELMINTHIC INFECTIONS

Most authors suggest that breeding activity and physiology of amphibians affect helminthic infections. Unfortunately no study has adequately tested these hypotheses.

Smith (1950) studied the seasonal changes of gonads in the European frog, Rana temporaria. He found that the weight of the gonads was greatest from August to February and lowest during the spawning period from March to July. Lees and Bass (1960) studied the effect of seasonal changes in the reproductive physiology of the host on helminths. They found that the level of parasitism in male frogs, during and immediately before the breeding season was considerably higher than in females. Their results also indicated that the presence of the female hormone, oestradiol, was responsible for the depression of the level of parasitism by helminths.

INFLUENCE OF HELMINTHS ON THE HOST: PATHOGENICITY OF HELMINTHIC INFECTIONS

Studies on pathology of helminthic infections in anurans are rare in the literature. Rankin (1937) suggested that few salamander parasites injure their hosts. The most potentially harmful types were acanthocephalans, and metacercariae designated as "Diplostomulum".

An investigation of pathogenicity of anuran helminths was conducted by Goodchild (1950). The bladder flukes _Gorgoderina attenuata_ (Stafford, 1905) and _Gorgodera amplicava_ (Looss, 1899) were found regularly in the bladder, Wolffian ducts, and encapsulated in the mesonephroi. Frogs parasitized with several hundred juvenile worms in their ureters were sluggish and sickly. With heavier infections the kidneys were hyperemic, purplish and traumatized. The author suggested that a heavy infection can be fatal.

METHODS AND MATERIALS

SURVEY AREA

The purpose of the survey in the summer of 1968 was to find the species of helminths occurring in *Rana pipiens* and *Bufo hemiophrys*. Therefore, a large area of the Delta Marshes was needed for sampling (Fig. 1). This area included the lakeshore and sandy beaches, the forested beach ridge, dry dirt roads, roadside ditches, damp meadows and various marsh basins.

In 1969, a more convenient survey area was chosen for the summer. This area, within a one-half mile radius of the University of Manitoba Field Station, has four types of habitats, characteristic of most sites sampled in the 1968 survey. The following is a description of the four habitats (Fig. 2). Plant names are taken from Scoggan (1957).

BEACH

The largest area sampled was the south shore of Lake Manitoba. A sandy beach extends 3-8 m. from the forested beach ridge to the water's edge. The first 3-8 m. of lake sediment consists of soft organic matter mixed with silt and sand. In this site the predominant plant species *Phragmites communis* Trin. var. *berlandieri* (Fourn.) Fern., *Typha latifolia* L., and *Scirpus* spp. were occurring in clumps. This area was subject to fluctuations in water level and

Fig. 1. Map showing areas surveyed in 1968 and 1969.

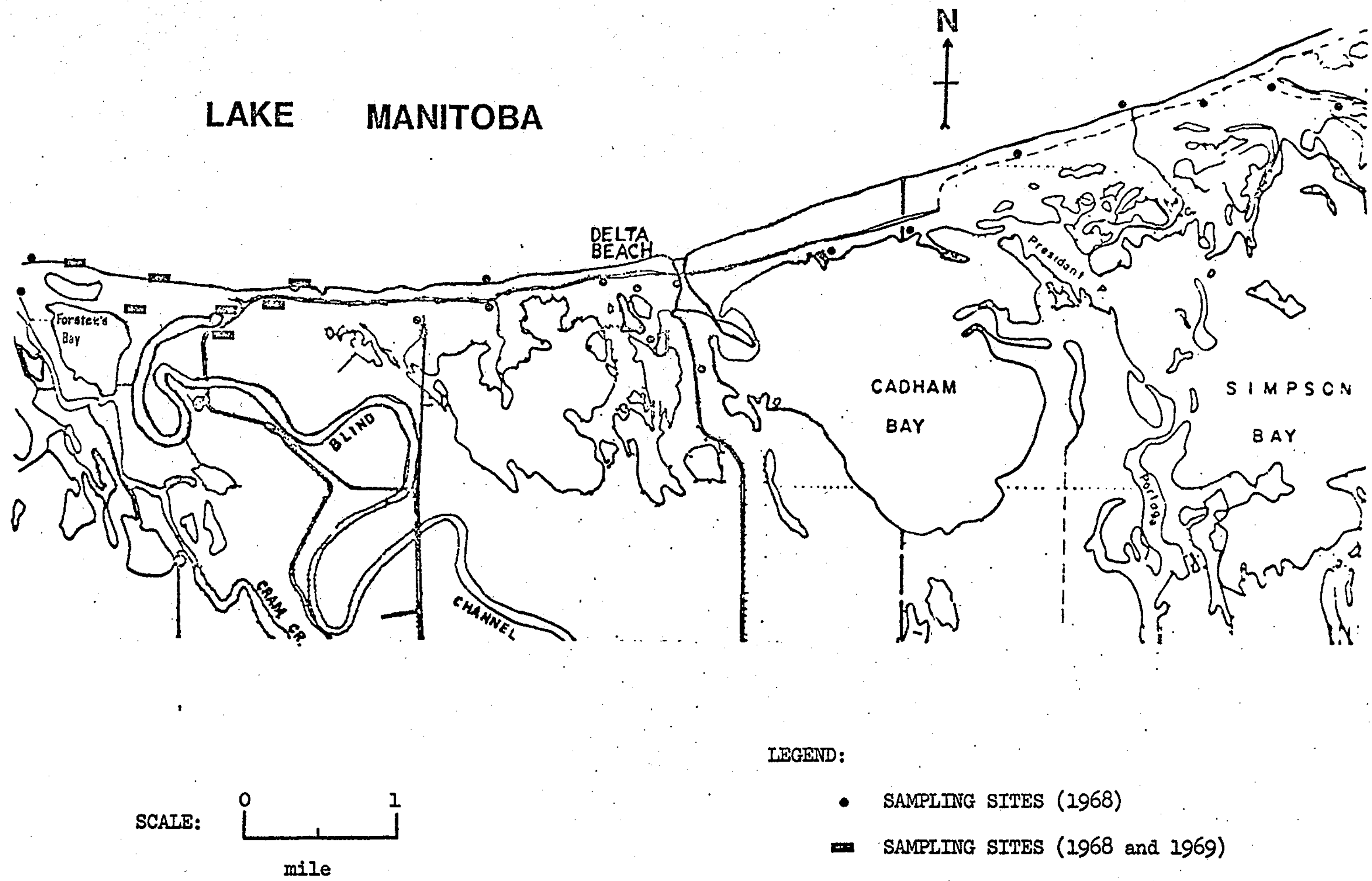
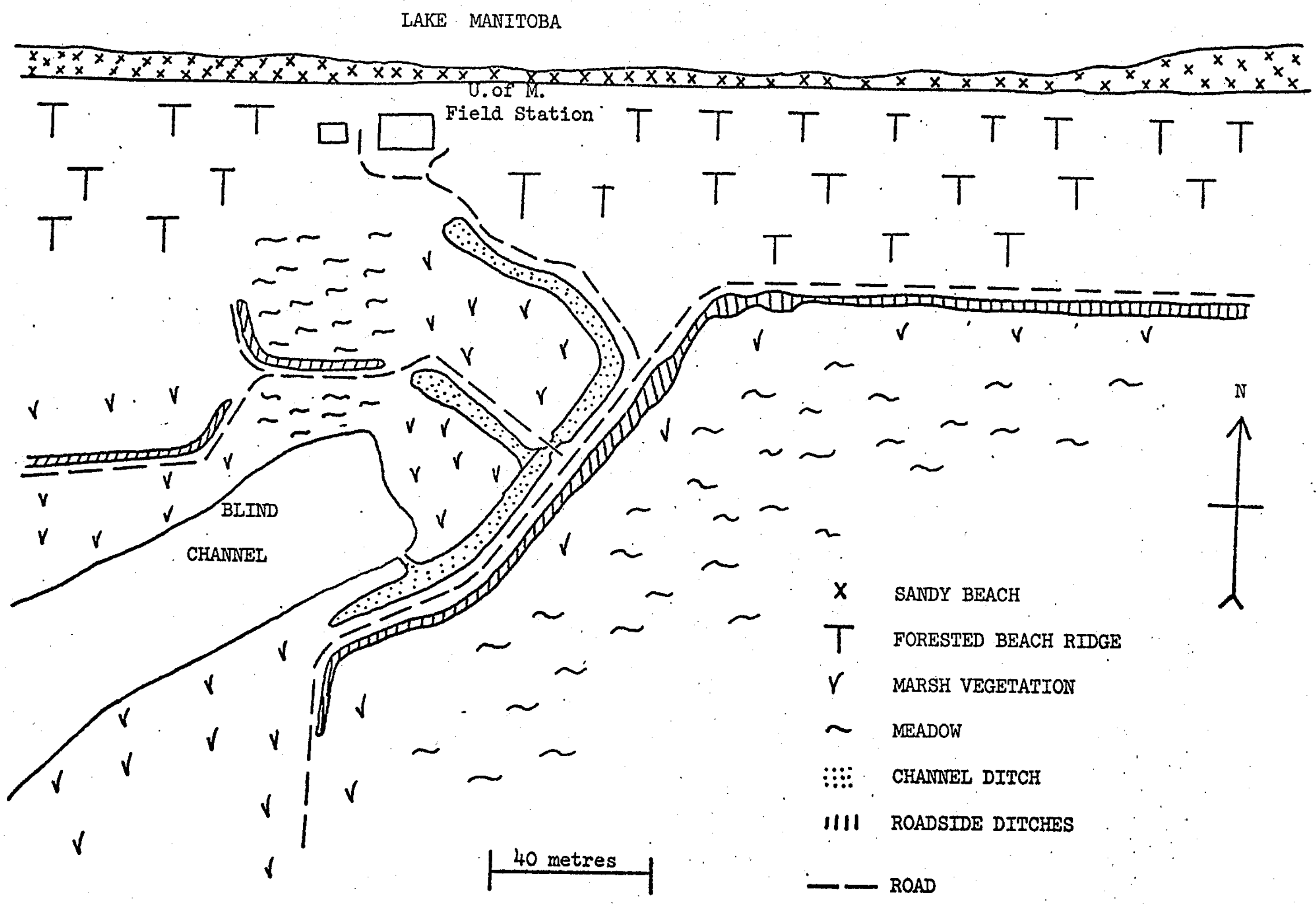

LAKE MANITOBA
N
DELTA BEACH
Forster's Bay
BLIND
CRAM CR.
CHANNEL
CADHAM BAY
President
SIMPSON BAY
Portage
SCALE: 0 1 mile
LEGEND:
SAMPLING SITES (1968)
SAMPLING SITES (1968 and 1969)

Fig. 2. Map showing habitats surveyed in 1969.


LAKE MANITOBA
U.of M.
Field Station
BLIND
CHANNEL
N
40 metres
SANDY BEACH
FORESTED BEACH RIDGE
MARSH VEGETATION
MEADOW
CHANNEL DITCH
ROADSIDE DITCHES
ROAD

was submerged or exposed with a north or south wind respectively. *Rana pipiens* were caught at this site in early spring and early fall. *Bufo hemiophrys* were taken here from mid-summer to September.

CHANNEL DITCH

This area is maintained as a permanent water basin by the influx of lake water via Cram Creek, Forster's Bay and Blind Channel (Figs. 1 and 2). Its mean depth is 0.75 m. and bottom sediments consist of soft organic debris. Pike, carp, stickleback, and two species of minnows are found in the ditch. The flora and fauna, which is the richest of the sites sampled, attained a peak in July. Both host species occurred in this habitat.

ROADSIDE DITCHES

These ditches are temporary water basins with maximum depths ranging from 0.3 - 1.0 m. *Phragmites communis*, *Scholochloa festucacea* (Willd.) Link., *Typha* spp., and *Agropyron* spp. are common plants of this habitat. Bottom composition ranges from soft organic debris to firm vegetation mats. *B. hemiophrys* were captured here during their breeding period. *R. pipiens* were abundant in ditches adjacent to the channel ditch (Fig. 2).

MEADOW

The meadow is a well drained habitat. The dominant plant species are Carices, *S. festucacea*, *Hordeum jubatum*, and *Agropyron* spp. Few leopard frogs were taken from this site.

SAMPLE METHOD

GENERAL

The size groups present in the anuran populations were not sampled regularly in 1968. The greater proportion of large anurans, compared with smaller or immature individuals, in the weekly sample, resulted in a sampling bias which was even greater parasitologically for it was found that larger hosts harbored greater numbers and variety of helminths.

The sampling procedure for the period April to September 1969, was designed to investigate the decrease in the intensity of helminthic infections from spring to autumn as it occurred in both hosts in 1968, but to avoid a bias to any particular size. This procedure was based on sampling of three arbitrary size groups at weekly intervals. These size categories, based on the length to the nearest millimetre from the tip of the snout to the vent (Wright and Wright, 1949), are listed below:

Size Class	*R. pipiens*	*B. hemiophrys*
A	≤ 69 mm.	≤ 40 mm.
B	70 - 85 mm.	41 - 50 mm.
C	≥ 86 mm.	≥ 51 mm.

Size class 'A' contained only sexually immature individuals. Both immature anurans and mature anurans comprised the 'B' class while size class 'C' contained only sexually mature individuals.

CAPTURE TECHNIQUE

In daylight, anurans were captured with a large hooped dip net or by hand. At night, they were caught by hand or dip net, with the aid of a portable 12 volt head lamp. Individuals were placed in polyethylene bags and brought to the laboratory for examination.

