A SEASONAL AND COMPARATIVE STUDY OF HELMINTH PARASITES IN NINE WISCONSIN AMPHIBIANS

By

Matthew G. Bolek

A Thesis Submitted in Partial Fulfillment of the

Requirements for the Degree of

Master of Science

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Biological Sciences

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Matthew G. Bolek

The University of Wisconsin-Milwaukee

Under the Supervision of Dr. James R. Coggins

Abstract

A seasonal and comparative study was conducted on helminth parasite populations and communities in amphibian hosts in order to elucidate ecological factors important in structuring these communities. Three hundred fifty amphibians of nine species including green frogs, northern leopard frogs, American toads, Cope's gray treefrogs, western chorus frogs, spring peepers, blue-spotted salamanders, spotted salamanders, and red-backed salamanders were collected during 1996 and 1997, from four ponds in Waukesha and Bayfield Counties, Wisconsin and examined for helminth parasites. Two of these species, the green frog and American toad were surveyed seasonally. Seasonally, helminth populations and communities were highly variable among the two hosts sampled. Most helminth species did not show significant differences in prevalence and mean intensities during the year, but due to the complexity of their life cycles a number of species showed seasonal variation in

location in the host and size differences over time which were related to recruitment period of these species.

In general, communities were depauparate and isolationist in nature. Of the nine amphibian species examined, a total of 3,889 nematodes, approximately 3,376 larval trematodes, approximately 3,317 larval cestodes, 784 adult trematodes, 13 monogeneans and one adult cestode were recovered, most of which were not host specific. Transmission dynamics and life cycles of parasites appeared to be important with skin penetrating nematodes and larval trematodes being the most common or the dominant species. Overall nematode abundance was highest in toads while larval trematode and cestode abundance was highest in green frogs, Cope's gray treefrogs and leopard frogs. Salamanders harbored fewer helminth species than anurans, with nematodes and larval trematodes being most common. Stomach content analysis revealed that toads were active foragers, which ate few prey items (primarily ants, beetles and mites), while Cope's gray treefrogs, leopard frogs, and green frogs were "sit and wait predators" with a broader diet of invertebrates. Although site characteristics played a role in producing variation in type and prevalence of helminths observed, host size, diet and feeding patterns were important in determining helminth parasites in amphibian hosts. This work represents seven new host records and 12 new locality records in Wisconsin amphibians.

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Major Professor Date

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I thank my advisor, Dr. James R. Coggins for his guidance throughout this project and his acceptance of my many other side projects. He allowed me the absolute freedom to conduct studies on natural history of herps and other invertebrates even though at times it must of seemed chaotic. I will always be grateful for his patience and support. I would like to thank my committee members, Drs. Dianne Seale and Timothy Ehlinger for their input and suggestions on the manuscript. Also, I would like to thank Dr. Susan Lewis for allowing me to collect green frogs at the Carroll College field station. A special thanks to Dr. Heather Owen for her help and guidance in scanning electron microscopy studies of numerous parasites.

My interests in parasitology and amphibian biology were fostered during my undergraduate career at Carroll College. I would like to thank my undergraduate mentors Dr. Jack Mellen and Dr. Leslie Zettergren for introducing me to these critters and taking the time to discuss numerous projects on their biology.

Thirdly, I would like to thank a number of graduate students whose input and help over the last few years were invaluable to the completion of this study and my understanding of scientific investigations. I would like to thank Dr. Randy Yoder for help in collecting salamanders and discussion of helminth amphibian biology; Henry Mier for help in arthropod identifications and discussions of their biology; Joseph Boxhorn and Dr. Thomas Slawski for their input and reviews on numerous talks and manuscripts during this study.

I would like to acknowledge my parents , Margaret and George Bolek, my brother Luke Bolek and grandmother, Maria Brykalska for their support and understanding over the past three years, without their help this work would have been impossible. Finally, I like to thank my girlfriend, Melissa Ewert, for her continuous moral support, understanding and sacrifices over the last three years.

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Introduction

Parasitism represents a complex web of interactions among organisms: the host, its parasites, its prey and its predators (Tinsley, 1996). Therefore, it is a symbiotic association between two or more organisms. The most widely used definition states that it is a symbiotic and intimate relationship between two organisms with one living on, off, and at the expense of the other (Roberts and Janovy, 1996). Although, this definition implies harm, Smyth (1962) and others (Esch and Fernandez, 1993) have stated that whether parasitic organisms are harmful or not is irrelevant to the metabolic concept of parasitism. Harm is a relative term that can not be quantified and pathogenicity may be a function of parasite density (Cheng, 1991; Esch and Fernandez, 1993). The definition of parasitism has varied depending on the author and their discipline, eg. naturalist, immunologist, endocytobiologist, yet all try to embrace the concept of the host parasite relationship, and their obligate symbiosis. Therefore the study of parasitology is the study of interactions among organisms, an ecological association.

Ecology deals with the study of interactions between and among organisms and their environment (Ricklefs, 1990). Parasitism is one of the most common ecological relationships. Price (1980) estimated that more than 50% of all plant and animal species are parasitic at some point in their life cycle. Just as importantly, it has been estimated that approximately 100% of all animals and plants serve as hosts for parasites (Esch and Fernandez, 1993). Therefore, parasitism is a very common ecological relationship which is represented in most species. To the parasite, the host represents a resource and a habitat where the parasite can grow and reproduce. Once produced, eggs

are released from the host into the external environment where they hatch, undergo development, and must find their way back in to another host (Fig. 1). Therefore, unlike most free living organisms, one of the major problems for parasites is for individuals of a particular species to find the correct host to propagate the next generation and complete the life cycle. This is a statistical problem of colonization, where parasites face spatial and temporal difficulties of transfer from one host to another, which must be overcome by enormous reproductive outputs and/or by exploiting complex ecological associations between successive hosts (Tinsley, 1990).

Community ecology deals with organisms of different species living in a certain habitat and attempts to interpret the effects of competition, predation, mutualism and parasitism as well as abiotic factors, in determining their community structure (Goater et al. 1987). Most ecological studies have been conducted on free-living animals with little attention placed on organisms such as parasites. As stated by Price (1980, 1984), "Small, highly specialized organisms are different in many aspects of their biology from larger, more generalized animals". Thus, communities of specialists such as helminth parasites may be organized in different ways from generalist communities. The complexity of parasitic life cycles and recruitment strategies make them very different from free-living organisms. Due to these differences, populations and communities of parasites can be studied at a number of hierarchical levels of organization (Esch and Fernandez, 1993). These have been defined as follows: 1.) Infrapopulation and Infracommunity-a population or community of parasites in an individual host. 2.) Metapopulation and Component Community-all the infrapopulations and

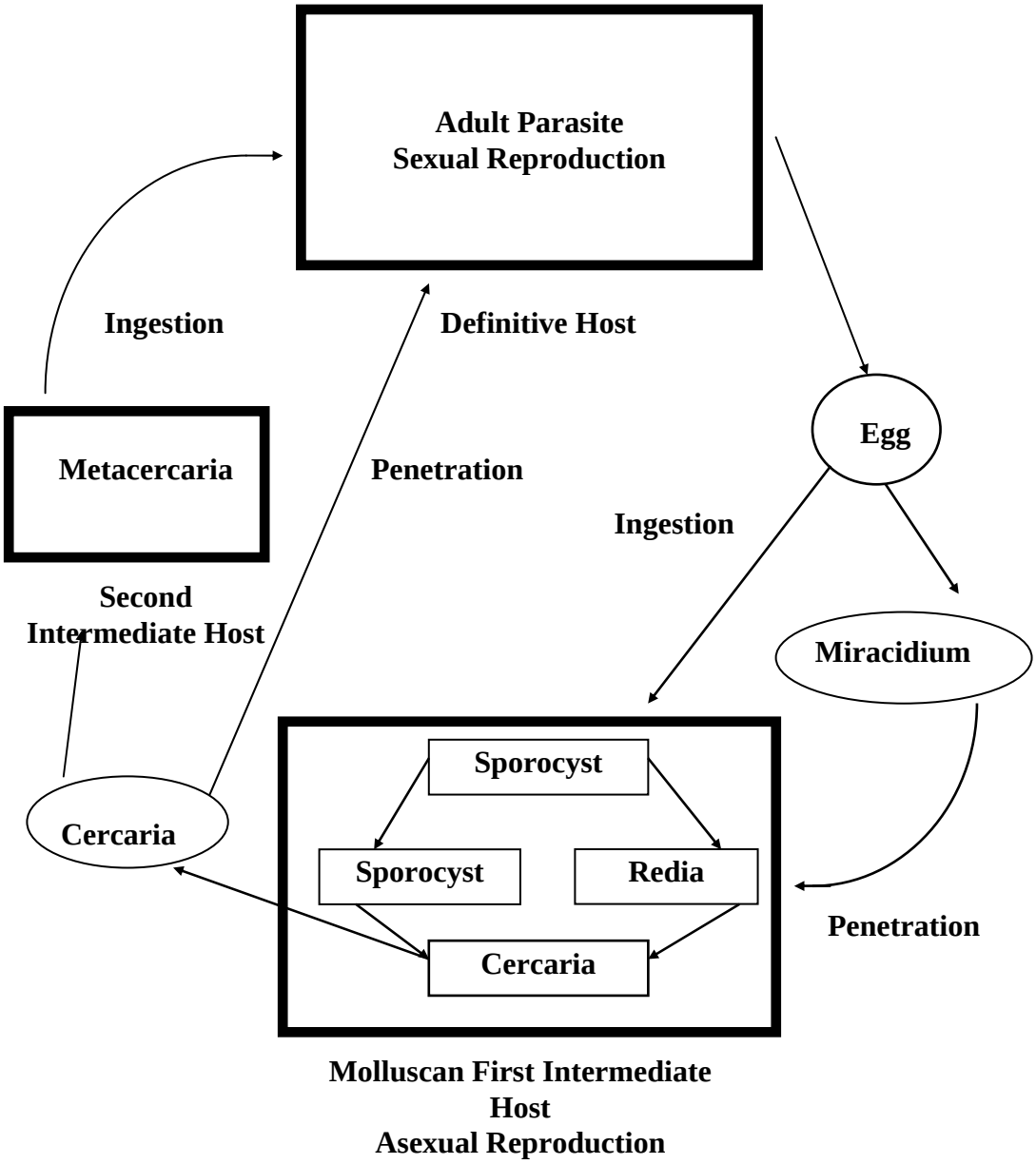

Figure 1. General life cycle of digenetic trematode. Bold rectangles represent hosts, ovals and circles represent free swimming or resting stages in the external environment.

infracommunities of parasites in a population of hosts in a given habitat. 3.) Suprapopulation and Compound Community- all the parasites of a given species (population) and all the parasite species (community), in all stages of development, within all hosts in an ecosystem. Despite these differences in structure and complexity, helminth parasites have been shown to possess certain unique qualifications that permit them to contribute significantly to certain concepts in community ecology. First, helminth communities have unambiguous boundaries the host. Second, since each host represents a separate and distinct habitat for a population or community of parasites, helminth communities may be replicated easily, resulting in the potential for a useful comparative approach (Aho, 1990). Therefore, parasitologists deal with the most clearly structured habitats of any ecological system (Price, 1990).

Currently, parasitic helminth community structure is viewed as occurring at two distinct forms, isolationist or interactive (Esch, et al, 1990). Isolationist communities are depauperate and are structured by random events, while interactive communities are species rich and the role of interactions and competition are important in structuring these communities (Esch, et al., 1990). Studies on freshwater fish, birds and a mammal by Kennedy et al. (1986) have helped develop predictions which can be important in determining helminth community structure. The following factors have been identified by these investigators as important in producing diverse helminth communities: 1.) The complexity of the hosts alimentary canal and/or its physiology (ectothermy/endothermy). 2.) Host vagility. 3.) A broad host diet. 4.) Selective feeding by a host on prey which serve as intermediate hosts for a wide variety of helminths. 5.) Exposure of a host to direct life-cycle helminths which enter by

penetration. Needed now are more studies on a broader range of hosts such as marine fish, amphibians, reptiles and mammals.

In general, the biogeography and ecology of helminth parasites of amphibians has been neglected, and many aspects remain poorly understood. More than fifty years ago Brandt (1936) stated: “It is remarkable that although frogs and toads have long been used as material for the study of parasites, there has been no systematic and comprehensive study of the relations of parasite infestation to the ages, habitats and habits of hosts”. Since that time a few interesting and important studies on the ecology of helminth parasites and their amphibian hosts have been conducted by Rankin (1937 a, b; 1938; 1939; 1945), Fishthal, (1955 a, b) and Walton (1938; 1940), yet there are relatively few compared to ecological studies on free-living organisms.

Amphibians represent excellent systems to explore patterns and processes influencing helminth community organization (Aho, 1990). They have invaded a multitude of habitats and exhibit a striking diversity of life history patterns, reproductive modes, body sizes, foraging modes and trophic relations. They provide a good comparative system for understanding ecological relationships in determining helminth species distribution and abundance. Also, because amphibians are ectotherms, they represent an interesting system for studying seasonal changes in helminth parasite populations and communities. Seasonal temperature cycles are associated with variation in parasite infection (Tinsley, 1990). In endothermic hosts, internal parasites are buffered from external temperature changes. While in ectothermic hosts, parasites may have much more information about the external environment (Tinsley, 1990).

Therefore, they may be more responsive to host biology and may make seasonal changes more noticeable.

Recently, a number of studies have concentrated on seasonal and comparative aspects of community structure of amphibian helminths (Coggins and Sajdak, 1982; Goater et al. 1987; Aho, 1990; Muzzall, 1990, 1991a, b; Muzzall and Peebles, 1991; Muzzall and Schinderle, 1992; Yoder and Coggins, 1996; Bolek, 1997a; McAlpine, 1997). Although very valuable, these contributions were mostly restricted in their analysis to infrapopulation community organization. From the findings of these workers and a recent review of helminth community structure by Esch, et al. (1990), three features have been consistently identified as important determinants of helminth distribution and abundance: geography or habitat, temporal, and host demography (Aho, 1990).

The ecology and natural history of Wisconsin amphibians has been well studied (Vogt, 1981). These species are locally abundant and are represented by seven families of 20 species representing a number of habitats and foraging strategies (Vogt, 1981). Therefore, they represent a good system for the study of helminth populations and communities. However, few studies on helminth parasites exist from this area (Williams and Taft, 1980; Coggins and Sajdak, 1982; Tiekotter and Coggins, 1982; Yoder and Coggins, 1996; Bolek, 1997a; Yoder, 1998). The present study of amphibian helminths was undertaken to address three important questions concerning the ecology of parasite populations and communities of Wisconsin amphibians. 1.) What species of helminths parasitize Wisconsin amphibians? 2.) Are there seasonal differences in helminth populations and community structure in amphibian hosts? 3.)

Is host biology important in determining the types of helminth parasites (specialists, generalist, direct or indirect life cycle parasites) specifically is host habitat, age/size, diet and sex important in determining helminth populations and communities?

Materials and Methods

Amphibian Species and Collecting Sites

Three hundred fifty amphibians of nine species including green frogs, northern leopard frogs, American toads, Cope's gray treefrogs, western chorus frogs, spring peepers, blue-spotted salamanders, spotted salamanders and red-backed salamanders, were collected during 1996 and 1997 from four locations in Waukesha and Bayfield Counties, Wisconsin (Table 1). All species were identified and represent species ranging from an aquatic to terrestrial habitat.

Seasonal Study

In order to address seasonal changes of helminth populations and community structure, two species, the green frog and American toad were surveyed seasonally. A total of 75 green frogs, *Rana clamitans* Rafinesque 1820, a semi-aquatic species were collected from April to October of 1996 at a small spring feed permanent pond located at the Carroll College field station in Genesee Depot, Waukesha County, Wisconsin (42°59'N, 88°21'W). Ten to fifteen frogs were collected monthly around the periphery of the pond by the use of a dip-net. A total of 47 American toads, *Bufo a. americanus* Holbrook 1836, a terrestrial species were collected from April to November of 1996 by driving two and a half mile sections of highway N and 67 (between 59 and ZZ) in Eagle Waukesha County, Wisconsin (42°54'N, 88°29'W) during the night and collecting individuals as they crossed roads.

Comparative Study and General Survey

To address the question whether host biology and their habitat is important in structuring helminth populations and communities three different areas were surveyed for amphibians and their parasites. All anurans were collected by hand or dip-net during the night or breeding chorus, or as they emerged from ponds during metamorphoses. Salamanders were collected by pitfall traps, overturning rocks and logs during the day or by dip-net during their breeding period.

Five species of anurans were collected during April to November of 1996 and April to June of 1997 from two adjacent ephemeral ponds and highways N and 67 in Eagle Waukesha County, Wisconsin (42°54'N, 88°29'W). These included 65 Cope's gray treefrogs, *Hyla chrysoscelis* Cope 1880, an arboreal species; 47 American toads, *B. a. americanus*, a terrestrial species; six western chorus frogs, *Pseudacris t. triseriata* Wied 1839, a terrestrial species; four spring peepers, *P. c. crucifer* Wied 1839, a semi-arboreal terrestrial species; and one northern leopard frog, *R. pipiens* Schreber 1782, a semi-aquatic species.

Because of the difficulty of identifying Cope's gray treefrog from the gray treefrog (*H. versicolor*), they were identified by mating call and mean erythrocyte length which was compared to a known *H. versicolor* population as described by Bolek (1997b). A statistically significant difference existed between the mean erythrocyte length of *H. chrysoscelis* (19.71 μm) and mean erythrocyte length of *H. versicolor* (25.07 μm, $P < 0.001$, two sample *t* test) (Appendix 1.).

Three species of adult and metamorphosed amphibians were collected during April to October of 1996 from an ephemeral pond located in Brookfield Waukesha County, Wisconsin (43°03'N, 88°04'W). These included 51 blue-spotted salamanders, *Ambystoma laterale* Hallowell 1856, a semi-fossorial species; 31 northern leopard frogs, *R. pipiens*, a semi-aquatic species; and 30 American toads, *B. a. americanus*, a terrestrial species.

At the third site, located at Pigeon Lake Field Station in Bayfield County, Wisconsin (46°18'N, 91°20'W), 20 adult red-backed salamanders, *Plethodon c. cinereus* Green 1818, a terrestrial species, and 20 spotted salamanders, *A. maculatum* Shaw 1802, a fossorial species were collected during May of 1996.

Animals were placed in plastic containers, transported to the laboratory, stored at 4°C, and euthanized in MS 222 (ethyl m-aminobenzoate methane sulfonic acid) within 72 hours of capture. Snout-vent length (SVL) and wet weight (WW) were recorded for each individual. Anurans and salamanders were classified as juveniles or adults based on size and knowing the length of these species at maturity (Bishop, 1943; Wright and Wright, 1949). Most amphibians were individually toe clipped, frozen and necropsied as time allowed, while others were necropsied immediately after euthanasia. At necropsy, the digestive tract, limb and body wall musculature, and internal organs were examined for helminth parasites. Each organ was individually placed in a petri dish and examined under a stereo microscope. The body cavity of all amphibians was

rinsed with distilled water into a petri dish and the contents examined for parasites. All individuals were sexed by gonad inspection during necropsy.

When amphibians were necropsied fresh, non-encapsulated immature and adult digenetic trematodes and cestodes were relaxed and killed by slowly warming them in staining dishes containing 0.25% saline, and fixed in alcohol-formaldehyde-acetic acid (AFA). Monogeneans were relaxed under slight cover slip pressure in the refrigerator, frozen and fixed in AFA. They were then stained with Aceto-Carmine, dehydrated in a graded ethanol series, cleared in xylene and mounted in Canada Balsam. Nematodes were killed in hot AFA, dehydrated to 70 % ethanol, cleared in glycerol and identified as temporary mounts. Tissue containing metacestodes were removed and fixed in 10% formalin, embedded in Paraplast, sectioned at 7µm, affixed to slides and stained with Harris' hematoxylin and eosin, and mounted in Canada Balsam. Certain species of trematodes were measured by the use of a calibrated ocular micrometer. When amphibians were frozen all worms were removed, fixed in AFA or formalin and stained and processed as above. All undigested stomach content was identified to Class or Order following Borror et al. (1989). Stomach content is reported as a percent, number of arthropods in a given class or order divided by total number of arthropods recovered.

Definitions

The following measures of parasitism were calculated for the various parasite species recovered from each amphibian species (Margolis et al., 1982): Prevalence, the percentage of infected amphibians in a sample; mean intensity, the mean number of worms per infected host, and abundance, the mean number of individuals of a particular

parasite species per amphibian examined including infected and non-infected individuals. Mean helminth species richness is the sum of helminth species per individual amphibian, including non-infected individuals, divided by the total sample size of a particular amphibian species. All values are reported as a mean $\pm$ one standard deviation. Indirect life cycle parasites are defined as parasites involving at least one other host other than the definitive host (trematodes and cestodes), while direct life cycle parasites are defined as ones that utilize only one host, the definitive host (monogeneans and most nematodes) (Tinsley, 1996).

Data Analyses

The Chi-square test for independence was used to compare differences in prevalence among different host species and sex or age. Yates' adjustment for continuity was used when sample sizes were low (Sokal and Rohlf, 1981). A single-factor, independent-measures ANOVA and Scheffe's post hoc test was used to compare among seasonal differences in mean intensity, mean helminth species richness, and among different species of host. When variances were heteroscedastic the Kruskal-Wallis test and the Kolmogorov-Smirnov two sample test were used (Sokal and Rohlf, 1981). The students *t*-test was used to compare differences in mean intensity and mean helminth species richness among sex or age of hosts. Approximate *t*-tests were used when variances were heteroscedastic (Sokal and Rohlf, 1981). Pearson's correlation was used to determine relationships among host snout-vent length and wet weight and abundance of helminth parasites, excluding larval platyhelminthes. Pearson's correlation was calculated for host snout-vent length and wet weight and helminth

species richness per individual amphibian. Because wet weight gave a stronger correlation then snout-vent length in each case, it is the only parameter reported.

Results

A. Seasonal Study

Rana clamitans

A total of 75 green frogs, 43 males and 32 females, were collected during April through October 1996. No significant difference existed in the number of male and female frogs collected throughout out the year ($\chi^2 = 7.01$, $P > 0.05$). The overall mean snout-vent length and wet weight of green frogs was 68.79 $\pm$ 10.75 mm (range 39.75-89.40) and 35.80 $\pm$ 15.49 g (5.35-75.64), respectively. There was no significant difference in mean length ($t = 0.10$, $P > 0.05$) or mean wet weight ($t = 0.24$, $P > 0.05$) in male and female frogs.

Seventy one (94%) of 75 *R. clamitans* were infected with helminth parasites. The component community of *R. clamitans* was composed of 12 species of helminths (five trematodes, two cestodes and five nematodes). Of these, eight had indirect life cycles, one had a direct life cycle and three were unknown. Overall mean helminth abundance, excluding larval platyhelminthes, was 16.5 $\pm$ 38 with most frogs infracommunities having 10 or fewer worms. Prevalence ranged from 80% for echinostome metacercariae to 1.3% for an unidentified adult cestode, *Foleyella* sp. and an encysted nematode. Mean intensity was not calculated for echinostome metacercariae because they could not be counted accurately, and overall abundance was reported as an estimate of encysted metacercaria counted on the surface of the kidneys. Values for overall prevalence, mean intensity and mean abundance are summarized in Table 2.

Although variable, statistically significant differences in prevalence or mean intensity of infection existed between male and female frogs for *Haematoloechus varioplexus* Stafford, 1902, *Halipegus occidualis* Stafford, 1905, echinostome metacercariae, *Mesocestoides* sp. Valunt, 1863, and *Cosmocercoides* sp. Harwood, 1930 (Table 3). Both *Haematoloechus varioplexus,* a lung trematode, and *Halipegus occidualis,* a trematode of the eustachian tubes, had significantly higher mean intensities in male frogs, while echinostome metacercaria occurred at a higher prevalence in males rather then female frogs. Female frogs had significantly higher mean intensities of both the cestode *Mesocestoides* sp. and the nematode *Cosmocercoides* sp..

Mean helminth species richness was 2.68 $\pm$ 1.29 species per frog. Multiple species infections were fairly common with zero, one, two, three, four and five species occurring in four, eight, 23, 20, 13 and seven hosts, respectively. No statistically significant differences were found between mean helminth species richness in male (2.76 $\pm$ 1.01) and female frogs (2.56 $\pm$ 1.52, $t = 0.66$, $P > 0.05$). A weak non-significant positive correlation was found between overall helminth abundance, excluding larval platyhelminthes, and wet weight ($r = 0.04$, $P > 0.05$, Fig. 2). Non-significant relationships were also observed for most helminth species, excluding larval platyhelminthes: unidentified adult cestode ($r = - 0.008$, $P > 0.05$), *Haematoloechus varioplexus* ($r = 0.10$, $P > 0.05$), *Halipegus occidualis* ($r = 0.188$, $P > 0.05$), *Glypthelmins quieta* ($r = -0.02$, $P > 0.05$), *Oswaldocruzia pipiens* ($r = -0.116$, $P > 0.05$), unidentified larval nematode ($r = -0.04$, $P > 0.05$), *Foleyella* sp. ($r = 0.20$, $P > 0.05$), and encysted nematodes ($r = -0.19$, $P > 0.05$). The nematode *Cosmocercoides* sp. had a

significant positive correlation with wet weight ($r = 0.306$, $P < 0.01$). A significant positive Pearson's correlation also existed between species richness and wet weight ($r = 0.31$, $P < 0.01$, Fig. 3). However, correlations between frog wet weight and species richness were not significant in May-June ($r = 0.31$, $P > 0.05$), July-August ($r= 0.01$, $P > 0.05$), and September-October ($r = 0.29$, $P > 0.05$) but was significant for the April collection ($r = 0.60$, $P < 0.02$). Stomach content analysis of *R. clamitans* revealed a broad range of aerial, terrestrial and aquatic invertebrates (Fig. 4). Sixteen different groups of invertebrates were recovered from stomach contents of green frogs, with coleopterans, gastropods and diplopodans making up the largest percentage.

Helminth parasites fall into two categories with respect to the dynamics of infection levels. Larval platyhelminths, which are usually found encysted in host tissue, represent a closed system where worm burdens tend to accumulate throughout the host's life without loss. Parasites that use amphibians as definitive hosts and occupy open organ systems have parasite infrapopulations and communities that will be determined by the balance between recruitment and loss. Therefore, larval platyhelminths were excluded from the seasonal analysis. Due to low sample sizes during some collection periods, data were pooled on a bimonthly bases in order for sample sizes to be 15 to 20 individuals.

The trematodes, *Haematoloechus varioplexus* and *Halipegus occidualis*, occurred throughout the year, with highest prevalences observed during the fall (September-October) collection (Fig. 5). The intestinal trematode, *G. quieta*, was first observed during mid spring (May-June) with a prevalence of 5 %. Prevalence for this species reached its maximum (30%) during summer (July-August) and decreased during

the fall collection (20%, Fig. 5). One frog was infected with an unidentified adult cestode in the May-June sample. Seasonal mean intensity of adult platyhelminthes followed similar patterns as prevalence (Fig. 6). No significant differences in seasonal mean intensity existed for any of the adult platyhelminthes recovered, *Halipegus occidualis* (Adjusted H = 2.02, $P > 0.05$), *Haematoloechus varioplexus* (F = 0.34, $P > 0.05$) or *G. quieta* ($t = 0.43$, $P > 0.05$).

Although intensity of *Haematoloechus varioplexus* did not vary significantly throughout the collection period, mean length of worms did (Fig. 7). Greatest mean length of worms (4.1 mm) was recorded in early spring (April), when all individuals were gravid adults, and reached its minimum length during mid spring (1.84 mm) when immature individuals were common. Statistically significant differences in mean length were observed for April and May-June collections, April and July-August collections, May-June and September-October collections, and July-August and September-October collections (F = 11.84, $P < 0.05$, single-factor, independent measure ANOVA; $P < 0.05$ for all pair-wise comparisons, Scheffe's test).

