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The intestinal platyhelminths of southwestern and central West Virginia Amphibia and relationships between the degree of infestation and habitat of amphibians and sex of host

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The intestinal platyhelminths of southwestern and central West Virginia Amphibia and relationships between the degrees of infestation and habitat of amphibians and sex of host.

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And finally, deep appreciation to Annie, my wife, for her constant encouragement and understanding during the endurance of this project.

CONTENTS

TABLES AND FIGURES

Page

ABSTRACT

In April and May, 1977, 397 (14 species) amphibians were collected from southwestern and central West Virginia and examined for parasites. Five species of trematodes and two species of cestodes were found from 93 hosts. The total of 397 amphibians was subdivided, for convenience, into six catagories of amphibians; aquatic male and female, terrestro-aquatic male and female, and terrestrial male and female. Nine of 24 (37.5%) female aquatic amphibians was the highest incidence found, while terrestro-aquatic females contained the highest mean number (6.0) worms per host. Hosts, incidence, and parasite loads of each platyhelminth specimen are given.

INTRODUCTION

The relationships between amphibians and their parasites and possible correlations which may exist between degree of infestations, ecology of host, and sex of host are problems on which little information has been available.

Phylogenetically, amphibians are a group of animals that are transitional between aquatic and terrestrial vertebrates. Each family may show varying degrees of transition. Some forms are wholly aquatic, others wholly terrestrial, while others are truly amphibious (Porter, 1972).

A review of the literature indicates that, with the exception of several writers, few of the North American amphibians have been examined for parasites. The report of Brachycoelium elongatum and B. obesum from Ambystoma opacum by Joy and Mills (1975), constitutes the only published material on adult platyhelminths parasitic in amphibians in West Virginia.

Leidy (1851) was the first to report trematodes from North American amphibians. Later that same year he recovered the cestode, Taenia pulchella n.sp., from Bufo americanus. He later (1855) reported Taenia dispar from both B. americanus and Rana pipiens. Looss (1899, 1902) made the first real attempt to classify amphibian parasites. Stafford (1900, 1902, 1903, 1905) was the first to attempt a systematic study of the helminths of North American

vertebrates. The next extensive works were by Cort (1912, 1915a), dealing with the classification and description of trematodes from the lungs and bladders of North American frogs.

Chandler (1923) and Holl (1928, 1928b) made important early contributions to the study of the trematode parasites of salamanders. Chandler worked with trematodes from the siren, Amphiuma means. Holl, in these early works, found new trematodes from the newt, Triturus (=Notophthalmus) viridescens. He later (1932) made a general survey of helminth parasites of fishes and amphibians.

Few instances of cestode infestations in amphibians in the early works have been found. LaRue (1914a) noted the hellbender, Cryptobranchus allegheniensis, as harboring Ophiotaenia cryptobranchi in Michigan. Thomas (1927) recovered a cestode, Bothriocephalus rarus, from the red spotted newt, Diemyctylus (=Notophthalmus) viridescens. Canavan (1928) reported the occurence of a cestode, Ophiotaenia lonnbergii, from the mudpuppy, Necturus maculosus. Zeliff (1932) recovered a new species of cestode, Crepidobothrium amphiumae, from Amphiuma tridactylum.

The literature on general surveys of amphibians for their parasites is even more meager than that on the individual species. Short papers, describing new species, or physiological experiments on particular amphibian parasites, form the major part of the work on this subject.

McCoy (1928) reported three life histories of trematodes

parasitic in amphibians in Missouri. Mann (1932) undertook a survey of parasites of North Carolina salamanders. Harwood (1932) made a survey of helminths occuring in reptiles and amphibians collected near Houston, Texas, although much of this work included nematodes as well as flatworms. Stunkard (1936) recovered trematodes from amphibians in New York, while trying to work out the morphology and life cycle of Plagitura parva. Ingles (1936) recovered species of trematodes and cestodes from a total of 264 California amphibians. Brandt (1936) reported 20 platyhelminths from 368 amphibians in North Carolina. The following year, Rankin (1937) obtained 12 platyhelminth species occuring in 1,010 amphibians collected in North Carolina. Crawford (1939) studied life histories of Colorado trematodes parasitizing amphibians. Manter (1939) recovered 10 trematode species from Florida amphibians.

More recently, other surveys have been done on a wide variety of amphibians and their helminth symbionts: Lehman (1960) in California; Waitz (1961) in Idaho; Parker (1941) in Florida; Rabalais (1970) in Louisiana; Bouchard (1970) in Maine; Najarian (1955) in Michigan; Fischthal (1955b) in New York; Lehman (1954, 1956) and Weatherly and Canaris (1961) in Oregon; Fischthal (1955a) in Pennsylvania; Frandsen and Grundman (1960) in Utah, and Cheng (1958) in Virginia.

Due to the paucity of literature pertaining to amphibian helminths from West Virginia, as well as, the absence of literature relating incidence of infestation and parasite

load to the sex of the infested amphibian, a need to do further work in this area was recognized. This paper represents the first attempt to recover helminths from West Virginia amphibians and to determine relationships of the incidence of infestation and parasite load with the habitat and sex of the infested amphibian.