EXAMINATION OF HOSTS

R. pipiens and *B. hemiophrys* were kept in 10 gallon aquaria with one-half inch of water covering the base. Storage of anurans in aquaria did not exceed 24 hours prior to dissection. Storage of hosts for longer periods was most effective when the frogs were killed and stored frozen in a refrigerator.

Host specimens were killed either by freezing or by placing them in a 32 oz. jar with cotton soaked in chloroform. After each specimen was killed (or thawed if frozen), it was weighed and the snout to vent length was measured to the closest millimetre. Prior to dissection each individual was examined externally for abnormalities or injuries, and the oral cavity was examined for helminths. The specimen was then placed ventral side up on a dissecting board, and a mid-ventral incision made from the cloaca to the throat. The skin and abdominal musculature were reflected to each side.

The heart was measured from base to apex with fine pointed forceps, and the distance between the points, in millimetres, was recorded as a measure of heart distention. A blood sample was taken directly from the heart and a blood smear made, to determine if any Foleyella spp. were present. The lungs, stomach, intestine, rectum and bladder were then excised and each examined separately for helminths under an Olympus dissecting microscope. Stomach contents were examined under the low power of the dissecting microscope, indentified, recorded, and preserved in 10% formalin. The helminths were counted and placed in separate vials for each host specimen.

PREPARATION OF HELMINTHS

TREMATODA AND CESTODA

Platyhelminths were placed into fresh 2.5% $MgCl_2$ solution and allowed to relax and die. After death they were compressed slightly between two microscope slides and fixed in F.A.A. (40% formaldehyde 10 cc., 95% ethanol 85 cc., glacial acetic acid 5 cc.), for at least 24 hours. Trematodes and cestodes were then washed to remove fixative, transferred to Petri dish with Ehrlich's haematoxylin or aceto-carmine dye and stained for 2 hours. If Ehrlich's haematoxylin was used the helminths were destained in acid alcohol and blued in running tap water. The specimens were then dehydrated in an ascending series of alcohol, cleared in xylene or cedarwood oil, and mounted in Permount.

NEMATODA

Nematodes were heat killed, fixed in 5% formalin for at least 24 hours, then placed in a 5% solution of glycerol in methanol in an uncorked 1 dram vial. The vial and contents were placed in an oven at 60°C for 30 minutes permitting the methanol to evaporate. The vial was next transferred to a dessicator for 24 hours to extract the remaining methanol and water, leaving pure glycerol. Excellent results were obtained by this method.

ACANTHOCEPHALA

Acanthocephala were relaxed and killed in a solution of 2.5% $MgCl_2$ and fixed in 5% formalin for 24 hours. They were washed in water to remove the fixative and the body wall was punctured 2 to 3 times with a sharp dissecting needle. The acanthocephalans were then placed in a Syracuse watchglass with aceto-carmine dye for 30 minutes. They were cleared and mounted in glycerol using the same method as in the nematode preparation. The puncturing of the body wall was done under the high power of a dissecting microscope and care taken not to disfigure the internal organs. This procedure allowed the aceto-carmine dye entrance into the body cavity, otherwise sealed by the cuticle.

IDENTIFICATION OF HELMINTHS

Helminths were identified using the Zoology of Tapeworms (Wardle and McLeod, 1952), the Nematode Parasites of Vertebrates

(Yorke and Maplestone, 1926), Systema Helminthum, Vol. I, II, III, and V (Yamaguti, 1958, 1959, 1961, and 1963), and original literature and drawings.

TABULATION OF DATA

The species, sex, size, weight, pathological appearance, food available for each anuran, and the presence and position of any helminths were recorded in a set of data sheets. (Appendix III.)

OBSERVATIONS

BIOLOGY OF *Rana pipiens*

GENERAL

The leopard frog is the most aquatic anuran species in the region studied. Its environment is fully aquatic during hibernation and breeding. Following the breeding period, the leopard frog alternates between aquatic and moist terrestrial sites, best described as a semi-aquatic environment.

The breeding period which begins just after the frogs' emergence from hibernation in Lake Manitoba, ends about mid May. Gonads were observed to be fully developed at the time of the frog's emergence from the lake. Ova were heavily pigmented at the animal poles and filled most of the abdominal cavity, and testes were large. The first spent female was found on May 12, 1969, while regenerating ovaries were first observed on July 10, 1969. Changes in testes size were not as readily observable. The maximum development of testes and ovaries was observed in the periods of April-May and August-September.

SIZE STRUCTURE OF SAMPLE

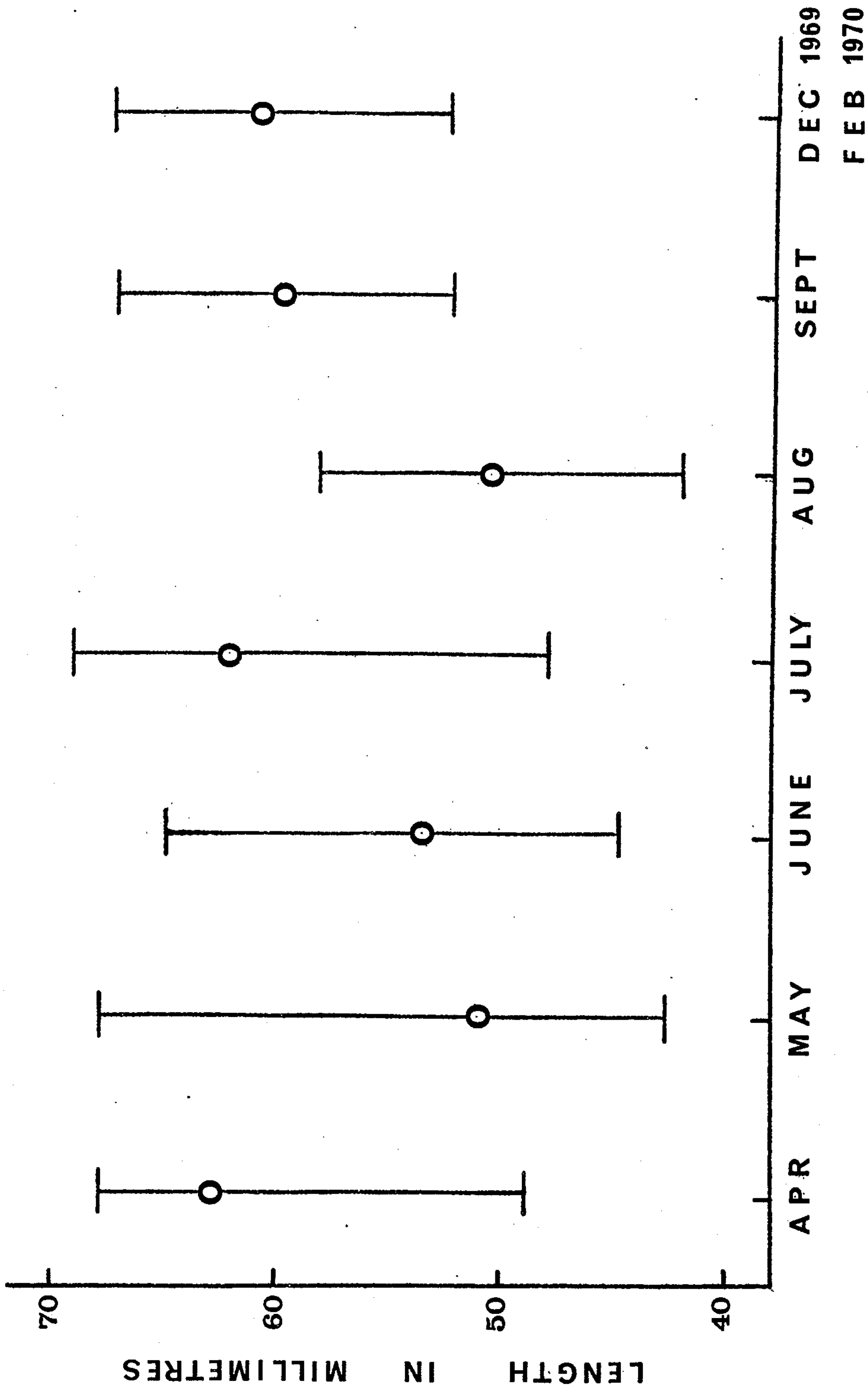
The three size categories were arbitrarily selected for the weekly samples during the April 1969 to February 1970 period (see p. 30). They reflect differences of maturity or age in the following ways.

Figure 3 shows the mean and range of lengths of leopard frogs in the 'A' size class during the 1969 sampling period. The general emergence of yearlings from hibernation did not occur until the first week of May. Thus the mean body length of the April sample was influenced by the larger frogs which had hibernated through their second winter. These individuals probably moved into the 'B' size class by May. The decline of the mean body length in August reflects the large numbers of newly transformed individuals entering the population. Leopard frogs which transformed at the end of July at 38 to 44 mm. in length, generally did not exceed 65 mm. in length by April of the following year.

Ovaries of frogs less than 75 mm. in length were immature during the breeding period, whereas females larger than 75 mm. had well developed ovaries and oviducts with darkly pigmented ova filling the abdominal cavity. The variation in size of testes was too small to be graded. Thus the size of frogs at maturity was deduced from observations of ovary development only.

Observations of growth rates of R. pipiens suggest that size class 'A' was composed primarily of yearlings. The age composition of size classes 'B' and 'C' could not be determined from the data available. Approximate age compositions were suggested as follows: size classes 'B' and 'C' contained sexually immature and sexually mature individuals which had spent more than one year in hibernation.

Fig. 3. Rana pipiens: mean body lengths and ranges of size class 'A' individuals during the period April 1969 - February 1970.


LENGTH IN MILLIMETRES
70
60
50
40
APR
MAY
JUNE
JULY
AUG
SEPT
DEC 1969
FEB 1970

FOOD

Table VIII shows the occurrence of various organisms in 206 stomachs of *R. pipiens*. The data indicate that leopard frogs in the Delta marshes feed chiefly on arthropods and to a lesser extent on snails and small vertebrates. The major part of the arthropod material was insectan. Among the wide variety of insects taken, adult beetles and flies were most common as food items while hemipterans were next. Predation on any one organism by frogs was influenced by the abundance of the prey in the frogs' habitat. For example, during chironomid emergences in June, July, and August, when thick mating swarms of midges were evident throughout the sampling area, large numbers of Diptera were found in the stomach contents.

The percentage of full stomachs containing aquatic organisms (i.e. aquatic larvae of dipterans, coleopterans and trichopterans; aquatic nymphs and adult hemipterans and also aquatic gastropods) is plotted against time in Fig. 4. The rapid decrease of aquatic organisms taken, from May to September, suggests that either the frogs spend less time in an aquatic environment as the summer progresses or there is a decrease in the number of aquatic organisms available and an increase in the number of non-aquatic organisms available for anurans as food during this period.

No significant correlations could be found between the kind or number of occurrence of food items and the size or sex of the frog.

All 34 stomachs from frogs captured during the hibernating period were macroscopically empty, and only one out of 18 stomachs in the month of April was full.

TABLE VIII.

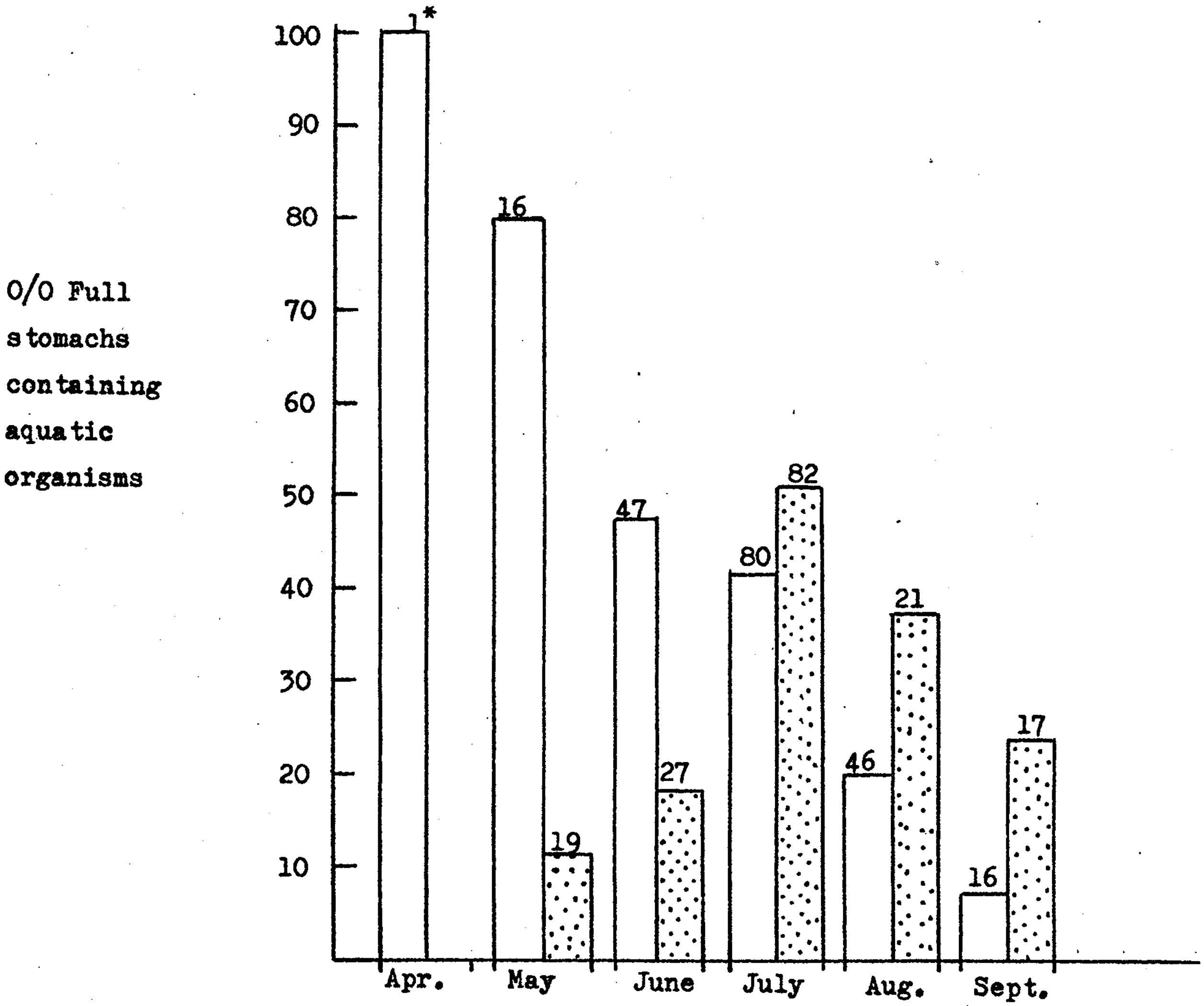
Stomach contents of <u>Rana pipiens</u>. All figures represent the number of stomachs containing the given organisms.