The nematodes, *Foleyella* sp. and an unidentified encysted nematode, were recovered infrequently as single infections during mid spring and fall collections (Fig. 8). While the nematodes *O. pipiens*, *Cosmocercoides* sp. and unidentified larval nematodes were first observed during mid spring and persisted until fall (Fig. 8), with prevalences being highest in summer for *O. pipiens* and mid spring and summer for *Cosmocercoides* sp. and larval nematodes. Mean intensities of these three species were variable and irregular throughout the collection period (Fig. 9). However, only *O. pipiens* exhibited statistically significant differences (Adjusted H = 9.45, $P < 0.05$) in

mean intensity. The two-sample Komogorov-Smirnov test revealed significant differences in mean intensities during the May-June and July-August collections ($P < 0.05$, Fig. 9). However, no significant differences were observed for *Cosmocercoides* sp. (Adjusted H = 0.629, $P > 0.05$) or larval nematodes (Adjusted H = 0.319, $P > 0.05$) throughout the year.

Mean helminth species richness fluctuated seasonally (Fig. 10), was lowest during early spring (1.53) and highest during the summer collection (3.1). Statistically significant differences in mean helminth species richness were observed for April and May-June collections, April and July-August collections, and April and September-October collections, ($F = 6.01$, $P < 0.05$, single-factor, independent measure ANOVA; $P < 0.05$ for all pairwise comparisons, Scheffe's test). No statistically significant differences in frog wet weight were observed during the year ($F = 0.37$, $P > 0.05$).

Bufo a. americanus

A total of 47 American toads, 28 males and 19 females, were collected. The overall mean snout-vent length and wet weight of toads was 56.60 $\pm$ 12.47 mm (range = 26.20-72.55) and 26.55 $\pm$ 13.54 g (range = 2.19-55.46), respectively. No significant difference existed in numbers of male and female toads collected throughout out the year ($\chi^2 = 1.72$, $P > 0.05$). Although female toads were larger (58.22 $\pm$ 15.39 mm) and heavier (30.47 $\pm$ 17.32 g) than males (55.50 $\pm$ 10.21 mm) and (23.89 $\pm$ 9.72 g), these differences in mean snout-vent length and wet weight were not significant ($t = 0.73$, $P > 0.05$, $t'_s = 1.50$, $P > 0.05$).

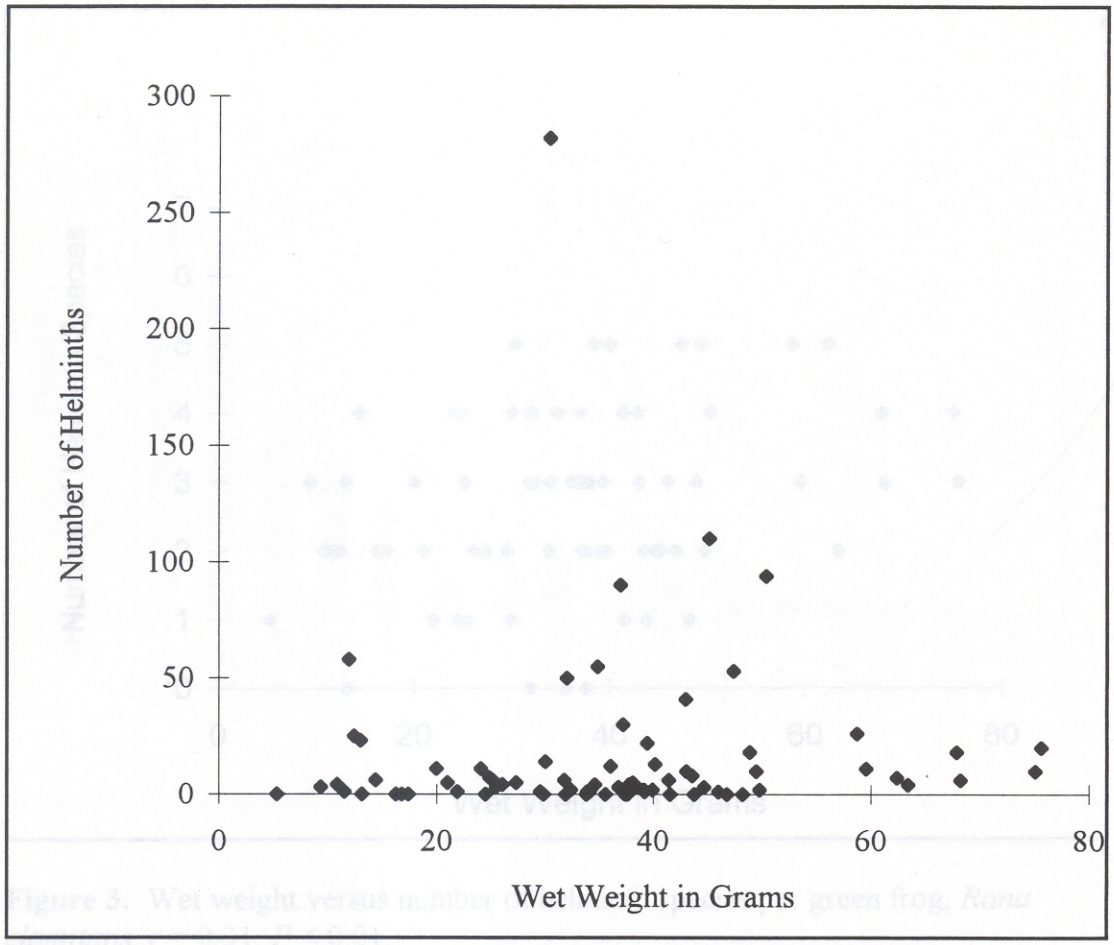

Figure 2. Wet weight versus total helminth abundance, excluding larval platyhelminthes, in green frogs, *Rana clamitans*, $r = 0.04$, $P > 0.05$.

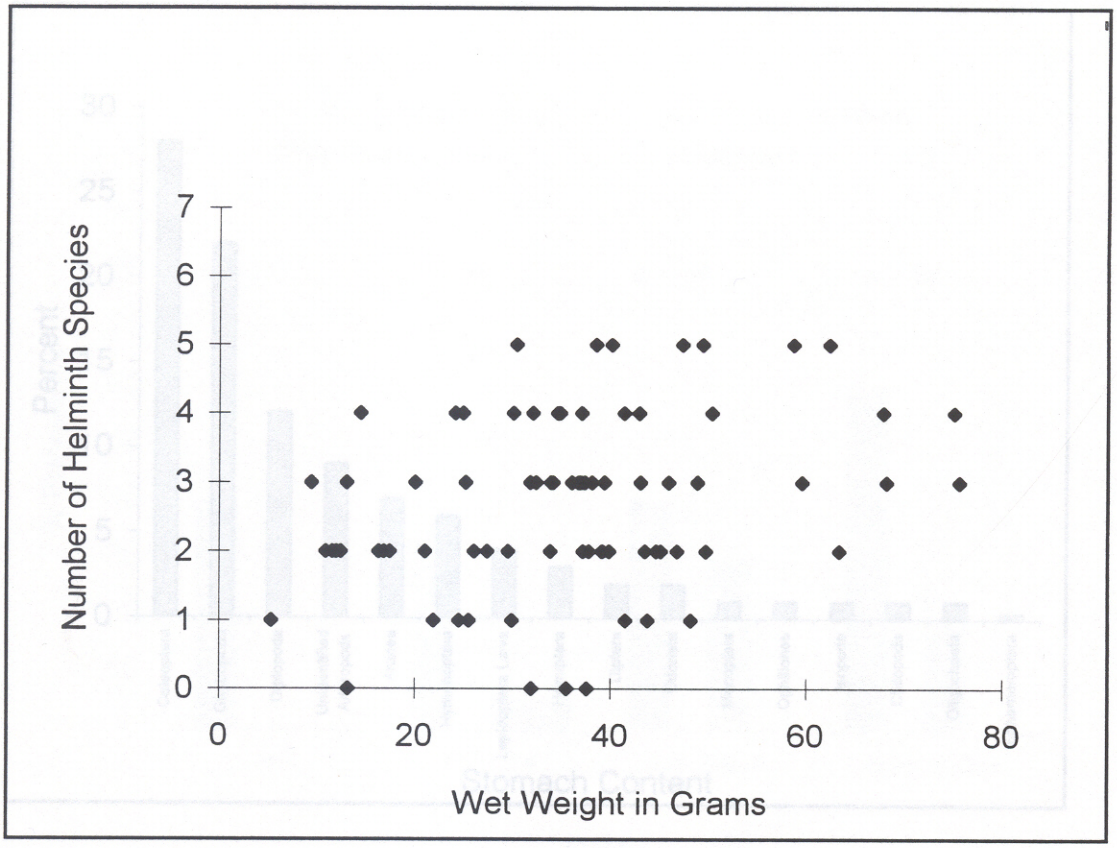

Figure 3. Wet weight versus number of helminth species per green frog, *Rana clamitans*, $r = 0.31$, $P < 0.01$.

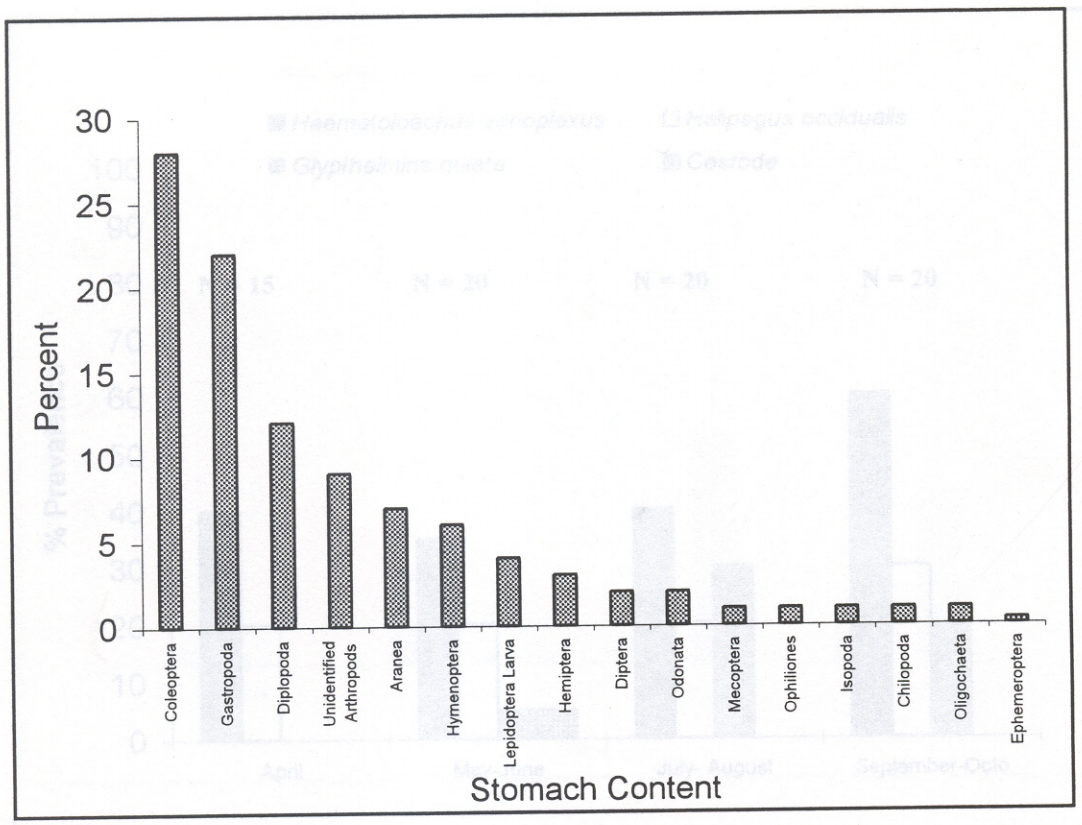

Figure 4. Undigested stomach contents of green frogs, *Rana clamitans*.

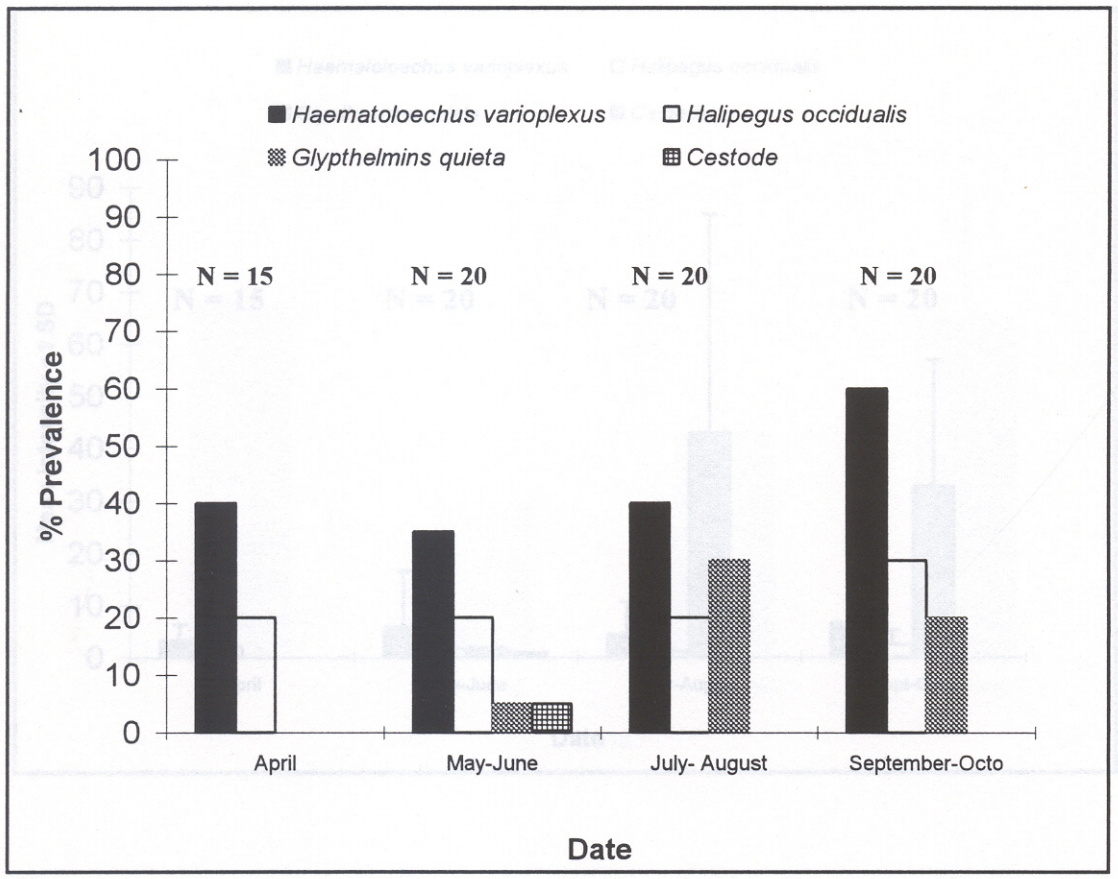

Figure 5. Seasonal prevalence of adult platyhelminthes in *Rana clamitans*. N equals sample size of frogs collected for each date.

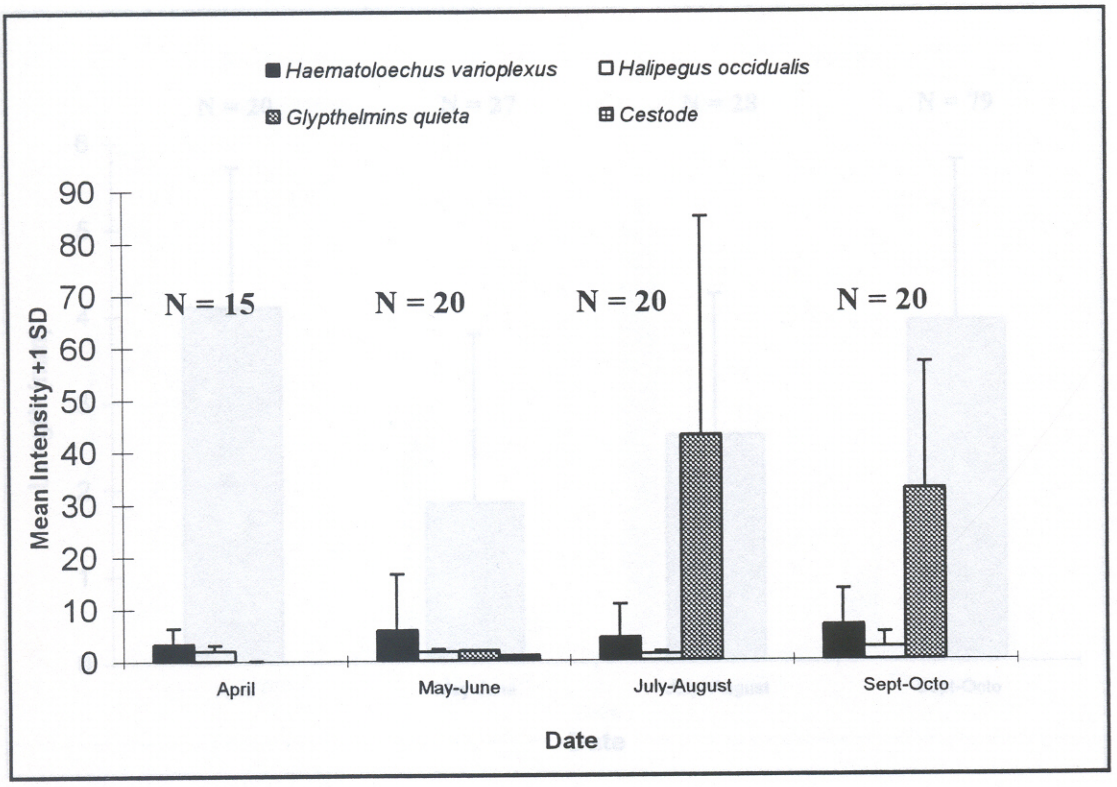

Figure 6. Seasonal mean intensity of adult platyhelminthes recovered in *Rana clamitans*. N equals sample size of frogs collected on each date.

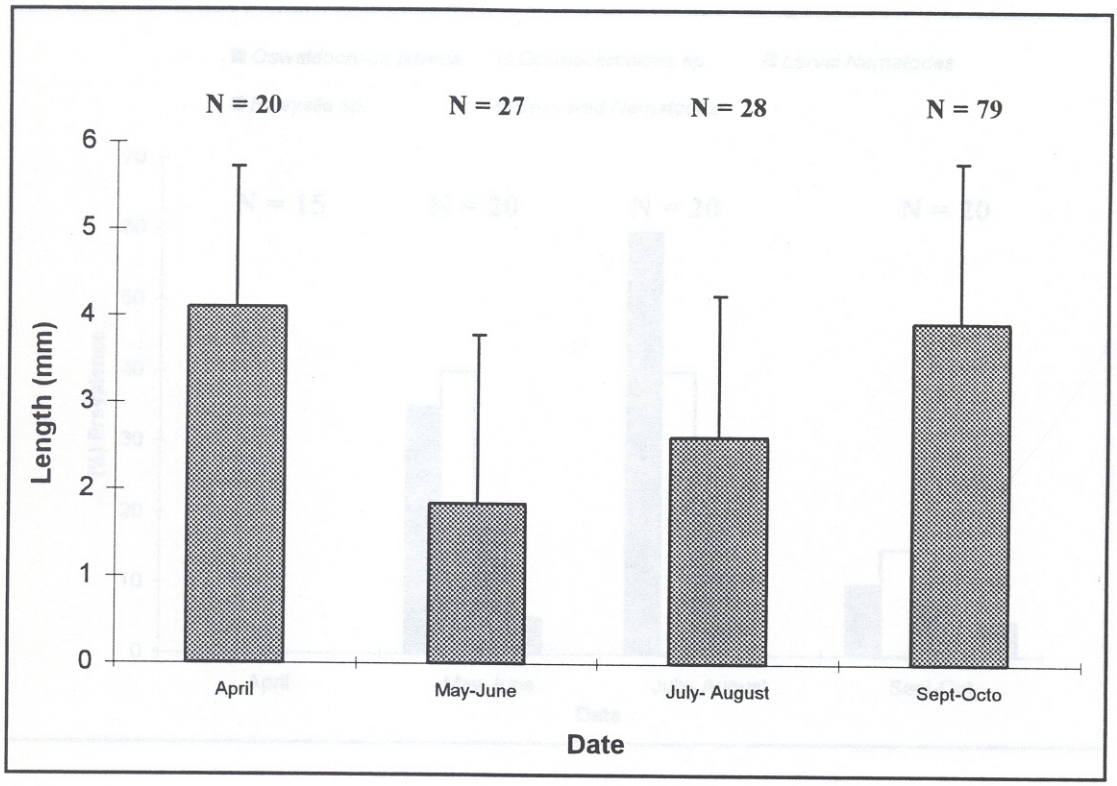

Figure 7. Average length of *Heamatoloechus varioplexus* from *Rana clamitans.* N equals number of worms measured from all frogs recovered on each date.

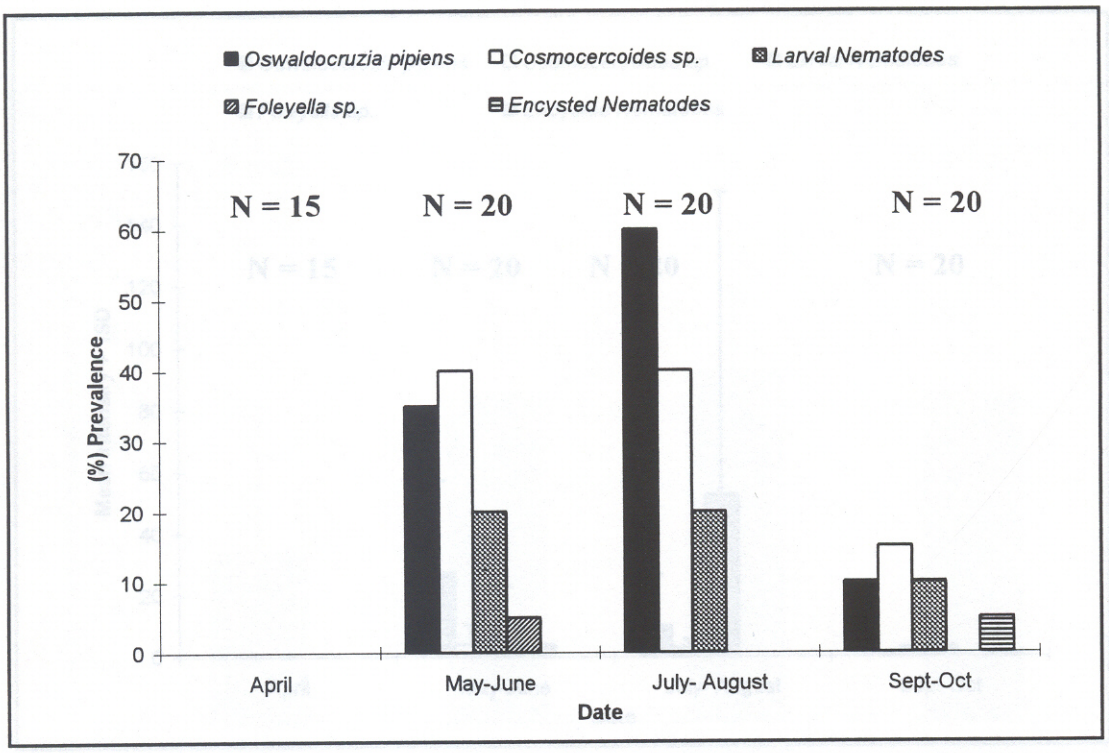

Figure 8. Seasonal prevalence of nematodes of *Rana clamitans*. N equals sample size of frogs collected on each date.

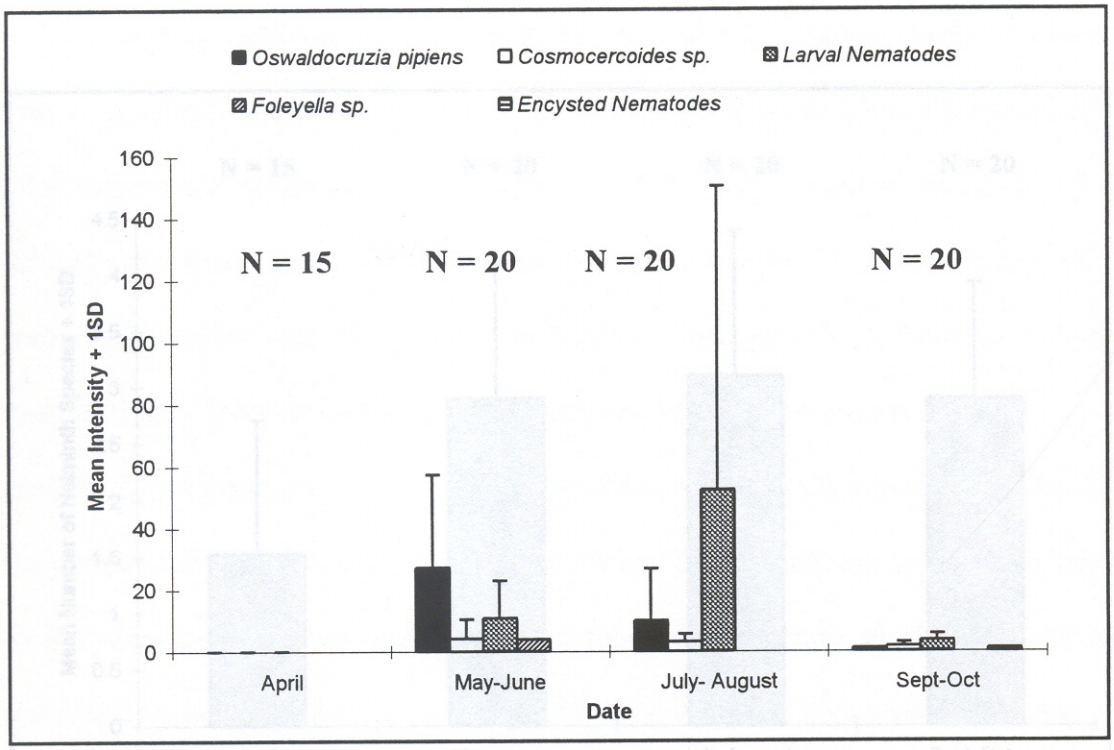

Figure 9. Seasonal mean intensity of nematodes of *Rana clamitans*. N equals sample size of frogs collected on each sample date.

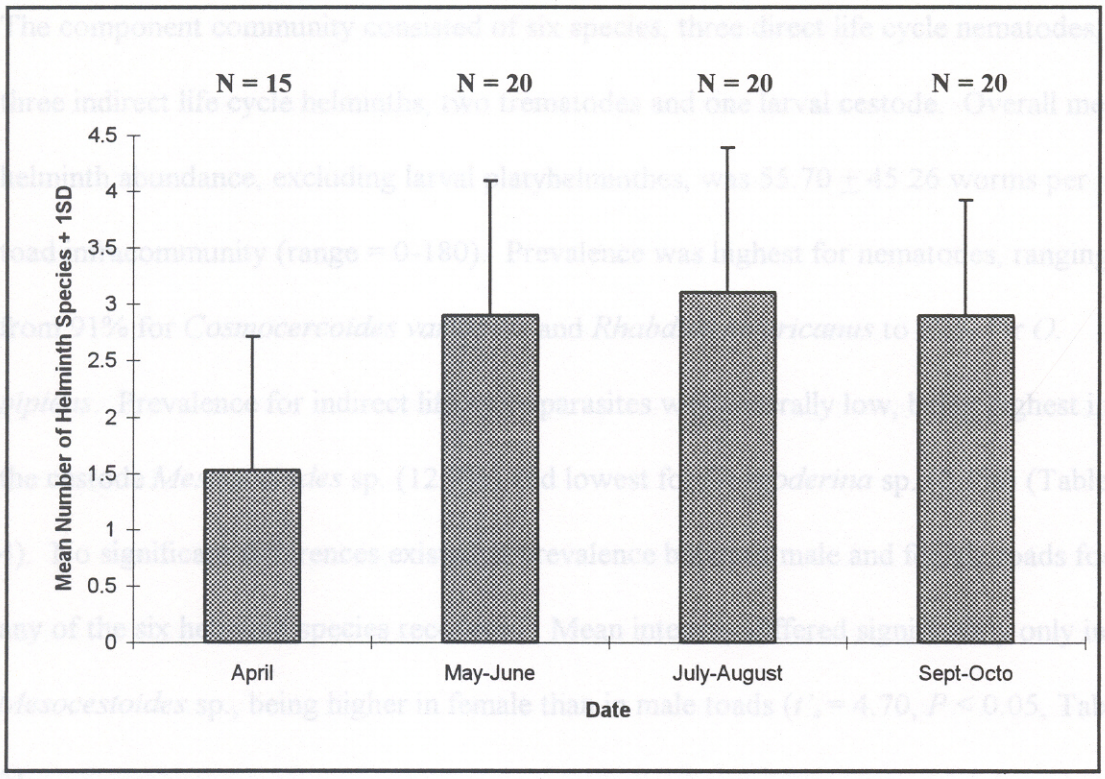

Figure 10. Mean helminth species richness in *Rana clamitans* during April through October 1996.