MATERIALS AND METHODS

Fourteen species of amphibians, including 397 individuals, from West Virginia were collected in the spring of 1977 from the following locations: Four Pole Creek, Greenbottom Swamp, Kanawavista Park, and Ona Pool, Cabell Co.; Spruce Knob Lake, Randolph Co.; Lake Stephen and Perdue Swamp, Wayne Co. (Table I.). These specimens were examined for platyhelminths. All individuals were collected by hand, at night, with a flashlight. The identification of the amphibians was based on Green's Amphibians and Reptiles in West Virginia.

All amphibians were anesthetized with either ether or novocaine, or pithed. Each amphibian was sexed, measured for snout-vent length in millimeters, weighed in grams, and examined immediately for platyhelminths. The digestive tract from the stomach to the large intestine was removed and segregated into separate bowls according to its anatomical regions; the stomach, the small intestine, and the large intestine. Each section was slit lengthwise to expose the mucosae and inner intestinal wall. A blunt probe was used to scrape the mucosae of the stomach and intestine. Trematode specimens were found lying either in the mucosae or attached to the intestinal wall. Cestodes were found attached to the intestinal wall. Cestodes were found attached to the intestinal wall. These were removed with forceps and placed in distilled water until relaxed.

The trematodes were placed in 10% formalin and flattened

between two glass slides. They were then placed in vials in 10% formalin until time for staining and mounting. After at least 72 hours in the fixative, they were washed in distilled water and stained in carmine alum in distilled water for 16 hours. They were then passed through an ethanol dehydration series, cleared with methyl salicylate, and mounted whole in permount on glass slides.

The cestodes were flattened between two glass slides and fixed in A.F.A. They were then placed in vials containing A.F.A. for 72 hours. They were washed in distilled water, 50%, and 70% ethanol. The cestodes were then stained for 3 hours in Semichon's acetic carmine in 70% ethanol. After destaining in 70% HCL/ethanol, they were passed through an ethanol dehydration series to 99%, cleared with methyl salicylate, and mounted whole in permount on glass slides.

The identification of the trematodes was based on Yamaguti's Systema Helminthum, volume one and Schell's How to Know the Tapeworm. The platyhelminth specimens were examined with the aid of an Olympus binocular microscope equipped with a calibrated ocular micrometer. All statistical calculations were made with the use of a Melcor 400 calculator.

Table I.

DATES	SITES	AMPHIBIANS
April, 1977.	Greenbottom Swamp	Ambystoma maculatum Bufo americanus Hyla crucifer Rana palustris Rana pipiens Rana sylvatica
	Kanawavista Park	Gyrinophilus porphyriticus Plethodon glutinosus Plethodon richmondi
	Ona Pool	Bufo americanus Rana palustris
	Perdue Swamp	Ambystoma maculatum Bufo americanus Hyla crucifer Notophthalmus viridescens Pseudacris brachyphona Rana catesbeiana Rana clamitans melanota Rana sylvatica
	Spruce Knob Lake	Notophthalmus viridescens
May, 1977.	Four Pole Creek	Eurycea bislineata
	Lake Stephen	Rana catesbeiana Rana clamitans melanota

HABITAT of AMPHIBIANS EXAMINED

Fourteen species of amphibians were examined in this study. It has been convenient to assign these species to three broad groups; those that are found primarily in water, (aquatic); those that are usually aquatic or terrestrial but do migrate to either land or water, (terrestro-aquatic); and those that rarely visit water, (terrestrial). The lack of balance between the number of different species examined is chiefly a reflection of their relative abundance. In the following notes on the habits of each species, the geographical range has been taken from Conant (1958).

Ambystoma maculatum — Terrestro-aquatic

This species is an early spring breeder which, under the stimulus of warm rains, sometimes makes mass migrations to woodland ponds. These can occasionally be found (from spring to autumn) beneath stones or boards in moist environments or during wet weather.

Range: Nova Scotia and Gaspe Peninsula to central Ontario; south to Georgia and East Texas.

Bufo americanus — Terrestro-aquatic

The habitats of this species are legion, spanning the gap from city back yards to mountain wildernesses. The requisites for this species seem to be shallow bodies of water in which to breed (temporary pools or ditches).

Range: Maritime Provinces to southeast Manitoba; south to Mississippi and northeast Kansas.

Eurycea bislineata — Terrestro-aquatic

This species is essentially a brookside salamander often found hiding under objects at the water's edge. Saturated areas or seeps are also favorite habitats. In warm wet weather, it may wander far out into nearby woodlands.

Range: Southern Quebec to Virginia, Tennessee, and eastern Illinois.

Gyrinophilus porphyriticus — Terrestro-aquatic

These are found in cool springs and mountain streams, but are also likely to be found in any wet depression, beneath logs, stones, or leaves in the surrounding forest.