Description	Hibernation Mar.1964 Dec.1969-Feb.1970	April 1969	May 1968 1969	June 1968 1969	July 1968 1969	Aug. 1968 1969	Sept. 1969	Total
Mammalia (<u>Sorex</u>)					1	2	1	4
Aves (<u>Troglodytes</u>)					1			1
Amphibia (<u>Rana</u>,<u>Bufo</u>)			1	3	6	7	2	19
Arthropoda		1	16	37	73	38	15	180
Arachnida		1	2	9	18	5	4	39
Crustacea		1		2	1		1	5
Insecta		1	16	37	72	37	11	174
Coleoptera		1	10	28	51	32	8	130
Diptera		1	3	23	37	9	2	75
Hemiptera			7	3	22	5	1	38
Hymenoptera				2	4	2	1	9
Homoptera				1	1	1		3
Trichoptera			1	3	5	1		10
Orthoptera					3			3
Lepidoptera					7	6	2	15
Ephemeroptera					2	1		3
Odonata					1			1
Collembola			1					1
Gastropoda			16	22	31	7	1	67
No. stomachs examined	34	18	42	64	109	81	31	379
No. stomachs full	0	1	16	47	80	46	16	206
No. stomachs with aquatic organisms	0	1	13	22	34	9	2	81

Fig. 4. Percentage of full stomachs containing aquatic organisms.

☐ *Rana pipiens*

▦ *Bufo hemiophrys*



* number of full stomachs

SEX RATIO

Females were more numerous than males. In the 1968 sampling period, out of 92 frogs greater than 69 mm. in body length, the sex ratio was 26:66 or 39:100. The females were generally larger with a mean length of 88 ± 1.5 mm.*, whereas the mean length of males was 81 ± 1.9 mm. A sampling bias in 1968, when large frogs made up most of the weekly sample was probably responsible for the unbalanced sex ratio of the sample.

The sex ratio of frogs greater than 69 mm. in body length, in the sampling period 1969-1970 was 55:78 or 71:100. The average length of female frogs was 88 ± 1.0 mm., whereas male frogs averaged 82 ± 0.8 mm. in length. The largest size class (size class 'C') had a sex ratio of 33:100. As the sex ratio in the middle size class (size class 'B') was 140:100 the resulting sex-ratio of the sample favoring females is due to larger frogs (size class 'C') being mainly females.

HIBERNATION AND MIGRATIONS

Frogs were observed migrating in large masses from the marsh basins to the beach of Lake Manitoba. Most of the migration must have occurred in September for frogs were rarely observed in the marsh at the end of September. Concentrations of frogs were observed on the beach and inshore waters of Lake Manitoba during September. Inshore observations at the end of September did not reveal the presence of many frogs and it was assumed that they were hibernating.

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* length ± standard error.

Fishermen at Delta have found R. pipiens lightly enmeshed in their gill nets during their offshore winter fishing through the ice. Catches of frogs were made in Lake Manitoba on March 3, 1964 as far as eleven miles off shore under three feet of ice. During winter many of the marsh basins are frozen to the bottom with an ice cover up to $4\frac{1}{2}$ feet thick. Oxygen tests of water in the Delta marsh, taken during March, 1964, showed 0.0-0.1 part per million.

Emergence from hibernation occurs during the last half of April when the ice has receded about a hundred yards from shore. R. pipiens was first observed emerging from Lake Manitoba on April 24th, 1969. Upon emergence the leopard frogs migrate over the beach ridge and into marsh basins where breeding ensues.

Sexually immature frogs measuring less than 70 mm. in length were seldom found during the first week of R. pipiens' emergence from the lake. The same size class (size class 'A') was found most abundant in the last week of September when larger and sexually mature frogs were least encountered. These observations agree with those of Noble (1931), that smaller and sexually immature frogs enter, and emerge from hibernation one to two weeks after the larger and sexually mature individuals.

BIOLOGY OF Bufo hemiophrys

GENERAL

The Canadian toad is the most terrestrial anuran in the region. The toads' environment is aquatic only during the breeding period.

Following this period, the toad alternates between a semi-aquatic environment and a terrestrial environment.

The breeding period was observed from the beginning of May to the end of June in 1969. Gonads were fully developed at the time of the toads' emergence from hibernation. The first spent female was found on July 1, 1969, and regenerated ovaries were first observed on July 22, 1969. Changes in testes size were too gradual to be graded, however a maximum size similar to that prior to breeding was attained by the end of August.

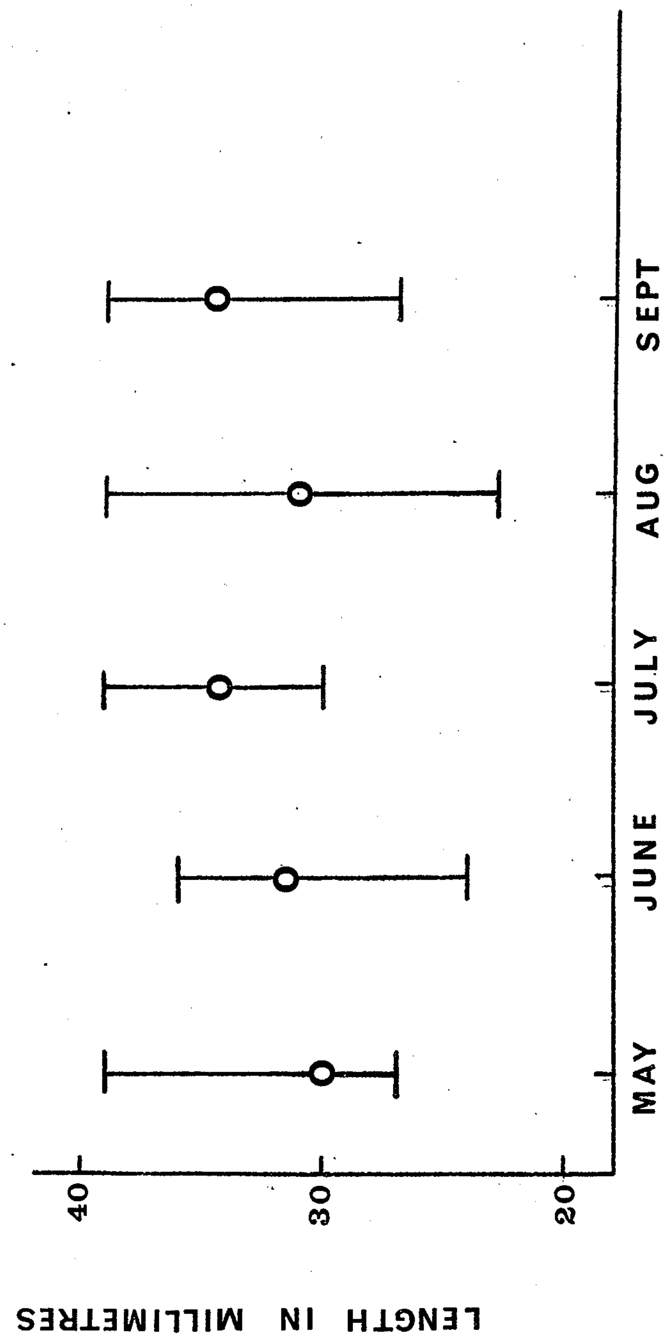
SIZE STRUCTURE OF SAMPLE

The three size categories were arbitrarily selected for the weekly samples collected from May to September, 1969 (see p. 30). They reflect differences of maturity or age in the following ways:

Figure 5 shows the means and ranges of toads in the 'A' size class during the 1969 sampling period. The decline of the mean length in August reflects the large numbers of newly transformed individuals entering the population. Toads which underwent transformation at the end of July at 19 to 24 mm. in length were thought not to exceed 35 mm. by May of the following year.

Ovaries of toads less than 43 mm. in length were immature during the breeding period, whereas females longer than 43 mm. had well developed ovaries and oviducts with darkly pigmented ova filling the abdominal cavity. The variation in size of testes was too small to be graded. Thus the size of frogs at maturity was deduced from observations of ovary development only.

Fig. 5. Bufo hemiophrys: mean body lengths and ranges of size class 'A' individuals during the period May - September 1969.


LENGTH IN MILLIMETRES
40
30
20
MAY
JUNE
JULY
AUG
SEPT

Observations of growth rates of B. hemiophrys suggest that size class 'A' was composed primarily of yearlings. The age composition of size classes 'B' and 'C' could not be determined from the data available. Approximate age compositions were suggested as follows. Size class 'B' contained both sexually immature and sexually mature individuals which had hibernated through two or more winters, while size class 'C' contained individuals which were all sexually mature and had probably spent three or more winters in hibernation.

FOOD

Table IX shows the occurrence of various organisms in 167 stomachs of B. hemiophrys. The data indicate that toads in the Delta marshes feed chiefly on arthropods and to a lesser extent on snails. The major part of the arthropod material consists of insects. Among the wide variety of insects taken, beetles and flies appear to be most common as food items while hymenopterans are next.

Like frogs, the toads show no preference for any food items. Their diet seems to be influenced by the abundance of a given organism.

The percentage of full stomachs containing aquatic organisms is plotted against time in Fig. 4. The relatively high percentage in July indicates that toads remain near water after breeding. The decline in the percentage of full stomachs with aquatic organisms in August and September is in agreement with observations that toads leave the aquatic environment of the marsh and inhabit the sandy beach and forested beach ridge of Lake Manitoba during this period.

TABLE IX.

Stomach contents of Bufo hemiophrys. All figures represent the number of stomachs containing the given organisms.

Description	May 1968 1969	June 1968 1969	July 1968 1969	Aug. 1968 1969	Sept. 1969	Total
Arthropoda	19	26	79	21	17	162
Arachnida	4	4	23	3	3	37
Crustacea			10			10
Insecta	19	26	79	18	17	159
Coleoptera	16	21	64	16	16	133
Diptera	4	10	55	4	5	78
Hemiptera	1	2	16	1	1	21
Hymenoptera	1	3	29	1	6	40
Homoptera			3	1	5	9
Trichoptera					1	1
Lepidoptera			1			1
Ephemeroptera			2			2
Odonata			4			4
Collembola	1		2			3
Gastropoda	1	5	19	3		28
No. stomachs examined	41	54	113	75	25	308
No. stomachs full	19	27	82	21	17	166
No. stomachs with aquatic organisms	2	5	42	8	4	61

No significant correlations could be found between the kind or number of occurrence of food items and size or sex of the toads.

Unlike R. pipiens, 50% of the toads examined during emergence from hibernation and breeding had food in their stomachs.

SEX RATIO

Of the toads over 40 mm. long, females were more numerous than males in the 1968 sample with a male to female ratio of 27:35 or 77:100. The females were generally larger with a mean length of 54 ± 1.1* mm., whereas the mean length of males was 49 ± 1.0 mm. A sampling bias in 1968, when weekly collections were mainly of large toads, was probably responsible for the unbalanced sex ratio of the sample. The sex ratio in the largest size class (size class 'C') was 11:27 or 41:100, and in the middle size class (size class 'B') was 24:8 or 300:100. The greater number of large toads sampled resulted in a sex-ratio in favor of females.

The sex ratio of toads greater than 40 mm. in body length, was 60:56 or 107:100 in the 1969 sample. The mean length of the females was 53 ± 0.9 mm., whereas the males averaged 49 ± 0.7 mm. in length. The sex ratio of the 'C' size class was 22:34 or 65:100. The sex ratio of size class 'B' was 38:23 or 165:100. The equal samples of the large toads (size class 'C') and the medium sized toads (size class 'B') resulted in the sex ratio of the sample approaching 1:1.

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* length ± standard error.

HIBERNATION

Toads were not found during their hibernation period. B. hemiophrys is known to burrow into sediments of well drained habitats (Breckenridge and Tester, 1961). As the toads do not hibernate in Lake Manitoba, but are found on the sandy beach and forested beach ridge during August and September, the most probable site of their hibernation is the forested beach ridge.

HELMINTHIC INFECTIONS

HELMINTHS FOUND

A list of helminths found in juvenile hosts (size class 'A') and in female and male hosts (size classes 'B' and 'C'), is presented in Table X. Of the eleven helminth species found, seven occurred in both hosts. Ophiotaenia saphena, Rhabdias bufonis, Rhabdias ranae, and Pomphorhynchus sp. occurred either in B. hemiophrys or R. pipiens, but not both.

IDENTIFICATION PROBLEMS

Lung flukes Haematoloechus medioplexus and H. similiplexus can be readily identified by the shape and arrangement of gonads, coiling of the uteri and the ratio of the diameters of oral sucker to acetabulum. On the other hand, identifications of the lung nematodes, Rhabdias bufonis and R. ranae, were doubtful largely because they were based only on body length.