Forty six (98%) of 47 toads were infected with one or more species of helminths. The component community consisted of six species, three direct life cycle nematodes, three indirect life cycle helminths, two trematodes and one larval cestode. Overall mean helminth abundance, excluding larval platyhelminthes, was 55.70 $\pm$ 45.26 worms per toad infracommunity (range = 0-180). Prevalence was highest for nematodes, ranging from 91% for *Cosmocercoides variabilis* and *Rhabdias americanus* to 87% for *O. pipiens*. Prevalence for indirect life cycle parasites was generally low, being highest in the cestode *Mesocestoides* sp. (12.7%) and lowest for *Gorgoderina* sp. (2.1%) (Table 4). No significant differences existed in prevalence between male and female toads for any of the six helminth species recovered. Mean intensity differed significantly only in *Mesocestoides* sp., being higher in female than in male toads (t'_s = 4.70, $P < 0.05$, Table 5).

Mean helminth species richness was 2.9 $\pm$ 0.9 species per toad. Multiple species infections were common, with zero, one, two, three, four and five species occurring in one, two, six, 30, seven and one host, respectively. No statistically significant differences were found in mean helminth species richness between male (3 $\pm$ 0.86) and female toads (2.8 $\pm$ 0.85, $t = 0.83$, $P > 0.05$). A significant positive correlation existed between wet weight and helminth species richness per toad ($r = 0.31$, $P < 0.05$, Fig. 11). This relationship became insignificant when a single uninfected toad was removed (r = 0.20, $P > 0.05$). A significant positive correlation existed between wet weight and overall helminth abundance, excluding larval platyhelminthes ($r = 0.47$, $P < 0.01$, Fig. 12), although this correlation was only significant for female toads ($r = 0.57$, $P < 0.01$) and not for males ($r = 0.20$, $P > 0.05$). Similar results were obtained for *C. variabilis* (r

= 0.41, $P < 0.01$) and *O. pipiens* ($r = 0.43$, $P < 0.01$) while no significant correlation was observed for *R. americanus* ($r = 0.27$, $P > 0.05$). When these analyses were performed separately for male and female hosts, significant positive correlations only occurred in female toads (*R. americanus*, $r = 0.54$, $P < 0.05$; *O. pipiens*, $r = 0.58$, $P < 0.01$; *C. variabilis*, $r = 0.52$, $P < 0.05$). Stomach content revealed that toads feed mostly on ants (98%) with beetles and other terrestrial arthropods representing a small portion of the diet (Fig. 13).

Due to low sample size during certain collection periods, data were pooled on a bimonthly basis in order for samples to be fifteen to sixteen toads per season. Larval helminths were not included in the analysis because they can accumulate throughout the amphibian's life. Only one male toad was infected with six *Gorgoderina* sp. during the early spring (April) collection. Seasonally, there was no significant difference in prevalence or mean intensity for any of the nematodes recovered (Table 6). Prevalence was highest during early spring for *O. pipiens* (93%), early summer (June-July) for *R. americanus* (100%), and late summer-early fall (August-September) for *C. variabilis* (94%). Mean intensities were higher during early summer for *C. variabilis* and *O. pipiens* and late summer-early fall for *R. americanus*. In toads, seasonally, there was a significant difference in location of *C. variabilis* ($\chi^2 = 556.02$, $P < 0.01$), and *R. americanus* ($\chi^2 = 232.73$, $P < 0.01$). *Cosmocercoides variabilis* occurred in greater numbers in the body cavity and lungs during the early spring and in the small and large intestines during early summer and late summer-early fall collections (Fig. 14). *Rhabdias americanus* were found primarily in the lungs during early spring and progressively increased in the body cavity during early summer and late summer-early

fall collections (Fig. 15). The nematode *O. pipiens* did not exhibit any seasonal variation in location, and was found in the small intestine at all times during the year. *Oswaldocruzia pipiens* was significantly correlated with *C. variabilis* ($r = 0.54$, $P < 0.01$), in both male ($r = 0.51$, $P < 0.01$) and female toads ($r = 0.58$, $P < 0.01$, Fig. 16).

Although mean species richness exhibited variability throughout the year, being highest in early spring (3.26 ± 0.79) and lowest during the late summer-early fall collection (2.62 ± 0.95), these differences were not statistical significant ($F = 2.3$, $P > 0.05$). Wet weight of toads showed similar seasonal variability but these differences were also not significant ($F = 2.99$, $P > 0.05$).

B. Comparative Study

Location - Eagle Waukesha Co.

A total of 123 amphibians of five species including the above 47 American toads, 65 Cope's gray treefrogs, six western chorus frogs, four spring peepers and one northern leopard frog were collected during April to November of 1996 and April to June of 1997 from two adjacent ephemeral ponds and highways N and 67 in Eagle Waukesha County, Wisconsin. The compound community consisted of at least 11 species of helminths, eight indirect life cycle parasites and three direct life cycle parasites. The single female northern leopard frog, *Rana pipiens*, had a snout-vent length of 63.6 mm and wet weight of 21.11 g and was infected with one direct life cycle parasite *O. pipiens*, one unknown

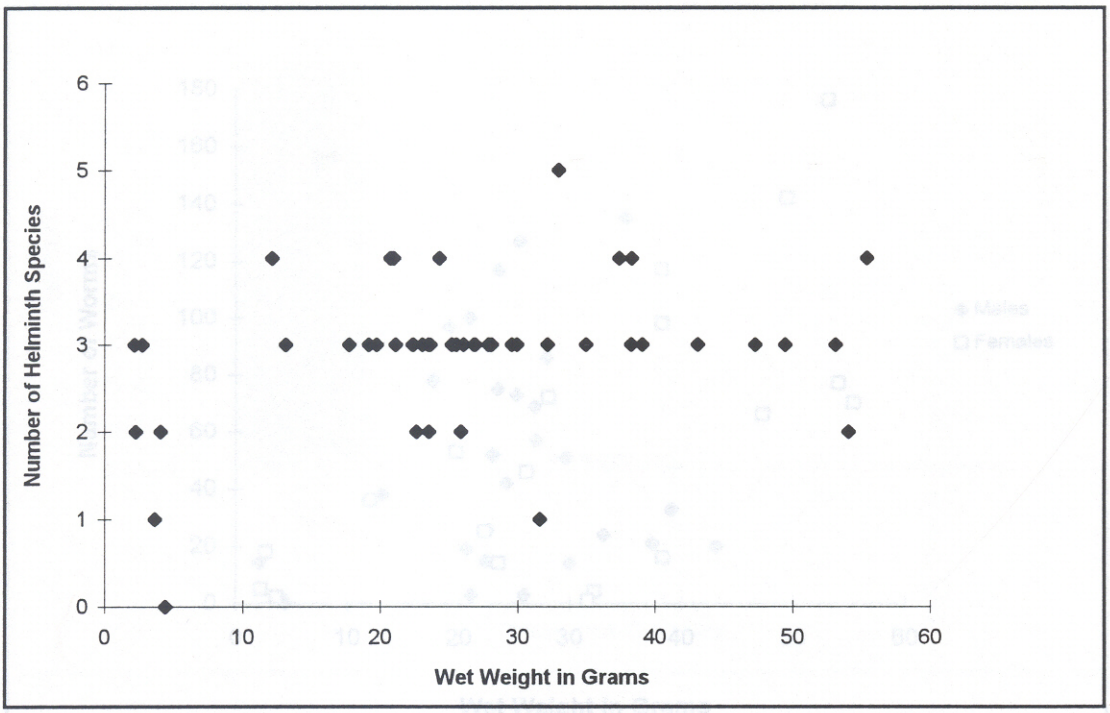

Figure 11. Wet weight versus number of helminth species per American toads, *Bufo a. americanus*, $r = 0.31$, $P < 0.05$.

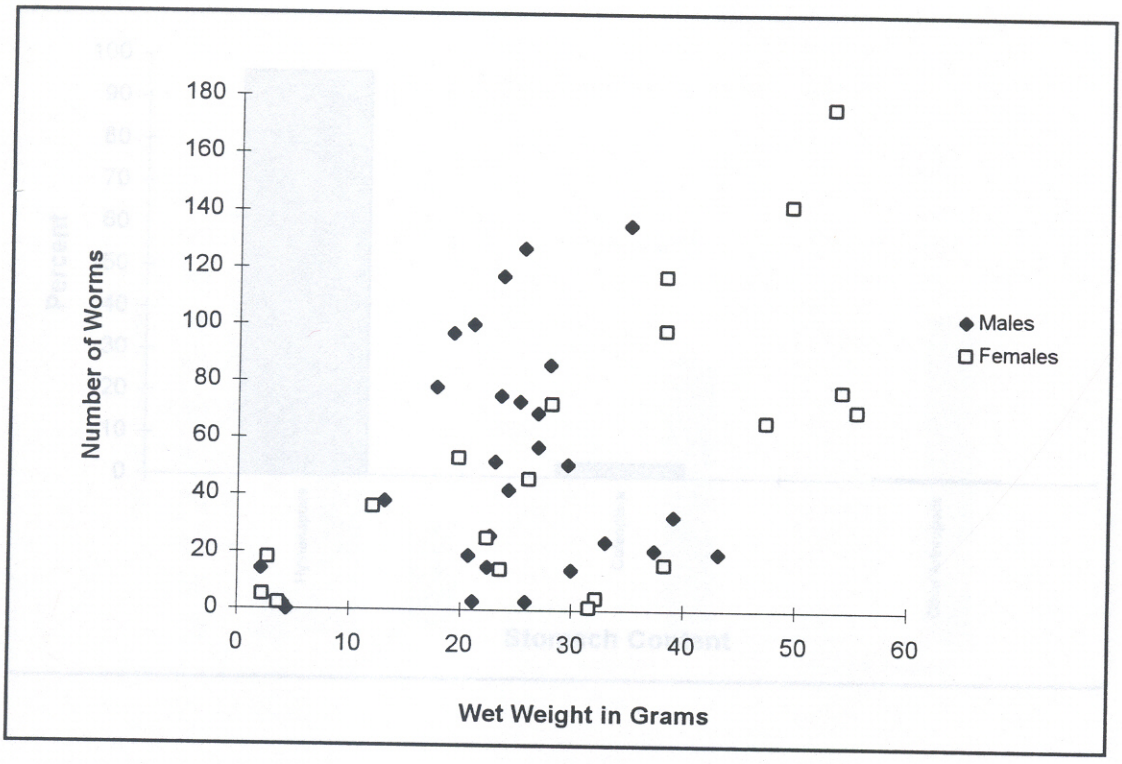

Figure 12. Wet weight versus total helminth abundance, excluding larval platyhelminthes, in American toads, *Bufo a. americanus,* overall $r = 0.47$, $P < 0.01$, female toads $r = 0.57$, $P < 0.01$ and male toads $r = 0.20$, $P > 0.05$.

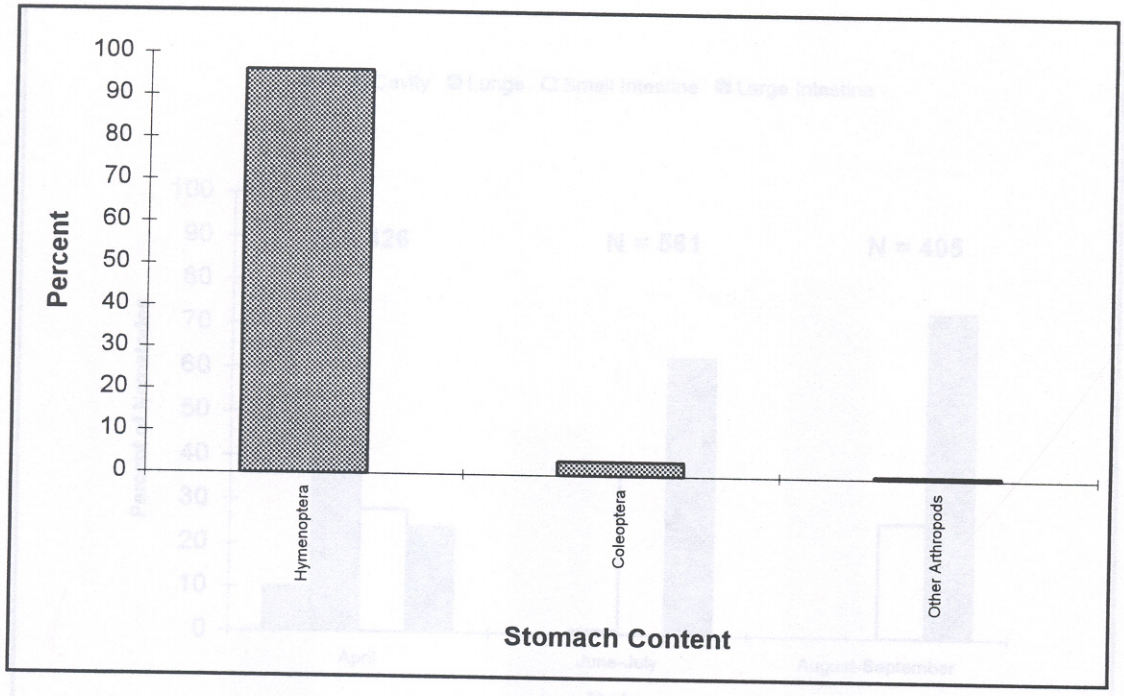

Figure 13. Undigested stomach content of American toads, *Bufo a. americanus*.

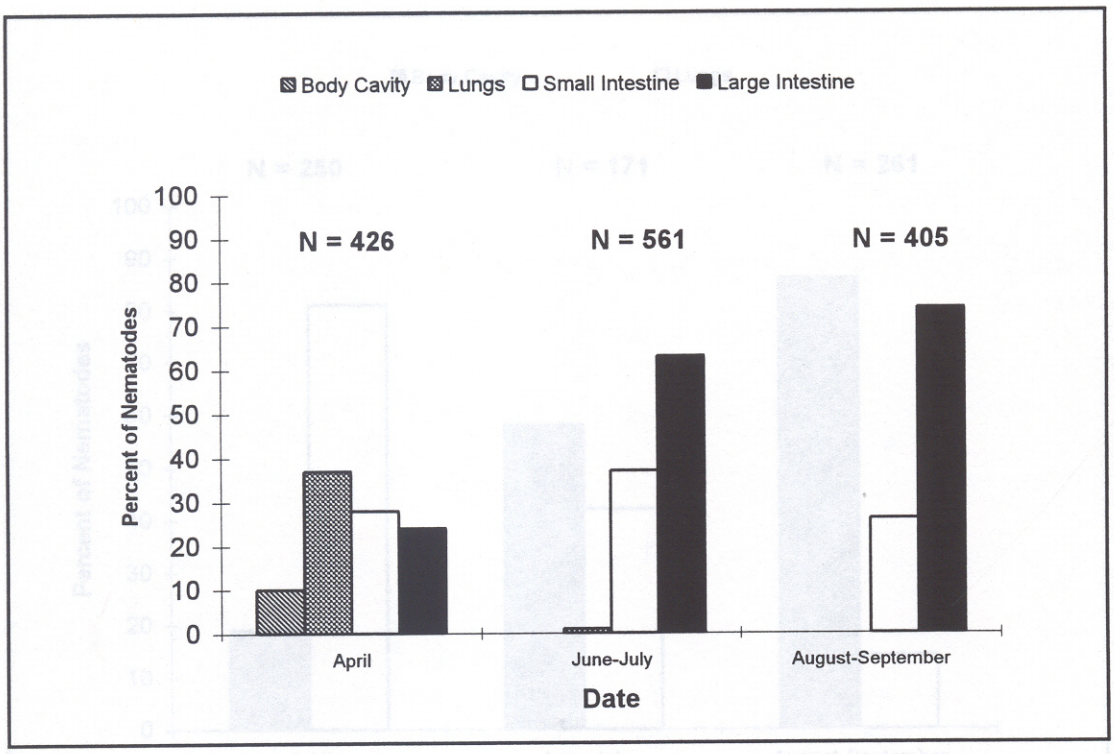

Figure 14. Seasonal distribution of the relative proportions of *Cosmocercoides variabilis* recovered in the body cavity, lungs, small intestine and large intestine of *Bufo a. americanus*. N equals number of nematodes recovered on each date.

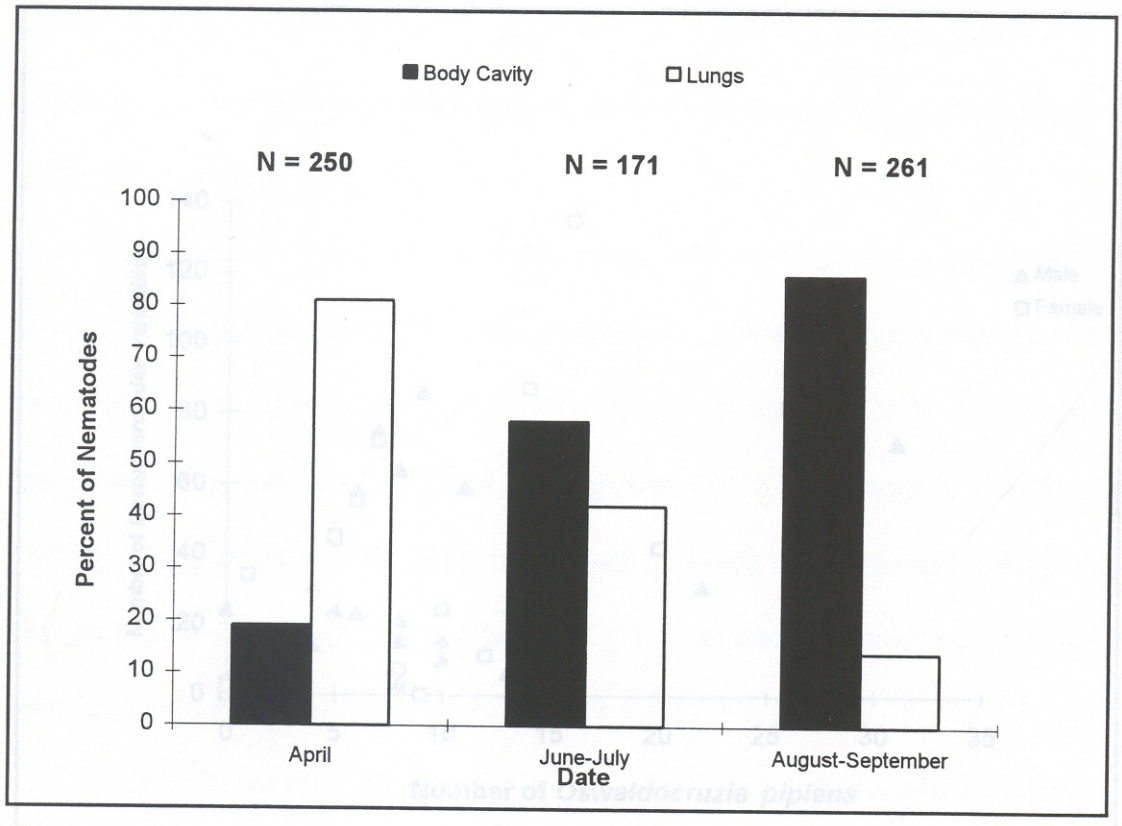

Figure 15. Seasonal distribution of the relative proportions of *Rhabdias americanus* recovered in the body cavity and lungs of *Bufo a. americanus.* N equals number of nematodes recovered on each date.

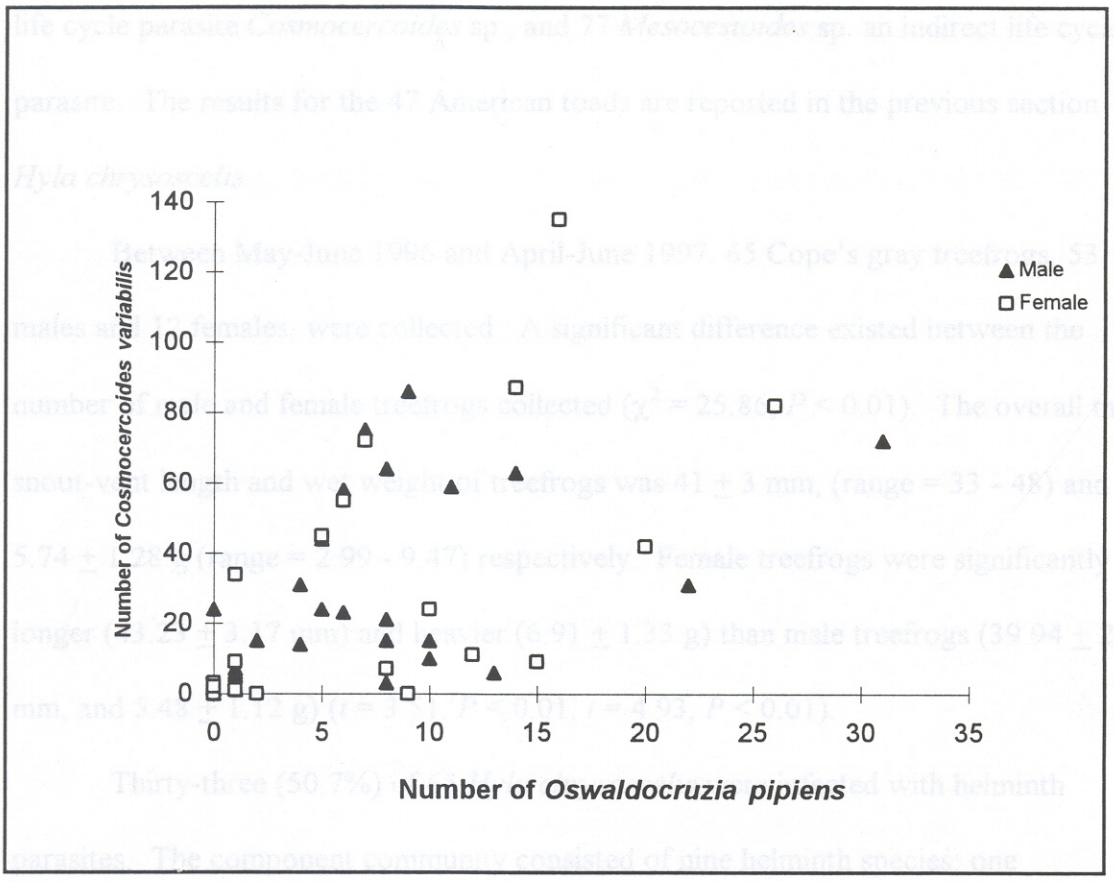

Figure 16. Number of *Oswaldocruzia pipiens* versus *Cosmocercoides variabilis* in *Bufo a. americanus*, overall $r = 0.54$, $P < 0.01$; male toads $r = 0.51$, $P < 0.01$ and female toads $r = 0.58$, $P < 0.01$.

life cycle parasite *Cosmocercoides* sp., and 77 *Mesocestoides* sp. an indirect life cycle parasite. The results for the 47 American toads are reported in the previous section.

Hyla chrysoscelis

Between May-June 1996 and April-June 1997, 65 Cope's gray treefrogs, 53 males and 12 females, were collected. A significant difference existed between the number of male and female treefrogs collected ($\chi^2 = 25.86$, $P < 0.01$). The overall mean snout-vent length and wet weight of treefrogs was 41 $\pm$ 3 mm, (range = 33 - 48) and 5.74 $\pm$ 1.28 g (range = 2.99 - 9.47) respectively. Female treefrogs were significantly longer (43.23 $\pm$ 3.17 mm) and heavier (6.91 $\pm$ 1.33 g) than male treefrogs (39.94 $\pm$ 2.88 mm, and 5.48 $\pm$ 1.12 g) ($t = 3.51$, $P < 0.01$, $t = 4.93$, $P < 0.01$).

Thirty-three (50.7%) of 65 *Hyla chrysoscelis* were infected with helminth parasites. The component community consisted of nine helminth species: one monogenian, three larval cestodes, three larval and adult trematodes, and two nematodes. Six of these are indirect life cycle parasites, while the two nematodes and monogenian have a direct life cycle. Overall mean helminth abundance, excluding larval platyhelminthes, was 2.95 $\pm$ 9.74 worms per infracommunity (range = 0-48). Prevalence ranged from 22% for *C. variabilis* to 1.5% for *G. pennsylvaniensis*, unidentified plerocercoides and cestode cysts. Mean intensity was highest for immature trematodes (42.3 $\pm$ 2.5) and lowest for *O. pipiens* (1.3 $\pm$ 0.5) (Table 7.). Mean intensity was not calculated for *Mesocestoides* sp. because they could not be counted accurately, and overall abundance was reported as an estimate of worm cysts. No significant difference existed in prevalence or mean intensity between male and female treefrogs.

Mean helminth species richness was 0.72 ± 0.85 species per treefrog. Multiple species infections were rare with zero, one, two, three and four species occurring in 32, 21, 11, zero, and one host, respectively. No statistically significant differences were found in mean helminth species richness between male (0.73 ± 0.88) and female treefrogs (0.66 ± 0.77, $t = 0.25$, $P > 0.05$). Also, no significant correlation existed in wet weight and overall helminth abundance, excluding larval platyhelminthes ($r = 0.01$, $P > 0.05$, Fig. 17) or wet weight and helminth species richness per treefrog ($r = 0.08$, $P > 0.05$, Fig. 18). Similar results were obtained for individual helminth species abundance and wet weight: *P. nearcticum* ($r = 0.03$, $P > 0.05$), *G. pennsylvaniensis* ($r = 0.22$, $P > 0.05$), unidentified immature trematodes ($r = -0.03$, $P > 0.05$), *C. variabilis* ($r = 0.02$, $P > 0.05$), and *O. pipiens* ($r = -0.01$, $P > 0.05$). Twenty-nine (45%) treefrogs had identifiable stomach contents, their diet consisted of insects, with coleopterans making up 43% of the diet followed by lepidoptera larvae (24%), unidentifiable insects (22%), and orthopterans (11%).

Pseudacris t. triseriata and *Pseudacris c. crucifer*

Six western chorus frogs (four males and two females) and four spring peepers (two males and two females), were collected during 1996 and 1997. The overall mean snout-vent length and wet weight of chorus frogs was 24 ± 1 mm (range = 22 - 25 mm) and 0.88 ± 0.19 g (range = 0.8 - 1.19). Average spring peeper snout-vent length was 20.06 ± 1.53 mm (range = 18.3-22) and wet weight was 0.61 ± 0.04 g (range = 0.56-0.64). Three species of helminths infected both species of frogs (Tables 8 and 9). When the statistical analyses were performed, these species were lumped because of the low

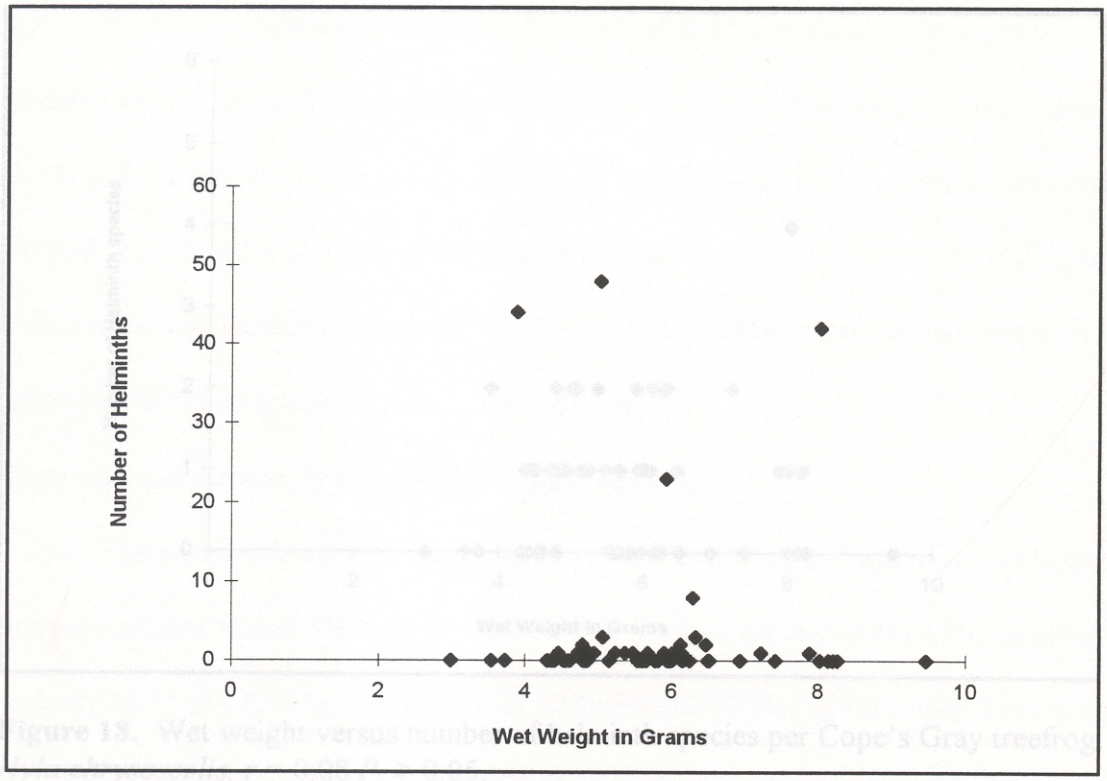

Figure 17. Wet weight versus total helminth abundance, excluding larval platyhelminthes, in Cope's Gray Treefrog, *Hyla chrysoscelis,* $r = 0.01$, $P > 0.05$.

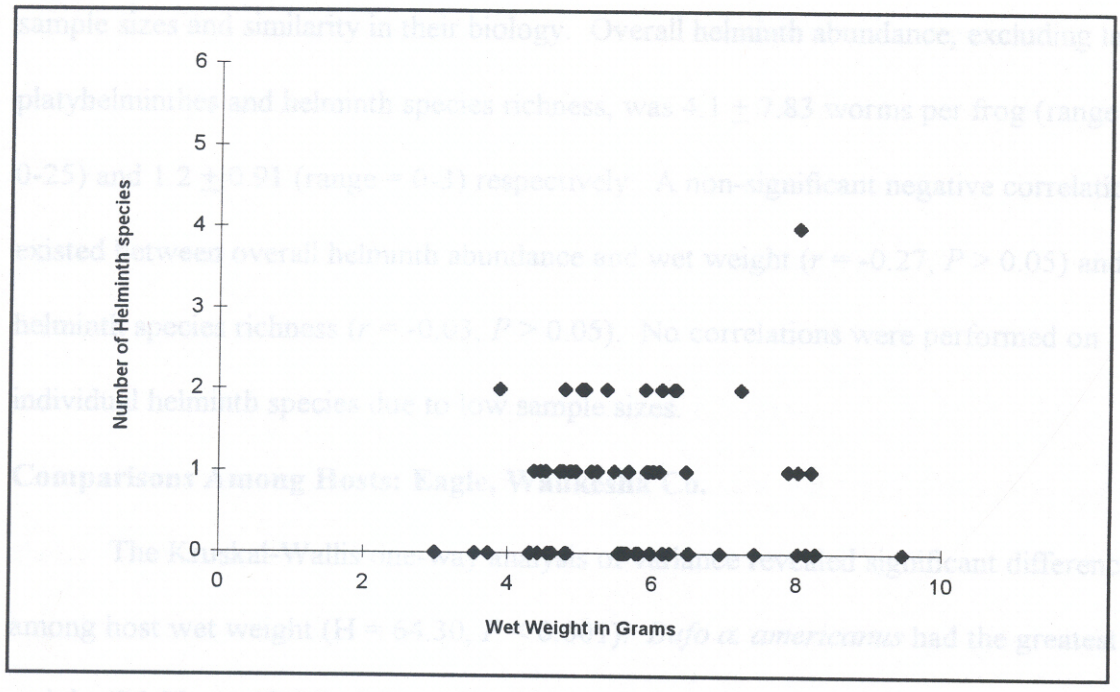

Figure 18. Wet weight versus number of helminth species per Cope's Gray treefrog, *Hyla chrysoscelis*, $r = 0.08$ $P > 0.05$.

sample sizes and similarity in their biology. Overall helminth abundance, excluding larval platyhelminthes and helminth species richness, was 4.1 $\pm$ 7.83 worms per frog (range = 0-25) and 1.2 $\pm$ 0.91 (range = 0-3) respectively. A non-significant negative correlation existed between overall helminth abundance and wet weight (r = -0.27, P > 0.05) and helminth species richness (r = -0.03, P > 0.05). No correlations were performed on individual helminth species due to low sample sizes.

Comparisons Among Hosts: Eagle, Waukesha Co.

The Kruskal-Wallis one-way analysis of variance revealed significant differences among host wet weight (H = 64.30, P < 0.001). *Bufo a. americanus* had the greatest weight (26.55 g $\pm$ 13.54), followed by *H. chrysoscelis* (5.74 g $\pm$ 1.28) and *Pseudacris* spp. (0.79 g $\pm$ 0.21). The Kolmogorov-Smirnov two-sample test revealed significant differences among all possible host species pairs, with toads having significantly greater wet weight than treefrogs (P < 0.001) and *Pseudacris* spp. (P < 0.01), and treefrogs being significantly greater in weight than *Pseudacris* spp. (P < 0.01).

Significant differences also existed in overall helminth prevalence between *B. a. americanus* 98%, and *H. chrysoscelis* 50.7%, (χ_{adj}^2 = 26.89, P < 0.01). No significant differences existed in overall prevalence between *H. chrysoscelis* and *Pseudoacris* spp., 80% (χ_{adj}^2 = 1.92, P > 0.05), or *B. a. americanus* and *Pseudacris* spp. (χ_{adj}^2 = 2.30, P > 0.05). The Kruskal-Wallis one-way analysis of variance revealed significant differences in helminth abundance (H = 37.45, P < 0.001, Fig. 19) among the three host groups. The single-factor, independent measure ANOVA revealed similar differences among hosts for helminth species richness (F = 89.53, P < 0.001, Fig. 20).

The Kolmogorov-Smirnov two-sample test revealed significant differences in overall parasite abundance between toads and treefrogs and toads and *Pseudacris* spp. ($P < 0.001$). Scheffe's post hoc test revealed similar significant differences in species richness between toads and treefrogs and toads and *Pseudacris* spp. ($P < 0.01$). When all hosts were combined, a significant positive correlation existed for wet weight and abundance ($r = 0.71$, $P < 0.01$, Fig. 21) and species richness ($r = 0.66$, $P < 0.01$).

When comparisons were made among host species and percent of indirect or direct life cycle parasites of individual component communities (Fig. 22), statistically significant differences were found ($\chi^2 = 2822$, $P < 0.001$). Toads had a higher relative abundance of direct life cycle nematodes (92%) with only 8% of the component community being indirect life cycle parasites. Treefrogs harbored higher relative abundances of indirect life cycle parasites (95%) with few direct life cycle nematodes, while *Pseudacris* spp. had more direct life cycle parasites than indirect.

Location - Brookfield Waukesha Co.

A total of 112 amphibians of three species including 30 American toads, 31 northern leopard frogs and 51 blue spotted salamanders were collected during April to October 1996 from a temporary pond in Brookfield Waukesha County, Wisconsin. The compound community consisted of at least ten species of helminths: five indirect life cycle parasites (four trematodes and one larval cestode), three direct life cycle parasites (three

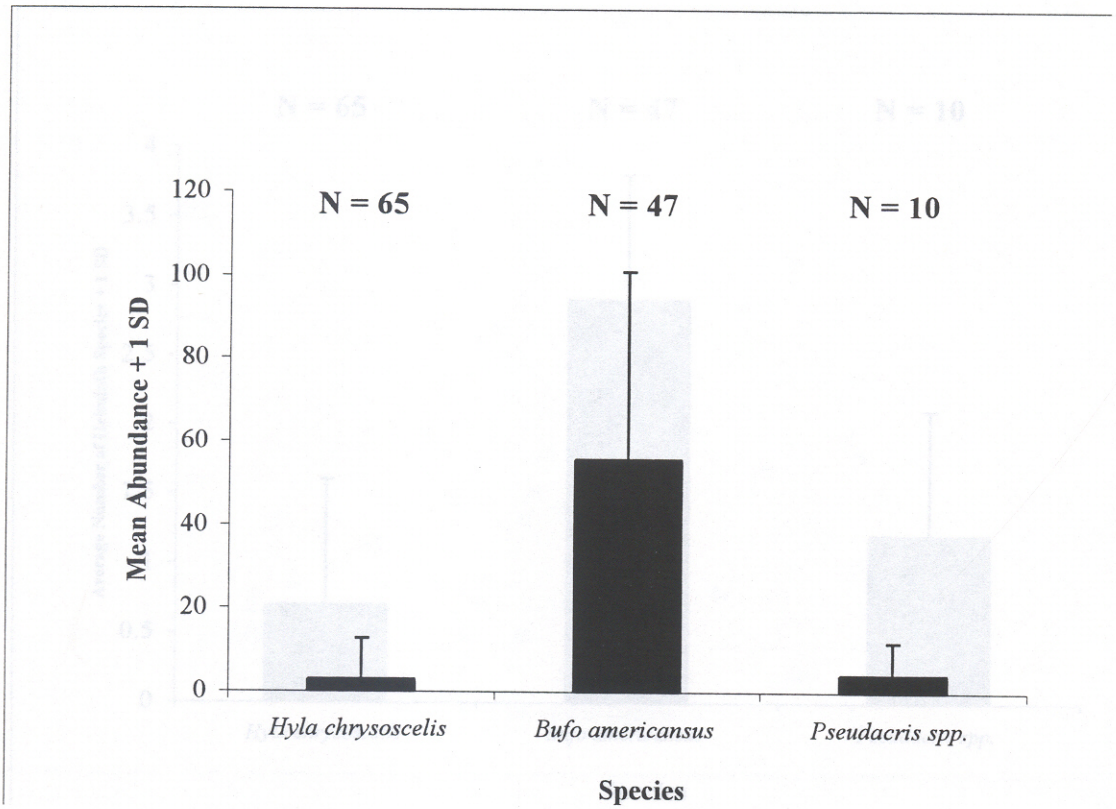

Figure 19. Mean helminth abundance among four species of amphibians. N equals number of frogs examined.

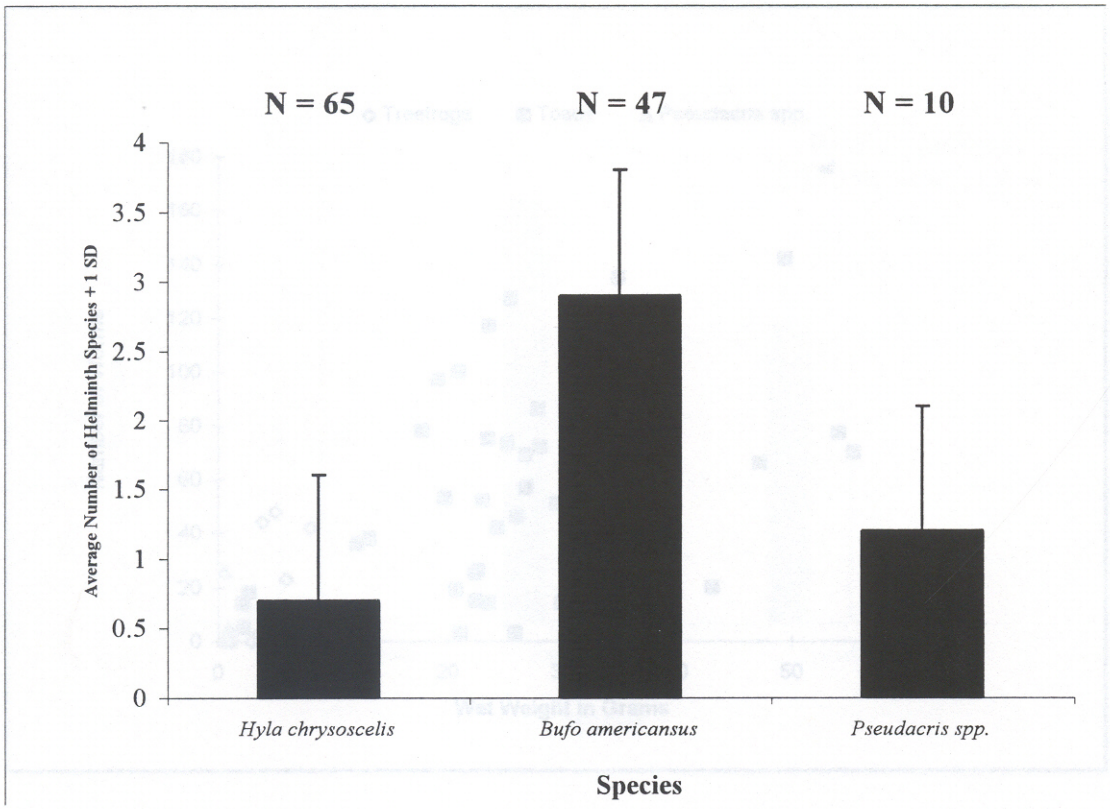

Figure 20. Mean species richness among four species of amphibians. N equals number of frogs examined.

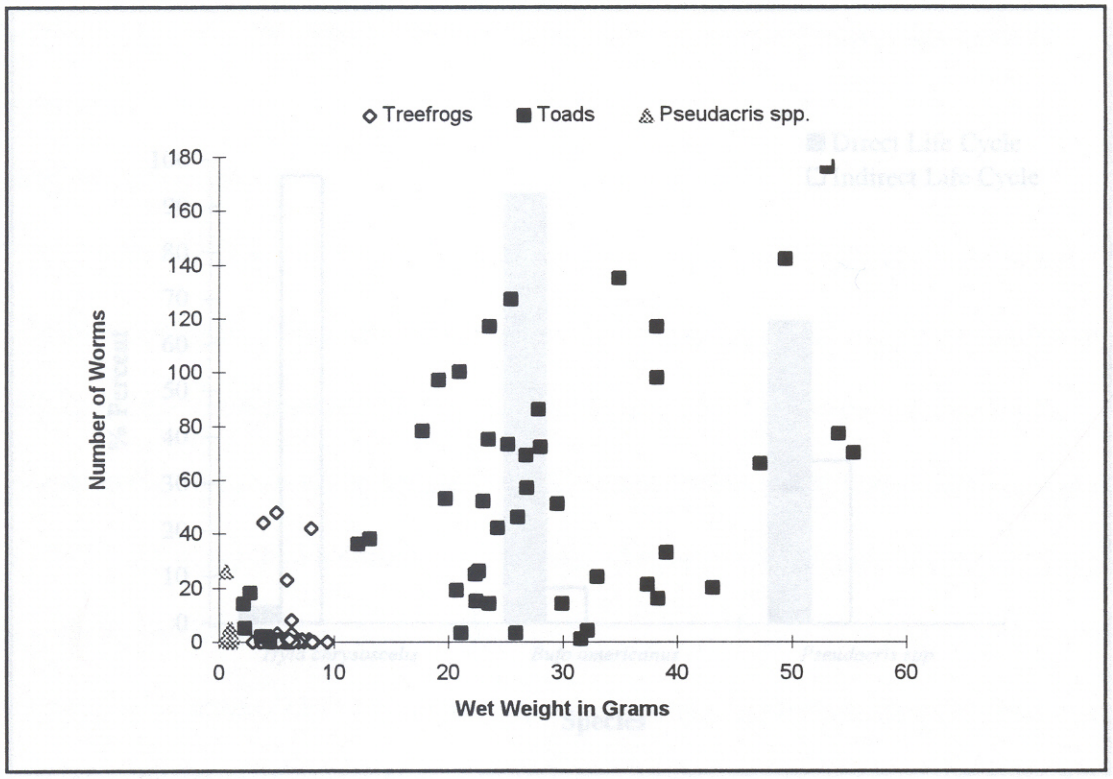

Figure 21. Combined wet weight for four species of amphibians versus total helminth abundance, excluding larval platyhelminthes, $r = 0.71$, $P < 0.01$.

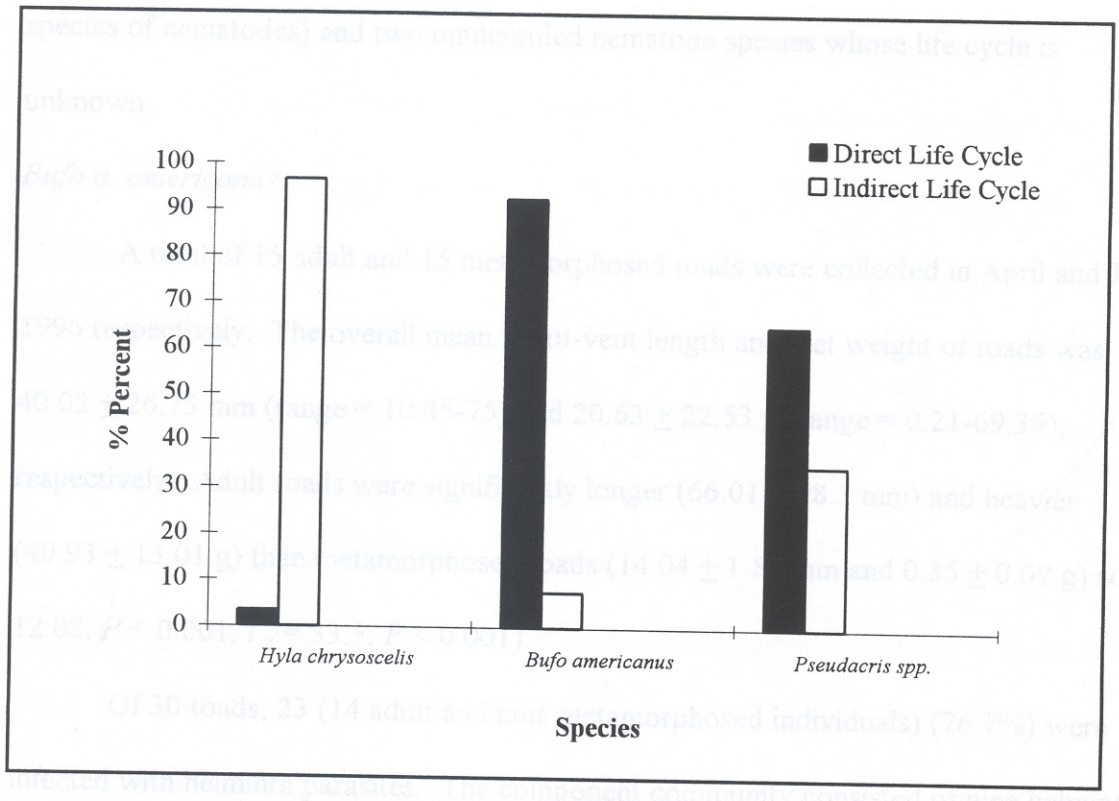

Figure 22. Percent of helminths with direct and indirect life cycles in individual component communities of four species of amphibians.

species of nematodes) and two unidentified nematode species whose life cycle is unknown.

Bufo a. americanus

A total of 15 adult and 15 metamorphosed toads were collected in April and July 1996 respectively. The overall mean snout-vent length and wet weight of toads was 40.02 $\pm$ 26.75 mm (range = 10.45-75) and 20.63 $\pm$ 22.53 g (range = 0.21-69.35), respectively. Adult toads were significantly longer (66.01 $\pm$ 58.3 mm) and heavier (40.93 $\pm$ 13.01 g) than metamorphosed toads (14.04 $\pm$ 1.54 mm and 0.35 $\pm$ 0.09 g) (t'_s = 12.08, $P < 0.001$, t'_s = 33.5, $P < 0.001$).

Of 30 toads, 23 (14 adult and nine metamorphosed individuals) (76.7%) were infected with helminth parasites. The component community consisted of nine helminth species: one larval cestode, one adult and two larval trematodes and five nematodes. Four of these were indirect life cycle parasites, while three nematodes were direct life cycle and two nematodes were unknown. Overall mean helminth abundance, excluding larval platyhelminthes, was 20.7 $\pm$ 48.95 worms per infracommunity (range = 0-189). Prevalence was highest for *R. americanus* and diplostomid metacercariae (40%) and lowest 3.3% for *Haematoloechus varioplexus* (Table 10.). Significant differences in prevalence occurred between adult and metamorphosed toads, with metamorphosed toads infected with only two species of metacercariae (Table 11.).

Mean helminth species richness was 1.97 $\pm$ 1.9 species per toad. Multiple species infections were common with zero, one, two, three, four, five and six species occurring in seven, nine, five, two, three, two and two toads, respectively. Statistically significant differences occurred in mean helminth species richness between adult 3.2 $\pm$

1.86 and metamorphosed toads 0.73 ± 0.7 (t'_s = 4.80, $P < 0.001$). A positive non-significant correlation existed for wet weight and total helminth abundance, excluding larval platyhelminthes ($r = 0.309$, $P > 0.05$). Similar results were obtained for commonly occurring helminth species *C. variabilis* ($r = 0.23$, $P > 0.05$), *O. pipiens* ($r = 0.32$, $P > 0.05$) and *R. americanus* ($r = 0.25$, $P > 0.05$) A positive significant relationship was found for wet weight and species richness ($r = 0.50$, $P < 0.01$). When these analyses were performed separately for adult toads, non-significant negative relationships were observed for total helminth abundance, excluding larval platyhelminthes ($r = -0.48$, $P > 0.05$), *C. variabilis* ($r = -0.48$, $P > 0.05$), *O. pipiens* ($r = -0.27$, $P > 0.05$), *R. americanus* ($r = -0.14$, $P > 0.05$) and species richness ($r = -0.41$, $P > 0.05$). A significant negative correlation occurred in wet weight versus species richness for metamorphosed toads ($r = -0.58$, $P < 0.05$). Only two adult toads had stomach content containing beetles while metamorphs fed on mites (Fig. 23).

Rana pipiens

A total of 20 adult and 11 metamorphosed northern leopard frogs were collected in April, October and July 1996, respectively. The overall mean snout-vent length and wet weight of leopard frogs was 56.73 ± 18.62 mm (range = 27.35-83.75) and 21.23 ± 15.36 g (range = 2.39-46.57). Adult leopard frogs had a significantly greater snout-vent length (68.77 ± 10.19 mm) and wet weight (30.72 ± 10.22 g) than metamorphosed individuals (34.85 ± 5.2 mm, 3.95 ± 1.2 g) (t'_s = 8.58, $P < 0.001$, t'_s = 10.26, $P < 0.001$).

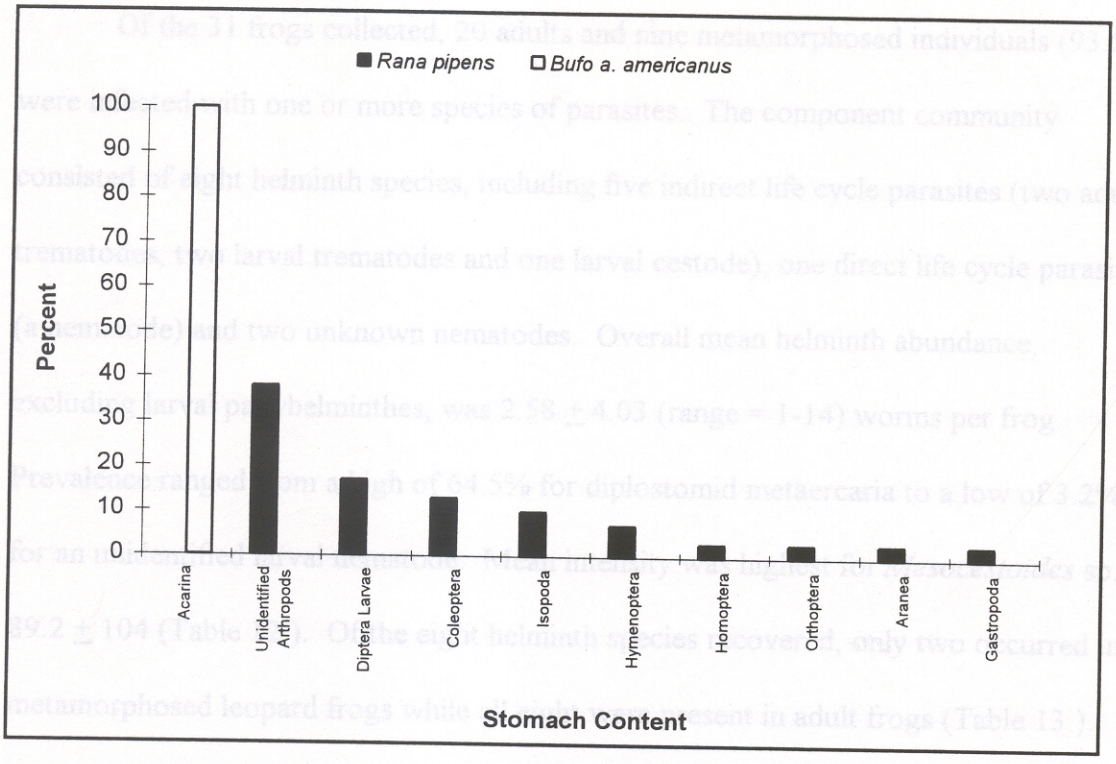

Figure 23. Undigested stomach contents of metamorphosed American toads, *Bufo a. americanus* and northern leopard frogs, *Rana pipiens.*

Of the 31 frogs collected, 20 adults and nine metamorphosed individuals (93.5%) were infected with one or more species of parasites. The component community consisted of eight helminth species, including five indirect life cycle parasites (two adult trematodes, two larval trematodes and one larval cestode), one direct life cycle parasite (a nematode) and two unknown nematodes. Overall mean helminth abundance, excluding larval paltyhelminthes, was 2.58 $\pm$ 4.03 (range = 1-14) worms per frog. Prevalence ranged from a high of 64.5% for diplostomid metaercaria to a low of 3.2% for an unidentified larval nematode. Mean intensity was highest for *Mesocestoides* sp. 89.2 $\pm$ 104 (Table 12.). Of the eight helminth species recovered, only two occurred in metamorphosed leopard frogs while all eight were present in adult frogs (Table 13.).

Species richness was variable among individuals, averaging 2.3 $\pm$ 1.3 species per frog. Multiple species infections were fairly common with zero, one, two, three, four, five and six species occurring in two, seven, nine, eight, four, zero and one host, respectively. Adult leopard frogs had a significantly higher number of species per individual (2.8 $\pm$ 1.2) than metamorphs (1.3 $\pm$ 0.8) ($t = 3.35$, $P < 0.01$). A positive non-significant correlation existed between wet weight and total helminth abundance, excluding larval platyhelminthes ($r = 0.20$, $P > 0.05$, Fig. 24), *O. pipiens* ($r = 0.15$, $P > 0.05$), *Gorgoderina attenuata* ($r = 0.19$, $P > 0.05$) and *Haematoloechus varioplexus* ($r = 0.24$, $P > 0.05$). Non-significant relationships were also observed when these analyses were performed on adult frogs for overall helminth abundance, excluding larval platyhelminthes ($r = -0.43$, $P > 0.05$), *O. pipiens* ($r = -0.25$, $P > 0.05$), *Gorgoderina attenuata* ($r = -0.02$, $P > 0.05$) and *Haematoloechus varioplexus* ($r = -0.01$, $P > 0.05$).

A significant positive correlation was found between wet weight and species richness for all frogs ($r = 0.48$, $P < 0.01$, Fig. 25), but was not significant in adult ($r = 0.09$, $P > 0.05$) or metamorphosed frogs ($r = -0.04$, $P > 0.05$). Only metamorphosed individuals contained stomach contents of nine different groups of invertebrates (Fig. 23).

Ambystoma lateral

Fifty-one blue-spotted salamanders, 31 adults and 20 metamorphs, were collected during April, May and August 1996 respectively. The overall mean snout-vent length and wet weight of salamanders was 42.68 $\pm$ 15.45 mm (range = 21.7-63.95) and 2.06 $\pm$ 1.58 g (range = 0.22-4.95) respectively. A significant difference existed in mean snout-vent length and wet weight of adult and metamorphosed salamanders, adults being larger (53.86 $\pm$ 8.1 mm) and heavier (3.14 $\pm$ 1.0 g) than metamorphs (25.36 $\pm$ 2.35 mm and 0.38 $\pm$ 0.11 g) ($t'_s = -11.9$, $P < 0.001$, $t'_s = -15.3$, $P < 0.001$).