TABLE X.

List of helminths found

Helminths		Location	Occurrence (X) of helminths in hosts					
			Rana pipiens			Bufo hemiophrys		
			juveniles	females	males	juveniles	females	males
Haematoloechus medioplexus	Stafford, 1902	lungs	X	X	X	X		
H. similiplexus	Stafford, 1902	lungs		X		X	X	X
Cephalogonimus americanus	Stafford, 1902	intestine	X	X	X	X	X	X
Diplodiscus temperatus	Stafford, 1905	rectum	X	X	X		X	
Gorgoderina attenuata	Stafford, 1902	bladder		X	X			X
Ophiotaenia saphena	Osler, 1931	intestine		X	X			
Rhabdias bufonis	Schrank, 1788	lungs				X	X	X
R. ranae	Walton, 1929	lungs	X	X	X			
Oswaldocruzia pipiens	Walton, 1929	intestine	X	X	X	X	X	X
Cosmocercoides dukae	Holl, 1928	rectum	X	X	X	X	X	X
Pomphorhynchus sp. (bulbocolli?)		intestine					X	X

Walton (1929), on the basis of material from U.S.A. and the Winnipeg region of Canada, found that adult lung nematodes of R. pipiens ranged from 3.5 to 4.5 mm. in length and that specimens of R. bufonis ranged from 11 to 13 mm. Walton measured fully mature females as indicated by the presence of rhabditoid embryos in the uterus. He observed that infective juveniles of R. ranae developed directly in the intestine of the host and did not develop from a free living sexual generation as do infective juveniles of R. bufonis. Walton concluded that, "Since the present description applies to material coming from a rather wide area in Temperate North America and also differs materially from specimens obtained from the toads of the same region, the new species has been designated as Rhabdias ranae."

My survey revealed that adult lung nematodes of R. pipiens ranged from 3.5 to 9.3 mm. in length while those from B. hemiophrys ranged from 7.5 to 16 mm. The overlap resulting from the greater size of the lung nematodes of R. pipiens and B. hemiophrys casts considerable doubt on the validity of body length as a diagnostic characteristic to separate R. ranae and R. bufonis. As no observations were made on the life history of the lung nematodes during this study, they are identified according to Walton's hypothesis that R. ranae occurs in R. pipiens and R. bufonis occurs in B. hemiophrys. Walton's hypothesis, certainly need further examination in light of my data.

Identification of the acanthocephalan, Pomphorhynchus was based on four specimens. The long neck with a bulbous anterior swelling (Appendix IV), places this worm in the genus Pomphorhynchus. The hooks are arranged in 12-14 longitudinal rows of 13-15 each. This number of hooks and range of rows falls within the limits given by Van Cleave (1919) for P. bulbocolli (Yamaguti, 1963), but more specimens need to be examined.

INCIDENCE IN ANURANS

General

The incidence of helminths in R. pipiens and B. hemiophrys during the period May 1968 to February 1970 is shown in Tables XI and XII. The terms 'extensity' and 'intensity' refer to the per cent of the hosts infected, and mean number of worms per host, respectively. Helminths occurred in 96.1% of the frogs with an intensity of 41.6 worms. Ninety-five per cent of the toads harbored a mean infection of 16.8 worms.

The greatest intensity of trematode infections was found in R. pipiens. The intensity and extensity of trematode infections in leopard frogs was 32.3 worms and 78.9% respectively. The mean intensity of trematode infections in B. hemiophrys was 1.2 worms and the extensity 28.2%.

The intensity and extensity of nematode infections were greater in B. hemiophrys than in R. pipiens. Toads harbored a mean of 15.5 nematodes with an extensity of 90%. The intensity and extensity of nematode infections in R. pipiens was 9.1 worms and 82.1%.

Cestodes and acanthocephalans were seldom encountered in the anurans studied. Cestodes were found only in R. pipiens whereas acanthocephalans were taken only from toads. A total of 55 tapeworms and 4 acanthocephalans were found.

TABLE XI.

Incidence of helminthic infections (1968,1969)

Helminths	349 *Rana pipiens* total helminths found	intensity	extensity	308 *Bufo hemiophrys* total helminths found	intensity	extensity
Trematoda						
Haematoloechus medioplexus	1547	4.4	49.5 ± 2.7*	1	0.003	0.3 ± 0.3
H. similiplexus	1	0.003	0.3 ± 0.3	368	1.2	26.8 ± 2.5
Cephalogonimus americanus	9242	26.5	45.6 ± 2.7	11	0.033	2.9 ± 0.9
Diplodiscus temperatus	478	1.4	22.2 ± 2.2	1	0.003	0.3 ± 0.3
Gorgoderina attenuata	19	0.5	3.1 ± 1.0	1	0.003	0.3 ± 0.3
Cestoda						
Ophiotaenia saphena	55	0.16	7.8 ± 1.4	---	---	---
Nematoda						
Rhabdias bufonis	---	---	---	691	2.3	57.0 ± 2.8
R. ranae	1507	4.3	56.8 ± 2.8	---	---	---
Oswaldocruzia pipiens	757	2.2	46.8 ± 2.7	2904	9.6	78.7 ± 2.3
Cosmocercoides dukae	904	2.6	39.3 ± 2.6	1085	3.6	52.0 ± 2.8
Acanthocephala						
Pomphorhynchus sp. (*bulbocolli* ?)	---	---	---	4	0.012	1.2 ± 0.6

$$\text{* standard error} = \sqrt{\frac{(100 - \%\text{ hosts infected})\%\text{ hosts infected}}{\text{total number of hosts}}}$$

TABLE XII.

Incidence of helminthic infections (1968,1969) cont'd.

Helminths	349 *Rana pipiens*			308 *Bufo hemiophrys*		
	total helminths found	intensity	extensity	total helminths found	intensity	extensity
All trematodes	11287	32.3	78.9 ± 2.2	374	1.24	28.2 ± 2.6
All cestodes	55	0.16	7.8 ± 1.4	---	---	---
All nematodes	3168	9.1	82.1 ± 2.0	4681	15.5	90.0 ± 1.7
All acanthocephalans	---	---	---	4	0.012	1.2 ± 0.6
All helminths	14510	41.6	96.1 ± 1.0	5059	16.8	95.0 ± 1.2

No filariids were found in the blood of the anurans.

The most frequently encountered trematode in R. pipiens was Cephalogonimus americanus. The intensity and extensity of infections was 26.5 worms and 45.6%. The intensities of H. medioplexus and D. temperatus infections were low with 4.4 and 1.4 worms respectively. The trematodes H. similiplexus and G. attenuata were rare in leopard frogs. The only frequently encountered fluke in B. hemiophrys was Haematoloechus similiplexus with an intensity and extensity of infection of 1.2 worms and 26.8%. The trematodes H. medioplexus, C. americanus, D. temperatus and G. attenuata were rare in toads.

The highest intensity and extensity of nematode infections in frogs was that of Rhabdias ranae with 4.3 worms and 56.8%. The intensities of Oswaldocruzia pipiens and Cosmocercoides dukae infections were low at 2.2 and 2.6 worms respectively.

The highest intensity and extensity of nematode infections in B. hemiophrys was that of O. pipiens with 9.6 worms and 78.7%. The intensities of Rhabdias bufonis and Cosmocercoides dukae infections were low at 2.3 and 3.6 worms respectively.

Intensity and Extensity of Helminthic Infections and Size of Host

The intensity and extensity of helminth infections in the three size classes of R. pipiens and B. hemiophrys sampled from May 1969 to February 1970 are presented in Table XIII.

Leopard frogs of the smallest size (class 'A'), harbored fewer helminths than either of the larger size classes. No cestodes were found in size class 'A' frogs. Trematode infections were considerably higher in the 'B' and 'C' classes. The intensity of nematode

TABLE XIII.

Intensity and extensity of helminthic infections and size of host
(202 *R. pipiens* sampled during the period April 1969- February 1970)
(177 *B. hemiophrys* sampled during the period May- September 1969)

Size class	Helminths	*Rana pipiens* intensity	*Rana pipiens* extensity	*Bufo hemiophrys* intensity	*Bufo hemiophrys* extensity
A	trematodes	4.9	55.5 ± 5.8	0.3	15.0 ± 4.6
	cestodes	---	---	---	---
	nematodes	8.1	73.6 ± 5.2	5.8	66.6 ± 6.1
	acanthocephalans	---	---	---	---
	all helminths	12.6	86.1 ± 4.1	6.0	86.6 ± 4.4
B	trematodes	28.9	86.9 ± 4.2	1.3	31.1 ± 5.9
	cestodes	0.03	2.8 ± 2.0	---	---
	nematodes	4.9	81.1 ± 4.7	13.0	95.0 ± 2.8
	acanthocephalans	---	---	0.06	4.9 ± 2.8
	all helminths	33.6	97.1 ± 4.0	14.2	96.7 ± 2.3
C	trematodes	21.4	92.8 ± 3.1	2.7	60.7 ± 6.5
	cestodes	0.01	1.4 ± 1.4	---	---
	nematodes	5.6	82.8 ± 4.5	31.8	100.0
	acanthocephalans	---	---	0.02	1.7 ± 1.7
	all helminths	28.4	98.5 ± 1.5	34.3	100.0

infections was highest in the 'A' size class while the extensity was higher in the 'B' and 'C' classes.

The intensity and extensity of infection shows a marked increase from the smallest to the largest size class of B. hemiophrys. No acanthocephalans were found in size class 'A'. The intensity and extensity of trematode and nematode infections increased with the size of the host.

Intensity and Extensity of Helminthic Infections by Months of the Year

The intensity of helminthic infections during the period May-August 1968 is shown in Figures 6 and 7. R. pipiens had high intensities of nematode and trematode infections in May and June, and low intensities in July and August. Cestodes were rarely encountered. B. hemiophrys had a similar trend of helminthic infections but with high intensities of nematode infections. The highest intensity of infections was in June with lower intensities in July and August.

Figures 8 and 9 show the intensity of helminthic infections during the period May 1969 - February 1970. The changes in intensities of nematode and trematode infections in R. pipiens show no similarity to the trends observed in 1968. Trematode infections were of a greater intensity than nematode infections and cestodes again were rarely encountered. The greatest intensity of helminthic infections occurred during June, July, and August. September, December, and February were months of lowest helminth intensity.

The intensity of helminthic infections of B. hemiophrys in 1969 is similar to that of 1968. The highest intensity occurred in

Fig. 6. Rana pipiens: intensity of helminthic infections by months of the period May-August 1968.

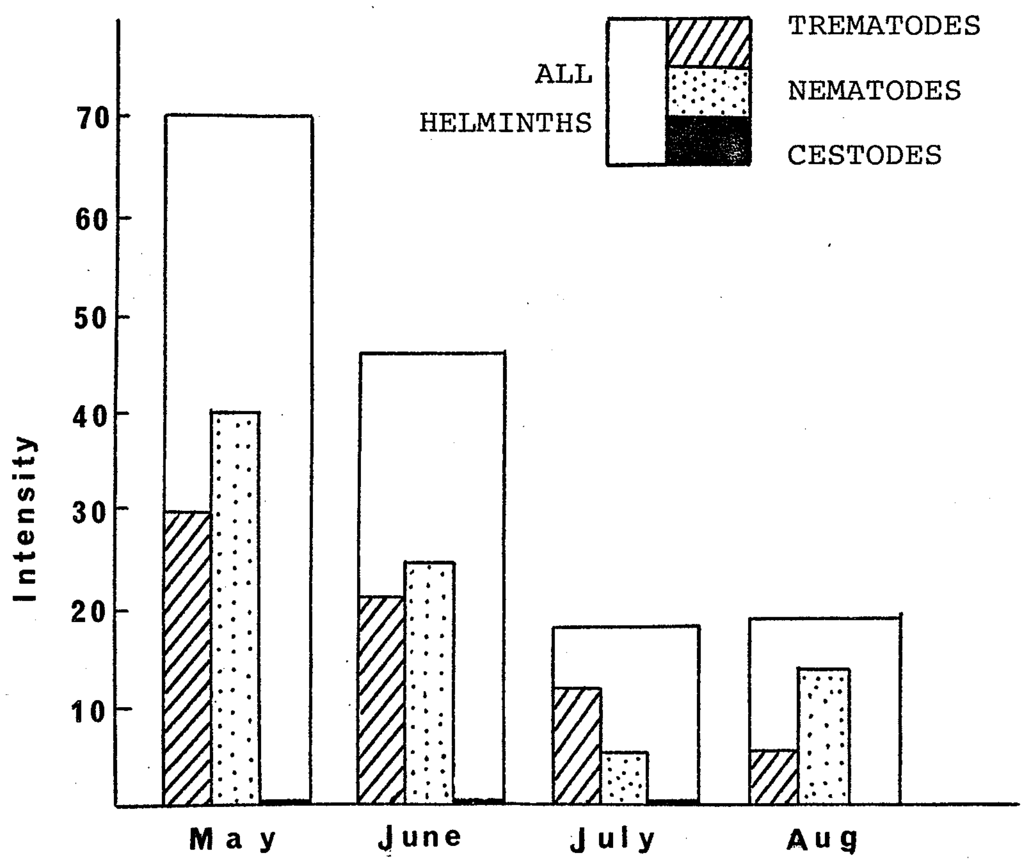
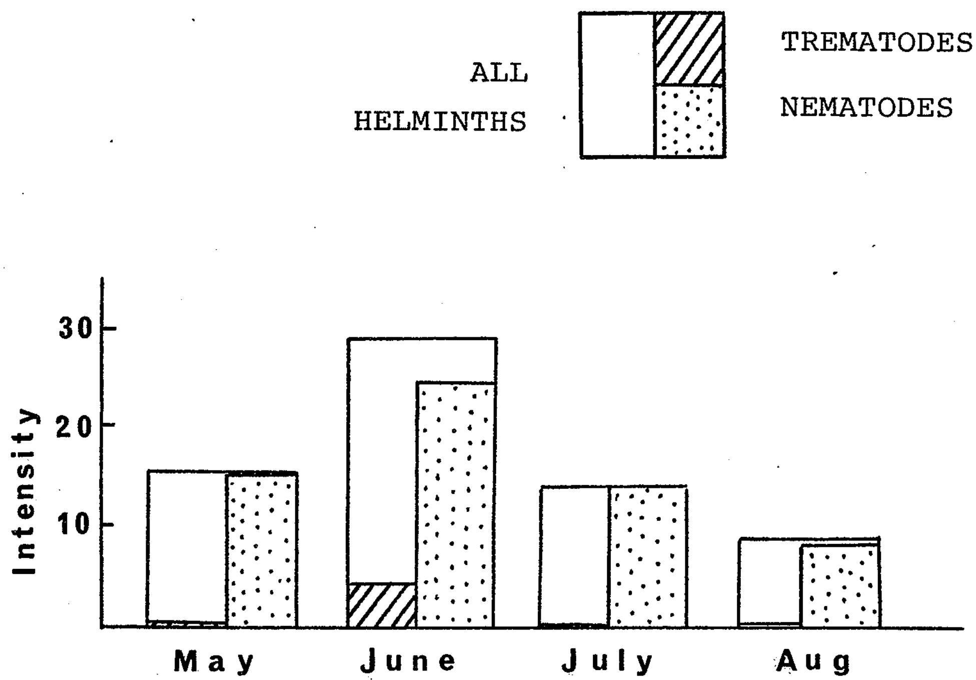

TREMATODES
ALL
HELMINTHS
NEMATODES
CESTODES
70
60
50
40
30
20
10
Intensity
May
June
July
Aug

Fig. 7. Bufo hemiophrys: intensity of helminthic infections by months of the period May-August 1968.



ALL
HELMINTHS
TREMATODES
NEMATODES
Intensity
30
20
10
May
June
July
Aug

Fig. 8. Rana pipiens: intensity of helminthic infections by months of the period April 1969 to February 1970.

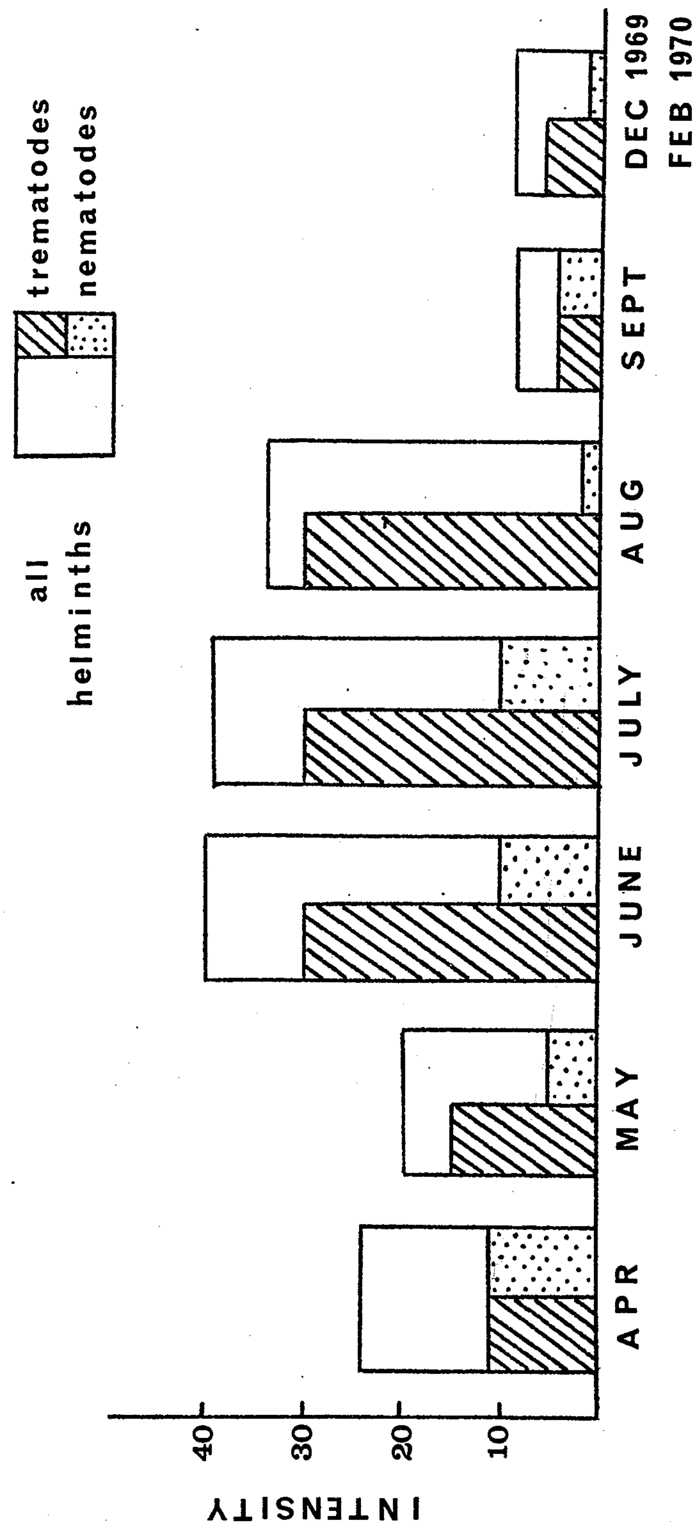
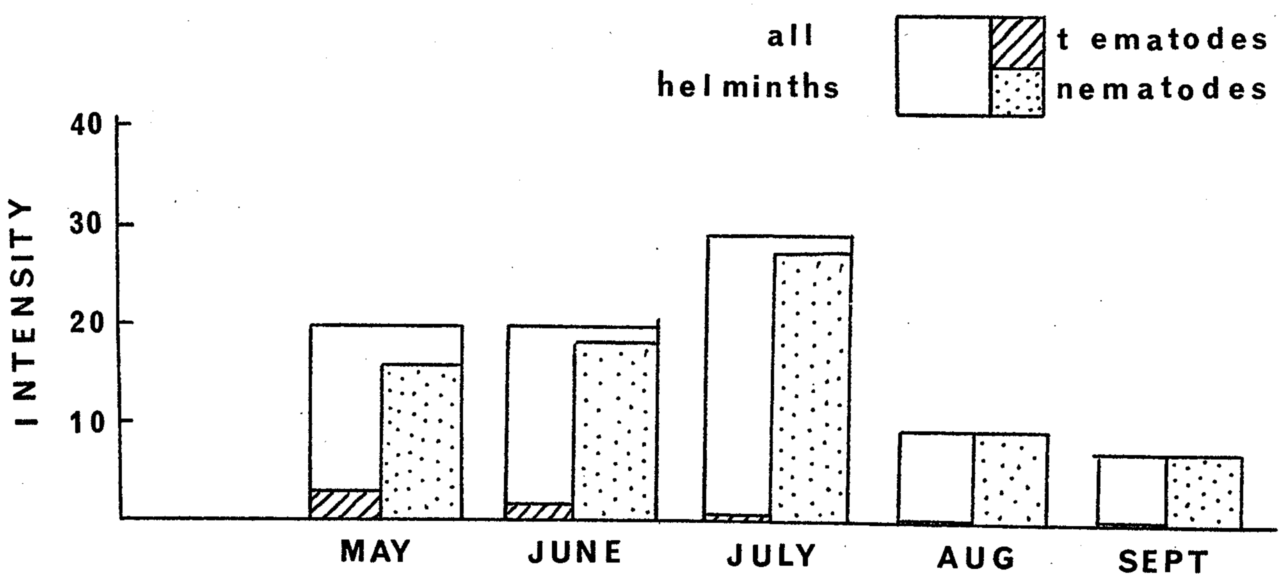

all helminths
trematodes
nematodes
INTENSITY
10
20
30
40
APR
MAY
JUNE
JULY
AUG
SEPT
DEC 1969
FEB 1970

Fig. 9. Bufo hemiophrys: intensity of helminthic infections by months of the period May-September 1969.


all helminths
t ematodes
nematodes
INTENSITY
40
30
20
10
MAY
JUNE
JULY
AUG
SEPT

July and the lowest in August and September. Thus the highest intensity occurred one month later in the 1969 sampling period.

Tables XIV to XVII show the monthly variations in the intensity and extensity of helminthic infections in the 1969 sampling period of R. pipiens and B. hemiophrys respectively. The greatest extensity of trematode infections in frogs occurred in spring, whereas nematodes were at the highest extensity in mid-summer. Trematodes were found in the greatest number of toads in May and June, whereas a high extensity of nematode infections was maintained throughout the period May-August.

Lung flukes of the genus Haematoloechus were least abundant during the early breeding of leopard frogs in May, and from August through hibernation. In B. hemiophrys, lung flukes were at their lowest intensity in mid-summer.

Cephalogonimus americanus and Diplodiscus temperatus were common only in leopard frogs and reached their highest intensity in the period June to August.

Lung nematodes of the genus Rhabdias were at a high intensity in both hosts immediately after their emergence from hibernation.

The greatest intensity and extensity of Oswaldocruzia pipiens infections occurred in June and July in R. pipiens, and June-August in B. hemiophrys.

Cosmocercoides dukae was found in both species of host. The parasite was most abundant in May and June in frogs, and June and July in toads.

TABLE XIV.

Rana pipiens: intensity of helminthic infections by months of the year

Helminths	Apr. 1969	May 1969	June 1969	July 1969	Aug. 1969	Sept. 1969	Dec. 1969-Feb. 1970
Trematoda							
Haematoloechus medioplexus	6.3	3.3	5.3	3.8	1.8	1.5	0.3
H. similiplexus	0.05	----	----	----	----	----	----
Cephalogonimus americanus	5.5	11.7	23.1	25.6	30.6	2.0	6.4
Diplodiscus temperatus	0.05	0.6	1.5	2.1	1.9	0.3	----
Gorgoderina attenuata	----	0.03	0.03	----	----	0.03	----
All trematodes	11.7	15.2	29.9	30.6	29.8	3.9	6.8
Nematoda							
Rhabdias ranae	5.9	2.7	3.5	2.9	0.4	1.6	0.8
Oswaldocruzia pipiens	3.2	1.7	4.0	4.2	0.3	0.8	1.1
Cosmocercoides dukae	0.8	0.8	2.5	1.7	0.9	1.9	----
All nematodes	11.4	5.0	10.7	8.9	1.7	4.7	1.9
Cestoda							
Ophiotaenia saphena	----	0.03	0.03	0.03	----	----	----
All cestodes	----	0.03	0.03	0.03	----	----	----
All helminths	23.5	20.6	40.3	39.3	34.0	8.5	8.7

TABLE XV.

Rana pipiens: extensity of helminthic infections by months of the year

Helminths	Apr. 1969	May 1969	June 1969	July 1969	Aug. 1969	Sept. 1969	Dec. 1969-Feb. 1970
Trematoda							
Haematoloechus medioplexus	77.7 ± 9.0	55.5 ± 8.3	55.5 ± 8.3	55.5 ± 8.3	37.7 ± 7.2	31.3 ± 8.3	33.3 ± 17.2
H. similiplexus	5.5 ± 5.4	----	----	----	----	----	----
Cephalogonimus americanus	72.1 ± 10.6	72.1 ± 7.5	72.1 ± 7.5	58.3 ± 7.4	53.3 ± 7.4	33.5 ± 8.5	44.4 ± 16.6
Diplodiscus temperatus	5.5 ± 5.4	22.2 ± 6.9	30.5 ± 7.7	27.7 ± 7.5	40.0 ± 7.3	22.6 ± 7.5	----
Gorgoderina attenuata	----	2.7 ± 2.7	2.7 ± 2.7	----	----	3.2 ± 3.2	----
All trematodes	94.4 ± 5.4	88.8 ± 5.3	80.5 ± 6.6	77.7 ± 7.3	71.1 ± 6.7	61.3 ± 8.7	55.5 ± 16.6
Nematoda							
Rhabdias ranae	61.0 ± 11.5	61.0 ± 8.1	74.9 ± 7.2	47.2 ± 8.3	28.9 ± 6.7	52.7 ± 8.9	55.5 ± 16.6
Oswaldocruzia pipiens	61.0 ± 11.5	31.3 ± 7.7	63.8 ± 8.0	69.4 ± 7.7	26.6 ± 6.6	29.7 ± 8.2	33.3 ± 17.2
Cosmocercoides dukae	11.1 ± 7.4	27.7 ± 7.5	52.7 ± 8.3	33.3 ± 7.8	28.8 ± 6.7	41.9 ± 8.9	----
All nematodes	77.7 ± 9.8	75.0 ± 7.2	97.2 ± 2.7	88.8 ± 5.3	58.8 ± 7.3	77.4 ± 7.5	66.6 ± 17.2
Cestoda							
Ophiotaenia saphena	----	2.7 ± 2.7	2.7 ± 2.7	2.7 ± 2.7	----	----	----
All cestodes	----	2.7 ± 2.7	2.7 ± 2.7	2.7 ± 2.7	----	----	----
All helminths	100.0	97.2 ± 2.7	100.0	97.2 ± 2.7	88.8 ± 4.7	83.8 ± 6.6	88.8 ± 10.5

TABLE XVI.