Thirty-five (68%) of 51, (18 adults and 17 metamorphs) were infected with one to three species of helminths. The component community consisted of three species, one indirect life cycle larval trematode and two unknown nematodes. Prevalence and mean intensity were highest for echinostome metacercariae (47% and 24 $\pm$ 17) and generally low for *Cosmocercoides* sp. and encysted nematodes (Table 14.). Metamorphs were infected with echinostome metacercariae while adults possessed all three helminth species (Table 15.).

Mean helminth species richness was low for this salamander, being 0.75 $\pm$ 0.6 species per individual with 16 salamanders infected with zero, 33 salamanders infected with one, and one salamander infected with two and three species respectively. There

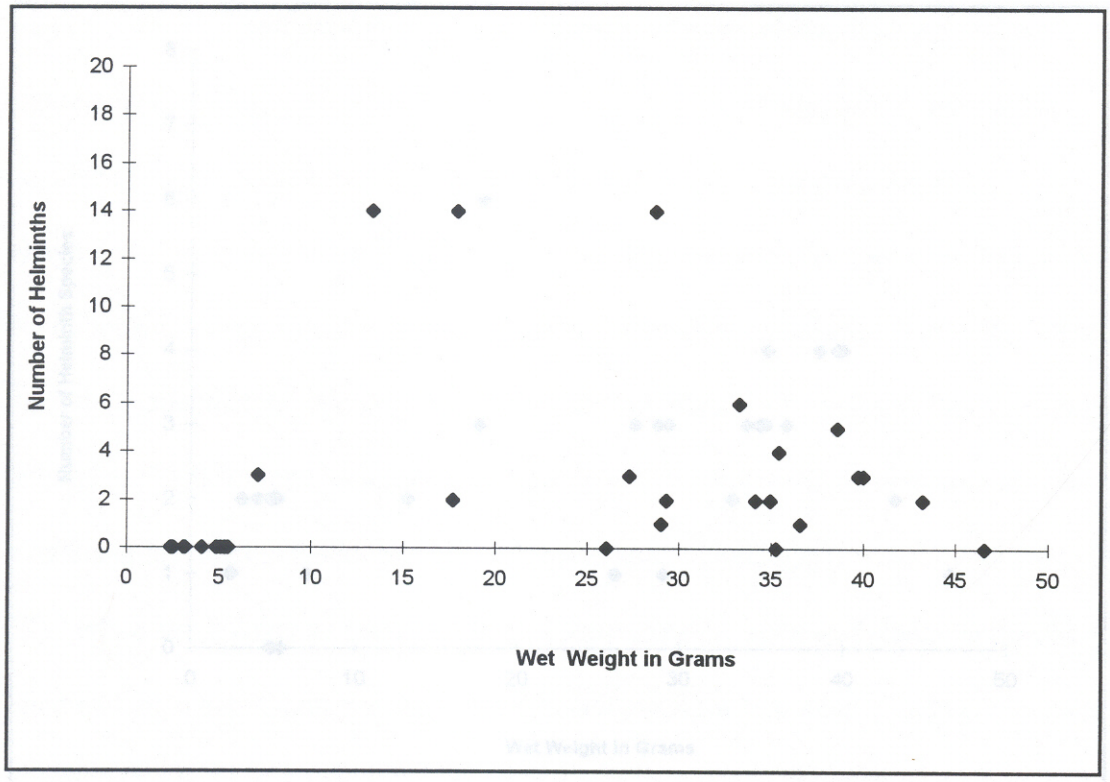

Figure 24. Wet weight versus total helminth abundance, excluding larval platyhelminthes, in northern leopard frogs, *Rana pipiens,* $r = 0.20$, $P > 0.05$.

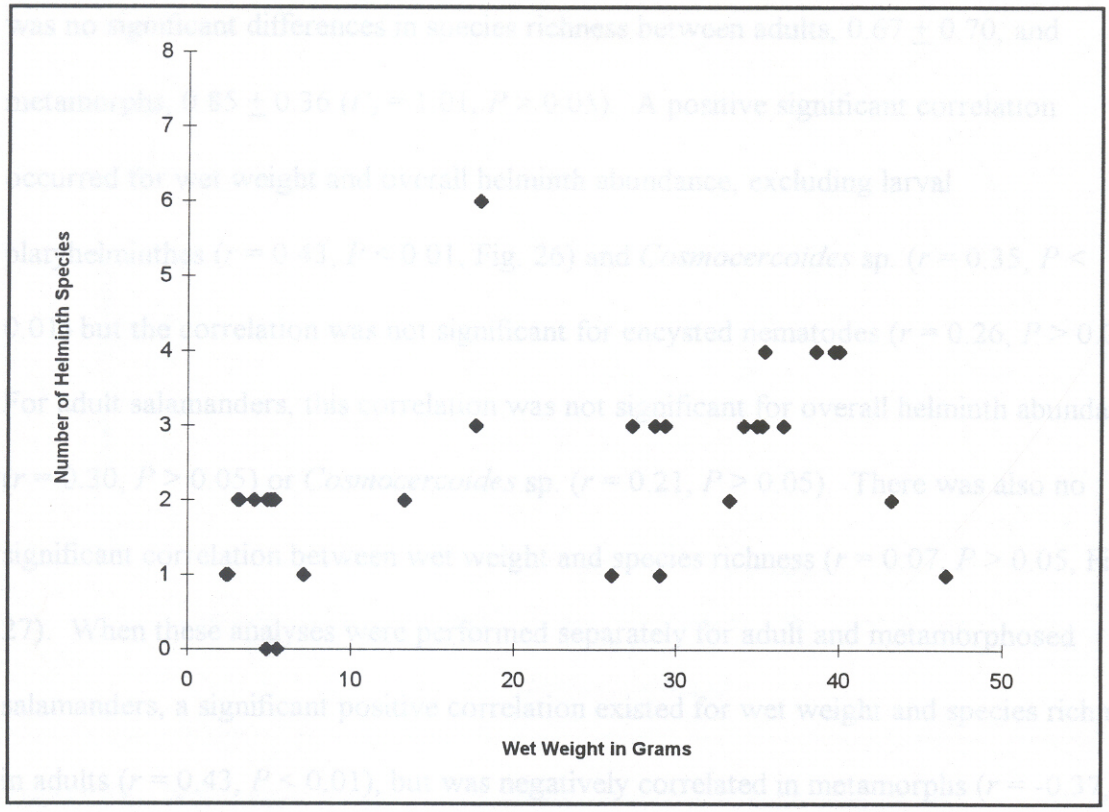

Figure 25. Wet weight versus number of helminth species per northern leopard frogs, *Rana pipiens*, $r = 0.48$, $P < 0.01$.

was no significant differences in species richness between adults, 0.67 ± 0.70, and metamorphs, 0.85 ± 0.36 (t'_s = 1.01, $P > 0.05$). A positive significant correlation occurred for wet weight and overall helminth abundance, excluding larval platyhelminthes ($r = 0.43$, $P < 0.01$, Fig. 26) and *Cosmocercoides* sp. ($r = 0.35$, $P < 0.01$) but the correlation was not significant for encysted nematodes ($r = 0.26$, $P > 0.05$). For adult salamanders, this correlation was not significant for overall helminth abundance ($r = 0.30$, $P > 0.05$) or *Cosmocercoides* sp. ($r = 0.21$, $P > 0.05$). There was also no significant correlation between wet weight and species richness ($r = 0.07$, $P > 0.05$, Fig. 27). When these analyses were performed separately for adult and metamorphosed salamanders, a significant positive correlation existed for wet weight and species richness in adults ($r = 0.43$, $P < 0.01$), but was negatively correlated in metamorphs ($r = -0.37$, $P > 0.05$). Most salamanders were collected during the breeding season or as they metamorphosed, therefore only two contained slugs in their stomach contents.

Comparisons Among Hosts: Brookfield, Waukesha Co.

The Kruskal-Wallis one-way analysis of variance revealed significant differences in wet weight, host age and species (H = 99.22, $P < 0.001$). The Kolmogorov-Smirnov two-sample tests showed that all possible host species pairs differed significantly ($P < 0.05$) in wet weight except for the following combinations: adult *B. a. americanus* and adult *R. pipiens*, adult *A. laterale* and metamorphosed *R. pipiens* and metamorphosed *A. laterale* and metamorphosed *B. a. americanus*. Adult *B. americanus* had the greatest weight (40.93 g ± 13.00) followed by adult *R. pipiens* (30.72 g ± 10.22), metamorphosed *R. pipiens* (3.95 g ± 1.24), adult *A. laterale* (3.15 g ± 1.03) ,

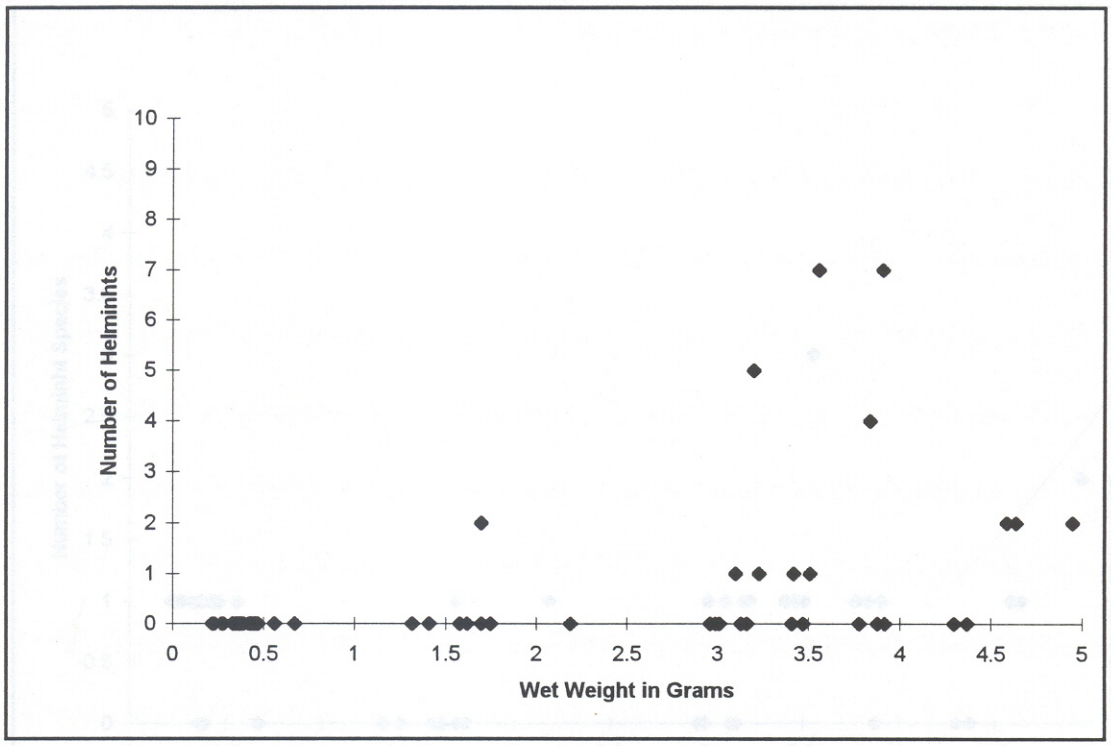

Figure 26. Wet weight versus total helminth abundance, excluding larval platyhelminthes, in blue-spotted salamander, *Ambystoma laterale*, $r = 0.43$, $P < 0.01$.

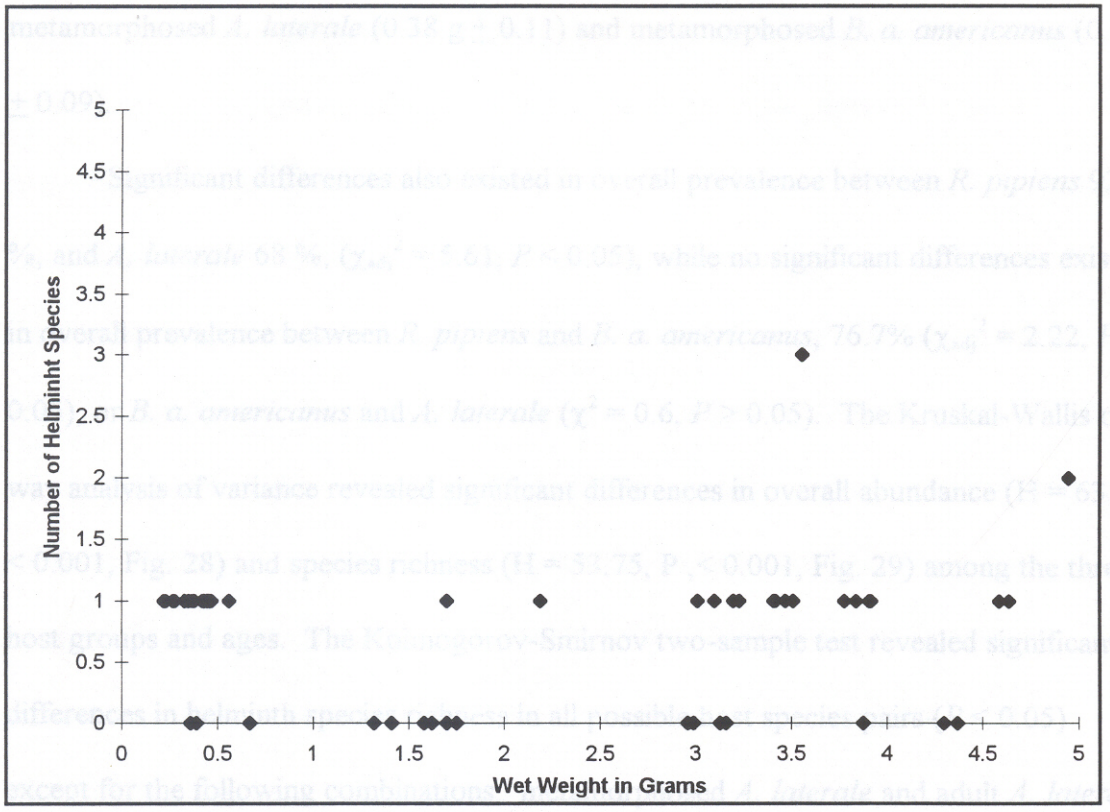

Figure 27. Wet weight versus number of helminth species per blue-spotted salamander, *Ambystoma laterale*, $r = 0.07$, $P > 0.05$.

metamorphosed *A. laterale* (0.38 g $\pm$ 0.11) and metamorphosed *B. a. americanus* (0.35 $\pm$ 0.09).

Significant differences also existed in overall prevalence between *R. pipiens* 93.5 %, and *A. laterale* 68 %, ($\chi_{adj}^2 = 5.61$, $P < 0.05$), while no significant differences existed in overall prevalence between *R. pipiens* and *B. a. americanus*, 76.7% ($\chi_{adj}^2 = 2.22$, $P > 0.05$), or *B. a. americanus* and *A. laterale* ($\chi^2 = 0.6$, $P > 0.05$). The Kruskal-Wallis one-way analysis of variance revealed significant differences in overall abundance (H = 63.48 < 0.001, Fig. 28) and species richness (H = 53.75, P ,< 0.001, Fig. 29) among the three host groups and ages. The Kolmogorov-Smirnov two-sample test revealed significant differences in helminth species richness in all possible host species pairs ($P < 0.05$) except for the following combinations: metamorphosed *A. laterale* and adult *A. laterale*, metamorphosed *B. a. americanus* and metamorphosed *A. laterale*, adult *B. a. americanus* and adult *R. pipiens*, metamorphosed *B. a. americanus* and adult *A. laterale* and metamorphosed *B. a. americanus* and metamorphosed *R. pipiens*. Kolmogorov-Smirnov two-sample test also revealed significant differences in overall helminth abundance excluding larval platyhelminthes for all possible host species pairs ($P < 0.05$) except for the following combinations: metamorphosed *R. pipiens* and metamorphosed *A. laterale*, metamorphosed *R. pipiens* and adult *A. laterale*, metamorphosed *B. a. americanus* and metamorphosed *R. pipiens*, metamorphosed *B. a. americanus* and adult *A. laterale* and metamorphosed *B. a. americanus* and metamorphosed *A. laterale*. When all hosts were combined, significant positive correlations existed for wet weight and abundance ($r = 0.31$, $P < 0.01$, Fig. 30) and for species richness ($r = 0.61$, $P < 0.01$).

Statistically significant differences were found when comparisons were made among host species and percent of indirect or direct life cycle parasites of adult amphibian individual component communities ($\chi^2 = 1015$, $P < 0.001$, Fig. 31). Toads had a higher relative abundance of direct life cycle nematodes (61 %) with only 37% of the component community being indirect life cycle parasites and one percent being unknown nematodes. Northern leopard frogs had a higher relative abundance of indirect life cycle parasites (96 %) only three percent direct life cycle parasites and one percent unknown nematodes, while blue-spotted salamanders had more indirect life cycle parasites 71.5 % than unknown nematodes 28.5 %. All infected metamorphosed salamanders, toads, and leopard frogs were infected by indirect life cycle larval trematodes.

Location - Bayfield Co.

A total of 20 red-backed salamanders, 15 males and five females, were collected by overturning rocks and logs during the day and 20 spotted salamanders, 15 males and five females, were collected by dip-net at an ephemeral pond in Bayfield County, Wisconsin in May 1996. The compound community consisted of at least three species of helminths, one indirect life cycle trematode and two direct life cycle nematodes.

Ambystoma maculatum

The mean snout-vent length and wet weight of *A. maculatum* was 76.15 $\pm$ 8.8 mm (range = 62.65-92.75) and 12.29 $\pm$ 4.46 g (range = 6.05-23.95). Eleven of 20 (55%) *A. maculatum* were infected with helminths. Nine (45%) were infected with the nematode *Batracholandros magnavulvaris* Rankin 1937 while three (15%) were infected

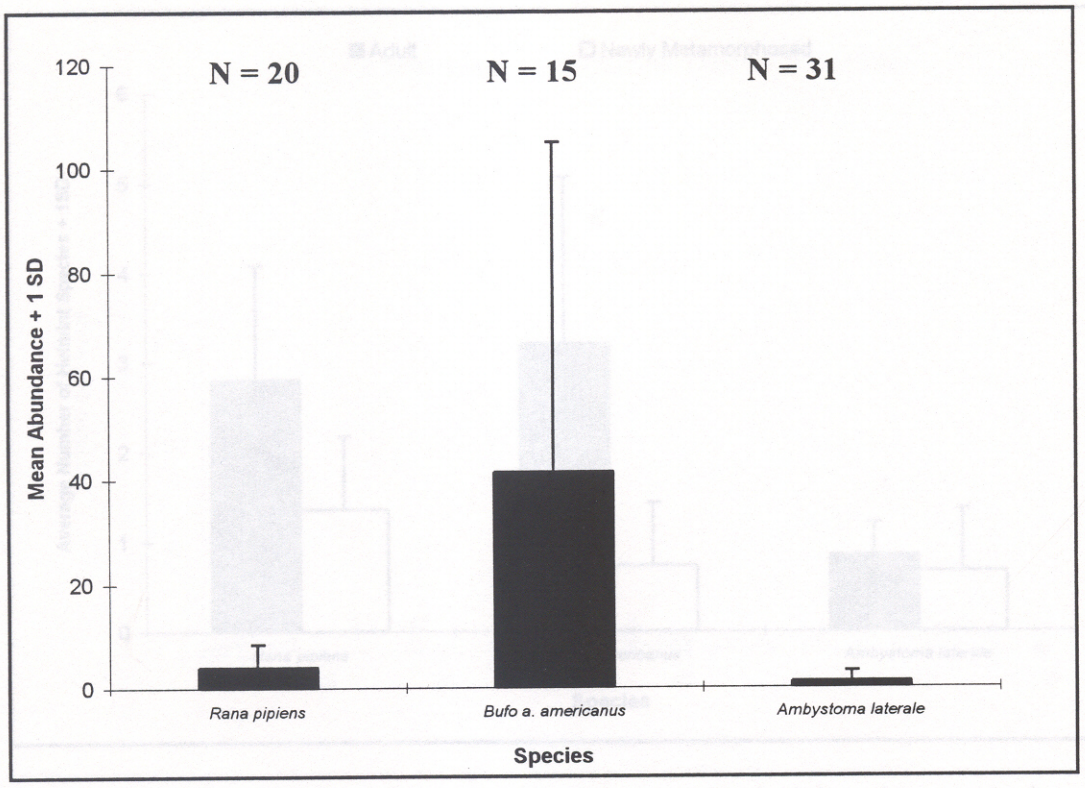

Figure 28. Mean helminth abundance among three species of adult amphibians. N equals number of amphibians examined.

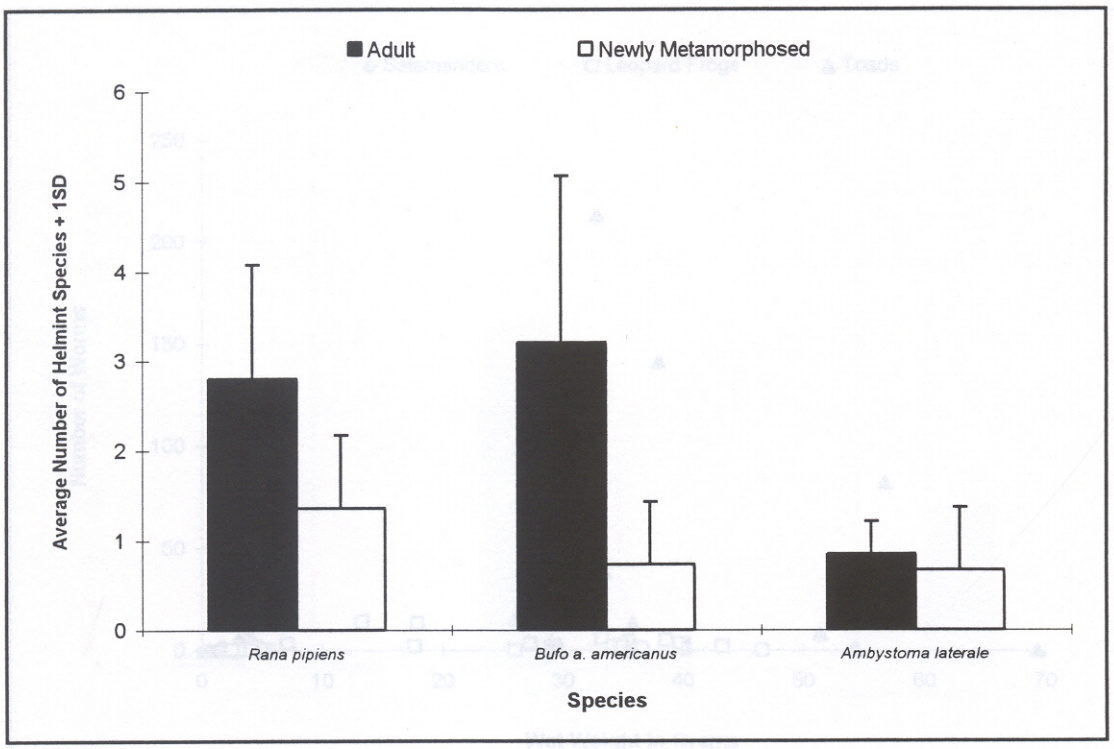

Figure 29. Mean speices richness among three species of adult and metamorphosed amphibians.

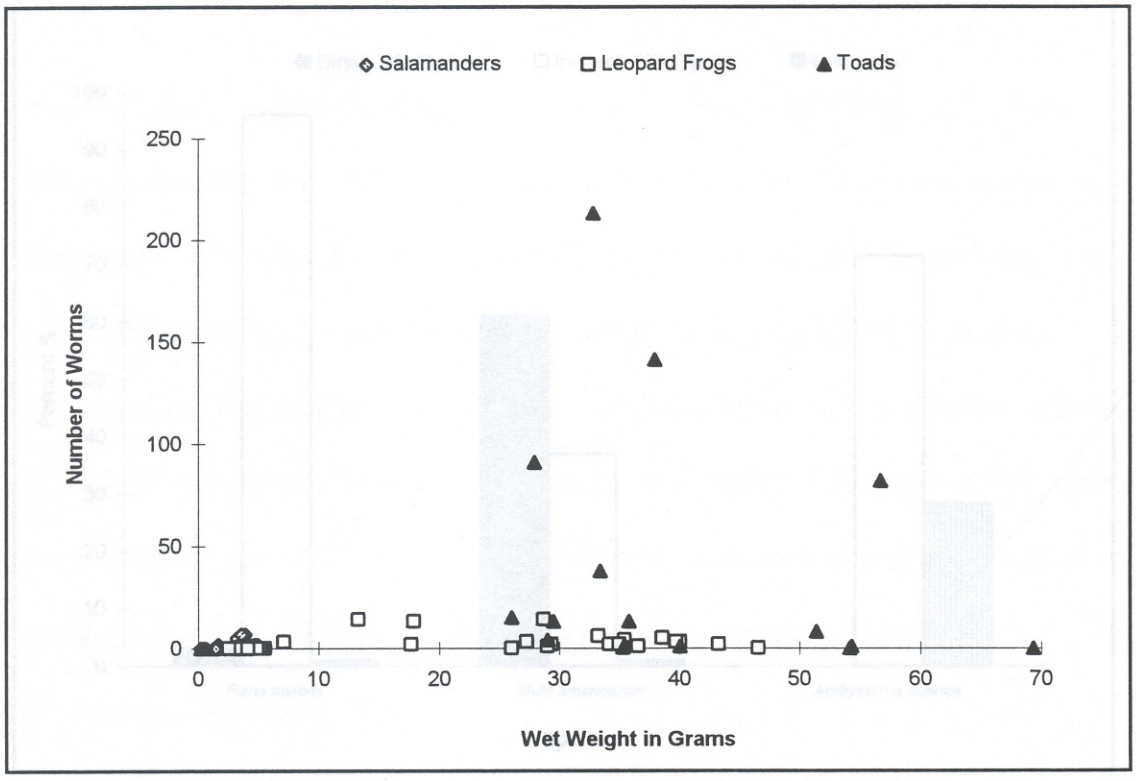

Figure 30. Wet weight versus total helminth abundance, excluding larval platyhelminthes for three species of amphibians, $r = 0.31$, $P < 0.01$.

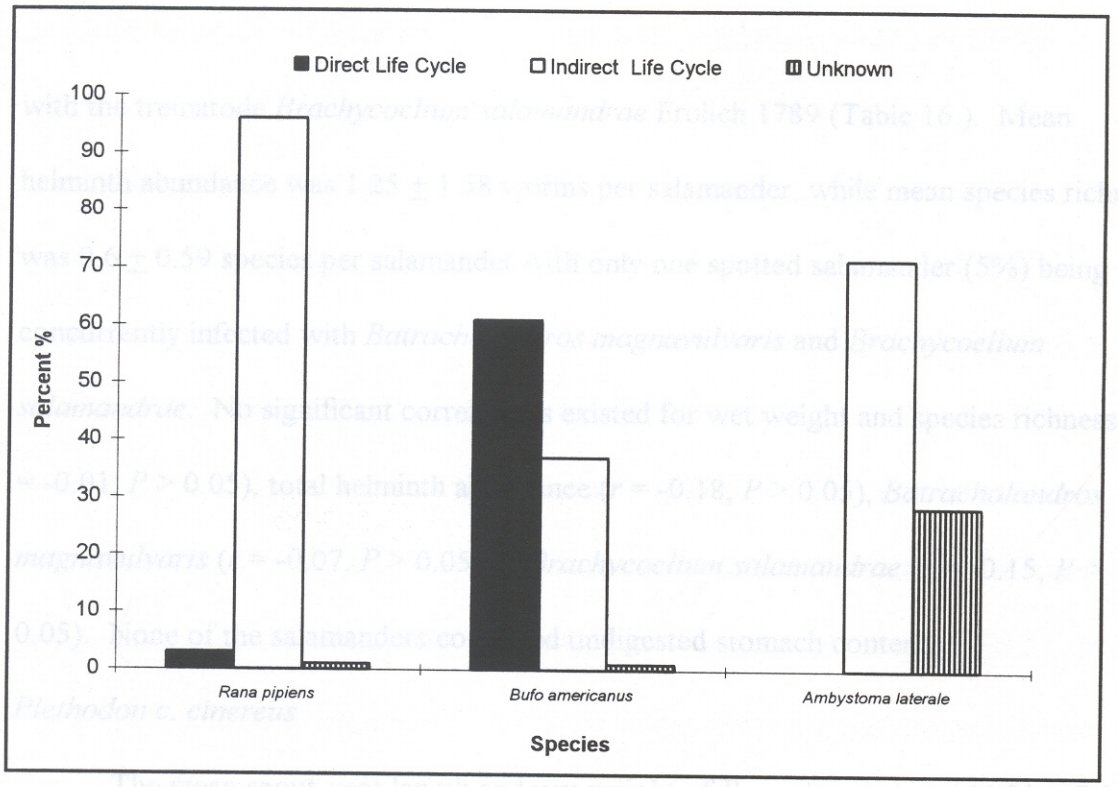

Figure 31. Percent of helminths with direct, indirect and unknown life cycles in individual component communities of three species of adult amphibians.

with the trematode *Brachycoelium salamandrae* Frolich 1789 (Table 16.). Mean helminth abundance was 1.25 ± 1.58 worms per salamander, while mean species richness was 0.6 ± 0.59 species per salamander with only one spotted salamander (5%) being concurrently infected with *Batracholandros magnavulvaris* and *Brachycoelium salamandrae*. No significant correlations existed for wet weight and species richness (r = -0.01, $P > 0.05$), total helminth abundance (r = -0.18, $P > 0.05$), *Batracholandros magnavulvaris* (r = -0.07, $P > 0.05$) or *Brachycoelium salamandrae* (r = -0.15, $P > 0.05$). None of the salamanders contained undigested stomach content.