Bufo hemiophrys: intensity of helminthic infections by months of the year

Helminths	May 1969	June 1969	July 1969	Aug. 1969	Sept. 1969
Trematoda					
Haematoloechus medioplexus	----	----	0.02	----	----
H. similiplexus	3.4	1.6	0.8	0.5	1.3
Cephalogonimus americanus	----	0.05	0.07	0.08	0.04
Diplodiscus temperatus	----	0.03	----	----	----
All trematodes	3.4	1.4	0.8	0.7	1.4
Nematoda					
Rhabdias bufonis	6.7	1.8	1.9	1.5	1.1
Oswaldocruzia pipiens	5.0	8.0	23.1	6.3	4.4
Cosmocercoides dukae	4.1	4.8	3.0	2.8	1.6
All nematodes	16.7	19.3	28.3	10.4	6.8
Acanthocephala					
Pomphorhynchus sp. (*bulbocolli?*)	----	----	0.02	0.06	0.04
All acanthocephalans	----	----	0.02	0.06	0.04
All helminths	20.2	20.5	29.1	11.3	7.6

TABLE XVII.

Bufo hemiophrys: extensity of helminthic infections by months of the year

Helminths	May 1969	June 1969	July 1969	Aug. 1969	Sept. 1969
Trematoda					
Haematoloechus medioplexus	----	----	2.2 ± 2.2	----	----
H. similiplexus	51.4 ± 8.4	41.6 ± 8.2	19.4 ± 5.9	22.2 ± 6.9	29.2 ± 9.3
Cephalogonimus americanus	----	2.7 ± 2.7	2.2 ± 2.2	5.5 ± 3.8	4.2 ± 4.1
Diplodiscus temperatus	----	2.7 ± 2.7	----	----	----
All trematodes	51.4 ± 8.4	44.4 ± 8.3	22.2 ± 6.2	30.5 ± 7.7	29.2 ± 9.3
Nematoda					
Rhabdias bufonis	80.1 ± 6.7	61.1 ± 8.1	38.8 ± 7.3	69.4 ± 7.7	45.8 ± 10.2
Oswaldocruzia pipiens	62.8 ± 8.3	88.8 ± 5.3	88.8 ± 6.2	80.5 ± 6.6	66.6 ± 9.6
Cosmocercoides dukae	77.1 ± 7.1	63.9 ± 8.0	60.0 ± 7.3	47.2 ± 8.3	20.8 ± 8.3
All nematodes	97.1 ± 2.8	94.4 ± 3.8	95.5 ± 3.1	88.8 ± 5.3	75.0 ± 8.8
Acanthocephala					
Pomphorhynchus sp. (*bulbocolli*?)	----	----	2.2 ± 2.2	5.5 ± 3.8	4.2 ± 4.1
All acanthocephalans	----	----	2.2 ± 2.2	5.5 ± 3.8	4.2 ± 4.1
All helminths	100.0	97.2 ± 2.7	97.8 ± 2.2	91.6 ± 4.6	79.2 ± 8.3

The acanthocephalan, Pomphorhynchus sp. though rare, occurred in B. hemiophrys from July to hibernation in September.

Intensity of Helminthic Infections and Sex of the Host

Table XVIII shows that the intensity of helminthic infections in the sexually mature anurans (size classes 'B' and 'C') is greater than in juvenile anurans (size class 'A'). No significant difference was found in the intensity of helminthic infections between males and females.

Hibernation and Parasitism

Two samples of Rana pipiens caught beneath the ice of Lake Manitoba were obtained for examination. The first sample of 25 leopard frogs caught in gill nets on March 3, 1964 (Table XIX) had a low intensity of helminthic infection of 11.4 worms, and a high extensity of 100%. Twenty-four of the 25 frogs were infected with nematodes. The intensity of the nematode infections were mainly due to Rhabdias ranae which had an intensity of 8.1 worms.

All trematode species that I found in 1968 and 1969 samples occurred in the museum sample of 1964. The intensity and extensity of trematode infections were low at 1.6 worms and 28%.

The second sample consisted of 9 frogs, three from each size class. These frogs were obtained from fishermen at Delta who had caught the frogs in gill nets under the ice of Lake Manitoba in December 1969 and February 1970. Eight of the nine frogs were parasitized by helminths, with the smallest frog (55 mm. in length) free of helminths. The intensity of helminthic infection was low at 8.7

TABLE XVIII

Intensity of helminthic infections and the sex, and size of the host species

Size Class	*Rana pipiens*				*Bufo hemiophrys*			
A	juveniles				juveniles			
	Sample size	69			Sample size	60		
	Mean	13.39			Mean	6.21		
	Variance	405.50			Variance	32.44		
	Standard dev.	20.13			Standard dev.	5.69		
	Standard error	2.42			Standard error	0.73		
B	males		females		males		females	
	N	38	N	28	N	38	N	23
	$\bar{X}$	32.23	$\bar{X}$	49.03	$\bar{X}$	15.07	$\bar{X}$	14.30
	S^2	7627.42	S^2	12848.70	S^2	191.64	S^2	225.03
	S	87.33	S	113.35	S	13.84	S	15.00
	S.E.	14.16	S.E.	21.42	S.E.	2.24	S.E.	3.12
C	males		females		males		females	
	N	17	N	50	N	22	N	34
	$\bar{X}$	30.29	$\bar{X}$	37.02	$\bar{X}$	24.27	$\bar{X}$	46.17
	S^2	579.34	S^2	2102.91	S^2	316.96	S^2	10312.51
	S	24.06	S	45.85	S	17.80	S	101.55
	S.E.	5.83	S.E.	6.48	S.E.	3.79	S.E.	17.41

TABLE XIX

Helminths from 25 Rana pipiens collected from Lake Manitoba on March 3,1964

Helminths	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	Total	Intensity
	Rana pipiens: individually numbered (1-25)*																										
Rhabdias ranae	5		1	14	2	6	14	9	15	10	4	8	8	10	22	17	5	4	1	6	1		29	9	3	203	
Oswaldocruzia pipiens	6		1		1			6			2			1		1	5	5				1		5		34	
Cosmocercoides dukae										1								1	7							9	
Haematoloechus medioplexus		1							1					2		7										11	
H. similiplexus																									1	1	
Diplodiscus temperatus																	1									1	
Cephalogonimus americanus																	21						5			26	
Gorgoderina attenuata			1																							1	
All trematodes																										40	1.6
All nematodes																										246	9.8
All helminths																										286	11.4

* catalogue numbers of the 25 leopard frogs obtained from the National Museum (Ottawa),corresponding to the numbers in this table are listed in Appendix V.

worms. Rhabdias ranae and Oswaldocruzia pipiens infections with low intensities of 0.8 and 1.1 worms respectively, were the only nematodes found.

Trematode infections were at a higher intensity than nematodes in the 1969-70 winter sample. The only two trematode species found, C. americanus and H. medioplexus, had intensities of infections at 6.4 and 0.3 worms respectively.

Multiple Species Parasitism

Tables XX and XXI give the frequency of the number of helminth species per host for the three size classes of R. pipiens and B. hemiophrys respectively. The mean number of helminth species harbored by larger hosts exceed those infecting smaller and probably younger hosts.

COMPATIBILITY

Statistical tests were made to determine if lung nematodes of the genus Rhabdias and lung flukes of the genus Haematoloechus show any interaction within the hosts studied. During the period April to September 1969, 202 Rana pipiens were autopsied. From 404 lungs examined, 160 were parasite free; 110 harbored R. ranae only; 90 harbored H. medioplexus only; and 44 harbored both helminth species. Table XXII shows the contingency X^2 test used to determine the presence of an interaction between the two helminth species. Yate's correction coefficient was used and no significant interaction found.

TABLE XX.

R. pipiens: multiple species infection*

Size class	No. of helminth species per host	Frequency	Mean
	0	10	
	1	20	
	2	19	
A	3	19	1.87
	4	2	
	5	2	
	6	0	
	0	2	
	1	5	
	2	16	
B	3	21	3.09
	4	15	
	5	7	
	6	3	
	0	1	
	1	5	
	2	20	
C	3	13	3.22
	4	18	
	5	9	
	6	4	

*collected in 1969

TABLE XXI.

B. *hemiophrys*: multiple species infection*

Size class	No. of helminth species per host	Frequency	Mean
	0	8	
	1	18	
A	2	22	1.68
	3	9	
	4	3	
	0	2	
	1	8	
B	2	22	2.41
	3	21	
	4	8	
	0	0	
	1	2	
C	2	16	2.93
	3	23	
	4	15	

* collected in 1969

TABLE XXII.

Rana pipiens: lung trematodes vs. lung nematodes
(figures represent number of lungs)

H. medioplexus	Rhabdias ranae present	absent	total
present	44	90	134
absent	110	160	270
total	154	250	404

$(Chi)^2 = 2.3723$

$(Chi)^2$ Yate's corrected = 2.0490

When the presence or absence of juvenile lung helminths was tested against the nature of the lung infection (i.e. trematodes, or nematodes, or both) it was found that where juveniles were absent the helminthic infection tended to be with nematodes or trematodes, but not both. Conversely, lungs with juvenile helminths tended to harbor both nematode and trematode infections. These results may be interpreted in a number of ways. The explanation which I suggest is that the presence of juvenile helminths in lungs infected by both trematodes and nematodes is related to a retardation of helminth development. Thus, the results in Table XXIII show a significant interaction between the helminth species.

Tables XXIV and XXV testing the presence of an interaction between lung helminths of B. hemiophrys agree with and thus support the above results.

PATHOGENICITY OF HELMINTHIC INFECTIONS

The helminths parasitizing the anurans caused no visible harm to their hosts.

Measures of Heart lengths were positively correlated with host body lengths. Neither heart distention nor the ratio of heart distention to body length indicated any correlation with the number of helminths in the lungs or total helminthic infection.

TABLE XXIII.

Rana pipiens: lung helminth infection vs. presence of juvenile helminths (figures represent number of lungs)

Helminth juveniles	Helminthic infection: both trematodes and nematodes	either trematodes or nematodes	total
present	18	27	45
absent	26	173	199
total	44	200	244

$(Chi)^2 = 18.0134$

$(Chi)^2$ Yate's corrected = 16.2372

TABLE XXIV.

Bufo hemiophrys: lung trematodes vs. lung nematodes
(figures represent number of lungs)

H. similiplexus	Rhabdias bufonis present	Rhabdias bufonis absent	total
present	37	38	75
absent	120	159	279
total	157	197	354

$(Chi)^2 = 0.9573$

$(Chi)^2$ Yate's corrected = 0.7183

TABLE XXV.

Bufo hemiophrys: lung helminth infection vs. presnce of juvenile helminths
(figures represent number of lungs)

Helminth juveniles	Helminthic infection: both trematodes and nematodes	Helminthic infection: either trematodes or nematodes	total
present	21	29	50
absent	16	129	145
total	37	158	195

$(\mathrm{Chi})^2 = 23.1883$

$(\mathrm{Chi})^2$ Yate's corrected = 21.2179

DISCUSSION

Five species of trematodes, four of nematodes, and one each of cestodes and acanthocephalans, were found in the 657 anurans examined. All the species of helminths found in B. hemiophrys are new host records. Oswaldocruzia pipiens from Rana pipiens is a new Canadian record.

More helminth species per host species were found in the anurans from the Delta marshes than in similar studies in the United States (Table XXVI). This was probably due to the larger host sample size in my study.

The factors affecting the ecological relationships of the host and its helminths are discussed separately below. It should be remembered that many factors interact to influence the host-parasite relationship.

HELMINTHS AND ENVIRONMENTS

The more aquatic R. pipiens harbored more helminths and helminth species than did the less aquatic B. hemiophrys. These observations support the findings of Brandt (1936), Rankin (1945), Bouchard (1951), Mazurmovich (1951), and Markov and Rogoza (1953) that less aquatic hosts have fewer parasites.

R. pipiens alternates between aquatic media and the damp ground surrounding the marsh basins. B. hemiophrys, though aquatic during its breeding period, alternates between the damp and the well

TABLE XXVI.

North American helminth surveys (north of Mexico)

Author	Host sample size	No. of host species	Geographical location	Number of helminth species found				
				Trematodes	Cestodes	Nematodes	Acanthocephalans	Total
Ingles, 1936	264	12	California	13	1	5	---	19
Brandt, 1936	368	6	North Carolina	11	2	13	1	27
Rankin, 1945	250	13	Massachusetts	6	1	4	---	11
Bouchard, 1951	195	9	Northern Maine	11	1	---	---	12
Najarian, 1955	102	9	Michigan	12	---	---	---	12
Lehmann, 1960	178	10	California	5	2	2	---	9
Waitz, 1961	167	14	Idaho	8	1	3	---	12

drained ground of the marsh, beach ridge, and sandy beach of Lake Manitoba. Rankin (1945), and Bouchard (1951) found that platyhelminth infections are associated with an aquatic environment as they require water for swimming and usually aquatic intermediate hosts for their development. The greater overlap of the leopard frog and platyhelminth environments in contrast to the lesser overlap, spatially and temporally, of the toad and platyhelminth environments, correlate with the higher extensity and intensity of platyhelminth infection in *R. pipiens*.

Nematode infections were associated with terrestrial environments by Brandt (1936), Mazurmovich (1951), Markov and Rogoza (1953), and Lees (1962). The greater extensity and intensity of nematode infections in *B. hemiophrys* than in *R. pipiens* supports the above conclusion associating nematodes with a terrestrial environment. The intensity level of nematode infections in *R. pipiens* may be the result of more frequent visits to a terrestrial environment by the host.

SIZE OF HOST AND HELMINTHIC INFECTIONS

Larger and presumably older frogs and toads harbored considerably greater number of helminths than smaller individuals. This agrees with the observations of Brandt (1936), and Rankin (1937) who studied salamander parasites. The greater number of helminths in large hosts could arise from greater exposure to helminthic infection through larger food intake. Another cause may be the carry over of parasites from year to year as noted in my observations of parasites in hibernating anurans.

The greater number of helminth species in larger anurans cannot be attributed to a difference in diet between larger and smaller hosts as the diet was similar in kind and number of occurrences of food items in all sizes of anurans. Accumulation of helminth species, together with behavioral and physiological properties of older hosts is probably reflected in a higher degree of multiple species infection in larger anurans.

SEX OF HOST AND HELMINTHIC INFECTIONS

A comparison of the intensity of helminthic infections in male and female anurans revealed no significant difference. Lees and Bass (1960) found that males of *Rana temporaria* harbored more helminths than did the females. These authors suggested that the female hormone, oestradiol, was responsible for the depression of the level of parasitization. My data contradict these findings. The greater intensity of infections in males studied by Lees and Bass may be the reflection of the larger size of the males and the positive correlation of host size with infection intensity.

SEASONAL VARIATION

The intensity of helminthic infections of *R. pipiens* and *B. hemiophrys* varies from month to month. In most cases the variations cannot be related to seasonal changes. An attempt was made to correlate the intensity of helminthic infections with the phases of activity of the hosts and the life cycles of the parasites.

The greatest intensity and extensity of the general helminthic infection in both hosts in 1969 occurred after their emergence from hibernation in late April, to mid-summer. Close examination of the data reveals that the high levels of parasitization during mid-summer reflect the intensities of C. americanus infections in leopard frogs and O. pipiens infections in toads. If we regard the intensity and extensity calculated on the basis of infections resulting from the remaining helminth species, the highest level of parasitization would occur in the spring. This agrees with the parasitism levels I found in 1968, and the indications in the literature, that the highest helminthic infection occurs during and shortly after the breeding period of the host. At the Delta marshes this period is from April to June.

Lung flukes of the genus Haematoloechus were found in least abundance from July to September. According to Krull (1930) Haematoloechus spp. require an aquatic gastropod and dragonfly nymph as their respective first and second intermediate host. The high intensity of lung fluke infections during the period April to June is probably due to new infections contracted at the time when the host is mainly aquatic and ingests more aquatic arthropods. The observed decrease of the intensity of lung fluke infections after June may be due to the loss of the older flukes which had over-wintered with the host and the decreased uptake of aquatic insects and intermediate stages of the parasite.

The intestinal fluke, Cephalogonimus americanus, is the most frequently encountered helminth in R. pipiens. The intensity of infection with this fluke is subject to erratic variation in the leopard frog. Lang (1968, 1969) found that C. americanus utilized an aquatic gastropod as its first intermediate host and tadpole or frog epidermis for its second intermediate host. Leopard frogs would become infected by ingestion of shed skin containing C. americanus cysts. Tadpoles and newly transformed frogs are abundant on the surface of many marsh basins from June to August. The overall high intensity of C. americanus infection during this time may result from the ingestion of infected tadpoles and newly transformed frogs as well as shed skins with encysted cercariae. Therefore, the factors probably responsible for the variation of C. americanus infections in leopard frogs are the life cycle of the fluke and the habits of the hosts.

The rectal fluke, Diplodiscus temperatus, generally causing infections of low intensity, was most frequently encountered in R. pipiens from June to August. As the life cycles of D. temperatus and C. americanus are similar and the intermediate hosts of both flukes are common in the marsh, the lower infection rate of D. temperatus in R. pipiens is probably due to the flukes' lower population density in the marsh.

The relative absence of C. americanus and D. temperatus from toads is noteworthy. It is probable that a dry skin, lacking a protective mucous coating, is not conducive to the survival of encysted cercariae of C. americanus and D. temperatus. Furthermore, my data do not indicate any predation on tadpoles by B. hemiophrys.

Therefore, the mechanisms utilized by C. americanus and D. temperatus for entry into a host are ineffective for the transmission of infections to B. hemiophrys.

Gorgoderina attenuata is rare in leopard frogs and in toads. The rare occurrence of the bladder fluke probably results from the absence of its first intermediate host, a bivalve mollusc of the genus Sphaerium, from the marsh basins. The occasional infection with G. attenuata was probably picked up in Lake Manitoba where Sphaeriids are known to occur.

Oswaldocruzia pipiens infections in the less aquatic B. hemiophrys were more prevalent after the host had left an aquatic environment. The intensity of O. pipiens infections in R. pipiens was lowest during the hosts' breeding period. Similar observations were made by Brandt (1936), Mazurmovich (1951), Markov and Rogoza (1953), and Lees (1962).

Infections with the lung nematode, Rhabdias ranae, showed a cyclic variation in R. pipiens during the 1969 sampling period. According to Walton (1929) the eggs of this worm develop into infective juveniles within the host. The cyclic variation of the intensity of infection may be the result of auto-infection of the host. The eggs of Rhabdias bufonis, a lung nematode of B. hemiophrys, are passed with feces and hatch into larvae of the free-living generation. The high infection rate in May suggests that infection is contracted in the moist habitats surrounding breeding sites.

HOST SPECIFICITY

Cheng (1964) defined host specificity as "the natural adaptability of a species of parasite to a certain species or group of hosts." He conceded that mechanisms responsible for host specificity are not completely known and the degree of specificity differs from species to species. In the present survey some degree of host specificity was found.

In all but two occasions the lung flukes H. medioplexus and H. similiplexus were found in their recognized and accepted hosts, R. pipiens and B. hemiophrys respectively. In the two exceptions the helminth species were of normal appearance and were readily identified. The evidence indicates that host specificity exists. The problem is to explain the mechanisms of this specificity in two hosts that often share the same habitat. The mechanisms of this specificity are undoubtedly complex and varied, and probably are contributed to by ecological and physiological factors.

The study of the specificity of Rhabdias ranae and Rhabdias bufonis in their respective hosts in this survey is made difficult by the uncertainty of their identification. As no experiments on the life cycles of these worms were conducted to verify the identification of the two species, the difference in body lengths of the nematodes in each host species may be influenced by the different internal environment of the hosts. If the nematodes are indeed R. ranae in R. pipiens and R. bufonis in B. hemiophrys, then a high degree of host specificity was found in this study. The mechanisms of the specificity could result from the association of the lung worm and the chemical constitution of the host's blood which it ingests.

MULTIPLE SPECIES PARASITISM

Many instances of multiple species parasitism were found in both hosts. R. pipiens harbored a maximum of six helminth species and B. hemiophrys harbored a maximum of four.

Dogiel (1964) stated: "that the entire quantitative and qualitative composition of the parasite population of various hosts is determined not only by the ecological factors and conditions which the host, as the micro-environment, has to offer, but also by the character of the interrelations between the individual species included in the parasitocoenosis (entire parasite population of one host). These interrelations are in some instances antagonistic, the presence of some species preventing the occurrence of members of another. When the relationship is of a synergistic character, one parasite increases the chances of the existence of the other."

When the observed number of cases of infection with two species of helminths was compared statistically with the number expected, only in the R. ranae-H. medioplexus and R. bufonis-H. similiplexus parasitocoenoses was a significant difference found between the number of expected and observed double infections. The contingency Chi^2 test indicated that the presence of both species of lung helminths increased the probability of juveniles of either species occurring in a lung. These results support the opinion of Krull (1930) that the presence of juvenile worms in mixed infections of lung nematodes and flukes related to some degree of retardation or inhibition in the development of the lung helminths. The correlation indicates the presence of antagonism in the lung nematode - lung fluke parasitocoenosis.

SUMMARY AND CONCLUSIONS

1. Eleven species of helminths were found in the present study, nine of them were found in both *Rana pipiens* (5 of trematodes, 3 of nematodes, and one of cestodes) and *Bufo hemiophrys* (5 of trematodes, 3 of nematodes, and one of acanthocephalans). All helminth species found in *B. hemiophrys* are new host records. *Oswaldocruzia pipiens* is a new Canadian record.
2. Seven helminth species occurred in both hosts; four were specific for a particular host. Evidence suggested that the separation of *Rhabdias bufonis* and *R. ranae* is doubtful. Five helminth species occurring in both hosts were rare in a particular host. *Haematoloechus medioplexus* and *H. similiplexus* seem to have separate hosts.
3. Anurans living mainly in a semi-aquatic environment are parasitized by more helminths and helminth species than those living mainly in a terrestrial environment.
4. Larger and presumably older hosts harbored more helminths and helminth species than smaller and younger hosts, probably due to the accumulation of helminths from year to year. No significant difference was found in the intensity of helminthic infections between male and female hosts.
5. The extensity and intensity of helminthic infections varied from month to month and could not be easily correlated with seasonal progression. Changes in the extensity and intensity of helminthic infections depended on the habits of the definitive host, and the

life cycles of the helminths.

6. Multiple species parasitism is common in the anurans surveyed. As many as six species of helminths were found in some specimens of *R. pipiens*, and four species in some specimens of *B. hemiophrys*.

7. Juvenile lung helminths were found in a greater number of lungs harboring both lung nematodes and trematodes, than in lungs harboring either lung flukes or lung nematodes. A statisical comparison of the expected and observed frequency of occurrence of juveniles in lungs harboring both trematodes and nematodes suggested the presence of antagonism in the *Rhabdias-Haematoloechus* parasitocoenosis.