Plethodon c. cinereus

The mean snout-vent length and wet weight of *P. c. cinereus* was 44.01 ± 7.01 mm (range = 29.55-51.75) and 1.02 ± 0.41 g (range = 0.41-1.68), respectively. Eight of 20 (40%) *P. c. cinereus* were infected with one or more *Batracholandros magnavulvaris, Brachycoelium salamandrae,* and *Rhabdias* sp.. The nematode *Rhabdias* sp. had the highest prevalence in *P. c. cinereus*, while *Brachycoelium salamandrae* had the highest mean intensity (Table 17). Mean helminth abundance was 0.65 ± 0.98 worms per salamander and mean species richness was 0.45 ± 0.6 species per salamander with only one red-backed salamander (5%) being concurrently infected with *Batracholandros magnavulvaris* and *Brachycoelium salamandrae*. As with spotted salamanders, no significant correlations existed for wet weight and species richness (r = -0.05, $P > 0.05$), total helminth abundance (r = -0.01, P > 0.05), *Batracholandros magnavulvaris* (r = 0.01, $P > 0.05$), *Brachycoelium salamandrae* (r =

-0.23, $P > 0.05$) or *Rhabdias* sp. ($r = 0.06$, $P > 0.05$). Seven of the salamanders contained mites in their stomachs.

Comparisons Among Hosts Bayfield Co.

A significant difference existed in wet weight between the two salamander species, with spotted salamanders being significantly heavier (12.29 $\pm$ 4.45g) than red-backed salamanders (1.02 $\pm$ 0.41) ($t'_s = 11.25$, $P < 0.001$). No significant differences occurred in overall prevalence of helminths ($\chi^2 = 0.88$, $P > 0.05$) or *Brachycoelium salamandrae* ($\chi_{adj}^2 = 0.00$, $P > 0.05$) between the two salamander species. A significant difference existed in prevalence of *Batracholandros magnavulvaris* ($\chi_{adj}^2 = 6.53.$, $P < 0.05$) and *Rhabdias* sp. ($\chi_{adj}^2 = 4.9$, $P < 0.05$) among the two hosts. No significant differences existed in total helminth abundance ($t = 1.43$, $P > 0.05$) or mean species richness ($t = 0.78$, $P > 0.05$) between the two host species. When comparisons were made among the two host species and percent of indirect or direct life cycle parasites in individual host component communities, no significant differences occurred ($\chi^2 = 0.04$, $P > 0.05$).

The four specimens of *Brachycoelium salamandrae* from *P. c. cinereus* and ten specimens from *A. maculatum* exhibited morphological variation. A statistically significant difference existed between the mean length, in mm $\pm$ 1SD (range), of trematodes recovered from *A. maculatum,* 2.82mm $\pm$ 0.30 (2.28-3.30), and *P. c. cinereus,* 1.26mm $\pm$ 0.47 (0.85-1.93) [one tailed $t = 2.8$, $P < 0.05$], while no such difference existed in mean width of trematodes from *A. maculatum,* 0.40mm $\pm$ 0.07 (0.28-0.50) and *P. c. cinereus* 0.34mm $\pm$ 0.12 (0.25-0.52) [one tailed $t = 0.74$, $P > 0.05$].

Other apparent differences existed in body shape, position of testes and distribution of vitellaria.

Discussion

Natural History and Ecology of *Rana clamitans*

Green frogs, *R. clamitans*, are large, semi-aquatic frogs inhabiting ponds, lakes, swamps and slow moving streams. They spend most of their time around the periphery of the water's edge. They occur from Newfoundland to western Ontario, south to eastern Oklahoma, southern Illinois, northern Georgia, and eastern North Carolina (Vogt, 1981). In Wisconsin, these frogs overwinter in freshwater, buried in the mud, and are active from early April through October (Vogt, 1981). They are largely "sit-and-wait" predators, feeding on any accessible prey of appropriate size including aerial, aquatic and terrestrial invertebrates. Larger individuals may even consume smaller frogs, turtles and snakes (Harding, 1997). Green frogs breed throughout the summer; males begin calling during early May and continue until August (Harding, 1997). Martof (1953) and Oldham (1967) have demonstrated that adult male green frogs establish a territory and may inhabit this area throughout the summer. They defend the breeding site from competitors and usually lay on the surface of the water calling. Female frogs only spend a brief time at the breeding site and typically are found on land and at the water's edge. After deposition, eggs float at the surface of the water where they hatch and develop. Tadpoles require 70 to 85 days for transformation and may overwinter before metamorphosing the next spring. Maximum size of frogs is reached in four to five years, and individuals in zoos have been known to live up to ten years (Harding, 1997).

Ecological Factors and the Parasite Fauna of *Rana clamitans*

Green frogs were one of the largest amphibians sampled in this study, having the greatest snout-vent length and second greatest wet weight of all anurans collected. The helminth parasites of Wisconsin green frogs were similar to other published reports (Campbell, 1967; Brooks, 1976; Williams and Taft, 1980; Muzzall, 1991; McAlpine, 1997). Green frogs had high overall helminth prevalence, with parasite infracommunities being dominated by indirect life cycle helminths, mostly larval and adult trematodes and cestodes. Of identified parasites, only one direct life cycle nematode *O. pipiens* was present. Most helminth species displayed a prevalence below 50% and/or low mean intensities below 30.

In the present study, most green frogs contained identifiable stomach contents containing mostly beetles, gastropods and diplopods. In total, 16 different groups of terrestrial, aerial and aquatic invertebrates comprised their stomach contents. These results appear similar to other investigators (Hamilton, 1948; Stewart and Sandison, 1972). Hamilton (1948) found that the principle food of green frogs collected form New York consisted of beetles, flies and grasshoppers with a total of 15 different items recovered from adult frogs and 20 different prey items recovered from various sized individuals.

The most common helminth recovered was an echinostome metacercaria. This larval trematode had a high overall prevalence (80%) and mean intensity of over 30 worms per frog. Digenetic trematodes in the family Echinostomatidae are widespread parasites of avian and mammalian definitive hosts and use amphibians and molluscs as second intermediate hosts. The metacercaria are commonly found in kidneys of

tadpoles and adult frogs (Martin and Conn, 1990). Amphibians acquire infections in water, where cercaria escape from the molluscan host and actively seek and penetrate into the kidneys of amphibians. Echinostome metacercaria have been reported previously in green frogs from New York by Martin and Conn (1990) and McAlpine (1997) from Canada, but this is the first report from Wisconsin green frogs.

Four other digeneans were recovered from green frogs: one larval stage, a diplostomid metacercaria encysted in the muscles, and three adult trematodes, *Haematoloechus varioplexus*, *Halipegus occidualis* and *G. quieta*. Frogs become infected with *Haematoloechus varioplexus*, a lung trematode, and *Halipegus occidualis*, an Eustachian tube trematode, by eating infected odonates (Krull, 1931; Macy et al., 1960; Dronen, 1975, 1978). *Glypthelmins quieta*, a trematode of the small intestine, was acquired when frogs ingested prey such as tadpoles, frogs and/or shed skin infested with metacercaria (Prudhoe and Bray, 1982). Therefore, diet was important in the transmission dynamics of these three helminth species in this study.

Haematoloechus varioplexus and *Halipegus occidualis* were recovered from frogs throughout the year, increasing in both prevalence and mean intensity during the fall collection, although not significantly. Both of these trematodes apparently overwintered in frogs, since adult flukes were recovered in April before frogs began feeding on odonates, and both persisted until October. Recently Wetzel and Esch (1997) have shown that the life span of *Halipegus eccentricus* (=*H. occidualis*) may be variable, with trematodes capable of maturing in as little as one week and being lost the following week. Due to the small number of these flukes being recovered in my study,

little can be said about their recruitment throughout the year. Krull (1931) estimated that the life span of *Haematoloechus medioplexus* averaged one year, while studies by Kennedy (1980) on *Haematoloechus* species have shown that trematodes can reach full length in only 60 days. The size differences observed for *Haematoloechus varioplexus* during the year may be significant in understanding recruitment of this species. The seasonal variation in length of *Haematoloechus varioplexus* suggests that adult worms are lost during early spring and that new infections begin during mid spring and continue throughout the year. These results are similar to Ward (1909) who observed lung flukes of *Rana pipiens* being lost during breeding and recruitment occurring throughout the year.

Glypthelmins quieta appeared in mid spring, increased during the summer and decreased during fall. Although no snails were examined for shedding cercaria during this study, the appearance and increased prevalence and mean intensity of *G. quieta* during the summer collection is likely a consequence of increased cercarial production by infected snails. The study of Snyder and Esch (1993) on trematode community structure in the pulmonate snail *Physa gyrina* agrees with this hypothesis. These investigators observed the highest prevalence of *G. quieta* cercaria shed during the June and October and lowest during April in North Carolina.

Two cestode species were recovered from green frogs during this study. The larval tetrathyridium of *Mesocestoides* sp. and one adult cestode which could not be identified because the scolex was lost from the one specimen recovered. The complete life cycles of *Mesocestoides* spp. are currently unknown; but a number of mammals,

amphibians and reptiles are known to serve as second intermediate hosts, while carnivorous mammals serve as definitive hosts. The tetrathyridian stage has been reported from a variety of mammals and reptiles but is rare in amphibians (McAllister and Conn, 1990). The first intermediate host for most species is unknown. Soldatova (1944) reported that the early larvae of *Mesocestoides lineatus* Goeze 1782, occurs naturally and experimentally in various species of orbatid mites; the first intermediate host is believed to be an arthropod. Although the life cycle of these two species of cestode are not known, diet of frogs is also believed to be important in their transmission dynamics.

Of the five species of nematodes recovered, only three were successfully identified. An unidentified encysted nematode and the filarial nematode *Foleyella* sp. occurred as single infections. The three most common species recovered were *Cosmocercoides* sp., *O. pipiens* and an unidentified larval nematode.

Nematodes in the genus *Foleyella* typically are found in the body cavity of *Rana* species. Adult worms release microfilaria into the bloodstream, mosquitoes serve as vectors, infecting frogs while feeding (Witenberg and Gerichter, 1944). *Rana clamitans* is apparently a new host record for *Foleyella* sp. (Esslinger, 1986; Baker, 1987). *Foleyella americana* Walton 1929, has been previously reported in Wisconsin leopard frogs by Walton (1929).

Oswaldocruzia pipiens is a common intestinal parasite of amphibians and reptiles (Baker, 1987). Adult worms live in the small intestine of the host. Infective J_3 larvae penetrate the host, enter the stomach within one to three days, and mature in the

small intestine (Baker, 1978b). This species has been reported in Wisconsin green frogs by Williams and Taft (1980) and from Canada by McAlpine (1997). Muzzall (1991b) did not find this nematode in a population of green frogs sampled from Michigan during July and August. Seasonal variation existed in prevalence and mean intensity of this species but was irregular. Nematodes appeared in mid spring and decreased in prevalence and mean intensity during the fall.

The nematode *Cosmocercoides* sp. was recovered from the large intestine of green frogs. Much confusion exists in the literature on the identification of *Cosmocercoides* species in amphibians and reptiles (Baker, 1987; Anderson, 1992). The nematode *C. dukae* has been reported from numerous gastropods, amphibians and reptiles in North America (Harwood, 1930; Ogren, 1953; Anderson, 1960; McGraw, 1968; Lewis, 1973; Baker, 1978a; Vanderburgh and Anderson, 1987a, b). Recently, it has been shown by Vanderburgh and Anderson (1987b) that *C. dukae* is a parasite of terrestrial molluscs with inadvertent occurrence in animals feeding upon terrestrial molluscs. *Cosmocercoides variabilis* is considered to be a parasite of amphibians. This nematode has a direct life cycle. It enters the host through skin penetration, molts in the lungs or body cavity, and matures in the intestine (Baker, 1978a; Vanderburgh and Anderson, 1987b). It has been suggested that this parasite may be restricted to certain amphibian groups, such as representatives of the Hylidae, Microhylidae and Bufonidae (Vanderburgh and Anderson, 1987a). The major difference in the two species is the number of rosette papillae per subventral row in males, with male *C. dukae* having 9-21 roset papillae, averaging 13-14, and *C. variabilis* 15-25, averaging 20 or 21. Because of

this overlap, and the presence of only five males out of 62 nematodes recovered, species identification was not possible. Interestingly, no worms were found in the lungs or body cavity of any green frogs, and *Cosmocercoides* sp. occurred in frogs in months when gastropods were commonly found in the stomach contents. I suspect that specimens of *Cosmocercoides* sp. recovered are *C. dukae*, although this can not be certain and therefore these specimens are referred to as *Cosmocercoides sp.*. This nematode showed similar increase in prevalence as *O. pipiens* appearing in mid spring and decreasing during the fall.

Differential infection among host sex and prevalence or mean intensity was observed for a number of helminth species. Male frogs had a significantly higher prevalence of echinostome metacercariae, and a significantly higher mean intensity of *Haematoloechus varioplexus* and *Halipegus occidualis*, than female frogs. Male green frogs are quite territorial during the breeding season, and defend their aquatic breeding site from potential competitors. Thus, unlike the females, they remain in the water for longer periods of time and may be exposed to echinostome cercaria for longer periods of time. Also, because males remain in a relatively small area of the pond during the breeding season, they may occur in a good microhabitat for becoming infected with digeneans. Recently, Wetzel and Esch (1997) in a seasonal study of *Halipegus occidualis* (= *H. projecta*, McAlpine and Burt, 1998) and *Halipegus eccentricus* (= *H. occidualis*, McAlpine and Burt, 1998) in green frogs suggested that certain areas of a pond may be hot spots for infection with digenetic trematodes. Therefore, male frogs in these “hot spots” may feed more often on emerging odonates containing up to 40

metacercaria of *Haematoloechus* and *Halipegus* species, explaining the higher mean intensities of these trematodes observed in male frogs (Wetzel and Esch, 1996).

Female frogs had significantly greater mean intensities of *Cosmocercoides* sp. and *Mesocestoides* sp. than males. Although *Cosmocercoides* sp. could not be identified to species in this host, both *C. variabilis* and *C. dukae* occur in a terrestrial habitat. Female frogs spend more time on the ground, and therefore have a higher probability of encountering these nematodes in a terrestrial habitat either by skin penetrating by *C. variabilis* or feeding on terrestrial molluscs which serve as hosts for *C. dukae*. Unfortunately, nothing can be stated about the transmission dynamics of *Mesocestoides* sp., and no conclusions can be drawn from this difference. The observed differences in host sex are due to ecological differences in their habitat preference throughout the year.

Significant positive relationships between wet weight and species richness were observed in green frogs. In this study, time of exposure was more important in developing richer helminth communities than was frog weight during the May-June, July-August and September-October collections. This is supported by significant differences in species richness over time and non-significant correlations between wet weight and species richness. These results differ from other studies. Observations on higher species richness with larger host size have been reported in green frogs and other *Rana* species by Muzzall (1991b) and McAlpine (1997). These investigators stated that older individuals may have a longer exposure time and possess more surface area for colonization by skin penetrating nematodes and digenean metacercariae. Also, bigger

frogs possess a greater gape and may feed on larger and wider number of intermediate hosts, then smaller individuals. These data support the island size hypothesis, which predicts larger hosts should support a higher species richness than smaller individuals (Holmes and Price, 1986). McAlpine (1997) also stated that aspects of host ecology, such as diet and habitat, and parasite transmission may confound any simple relationship between the diversity of helminth communities and size of hosts. Data from this study suggests that time of transmission may also have a similar confounding effect.

In general, the helminth fauna of green frogs was depauperate and dominated by indirect life cycle parasites. Host diet and their aquatic habitat was important in the transmission dynamics of these species. Host size, sex, and time of collection were also important factors in structuring helminth communities of green frogs and may mask any simple explanations.

Natural History and Ecology of *Bufo a. americanus*

Bufo a. americanus occurs from Labrador and Hudson Bay to eastern Manitoba, south to eastern Oklahoma and the coastal plains and is distributed throughout Wisconsin (Vogt, 1981). American toads are large, thick-bodied terrestrial anurans that are found around marshes, oak savannas, semi-open coniferous and deciduous forests and agricultural areas (Vogt, 1981). Female toads are significantly larger than males. This species has a short explosive breeding season during late April and early May which may last up to ten days. Most toads do not begin breeding until they are three or four years old (Dickerson, 1906). The eggs are laid in long strands of 4,000 to 20,000

eggs, hatch into tadpoles and develop quickly with new metamorphs emerging during July through late June (Vogt, 1981; Harding, 1997). After breeding, adults move out into open woodlands, prairies and agricultural areas, and are most active on humid or rainy nights (Vogt, 1981).

Toads are considered active foragers, differing from most anurans that are sit-and-wait predators (Seale, 1987). These amphibians have a number of adaptations well suited for a terrestrial life and their active forager feeding mode. Toads have a well developed seat patch in the pelvic region and sculptured skin surface channels for water absorption in terrestrial environments. Toads also posses well developed lungs for higher aerobic respiration and poison glands to deter predators. These adaptations allow them sustained movement and greater vagility in a terrestrial habitat unlike most *Hyla* and *Rana* species (Szarski, 1972; Vogt, 1981; Seale, 1987; Stebbins and Cohen, 1995). They feed on a wide range of invertebrates, their diet reflecting what is most abundant in the environment (Vogt, 1981; Harding, 1997). Although they are considered active foragers, individuals may sit and feed for an hour or more in one area (Dickerson, 1906). American toads can survive over 10 years in the wild and have been known to live up to 36 years in captivity although most breeding individuals range in age from two to five years (Dickerson, 1906; Acker, et al., 1986; Harding, 1997).

Ecological Factors and the Parasite Fauna of *Bufo a. americanus*

Toads were the second largest species and most terrestrial of the anurans collected. Helminth parasites of Wisconsin toads were similar to other published reports (Bouchard, 1951; Odlaug, 1954; Ulmer, 1970; Ulmer and James, 1976; Williams and Taft, 1980; Coggins and Sajdak, 1982; Joy and Bunten, 1997). The

component community of these amphibians consisted of six species: three direct life cycle nematodes and three indirect life cycle platyhelminthes. Toad infracommunities were dominated by three species of skin-penetrating nematodes with high overall prevalence of 87% to 91%, with few toads infected by indirect life cycle parasites such as *Mesocestoides* sp., *Gorgoderina* sp. and echinostome metacercariae.

Bladder flukes in the genus *Gorgoderina* are common parasites of amphibians, but few life cycle studies are known (Prudhoe and Bray, 1982). The life cycles of *Gorgoderina attenuata* and *G. vitrlliloga* have been determined. For these species, amphibians acquire infection by feeding on insect larvae or tadpoles, the worm excysts in the stomach and migrates to the kidneys and bladder (Smyth and Smyth, 1980). The low observed prevalence (2.1%) and intensity (6) of *Gorgoderina* sp. was not surprising; stomach content analysis of *B. a. americanus* revealed few types of arthropods. This is characteristic of actively foraging species, such as toads. Ants made up the largest portion of the diet (98 %), with beetles and other terrestrial arthropods making up only 2%. Few studies exist for stomach contents of American toads. Kirkland (1904) also found that ants and beetles made up the greatest portion of the diet of 149 toads from New England. These results are similar to other investigations on diet of *Bufo* species (Toft, 1981; Collins, 1993; Indraneild and Martin, 1998). Ants and beetles appear to be an important food item in the diet of toads. Toft (1981) reported that ants made up 64% to 91% of the arthropods consumed by three South American species, while Collins (1993) mentioned ants and beetles being important items in the diet of five species of *Bufo* from Kansas. Because most trematodes utilize aquatic or semi-aquatic arthropods as intermediate hosts, these

observations may indicate why toads usually have low species richness and prevalence of adult trematodes (Williams and Taft, 1980; Coggins and Sajdak, 1982; McAllister et al., 1989; Goldberg and Bursey, 1991a, b; Goldberg et al., 1995; Goldberg and Bursey, 1996; Bursey and Goldberg, 1998).

The most commonly occurring nematode in toads was *Cosmocercoides variabilis*, with a total of 1,392 worms recovered. Adult worms reside in the large intestine of their hosts. Eggs pass out with the feces and develop to J_3 infective larvae. This nematode has a direct life cycle; they enter the host through skin penetration, molt in the lungs or body cavity, and mature in the intestine (Baker, 1978a; Vanderburgh and Anderson, 1987a). Vanderburgh and Anderson (1987c) studied the seasonal transmission of this species in American toads from Ontario. They observed J_4 larvae in the lungs during the breeding season and adult worms in the rectum of toads throughout the year. They suggested that toads may acquire *C. variabilis* soon after emerging in the spring and that transmission may decline during summer and fall. However, they stated that this may have been an artifact of sampling because all toads collected after the breeding season were from another location and may have had a lower prevalence and mean intensities of this species. They also observed larvae in the lungs of five toads collected in October during the following year and concluded that transmission probably occurs throughout the year.

Data from the present study suggests that the breeding period may be important in transmission of this species in adult toads. During the present study, all toads were collected from the same general location and had a high prevalence and high mean intensities throughout the year. Although not significant, mean intensity increased after

the breeding season and decreased during the late summer-early fall collection. Ten percent of worms recovered during April were located in the body cavity, while 37% were located in the lungs with 28% and 24% being located in the small and large intestine. Subsequent sampling revealed that only one toad collected during June had six larvae in the lungs, with all other worms being recovered from the small and large intestine. Baker's (1978a) studies on the life cycle of *C. dukae* (= *C. variabilis*) in toads revealed that eight to 10 days are required for larvae to reach the lungs and longer than 30 days at 14-18°C to migrate to the rectum and develop to a gravid stage. My observations suggest that toads become infected during the breeding season and there appears to be a decline during summer and early fall. Unfortunately, no toads were collected during October; therefore it is not known if infection may occur during the fall (but see below). All adult female worms recovered were gravid throughout the year, indicating that eggs were being produced from April through September.

The second most frequently recovered nematode was *R. americanus*, primarily a parasite of toads (Baker, 1979a; Baker, 1987). *Rhabdias* species are protandrous hermaphrodites which reside in the lungs of their hosts. Here eggs are released and pass up the respiratory system, hatch in the rectum, and are released as J_1 larvae that undergo heterogonic development. Infective J_3 larvae escape from females and infect toads by skin penetration. Larvae migrate into the body cavity of the host where they develop to sub-adults and must invade the lungs to mature and produce eggs (Baker, 1979a).

There have been few studies on the seasonal occurrence of *Rhabdias* species in amphibians (Lees, 1962; Plasota, 1969; Baker, 1979b). Lees (1962) studied the seasonal occurrence of *Rhabdias bufonis* in its host *Rana temporaria* in England, and Baker (1979b) studied the seasonal occurrence of *Rhabdias ranae* in the wood frog, *Rana sylvatica,* in Canada. Prevalence and intensities in these species were lowest during summer and highest in spring and early fall. Plasota (1969) also observed a decrease in prevalence of adult *Rhabdias bufonis* in the lungs of *Rana terrestris* during the summer months in Poland. Baker (1979b) observed many subadult worms in the body cavity of wood frogs during late summer and early fall, with no worms being found in the body cavity during early spring and few in the fall. On the contrary, worms occurred in the lungs during early spring and fall, while few were found in the lungs during late summer and early fall. He concluded that transmission of this species occurs throughout the summer and early fall, with worms maturing in the lungs during the fall and overwintering in their hosts. My results are consistent with these earlier studies of seasonal distribution of *Rhabdias* species.

During this study, no significant differences in prevalence or mean intensity were observed throughout the collection period, although the number of worms and their location (lungs or body cavity) varied during the year. Most *R. americanus* recovered in April occurred in the lungs, with numbers of worms in the lungs decreasing during early summer (June-July) and late summer-early fall (August-September). In contrast, the number of worms in the body cavity increased during the June-July and August-September collections. Transmission of this species occurs during the summer and early fall, with worms overwintering in their host.

Oswaldocruzia pipiens also had high prevalence but lower mean intensities than the other two common nematodes recovered in toads. Because of its fast migration, reaching the stomach and small intestine within one to three days of infection, differences in location of these worms within the host were not observed. Prevalence and mean intensity were variable, but not significant over the course of this study. Baker (1978b), in a seasonal studied of *O. pipiens* in wood frogs, observed peak prevalence and intensity during spring (May-June) and early fall (September-October) in Ontario, Canada. He stated that worms overwintered in the host and that transmission occurred in early spring with an initial decline during early summer and continued during summer and early fall. A significant positive correlation existed for this species and *C. variabilis*. These data suggest that toads became infected with *O. pipiens* and *C. variabilis* during the same time and place. The spring breeding period may also be important in the recruitment of this species in toads, as it is in wood frogs. Because toads were not collected during October it is not known if recruitment occurs during this time. If infection occurs during the fall, as Baker's data for wood frogs imply, it may also occur for *C. variabilis.*

Significant, positive relationships between wet weight and species richness and abundance were also observed in toads. However, significant relationships between wet weight and abundance were only observed in female toads. Female toads were larger than males and may provide a greater surface area for colonization by skin penetrating nematodes. Similar observations were reported by McAlpine (1997) for female leopard frogs which were significantly bigger than males. Although species richness also

showed a significant positive relationship with wet weight once the single noninfected individual was removed, no significant relationships were observed. Therefore, no conclusions can be drawn from this relationship. Seasonal variance in species richness was not significant in this species, with toad infracommunities being dominated by three skin penetrating nematodes throughout the year.

Transmission of helminths with direct life cycles was more significant in toads than other anurans such as semi-aquatic *Rana* species. The toad's terrestrial habitat and diet of ants and beetles may be important in excluding transmission of adult and larval trematodes, with infracommunities being dominated by skin penetrating nematodes with direct life cycles.

Natural History and Ecology of *Hyla chrysoscelis*

Cope's gray treefrog, *Hyla chrysoscelis*, is a large treefrog occurring in prairie ponds, oak savannas, dry and dry-mesic northern hardwoods and lowland forests and has been distinguished from the eastern gray treefrog *H. versicolor* (Ralin, 1968). Due to the similarity between these two species, different ranges have not been determined and little information on the natural history of *H. chrysoscelis* is available (Ralin, 1968; Jaslow and Vogt, 1977). The composite range covers most of the eastern United States from Ontario and southern Maine to the Gulf of Mexico (Vogt, 1981). Breeding occurs at temporary ponds, swamps and floodings in late spring. Males of this species usually begin calling in early May and continue through late June, females deposit up to 2,000 eggs in small clusters of 10 to 40. Tadpoles metamorphose within six to eight weeks (Harding, 1997). Very few studies exist on the diet of these treefrogs. Ralin (1968)

reported the diet of *H. chrysoscelis* to consist entirely of insects, with coleopterans making up the bulk of the diet, with some lepidopterans, hymenopterans and formica also being consumed. The natural life span of this species is unknown but individuals have been known to survive over seven years in captivity (Collins, 1993).

Ecological Factors and the Parasite Fauna of *Hyla chrysoscelis*

Helminth parasites of Cope's gray treefrogs are poorly known. Brooks (1976) conducted a survey on the platyhelminthes of Nebraska amphibians and examined 57 Cope's gray treefrogs; other than his study, nothing is known about their helminth fauna. The component community of these treefrogs consisted of nine species: three direct life cycle parasites and six indirect life cycle parasites. Overall helminth prevalence was lower than in all other anurans examined.

Polystoma nearcticum Paul, 1938 infected the urinary bladder of *H. chrysoscelis*. This species has been reported previously from *H. versicolor* from Minnesota and from *H. cinerea* Schneider, 1799, the green treefrog, from Florida (Paul, 1938). Brooks (1976), in his survey of Nebraska amphibians, found no *H. chrysoscelis* infected with this monogenean. I report *H. chrysoscelis* as a new host record, and Wisconsin a new locality record, for *P. nearcticum*. Adults of this species are found in the urinary bladder of their host. Eggs are released in the urine of the frog during the breeding period of their hosts. Onchomiracidia (larval stage) hatch and attach to the gills of small tadpoles where they feed and develop to maturity. As tadpoles metamorphose worms migrate to the cloaca and enter the urinary bladder

where they develop to sexual maturity (Paul, 1938). An aquatic habitat is an essential requirement for the transmission of this direct life cycle species.

In this study, one Cope's gray treefrog was infected with *G. pennsylvaniensis*. *Glypthelmins pennsylvaniensis* is a common parasite of the small intestine of *Pseudacris* species. Frogs become infected by cercaria during the tadpole stage. During metamorphosis, frogs shed and consume their skins, becoming infected (Cheng, 1961). This trematode has been reported from Wisconsin spring peepers, *P. c. crucifer*, by Coggins and Sajdak (1982) and Yoder and Coggins (1996), but is the first report from Cope's gray treefrogs.

Three Cope's gray treefrogs were infected with an unidentified immature digenean located in the small intestine. These trematodes were small, showed some development of testes and ovary but I could not distinguish vitellaria, genital pore or uterus. Treefrogs were also infected at low prevalence with an unidentified metacercaria located in the leg muscles and body cavity, which was presumably acquired during the tadpole stage or breeding period of the host. Other metacercariae have been previously reported at low prevalence from leg muscle and body cavity musculature in other *Hyla* adults (Ulmer, 1970).

Ten Cope's gray treefrogs were infected with numerous metacestodes. Eight of the 10 frogs were heavily infected with tetrathyridia of *Mesocestoides* sp.. These were encapsulated in the intestine, liver and musculature, while a few were also found free in the body cavity. Three of the frogs harbored heavy infections under the skin of the hind legs with as many as 300 metacestodes per leg. These frogs displayed small abrasions on the outer surface of the skin which appeared red or pink in color instead of the usual

cream white color. While a number of Bufonidae and Ranidae species have been reported to be infected with *Mesocestoides* sp. (see McAllister et al., 1989; McAllister and Conn, 1990; McAllister et al. 1995), only one hylid is known to be infected with this metacestode (McAllister, 1987). Therefore, Cope's gray treefrog is a new host record. Two other Cope's gray treefrogs were infected with other unidentified metacestodes. One frog harbored 14 plerocercoids, 13 in the body cavity and one in a lung. All possessed a tetracetabulate scolex with an apical organ. The other frog possessed seven cestode cysts on the outer mesentery of the stomach. These lacked an excretory antrum and did not appear to be *Mesocestoides* sp.. Plerocercoids and other cestode cysts have been previously reported from *H. versicolor* in Missouri and other Hylidae species from North Carolina (Brandt, 1936; Shannon, 1988). Frogs probably become infected with these species by feeding on intermediate hosts.

Two species of skin-penetrating nematodes, *Oswaldocruzia pipiens* and *Cosmocercoides variabilis*, infected Cope's gray treefrog. Four male and one gravid female *O. pipiens* were found within the small intestine of four Cope's gray treefrogs but probably do not represent patent infections in this host. Cope's gray treefrog is a new host record for *O. pipiens*.

A total of 46 *C. variablis* (16 males, 20 females and 10 J_4 larvae) were recovered from 14 Cope's gray treefrogs. All males in the present study possessed 17-23 rosette papillae, averaging 19.6. Measurements of gubernaculum length of males, esophagus length and bulb width for males and females fell in the range of *C. variabilis* as given by Vanderburgh and Anderson (1987a) for toads. J_4 larvae were also located in the lungs and small intestine and all adult females were gravid with developing

larvae in the eggs. Since the stomach contents of Cope's gray treefrog from this study and previous work (Ralin, 1968) consisted mostly of insects, molluscs probably play an insignificant role, if any, in their diet. Therefore, it is felt that the specimens collected in the present study are *C. variabilis* and not *C. dukae*. This is the first report of this nematode from Cope's gray treefrog.

There was no effect of host weight on helminth species richness or abundance in this anuran. Although female treefrogs were significantly larger and heavier, no differences were observed for host sex and parasite community structure. The breeding pond probably serves as the most important site of infection with helminths for treefrogs, while diet plays a lesser role. Brandt (1936) suggested that an arboreal habitat is less conducive to metazoan parasitism than are terrestrial or aquatic habitats. The arboreal nature of Cope's gray treefrogs probably has an effect on parasite colonization occurring during the tadpole stage or breeding period of these frogs. Of the nine parasites recovered, four have been reported to have their life cycles synchronized to the amphibians, tadpole stage and their metamorphosis and emergence from the pond (Brandt, 1936; Paul, 1938; Cheng, 1961; Sullivan and Byrd, 1970; Baker, 1978b). The three metacestode species recovered were probably acquired by frogs feeding on intermediate hosts. Stomach content analysis suggested that the diet of these frogs probably is less diverse than other Wisconsin anurans (Vogt, 1981). Accordingly, parasites dependent on intermediate hosts were found at a lower prevalence in these hosts than other Wisconsin frogs (Williams and Taft, 1980; Coggins and Sajdak, 1982; Yoder and Coggins, 1996).

Natural History and Ecology of *Pseudacris t. triseriata* and *Pseudacris c. crucifer*

The western chorus frog, *Pseudacris t. triseriata*, is a small terrestrial treefrog that ranges from the east end of Lake Ontario, west to central Minnesota and south through Kansas and Oklahoma, with disjunct populations in New Mexico and Arizona (Vogt, 1981). The northern spring peeper, *Pseudacris c. crucifer*, is also a small semi-arboreal treefrog, found from New Brunswick west to eastern Manitoba, south to eastern Texas and Georgia (Vogt, 1981). Both species are common throughout the state and are the first early spring breeding treefrogs. Eggs are attached to underwater vegetation, and tadpoles develop and transform into froglets within 45 to 90 days. After breeding, spring peepers disperse into woodlands and old fields, while chorus frogs remain around their breeding site throughout the year. Both species feed on the ground and other small vegetation on a wide range of small invertebrates including mites, beetles, ants, files and moths (Whitaker, 1971; Vogt, 1981). Both species reach sexual maturity within one year and probably do not live longer than three years (Harding, 1997).

Ecological Factors and the Parasite Fauna of *Pseudacris t. triseriata* and *Pseudacris c. crucifer*

The two *Pseudacris* species were the smallest anurans collected during this study. A number of reports on the helminth fauna exists for the spring pepper and western chorus frog (Rankin, 1945; Odlaug, 1954; Brooks, 1976; Ashton and Rabalais, 1978; Coggins and Sajdak, 1982; Muzzall and Peebles, 1991; Joy and Dowell, 1994, Joy et al., 1996; Yoder and Coggins, 1996). A total of four species of helminths were

recovered from these two treefrogs. Three species of helminths comprised the component community of western chorus frogs, and three species occurred in spring peepers. Two species recovered had indirect life cycles and two had direct life cycles, with the nematode *C. variabilis* and trematode *G. pennsylvaniensis* having the highest prevalence and mean intensity. Only one spring peeper was infected with one *O. pipiens* and one chorus frog with six unidentified metacercaria. Wisconsin is a new locality record for *C. variabilis* and *G. pennsylvaniensis* in Wisconsin chorus frogs. All parasites recovered actively search and penetrate into these frogs at some period in their life cycle and therefore diet is probably unimportant in recruitment of helminth species. Although sample sizes were small, no correlation existed between weight and species richness or abundance for these treefrogs.

Comparisons Among Host, Eagle, Waukesha Co.

When comparisons were made among American toads, Cope's gray treefrogs and *Pseudacris* species, significant differences were observed in overall prevalence, species richness and abundance at this location. Toads had a greater overall prevalence, mean abundance and species richness among the four hosts examined. American toads are very different in their basic biology from the other species examined at this location. Toads were significantly bigger, more terrestrial, more vagile, and have longer life span than treefrogs or *Pseudacris* species. Therefore, these differences in basic biology may make toads more susceptible to parasitism in terms of becoming infected by skin penetrating nematodes and tending to accumulate parasites over time. In fact when all

amphibian species were grouped, a positive significant correlation existed for wet weight and abundance and species richness, indicating that the size of the host species was important in parasite colonization. These data support the island size hypothesis which was reviewed by Holmes and Price (1986) for parasitic systems. These investigators stated that larger hosts should have richer communities and higher abundance of parasites than smaller individuals and is supported by data presented in this study.

Diet of host, although not as significant, was also important in structuring helminth communities at this location. American toads feed on terrestrial arthropods, while treefrogs feed on a number of terrestrial, aerial and arboreal invertebrates. These differences probably reflect habitat differences rather than actual food preference. Due to these differences in diet, toads contained fewer helminth species that utilize intermediate hosts, compared to treefrogs. As sit and wait predators treefrogs may have a wider exposure to prey that serve as intermediate hosts explaining the higher number of cestode and trematode species recovered from these hosts.

Natural History and Ecology of *Rana pipiens*

Leopard frogs are considered semi-terrestrial or semi-aquatic species and use shallow water habitats. Where suitable damp cover is available, they can be found long distances from water. These frogs occur in New England, New York, Pennsylvania, Ohio, Indiana, northern Kentucky and northern Illinois, and are found throughout all of Wisconsin (Vogt, 1981). They hibernate in permanent streams and lakes and migrate to woodland breeding ponds in early April. Leopard frogs display explosive breeding, and

may lay up to 6,500 eggs. New metamorphs emerge during late July and August (Harding, 1997). After the first week in June, adults usually disperse from water into meadows and fields (Vogt, 1981). Leopard frogs feed on a wide range of invertebrates, and like green frogs are considered to be sit-and-wait predators. Their diet consists of insects, spiders, slugs, earthworms and other invertebrates, and have been shown to have similar prey size as green frogs (McAlpine and Dilworth, 1989). Adults may occasionally feed on small frogs (Oldfield and Moriarty, 1994). Longevity is reported to be up to nine years, but most frogs in Wisconsin only live for two to three years (Leclair and Castanet, 1987; Harding, 1997).

Ecological Factors and the Parasite Fauna of *Rana pipiens*

A number of studies on helminth parasites of northern leopard frog exist (Hollis, 1972; Ashton and Rabalais 1978; Williams and Taft, 1980; Coggins and Sajdak, 1982; McAlpine, 1997). The component community of leopard frogs consisted of eight species, with one direct life cycle parasite, five indirect life cycle parasites and two unknown nematodes. Leopard frogs shared seven species with toads and two species with blue-spotted salamanders at this location.

In terms of abundance, the most commonly recovered helminth in this study was the larval tetrathyridium of *Mesocestoides* sp., and represents the highest prevalence ever reported for these parasites from amphibian hosts (McAllister and Conn, 1990). This species was only found in adult frogs, however. Because the life cycle is not known, nothing can be stated about the transmission dynamics of this helminth, although diet of frogs is probably important in its transmission.

The most common trematodes recovered were diplostomid metacercaria and mesocercaria. Numerous larval trematodes were found encysted in muscle tissue and free in the body cavity of adult and metamorphosed frogs. Adult parasites of this group occur in the enteric system of birds and mammals and use fish and amphibians as second intermediate hosts (Prudhoe and Bray, 1982). Actively seeking cercaria infect frogs and tadpoles by direct penetration in an aquatic habitat.

Three other digenetic trematodes infected these hosts. Larval echinostome metacercariae were found in the kidneys and body cavity, and adult *Haematoloechus varioplexus* and *Gorgoderina attenuata* were located in the lungs and bladder respectively. The life cycle of *G. attenuata* is known. Frogs become infected by feeding on tadpoles, smaller frogs or dragonflies containing metacercariae (Smyth and Smyth, 1980). *Haematoloechus varioplexus* is acquired by frogs feeding on odonate intermediate hosts (Dronen, 1975). Therefore, diet of hosts is important in the transmission dynamics of theses two species, with leopard frogs feeding on a wide range of aerial, terrestrial and semi-aquatic invertebrates.

Three species of nematodes were recovered from leopard frogs, but only one was successfully identified. *Oswaldocruzia pipiens*, a skin-penetrating nematode, occurred frequently in adult leopard frogs, but was absent from metamorphosed individuals. Because little time past since froglets metamorphosed and sampling, time of exposure may have been limited for *O. pipiens* infections. In a seasonal study of *O. pipiens* in wood frogs, Baker (1978b) stated that young of the year frogs acquire nematode infection during late summer as the ponds dry up and frogs emerge. Three

adult frogs were infected with encysted nematodes and one with an unidentified larval nematode.

Adult leopard frogs had higher helminth species richness and higher abundance than metamorphs. This resulted from older frogs having a larger gape size and feeding on a larger number of intermediate hosts. Similarly, adult frogs had a longer period for infection and more surface area for colonization by skin-penetrating nematodes.

Ecological Factors and the Parasite Fauna of *Bufo a. americanus*

The component community of toads from this location was similar to leopard frogs, consisting of nine species: three trematodes, one cestode and five nematodes. Three species had a direct life cycle while four were indirect life cycle parasites and two nematode species are unknown. Adult toad infracommunities from this location were also dominated by three species of skin penetrating nematodes while metamorphosed toadlets were infected by two species of metacercariae.

The most commonly occurring helminth in adult toads was the lung nematode *R. americanus*. These nematodes were recovered from the lungs and body cavity with most individuals found in the lungs. Also recovered from adult toads at high prevalence (> 50%) were *O. pipiens* and *C. variabilis*. Adult worms were recovered from the small and large intestines. *Cosmocercoides variabilis* larvae were also recovered from the lungs, suggesting that toads were becoming infected during the breeding season.

Three larval platyhelminthes were also recovered from toads at this location. Both echinostome and diplostomulum metacercariae were located in the muscle, body cavity and kidneys of metamorphs and adult toads, while the cestode *Mesocestoides* sp.

only occurred in adult toads. One adult *Haematoloechus varioplexus* was recovered from a single toad. This individual toad probably fed on an infected odonate to obtain the infection. This is the first report of *Haematoloechus varioplexus* and echinostome metacercaria in Wisconsin toads.

As in leopard frogs, adult toads had higher species richness and abundance than metamorphosed toadlets. Adult toads had a larger gape size and fed on larger prey while metamorphs fed on small prey such as mites. Gape size therefore prevented metamorphs from feeding on large odonates and other arthropods which served as intermediate host for digeneans and cestodes. Also, adult toads were larger, therefore possessed more surface area then metamorphs, had greater vagility and a longer time to become infected by skin penetrating nematodes.

Natural History and Ecology of *Ambystoma laterale*

Ambystoma laterale is a small semi-fossorial species, which is found throughout Wisconsin and inhabits both deciduous and coniferous forests, from moist bottomlands to dry uplands (Vogt, 1981). They occur from Nova Scotia west through New England, Quebec, northern New York, Ontario, Michigan and parts of Ohio and Indiana (Vogt, 1981). Blue-spotted salamanders are spring breeders that migrate to breeding ponds in late March or early April (Vogt, 1981; Bolek, 1998). Females lay approximately 225 eggs within two days of mating, attaching them individually to sticks, leaves, and other submerged objects (Vogt, 1981; Bolek, 1998). Metamorphs usually emerge during late July and early August and mature in about two years. After breeding, adults move

away from the ponds and may be found above ground throughout the year, under logs, rocks and other forest floor litter feeding on invertebrates.

Blue-spotted salamanders have a broad prey base and do not concentrate on any specific prey items. In a previous study on *A. laterale*, stomach content analysis revealed a large number of invertebrate species (Bolek, 1997a). Terrestrial isopods, gastropods and oligochaetes made up the largest portion of the diet, respectively, followed by insects, chilopods, diplopods, and other arthropods. These results are similar to other investigators (Minton, 1972; Gilhen, 1974). Gastropods appear to be an important food item in the diet of the blue-spotted salamander. Gilhen (1974) recorded slugs as the most common food item recovered from salamanders in Nova Scotia, while they were the second most common item found by Bolek (1997a). The potential life span is unknown, but adult individuals have been known to live for at least five years in captivity and probably have a relatively long life span as do other Ambystomids (Pfingsten and Downs, 1989; Petranka, 1998; personal observations).

Ecological Factors and the Parasite Fauna of *Ambystoma laterale*

The only published reports of helminths from the blue-spotted salamander *Ambystoma laterale* are those of Coggins and Sajdak (1982) who examined 26 specimens from Wisconsin, Muzzall and Schinderle (1992) on the helminths of *A. laterale* from southern Michigan and Bolek (1997a) who examined 83 salamanders from Wisconsin. Blue-spotted salamanders contained the fewest helminth species recovered from this location, with few individuals being concurrently infected with two or three species. These hosts shared two species of helminths with toads and leopard

frogs. The component community consisted of three species: one indirect life cycle parasite and two unknown.

Echinostome metacercaria had the highest prevalence and mean intensity in blue-spotted salamanders. Prevalence and mean intensities of this helminth were significantly lower in adult salamanders compared to metamorphosed individuals. This is not unexpected since the transmission of this species is in an aquatic habitat. Adult salamanders are fossorial and only spend a few weeks in the breeding ponds during the early spring, limiting their exposure to cercariae. To my knowledge this is the first record of echinostome metacercaria in Wisconsin blue-spotted salamanders. Muzzall and Schinderle (1992) reported echinostome metacercaria in blue-spotted salamanders from Michigan.

The nematode *Cosmocercoides* sp. had the highest prevalence in adult salamanders, with salamanders acquiring infection in a terrestrial habitat. Of the 25 nematodes recovered only nine were adults, three of which were males. Of the six adult females recovered none were gravid. Interestingly, one larvae nematode was located in the lungs of one salamander and may represent *C. variabilis*. Blue-spotted salamanders feed heavily on molluscs at this location (Bolek, 1997a) and the possibility exists that *C. dukae* may also be present. Due to the low number of male nematodes recovered and mollusks being a common food item in this host, species identification could not be determined. *Cosmocercoides dukae* has been previously reported in blue-spotted salamanders by Coggins and Sajdak (1982) and Bolek (1997a). The third species of helminth, an unidentified encysted nematode which infected blue-spotted salamanders,

was shared among the three hosts. Unfortunately, nothing can be said about transmission dynamics of this species.

Unlike toads and leopard frogs, metamorphosed and adult salamanders had similar species richness. This was due to metamorphs being heavily infected with echinostome metacercarial and adults harboring three species at low prevalence. Size of host and species richness and abundance was variable in salamanders with most relationships being positive but not significant. This was not unexpected due to the low number of species and abundance of parasites infecting these salamanders.

Comparisons Among Hosts: Brookfield, Waukesha Co.

In comparing overall prevalence, helminth abundance, and species richness among adult and newly metamorphosed toads, leopard frogs and salamanders significant differences were observed. Adult blue-spotted salamanders had significantly lower prevalence, mean abundance, and species richness then the adult anurans. This species differs in its biology from toads and leopard frogs. *Ambystoma laterale* is much smaller, more fossorial, has a small gape size and lowest vagility. The low species richness observed corresponds to small gape size and low vagility in this host. Salamanders have less surface area for skin-penetrating nematodes, and do not feed on aerial and semi-aquatic insects which serve as hosts for digenetic trematodes. Anurans exhibit greater vagility in that they can travel greater distances between aquatic and terrestrial environments and feed on a wider range of prey contributing to a richer helminth fauna.

The two adult anuran species showed no differences in overall prevalence and mean species richness, while adult toads had significantly higher helminth mean abundance. Leopard frogs from this location were dominated by indirect life cycle parasites, while toad infracommunities contained substantially more nematodes. *Rana pipiens*, a semi-aquatic sit-and-wait predator, feeds on a number of aquatic and semi-aquatic insects that serve as hosts for digeneans such as *Haematoloechus varioplexus* and *Gorgoderina attenuata*. Occurring in an aquatic habitat for a substantial part of the year may also have exposed these frogs to larval digenians. In contrast, toads only enter the water for a short period to breed, feeding on terrestrial arthropods during the year. They therefore are less likely to be exposed to parasites with aquatic life stages such as cercaria or metacercaria in intermediate hosts. The toad's feeding strategy of sitting in one area for up to an hour gorging itself may expose it to skin penetrating nematodes. Size appeared to be important in terms of species richness with both adult leopard frogs and toads not differing in wet weight or species richness.

The differences in abundance observed in toads and leopard frogs may be due to the presence of *R. americanus*, the most abundant nematode recovered, which was restricted to toads. Because larval platyhelminthes could not be counted accurately, they were excluded from abundance analysis and may have shown similar relationships in leopard frogs as nematodes did in toads.

Natural History and Ecology of *Ambystoma maculatum*

The spotted salamander, *Ambystoma maculatum*, is a large, robust fossorial species of mole salamander, that inhabits mesic forests throughout northern Wisconsin (Vogt, 1981). They are found from south-central Ontario to Nova Scotia, south to

Georgia and eastern Texas (Vogt, 1981). Spotted salamanders breed during April to mid May, in vernal or permanent woodland ponds. Eggs are deposited as large globular masses or several small clusters on sticks and other submerged vegetation. These salamanders need at least 60 days to metamorphose and may overwinter in ponds, metamorphosing in late fall or the following spring. Adults of this species are rarely seen after the spring breeding season, spending much time in burrows underground. They feed on worms snails, slugs, insects and other invertebrates (Harding, 1997). These salamanders have a relatively long life span and may live for over 20 years (Pfingsten and Downs, 1989).

Natural History and Ecology of *Plethodon c. cinereus*

The red-backed salamander, *Plethodon c. cinereus,* is one of the smallest woodland species of lungless salamanders. It occurs throughout the northeastern United States and southeastern Canada, with disjunct populations in Ontario, Minnesota, Missouri, Arkansas, Oklahoma, Louisiana, and Georgia (Vogt, 1981). This species is the only true terrestrial amphibian in Wisconsin with eggs developing on land. It also inhabits mesic forests throughout northern Wisconsin. These salamanders feed on a wide range of small invertebrates including insects, mites, isopods and centipedes. Longevity of this species is unknown but they have been known to live for up to five years in captivity and potentially may live up to 20 years as other Plethodontids (Harding, 1997).

Ecological Factors and the Parasite Fauna of *Ambystoma maculatum* and *Plethodon c. cinereus*

Although parasites of the spotted salamander, *Ambystoma maculatum* and the red-backed salamander, *Plethodon c. cinereus* have been studied by several authors (Chitwood, 1933; Rankin, 1937a, b, 1938, 1945; Rankin and Hughes 1937; Fischthal, 1955a, b; Cheng, 1958, 1960; Cheng and Chase, 1960; Ernst, 1974; Rosen and Manis, 1976; Dunbar and Moore, 1979; Bursey and Schibli, 1995), few studies are known from the Great Lakes area (Meserve, 1943; Coggins and Sajdak, 1982; Muzzall, 1990).

The helminth component communities of these salamander hosts were species poor. Spotted salamanders component community consisted of two species while red-backed salamanders component community consisted of three species. Only one spotted salamander and one red-backed salamander were concurrently infected with two species. No relationships were observed for host size and species richness or abundance in both hosts. Of the parasites recovered, two had a direct life cycle and one had an indirect life cycle.

The nematode *Batracholandros magnavulvaris* exhibits little host specificity, infecting plethodontids (Rankin, 1937b; Schad, 1960, 1963; Dyer et al., 1980; Goater et al., 1987; Muzzall, 1990; Joy et al., 1993; McAllister et al., 1995; Bursey and Schibli, 1995) as well as salamandrids (Rankin, 1937b; Baker, 1987) and ambystomids (Muzzall and Shinderle, 1992). In this study, 16 female *B. magnavulvaris* were recovered from both salamander species, 15 from *A. maculatum* and one from *P. c. cinereus*. Finding all female nematodes in salamanders has also been reported by other investigators (Rankin, 1937b; Walton, 1940; Fishthal 1955a; Joy et al., 1993).

Ambystoma maculatum is a new host record for *B. magnavulvaris* and Wisconsin is a new locality record for this nematode in *P. c. cinereus*. Although both

species of salamanders in this study are considered terrestrial, only *A. maculatum* returns to water during its reproductive season. *Plethodon c. cinereus* is totally terrestrial with reproduction and development occurring on land (Vogt, 1981). This prevalence data of *B. magnavulvaris* in *A. maculatum* (45%) and *P. c. cinereus* (5%) agree with reports by Dunbar and Moore (1979) and Joy et al. (1993) that more aquatic salamanders are more likely to be infected with this nematode than are terrestrial species of salamanders. Although the life cycle of this oxyurid nematode is unknown, members of the order are strictly monoxenous and transmission is probably direct (Anderson, 1992).

Trematodes in the genus *Brachycoelium* are common parasites of salamanders (Dyer et al., 1980; Goater et al., 1987; Muzzall, 1990; McAllister et al., 1995; Bursey and Schibli, 1995), but there has been controversy surrounding the assignment of species to this genus. Rankin (1938) reduced all known species to *B. salamandrae*. He concluded that heavy infections produce many small flukes, whereas light infections were usually made up of larger specimens. Other investigators (Parker, 1941; Cheng, 1958, 1960; Cheng and Chase, 1960; Couch, 1966) disagreed and described 13 species based on such morphological characteristics as body length and shape, length of esophagus, position of testes and distribution of vitellaria. Although the trematodes collected from *A. maculatum* and *P. c. cinereus* exhibited morphological variation, due to the low number of trematodes recovered I have adapted a conservative approach suggested by McAllister et al. (1995) to report *B. salamandrae* for North American

salamanders until a revision of this genus has been completed. Salamanders probably acquire fluke infection by feeding on molluscs (Prudhoe and Bray, 1982).

Rhabdias sp. was the most frequently found parasite in *P. c. cinereus*. Six red-backed salamanders (30%) were found infected with a total of eight specimens of these nematodes recovered from the body cavity. *Rhabdias spp.* have been reported previously from a wide variety of salamander species (Chitwood, 1933; Walton, 1938, 1940; Lehmann, 1954; Landewe, 1963; Dyer and Peck, 1975; Price and St. John, 1980; Coggins and Sajdak, 1982; Muzzall and Schinderle, 1992). However, the finding of this nematode in *P. c. cinereus* was unexpected, since *P. c. cinereus* is a lungless salamander and should not be expected to harbor lung parasites. The study of Baker (1979a) on *Rhabdias* revealed that sub-adult nematodes must invade the lungs if they are to mature and produce eggs, but numerous sub-adults can be found in the body cavity. All nematodes recovered in the present study were non gravid sub-adults from the body cavity and are probably accidental infections in this host.

These results support previous work on salamander helminths, indicating that they are not strongly host specific; rather, their distribution can be correlated with habitat preference and diet of the host (Fischthal, 1955a, b; Dunbar and Moore, 1979; Coggins and Sajdak 1982; Goater et al., 1987). Recently, Kleeberger and Werner (1982, 1983) showed that in a northern hardwood forest, similar to the present study area, *A. maculatum* spent 72% of its time underground, 21% under decaying logs and 7% under wet leaf litter, while *P. c. cinereus* in the same area spent 61% of its time above the soil primarily in the litter layers and decaying logs. Thus, the differences in

the parasite prevalence and species found in the two hosts may be due to the parasites encountering only a limited number of potential hosts under natural conditions, giving the appearance of a much narrower host specificity (Kennedy, 1975). To my knowledge, *A. maculatum* is a new host record for *B. magnavulvaris* and Wisconsin is a new locality record for this nematode in both salamander species.