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APPENDIX I

CLASSIFICATION OF HELMINTHS FOUND

TREMATODA

ORDER: DIGENEA VAN BENEDEN, 1858

SUBORDER: PROTOSTOMATA ODHNER, 1905

FAMILY: PLAGIORCHIIDAE WARD, 1917

1. *Haematoloechus medioplexus* Stafford, 1902

   in *Rana pipiens* - lungs

   in *Bufo hemiophrys* - lungs

2. *Haematoloechus similiplexus* Stafford, 1902

   in *Rana pipiens* - lungs

   in *Bufo hemiophrys* - lungs

FAMILY: CEPHALOGONIMIDAE NICOLL, 1905

3. *Cephalogonimus americanus* Stafford, 1902

   in *Rana pipiens* - intestine

   in *Bufo hemiophrys* - intestine

FAMILY: PARAMPHISTOMATIDAE FISCHOEDER, 1901

4. *Diplodiscus temperatus* Stafford, 1905

   in *Rana pipiens* - rectum

   in *Bufo hemiophrys* - rectum

FAMILY: GORGODERIDAE LOOSS, 1901

5. *Gorgoderina attenuata* Stafford, 1902

   in *Ranapipiens* - bladder

   in *Bufo hemiophrys* - bladder

CESTODA

ORDER: PROTEOCEPHALIDEA MOLA, 1928

FAMILY: PROTEOCEPHALIDAE LA RUE, 1911

6. Ophiotaenia saphena Osler, 1931

   in Rana pipiens - intestine

NEMATODA

ORDER: RHABDIASIDEA RAILLET, 1916

FAMILY: RHABDIASIDAE RAILLET, 1915

7. Rhabdias bufonis Schrank, 1788

   in Bufo hemiophrys - lungs

8. Rhabdias ranae Walton, 1929

   in Rana pipiens - lungs

ORDER: STRONGYLIDEA DIESING, 1851

FAMILY: TRICHOSTRONGYLIDAE LEIPER, 1912

9. Oswaldocruzia pipiens Walton, 1929

   in Rana pipiens - intestine

   in Bufo hemiophrys - intestine

10. Cosmocercoides dukae Holl, 1929

   in Rana pipiens - rectum

   in Bufo hemiophrys - rectum

ACANTHOCEPHALA

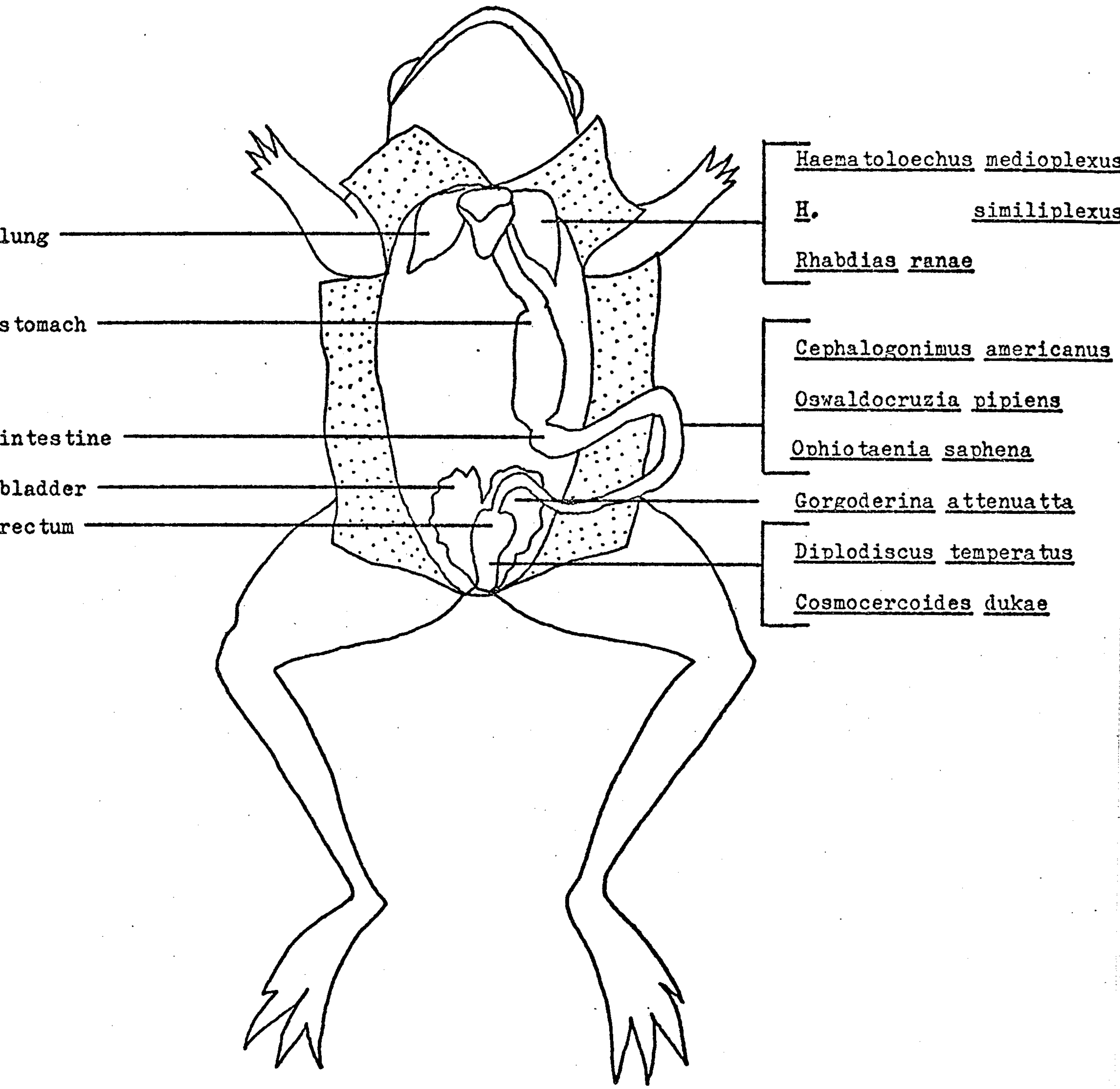
ORDER: PALAEACANTHOCEPHALA MEYER, 1931

FAMILY: POMPHORHYNCHIDAE YAMAGUTI, 1939

11. Pomphorhynchus sp. (bulbocolli?)

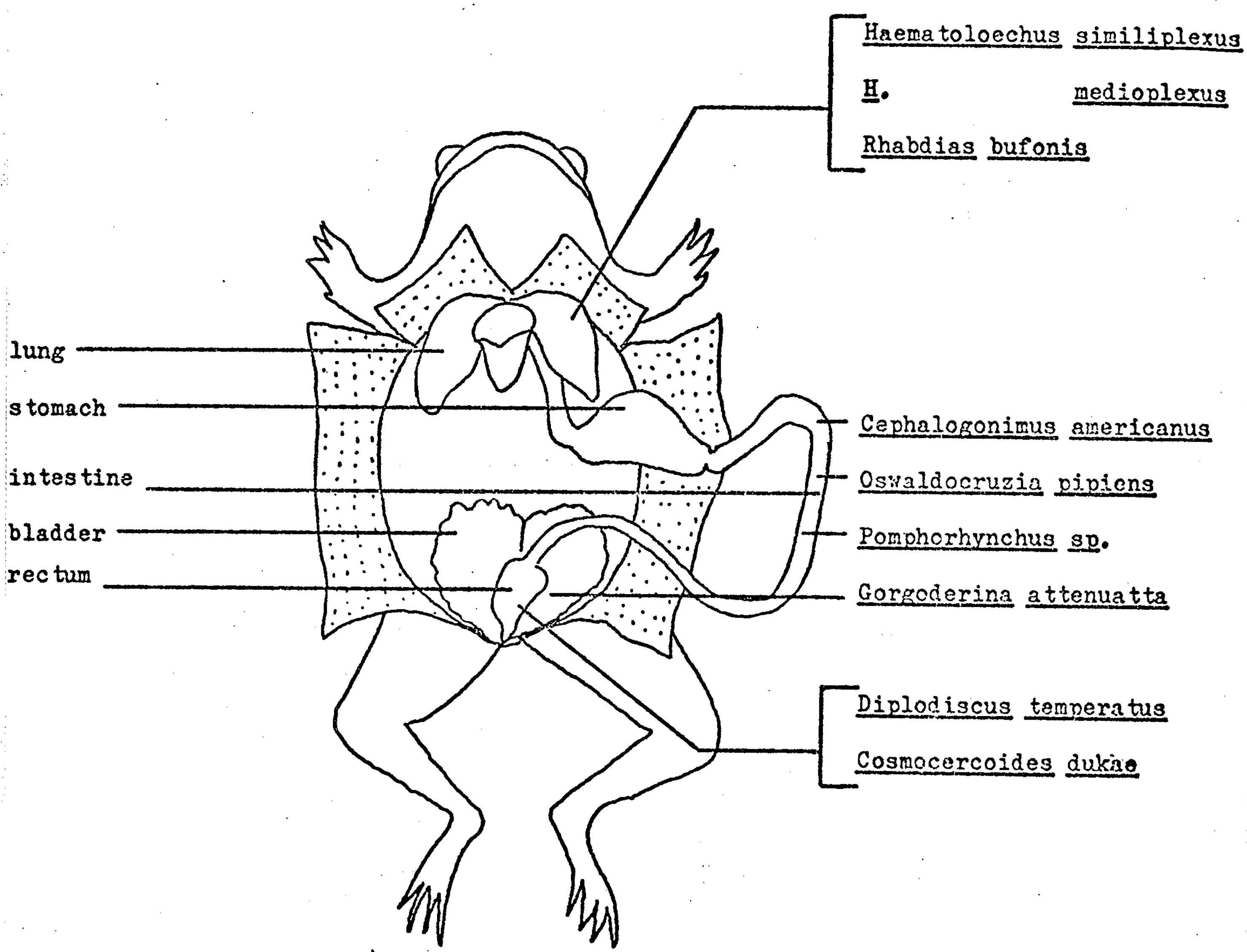
   in Bufo hemiophrys - intestine

APPENDIX II



Dissection of *Rana pipiens* as per Croll(1966)

showing location of helminths.



Dissection of Bufo hemiophrys as per Croll(1966) showing location of helminths.

APPENDIX III

DATA COLLECTION SHEETS

Collector: L.J. Hlynka

No: ____________

Date: ____________

Habitat: ____________

Station: ____________

Temp: Air: ____________

Water: ____________

Weather: ____________

Host: ____________

Sex: ____________

Length (mm.): ____________

Weight: ____________

Age: ____________

# of Parasites: ____________

Location: Ext. Int.

Host structure infected: (% total infection)

Remarks:

Food Available: ____________

Stomach Contents: ____________

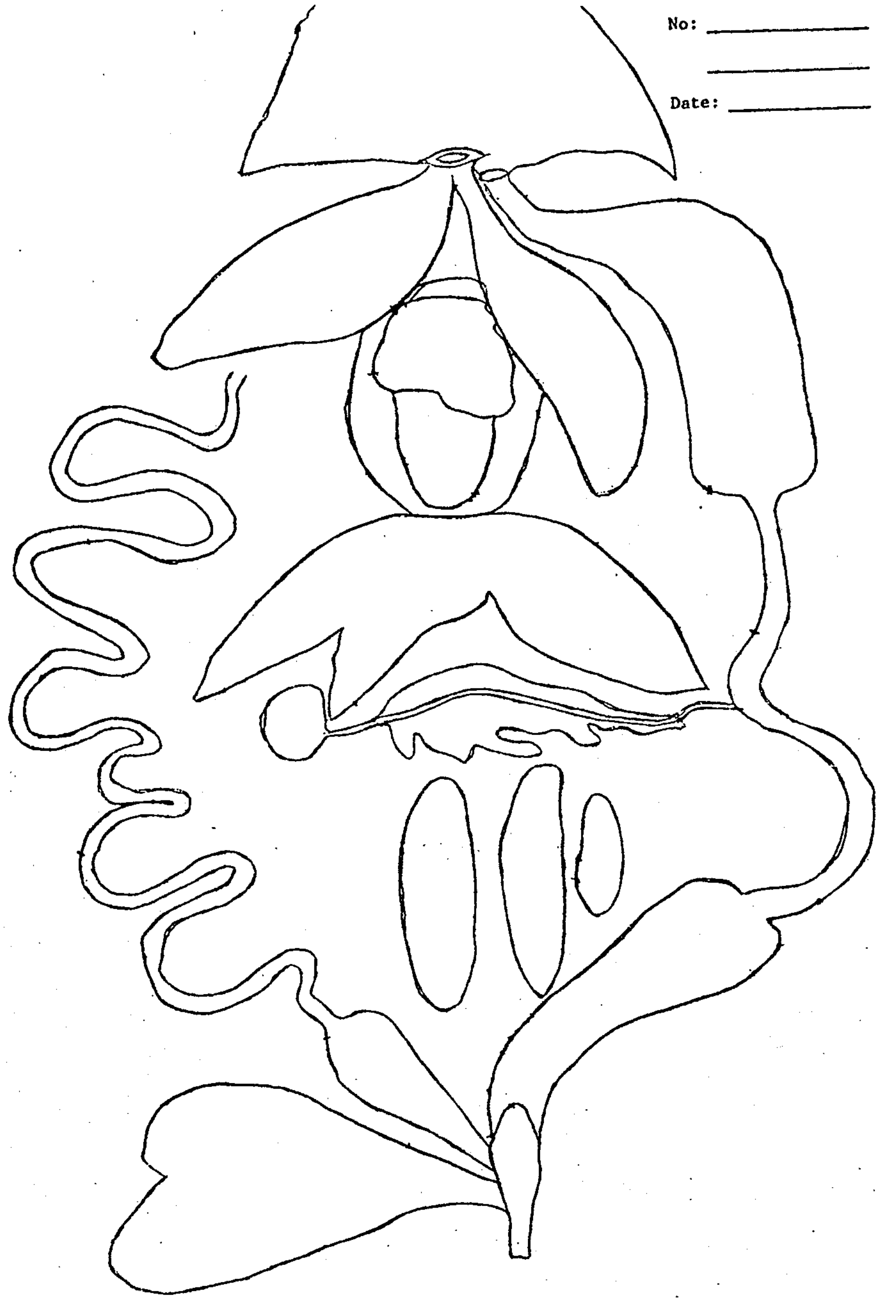
Pathology: ____________

Identification: ____________

No: ______________

____

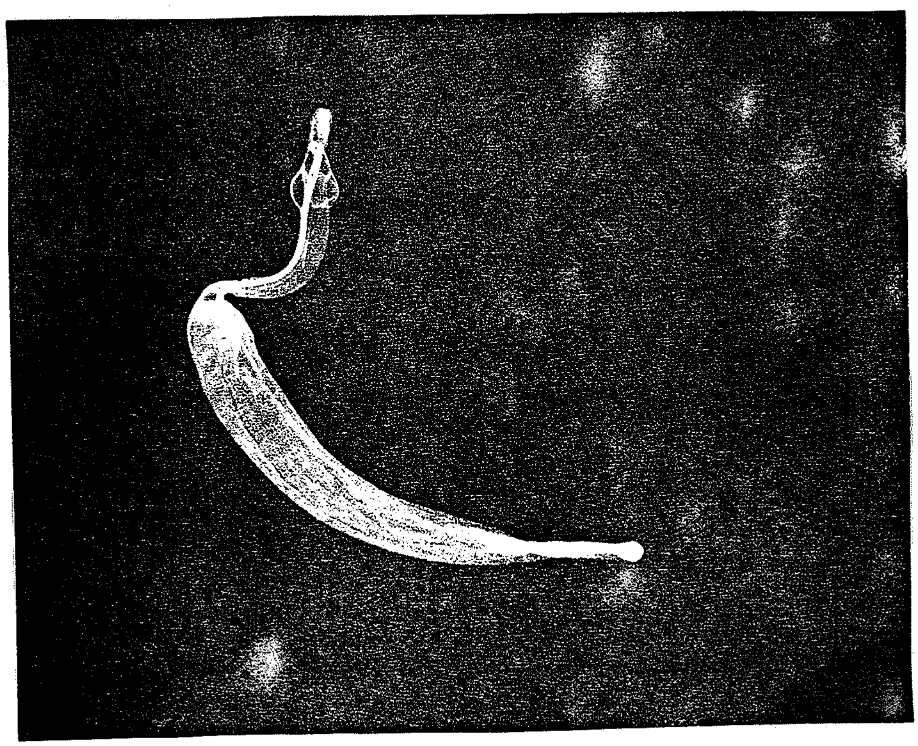
Date: ______________



APPENDIX IV

One worm of the genus *Pomphorhynchus* was found in each of four *Bufo hemiophrys* specimens. This genus usually parasitizes fish. The four toads infected with *Pomphorhynchus* sp. were captured at the interphase of the sandy beach and water of Lake Manitoba where amphipods of the genus *Gammarus* harboring acenthellas may have been eaten.

Figure A. shows the general appearance of the *Pomphorhynchus* sp. found in *B. hemiophrys*.



APPENDIX V

NATIONAL MUSEUM CATALOGUE NUMBERS

re: *Rana pipiens* specimens from Lake Manitoba, March 3, 1964.

(25 *Rana pipiens*, courtesy Francis Cook, Curator of Herpetology)

Specimen	Catalogue #	Specimen	Catalogue #
1	7679-2	14	7680-7
2	7679-4	15	7680-8
3	7679-5	16	7679-1
4	7679-6	17	7679-9
5	7679-7	18	7679-10
6	7680-1	19	7679-11
7	7680-2	20	7679-15
8	7680-3	21	7679-17
9	7680-4	22	7679-14
10	7680-6	23	7679-12
11	7679-8	24	7679-13
12	7679-3	25	7679-16
13	7680-5		