Conclusions

Helminth communities of amphibians examined in this study were depauperate and isolationist in nature. On average, most amphibian species harbored three or fewer parasite species. Seasonally, helminth populations and communities were highly variable among the two hosts sampled. Most helminth species did not show significant seasonal variation in prevalence and mean intensities, a number of species showed seasonal variation in location within the host and size differences over time. This variability seems to be a consequence of the complexity of the helminth life cycles, and recruitment period of these species. These data indicate that investigators working on seasonal variation of helminth parasites in ectothermic hosts may need to pay closer attention to these differences in distribution within the host, other then the standard measurements of parasitism.

Host biology appears to be important in structuring helminth communities in these hosts. Semi-aquatic sit-and-wait predators such as *Rana* species are dominated by indirect life cycle parasites associated with an aquatic habitat, in contrast, terrestrial active foragers such, as *Bufo a. americanus,* are dominated by direct life cycle skin penetrating nematodes associated with a terrestrial habitat. Arboreal and fossorial species have very depauparate communities compared to other hosts. Diet of hosts

appeared to be important in structuring helminth communities and probably reflects habitat differences and gape size of hosts examined. Terrestrial species such as toads may feed less often on semi-aquatic arthropods which serve as intermediate hosts for trematode species, while semi-aquatic species and sit-and-wait predators may be exposed to such parasites more often. Size of hosts, although variable, appeared to be an important factor in helminth abundance and species richness. Adult leopard frogs, green frogs and toads were similar in size and had similar species richness while smaller anurans such as Cope's gray treefrogs and *Pseudacris* species had less diverse communities. The role of host sex was very variable among species, and appeared to reflect size and habitat differences among the sexes. Host sex, per se, did not appear to be important in structuring helminth communities.

Component communities of salamander hosts were less diverse then the anurans, with few salamanders having concurrent infections of two or more helminth species. These differences may be attributed to anurans being bigger and exhibiting higher vagility then caudatans. As stated by Muzzall (1991b), frogs may occupy more diverse habitats and feed on a wider variety of aerial, terrestrial and aquatic invertebrates than salamanders, contributing to a more diverse helminth fauna.

Most helminth species did not show strict host specificity and have been reported in a wide range of amphibians (Prudhoe and Bray, 1982; Baker, 1987). As stated by McAlpine (1997), transmission dynamics of helminth parasites may also be important factors in structuring helminth communities. Dominant species of parasites in most cases were skin-penetrating nematodes or larval digeneans that actively seek out the amphibian host. The most common adult trematodes were *Glypthelmins*

species, which have life cycles that utilize the amphibian as both an intermediate and definitive host, by encysting in the host and being ingested with the hosts shed skin. Helminths which utilized intermediate hosts such as adult trematodes and cestodes were recovered less frequently indicating the importance of life cycles with direct transmissions. Finally, this work represents seven new host records and 12 new locality records for Wisconsin amphibians and contributes significantly to the biogeography of amphibian helminths of the midwest.

Table 1. Sampling sites and number of nine species of amphibians collected in Wisconsin during 1996 and 1997.

Site	Species	N
Genesee Depot, Waukesha Co. (Pond 1)		
	Rana clamitans melanota	75
Eagle, Waukesha Co. (Pond 2)		
	Hyla chrysoscelis	65
	Bufo a. americanus	47
	Pseudacris t. triseriata	6
	Pseudacris c. crucifer	4
	Rana pipiens	1
Brookfield, Waukesha Co. (Pond 3)		
	Rana pipiens	31
	Bufo a. americanus	30
	Ambystoma laterale	51
Pigeon lake, Bayfield Co. (Pond 4)		
	Plethodon c. cinereus	20
	Ambystoma maculatum	20

Table 2. Helminth parasites of *Rana clamitans.* N = 75.

Species	Prevalence Number (%)	Mean Intensity ± 1SD (range)	Mean Abundance ± 1SD	Number of Worms Recovered	Location
Trematoda					
Haematoloechus varioplexus	33 (44)	5.3 ± 7 (1-30)	2.3 ± 5.4	176	Lungs
Halipegus occidualis	17 (22.6)	1.9 ± 1.7 (1-8)	0.4 ± 1.1	33	Eustachian Tubes
Glypthelmins quieta	11 (14.6)	35.5 ± 34.7 (1-110)	5.2 ± 18	391	Small Intestine
Echinostome metacercariae	60 (80)	NC‡	NC	> 1,770	Kidneys, Body Cavity
Diplostomid metacercariae*	12 (16.0)	35 ± 41 (1-100)	5.7 ± 20.7	420	Leg Muscles
Cestoda					
Unidentified Adult Cestode	1 (1.3)	1 (1)	0.01 ± 0.1	1	Small Intestine
Mesocestoides sp.*	14 (18.6)	31.9 ± 22 (12-86)	5.9 ± 15.3	446	Leg Muscles
Nematoda					
Oswaldocruzia pipiens	21 (28)	14.7 ± 23 (1-90)	4.1 ± 13.7	310	Small Intestine
Cosmocercoides sp.	19 (25)	3.3 ± 4.3 (1-19)	0.8 ± 2.6	62	Large Intestine
†*Foleyella* sp.	1 (1.3)	4 (4)	0.05 ± 0.5	4	Body Cavity
Larval Nematode	9 (12)	25.9 ± 61 (1-200)	3.5 ± 23	259	Large Intestine
Encysted Nematode	1 (1.3)	1 (1)	0.01 ± 0.1	1	Small Intestine

* = Under estimate, ‡ = not counted, † = new host record.

Table 3. Prevalence and mean intensity of helminth parasites in male and female green frogs *Rana clamitans*.

Species	Measure of Parasitism	Males N = 43	Females N = 32	Statistic	*P*
Trematoda					
Haematoloechus varioplexus	Prevalence	46.5 (20/43)	40.6 (13/32)	$\chi^2 = 0.24$	$P > 0.05$
	Mean Intensity ± 1SD	7 ± 8.6	2.8 ± 2.2	$t'_s = 3.07$	$P < 0.05$
Halipegus occidualis	Prevalence	23.3 (10/43)	21.9 (7/32)	$\chi^2 = 0.02$	$P > 0.05$
	Mean Intensity ± 1SD	2.3 ± 2.1	1.4 ± 0.5	$t'_s = 2.71$	$P < 0.05$
Glypthelmins quieta	Prevalence	11.6 (5/43)	18.8 (6/32)	$\chi^2 = 0.46$	$P > 0.05$
	Mean Intensity ± 1SD	21.8 ± 27.9	47 ± 38.1	$t = 1.35$	$P > 0.05$
Echinostome metacercariae	Prevalence	90.7 (39/43)	65.6 (21/32)	$\chi^2 = 5.72$	$P < 0.05$
	Mean Intensity ± 1SD	NC‡	NC		
Diplostomid metacercariae*	Prevalence	18.6 (8/43)	12.5 (4/32)	$\chi^2_{adj} = 0.16$	$P > 0.05$
	Mean Intensity ± 1SD	45.3 ± 46.1	14.5 ± 23.7	$t = 1.23$	$P > 0.05$
Cestoda					
Unidentified Adult Cestode	Prevalence	2.3 (1/43)	0 (0/32)	$\chi^2_{adj} = 0.02$	$P > 0.05$
	Mean Intensity ± 1SD	1	0		

Table 3. Prevalence and mean intensity of helminth parasites in male and female green frogs *Rana clamitans* (cont'd).

Species	Measure of Parasitism	Males N = 43	Females N = 32	Statistic	*P*
Cestoda					
Mesocestoides sp.*	Prevalence	23.3 (10/43)	12.5 (4/32)	$\chi^2_{adj} = 0.78$	$P > 0.05$
	Mean Intensity ± 1SD	24.9 ± 16.2	49.3 ± 24.8	$t = 6.69$	$P < 0.05$
Nematoda					
Oswaldocruzia pipiens	Prevalence	27.9 (12/43)	28.1 (9/32)	$\chi^2 = 0.00$	$P > 0.05$
	Mean Intensity ± 1SD	16.6 ± 25.8	12.2 ± 19.6	$t = 0.44$	$P > 0.05$
Cosmocercoides sp.	Prevalence	20.9 (9/43)	31.3 (10/32)	$\chi^2 = 1.00$	$P > 0.05$
	Mean Intensity ± 1SD	2 ± 1.7	4.4 ± 5.6	$t'_s = 3.81$	$P < 0.05$
Foleyella sp.	Prevalence	0 (0/43)	3.1 (1/32)	$\chi^2_{adj} = 0.02$	$P > 0.05$
	Mean Intensity ± 1SD	0	4		
Larval Nematode	Prevalence	9.3 (4/43)	18.8 (6/32)	$\chi^2_{adj} = 1.67$	$P > 0.05$
	Mean Intensity ± 1SD	11.5 ± 11.2	35.5 ± 80.6	$t'_s = 1.67$	$P > 0.05$
Encysted Nematode	Prevalence	0 (0/43)	3.1 (1/32)	$\chi^2_{adj} = 0.02$	$P > 0.05$
	Mean Intensity ± 1SD	0	1		

* = Under estimate, ‡ = not counted.

Table 4. Helminth parasites of *Bufo a. americanus.* N = 47.

Species	Prevalence Number (%)	Mean Intensity ± 1SD (range)	Mean Abundance ± 1SD	Number of Worms Recovered	Location
Trematoda					
Echinostome metacercariae*	3 (6.3)	13 ± 19 (1-35)	0.8 ± 5.1	39	Kidneys, Body Cavity
Gorgoderina sp.	1 (2.1)	6 ± 0 (6)	0.1 ± 0.9	6	Bladder
Cestoda					
Mesocestoides sp. *	6 (12.7)	25.8 ± 22 (12-70)	3.3 ± 11.3	155	Leg Muscles
Nematoda					
Oswaldocruzia pipiens	41 (87)	8.5 ± 7 (1-31)	7.4 + 7.1	349	Small Intestine
Cosmocercoides variabilis	43 (91)	32.3 ± 31.5 (1-135)	29.6 ± 31.5	1,392	Lungs, Body Cavity, Large and Small Intestine
Rhabdias americanus	43 (91)	15.8 ± 17.9 (1-75)	14.5 ± 17.7	682	Lungs, Body Cavity

* = Under estimate.

Table 5. Prevalence and mean intensity of helminth parasites in male and female toads *Bufo a. americanus.*

Species	Measure of Parasitism	Males N = 28	Females N = 19	Statistic	*P*
Trematoda					
Echinostome metacercariae*	Prevalence	10.7 (3/28)	0 (0/19)	$\chi^2_{adj} = 0.75$	$P > 0.05$
	Mean Intensity $\pm$ 1SD	13 $\pm$ 19	0		
Gorgoderina sp.	Prevalence	3.6 (1/28)	0 (0/19)	$\chi^2_{adj} = 0.04$	$P > 0.05$
	Mean Intensity $\pm$ 1SD	6	0		
Cestoda					
Mesocestoides sp.*	Prevalence	10.7 (3/28)	15.8 (3/19)	$\chi^2_{adj} = 0.00$	$P > 0.05$
	Mean Intensity $\pm$ 1SD	32.7 $\pm$ 32.6	19 $\pm$ 4.6	$t'_s = 4.70$	$P < 0.05$
Nematoda					
Oswaldocruzia pipiens	Prevalence	89.3 (25/28)	84.2 (16/19)	$\chi^2_{adj} = 0.00$	$P > 0.05$
	Mean Intensity $\pm$ 1SD	7.8 $\pm$ 6.9	9.56 $\pm$ 7.3	$t = 0.76$	$P > 0.05$
Cosmocercoides variabilis	Prevalence	96.4 (27/28)	84.2 (16/19)	$\chi^2_{adj} = 0.88$	$P > 0.05$
	Mean Intensity $\pm$ 1SD	28.7 $\pm$ 26.3	38.6 $\pm$ 38.9	$t = 1.00$	$P > 0.05$
Rhabdias americanus	Prevalence	89.3 (25/28)	94.7 (18/19)	$\chi^2_{adj} = 0.02$	$P > 0.05$
	Mean Intensity $\pm$ 1SD	16.6 $\pm$ 17.3	14.8 $\pm$ 19.2	$t = 0.32$	$P > 0.05$

* = Under estimate.

Table 6. Seasonal prevalence and mean intensity ± 1 SD of three species of nematodes in *Bufo a. americanus*

Species		April	June-July	Augu.-Sept.	Statistic	*P*
Cosmocercoides variabilis	Prevalence	93% (14/15)	88% (14/16)	94% (15/16)	$\chi 2 = 0.5$	$P > 0.05$
	Mean Intensity ± 1SD	30.4 ± 28.5	40 ± 31.7	27 ± 34.6	F = 0.65	$P > 0.05$
Oswaldocruzia pipiens	Prevalence	93% (14/15)	88% (14/16)	81% (13/16)	$\chi 2 = 1.1$	$P > 0.05$
	Mean Intensity ± 1SD	7 ± 6.7	10 ± 8.1	8.3 ± 6.1	F = 0.63	$P > 0.05$
Rhabdias americanus	Prevalence	93% (14/15)	100% (16/16)	81% (13/16)	$\chi 2 = 3.7$	$P > 0.05$
	Mean Intensity ± 1SD	17.8 ± 21.6	10.7 ± 11.2	20 ± 19.8	F = 1.12	$P > 0.05$

Table 7 Helminth parasites of *Hyla chrysoscelis.* N = 65.

	Prevalence Number (%)	Mean intensity ± 1 SD (range)	Meand Abundance ± 1 SD	Number of Worms Recovered	Location
Monogenea					
†*Polystoma nearcticum*	10 (15)	1.3 ± 0.7 (1-3)	0.2 ± 0.5	13	Urinary Bladder
Trematoda					
†*Glypthelmins pennsylvaniensis*	1 (1.5)	1	0.02 ± 0.1	1	Small Intestine
Unidentified immature trematode	3 (5)	42.3 ± 2.5 (39-45)	2 ± 9	127	Small Intestine
Unidentified metacercariae	5 (8)	16.6 ± 29.6 (1-69)	1.3 ± 8.7	84	Leg Muscles, Body Cavity
Cestoidea					
Unidentified plerocercoid	1 (1.5)	14	0.2 ± 1.7	14	Body Cavity, Lungs
Unidentified cestode cyst	1 (1.5)	7	0.1 ± 0.9	7	Stomach
†*Mesocestoides* sp.	8 (12)	NC	NC	>1,150	Body Cavity, Small Intestine, Large Intestine, Liver
Nematoda					
†*Cosmocercoides variabilis*	14 (22)	3.3 ± 5.9 (1-23)	0.7 ± 3	46	Small Intestine, Large Intestine, Lungs
†*Oswaldocruzia pipiens*	4 (6)	1.3 ± 0.5 (1-2)	0.08 ± 0.3	5	Small Intestine

NC = not counted, † = new host record.

Table 8. Helminth parasites of *Pseudacris t. triseriata.* N = 6.

	Prevalence Number (%)	Mean intensity ± 1 SD (range)	Mean Abundance ± 1 SD	Number of Worms Recovered	Location
Trematoda					
Glypthelmins pennsylvaniensis	3 (50)	2 ± 1.7 (1-4)	1 ± 1.5	7	Small Intestine
Unidentified metacercariae	1 (17)	6 (6)	1 ± 2.4	6	Leg Muscles, Body Cavity
Nematoda					
Cosmocercoides variabilis	4 (67)	1.5 ± 0.6 (1-2)	1 ± 0.9	6	Small and Large Intestine

Table 9. Helminth parasites of *Pseudacris c. crucifer*. N = 4.

	Prevalence Number (%)	Mean intensity ± 1 SD (range)	Mean Abundance ± 1 SD	Number of Worms Recovered	Location
Trematoda					
Glypthelmins pennsylvaniensis	2 (50)	1.5 ± 0.7 (1-2)	0.75 ± 0.95	3	Small Intestine
Nematoda					
Cosmocercoides variabilis	1 (25)	25 (25)	6.25 ± 12.5	25	Small and Large Intestine
Oswaldocruzia pipiens	1 (25)	1 ± 0 (1)	0.25 ± 0.5	1	Small Intestine

Table 10. Helminth Parasites of *Bufo a. americanus.* N = 30.

Species	Prevalence Number (%)	Mean Intensity ± 1SD (range)	Mean Abundance ± 1SD	Number of Worms Recovered	Location
Trematoda					
Haematoloechus varioplexus	1 (3.3)	1 (1)	0.03 ± 0.18	1	Lungs
Echinostome metacercariae*	9 (30)	7 ± 3.6 (3-13)	2.1 ± 3.8	63	Kidneys, Body Cavity
Diplostomid metacercariae*	12 (40)	3.4 ± 1.8 (1-7)	1.4 ± 2.0	41	Leg Muscles Body Cavity
Cestoda					
Mesocestoides sp. *	5 (16.7)	66 ± 22.6 (39-93)	11 ± 26.4	330	Leg Muscles Body Cavity
Nematoda					
Oswaldocruzia pipiens	8 (26.7)	24.1 ± 18.2 (6-56)	6.4 ± 14.1	193	Small Intestine
Cosmocercoides variabilis	8 (26.7)	5.8 ± 4.8 (1-15)	1.5 ± 3.5	46	Lungs, Body Cavity, Large Intestine
Rhabdias americanus	12 (40)	31.7 ± 55.7 (1-189)	12.7 ± 37.7	380	Lungs, Body Cavity
Encysted Nematode	3 (10)	3 ± 2 (1-5)	0.3 ± 1.1	9	Intestines
Unidentified Larval Nematode	2 (6.7)	1 ± 0 (1)	0.06 ± 0.3	2	Large Intestine

* = Under estimate.

Table 11. Prevalence and mean intensity of helminth parasites in adult and metamorphosed toads *Bufo a. americanus.*

Species	Measure of Parasitism	Adult N = 15	Metamorphs N = 15	Statistic	*P*
Trematoda					
Haematoloechus varioplexus	Prevalence	6.7 (1/15)	0 (0/15)	$\chi^2_{adj} = 0.00$	$P > 0.05$
	Mean Intensity ± 1SD	1			
Echinostome metacercariae*	Prevalence	20 (3/15)	40 (6/15)	$\chi^2_{adj} = 1.07$	$P > 0.05$
	Mean Intensity ± 1SD	10 ± 2.6	5.5 ± 3.6	$t = 2.08$	$P > 0.05$
Diplostomid metacercaria*	Prevalence	46.6 (7/15)	33.3 (5/15)	$\chi^2 = 0.55$	$P > 0.05$
	Mean Intensity ± 1SD	3 ± 2.6	4 ± 2	$t = 0.93$	$P > 0.05$
Cestoda					
Mesocestoides sp.*	Prevalence	33 (5/15)	0 (0/15)	$\chi^2_{adj} = 3.84$	$P = 0.05$
	Mean Intensity ± 1SD	66 ± 22.6			
Nematoda					
Oswaldocruzia pipiens	Prevalence	53.3 (8/15)	0 (0/15)	$\chi^2_{adj} = 8.35$	$P < 0.05$
	Mean Intensity ± 1SD	24.1 ± 18.2			
Cosmocercoides variabilis	Prevalence	53.3 (8/15)	0 (0/15)	$\chi^2_{adj} = 8.35$	$P < 0.05$
	Mean Intensity ± 1SD	5.8 ± 4.8			
Rhabdias americanus	Prevalence	80 (12/15)	0 (0/15)	$\chi^2_{adj} = 16.8$	$P < 0.05$
	Mean Intensity ± 1SD	31.7 ± 55.7			
Encysted Nematode	Prevalence	20 (3/15)	0 (0/15)	$\chi^2_{adj} = 1.48$	$P > 0.05$
	Mean Intensity ± 1SD	3 ± 2			
Immature Nematode	Prevalence	13.3 (2/15)	0 (0/15)	$\chi^2_{adj} = 0.54$	$P > 0.05$
	Mean Intensity ± 1SD	1 ± 0			

* = Under estimate.

Table 12. Helminth Parasites of *Rana pipiens.* N = 31.

Species	Prevalence Number (%)	Mean Intensity ± 1SD (range)	Mean Abundance ± 1SD	Number of Worms Recovered	Location
Trematoda					
Haematoloechus varioplexus	5 (16.1)	2.2 ± 1 (1-4)	0.4 ± 0.9	11	Lungs
Gorgoderina attenuata	5 (16.1)	2.8 ± 2.4 (1-7)	0.5 ± 1.4	14	Bladder
Echinostome metacercariae*	13 (41.9)	13 ± 10.5 (6-39)	5.4 ± 9.3	170	Kidneys, Body Cavity
Diplostomid metacercariae*	20 (64.5)	10.9 ± 7.8 (1-35)	7 ± 8.1	217	Leg Muscles Body Cavity
Cestoda					
Mesocestoides sp. *	13 (41.9)	89.2 ± 104 (12-378)	37.4 ± 79.6	1,159	Leg Muscles Body Cavity
Nematoda					
Oswaldocruzia pipiens	11 (35.5)	3.6 ± 4 (1-14)	1.3 ± 2.9	40	Small Intestine
Encysted Nematode	3 (9.7)	2.3 ± 0.6 (2-3)	0.2 ± 0.7	7	Intestines, Stomach
Unidentified Larval Nematode	1 (3.2)	9 (9)	0.3 ± 1.6	9	Large Intestine

* = Under estimate.

Table 13. Prevalence and mean intensity of helminth parasites in adult and metamorphosed northern leopard frogs, *Rana pipiens.*

Species	Measure of Parasitism	Adult N = 20	Metamorphs N = 11	Statistic	*P*
Trematoda					
Haematoloechus varioplexus	Prevalence	25 (5/20)	0 (0/11)	χ^2_{adj} =1.69	*P* > 0.05
	Mean Intensity ± 1SD	2.2 + 1			
Gorgoderina Attenuate	Prevalence	25 (5/20)	0 (0/11)	χ^2_{adj} = 1.69	*P* > 0.05
	Mean Intensity ± 1SD	2.8 + 2.4			
Echinostome metacercariae*	Prevalence	35 (7/20)	54.5 (6/11)	χ^2 = 1.11	*P* > 0.05
	Mean Intensity ± 1SD	9.3 ± 1.7	17.6 ± 14.6	t'_s = 1.39	*P* > 0.05
Diplostomid metacercaria*	Prevalence	55 (11/20)	81.8 (9/11)	χ^2_{adj} =1.21	*P* > 0.05
	Mean Intensity ± 1SD	10 ± 5.6	13 ± 10.3	*t* = 0.839	*P* > 0.05
Cestoda					
Mesocestoides sp.*	Prevalence	65 (13/20)	0 (0/11)	χ^2_{adj} = 9.79	*P* < 0.05
	Mean Intensity ± 1SD	89 ± 104			
Nematoda					
Oswaldocruzia pipiens	Prevalence	55 (11/20)	0 (0/11)	χ^2_{adj} = 7.13	*P* < 0.05
	Mean Intensity ± 1SD	3.6 ± 4			
Encysted Nematode	Prevalence	15 (3/20)	0 (0/11)	χ^2_{adj} = 0.51	*P* > 0.05
	Mean Intensity ± 1SD	2.3 ± 0.6			
Immature Nematode	Prevalence	5 (1/20)	0 (0/11)	χ^2_{adj} = 0.09	*P* > 0.05
	Mean Intensity ± 1SD	9			

* = Under estimate.

Table 14. Helminth parasites of *Ambystoma laterale* N = 51.

Species	Prevalence Number (%)	Mean Intensity ± 1SD (range)	Mean Abundance ± 1SD	Number of Worms Recovered	Location
Trematoda					
Echinostome metacercariae*	24 (47)	24 ± 17 (8-68)	11 ± 17	566	Kidneys, Body Cavity
Nematoda					
Cosmocercoides sp.	10 (19.6)	2.5 ± 2.12 (1-7)	0.49 ± 1.35	25	Lungs, Body Cavity, Large Intestine
Encysted Nematode	4 (7.8)	2.5 ± 2.3 (1-6)	0.2 ± 0.9	10	Intestines

* = Under estimate.

Table 15. Prevalence and mean intensity of helminth parasites in adult and newly metamorphosed *Ambystoma laterale*.

Species	Measure of Parasitism	Adult N = 31	Metamorphs N = 20	Statistic	*P*
Trematoda					
Echinostome metacercariae*	Prevalence	22.6 (7/31)	85 (17/20)	$\chi^2_{adj} = 16.58$	$P < 0.01$
	Mean Intensity $\pm$ 1SD	12.6 $\pm$ 2.6	28.1 $\pm$ 18.3	$t'_s = 3.76$	$P < 0.05$
Nematoda					
Cosmocercoides sp.	Prevalence	32.3 (10/31)	0 (0/20)	$\chi^2_{adj} = 6.10$	$P < 0.025$
	Mean Intensity $\pm$ 1SD	2.5 $\pm$ 2.1			
Encysted Nematode	Prevalence	12.9 (4/31)	(0/20)	$\chi^2_{adj} = 1.29$	$P > 0.05$
	Mean Intensity $\pm$ 1SD	2.5 $\pm$ 2.4			

* = Under estimate.

Table 16. Helminth parasites of *Ambystoma maculatum* N = 20.

	Prevalence Number (%)	Mean intensity ± 1 SD (range)	Mean Abundance ± 1 SD	Number of Worms Recovered	Location
Trematoda					
Brachycoelium salamandrae	3 (15)	3.3 ± 1.5 (2-5)	0.5 ± 1.3	10	Small and Large Intestine
Nematoda					
† *Batracholandros magnavulvaris*	9 (45)	1.7 ± 1.1 (1-4)	0.8 ± 1.1	15	Small and Large Intestine

† = new host record.

Table 17. Helminth parasites of *Plethodon c. cinereus* N = 20.

	Prevalence Number (%)	Mean intensity ± 1 SD (range)	Mean Abundance ± 1 SD	Number of Worms Recovered	Location
Trematoda					
Brachycoelium salamandrae	2 (10)	2 (2)	0.2 ± 0.6	4	Small and Large Intestine
Nematoda					
Batracholandros magnavulvaris	1 (5)	1 (1)	0.05 ± 0.2	1	Small and Large Intestine
Rhabdias sp.	6 (30)	1.3 ± 0.8 (1-3)	0.4 ± 0.8	8	Body Cavity

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Appendix 1. Length and width of erythrocytes (RBC) of three *Hyla chrysoscelis* from Eagle, Waukesha Co. Wisconsin and one *Hyla versicolor* from Saukville, Ozaukee Co. Wisconsin. All frogs were identified by mating call and collected from locations where only single species were heard calling.

	Hyla chrysoscelis N = 3		*Hyla versicolor* N = 1	
Number	Erythrocyte Length	Erythrocyte Width	Erythrocyte Length	Erythrocyte Width
1	19.00 μm	15.00 μm	27.50 μm	15.00 μm
2	21.50 μm	15.00 μm	25.75 μm	14.75 μm
3	19.75 μm	14.75 μm	25.00 μm	14.50 μm
4	22.50 μm	15.25 μm	24.00 μm	14.75 μm
5	20.25 μm	15.00 μm	24.75 μm	15.00 μm
6	19.50 μm	12.75 μm	24.75 μm	15.25 μm
7	21.00 μm	15.00 μm	25.00 μm	14.25 μm
8	18.25 μm	15.25 μm	28.50 μm	15.50 μm
9	17.75 μm	15.00 μm	24.50 μm	17.25 μm
10	19.50 μm	15.00 μm	24.50 μm	16.00 μm
11	20.75 μm	12.25 μm	24.25 μm	15.00 μm
12	18.25 μm	13.25 μm	24.00 μm	15.50 μm
13	19.75 μm	12.75 μm	24.75 μm	15.00 μm
14	18.25 μm	12.50 μm	24.50 μm	15.25 μm
15	24.25 μm	12.75 μm	24.25 μm	15.50 μm
Average*	19.72 $\pm$ 1.33 μm		25.07 $\pm$ 1.28 μm	

* = significant differences between *H. chrysoscelis* and *H. versicolor* RBC length, two tailed *t* test, $t = 11.20$ $P < 0.0001$. Slides of dry RBC smears were deposited in the Milwaukee Public Museum along with one *H. chrysoscelis* voucher (MPM 29589).