Range: Southern Maine and southern Quebec to northern Alabama.

Hyla crucifer — Terrestro-aquatic

This species is a habitant of woodlands, being especially abundant in areas of brushy second growth or cutover woodlots if these are near small, temporary ponds or swamps.

Range: Maritime Provinces to northern Florida; west to eastern Manitoba and eastern Texas.

Notophthalmus viridescens — Aquatic

The most frequent habitats of this species are ponds, small lakes, marshes, ditches, quiet portions of streams, or other shallow permanent or semipermanent bodies of water.

Range: Maritime Provinces to Great Lakes and south to Apalachicola drainage to Florida.

Plethodon glutinosus — Terrestrial

Moist wooded ravines or hillsides are desired habitats.

Range: Central New York to central Florida and west to Missouri, Oklahoma, and Texas.

Plethodon richmondi — Terrestrial

Wooded slopes of valleys and ravines are preferred. They shun hilltops and occur only rarely on valley floors.

Range: Central Pennsylvania to eastern Indiana; south to western Virginia and eastern Kentucky.

Pseudacris brachyphona — Terrestro-aquatic

This is a woodland species ranging upward to elevations of at least 3500 feet, occurring chiefly on forested slopes and hilltops and often long distances from water.

Range: Southwestern Pennsylvania and southwestern Ohio to central Alabama.

Rana catesbeiana — Terrestro-aquatic

This species prefers larger, slow bodies of water. It is usually seen at water's edge or amidst vegetation.

Range: Nova Scotia to central Florida; west to Wisconsin and Nebraska and south through the Great Plains.

Rana clamitans melanota — Terrestro-aquatic

This species is characteristically a frog of brooks and small streams, but can also be found almost anywhere there is shallow, fresh water.

Range: Maritime Provinces to North Carolina; west to Minnesota and eastern Oklahoma.

Rana palustris — Terrestro-aquatic

This is typically a species of cool water, sphagnum bogs, rocky ravines, and meadow streams, but it also occupies a wide variety of other habitats. It wanders well out into grassy fields or weed covered areas in the summer.

Range: Maritime Provinces to the Carolinas; west to Wisconsin and southeast Texas; distribution spotty, especially toward the south and in the prarie portions of Ohio, Indiana, and Illinois.

Rana pipiens — Terrestro-aquatic

This is a frog of brooks and small streams, but it often wanders well away from water in the summer.

Range: Southern Labrador to extreme southern District of Mackenzie; south through uplands to northern Georgia; west to Pacific states, and south, in the west, into Mexico.

Rana sylvatica — Terrestro-aquatic

This species is usually encountered in or near moist wooded areas in the United States and southern Canada. It wanders considerable distances from water.

Range: Labrador to Alaska; south in the East to southern Appalachians. Isolated colonies in Ozarks, Kansas, Colorado, Wyoming, and Idaho.

HABITAT of PARASITES ENCOUNTERED

Trematoda

1. Brachycoelium elongatum Cheng, 1958.

   Hosts: Ambystoma maculatum, Rana palustris, Rana sylvatica.

   Location: Small intestine.

2. Brachycoelium obesum Nicoll, 1914.

   Hosts: Ambystoma maculatum, Rana sylvatica.

   Location: Small intestine.

3. Glypthelmins quieta (Stafford, 1900) Stafford, 1905.

   Hosts: Bufo americanus, Hyla crucifer.

   Location: Small intestine.

4. Megalodiscus temperatus (Stafford, 1905) Harwood, 1932.

   Hosts: Rana catesbeiana, Rana clamitans melanota, Rana sylvatica.

   Location: Large intestine.

5. Plagitura parva Stunkard, 1932.

   Host: Notophthalmus viridescens.

   Location: Small intestine.

Cestoda

1. Bothriocephalus rarus Thomas, 1937.

   Host: Notophthalmus viridescens.

   Location: Small intestine.

2. *Cylindrotaenia americana* Jewell, 1916.

Host: *Rana catesbeiana*.

Location: Small intestine.

Infestation Related to Habitat of Amphibia

During a seven week period, a total 397 amphibians were collected from southwestern and central West Virginia and examined for intestinal flatworms. This total was subdivided, for convenience, into three catagories of amphibians; aquatic, terrestro-aquatic, and terrestrial. Most of the amphibia collected, a total of 266, were catagorized as terrestro-aquatic, while 93 and 38 were considered aquatic and terrestrial, respectively. Sixty-three of 266 (23.7%) terrestro-aquatic individuals harbored intestinal flatworms. This group had a mean of 5.9 worms per host (w/h), which was the highest parasite burden of the three groups of amphibians. Thirty of 93 (32.2%) aquatic amphibians were parasitized by intestinal flatworms and had a mean of 3.9 worms per host (parasite load). The terrestrial amphibians were not parasitized by intestinal flatworms.

Terrestro-aquatic Habitat

Within the terrestro-aquatic group, there were 11 species of amphibia collected. One species, *Hyla crucifer*, had the highest incidence of parasitism. Twenty-two of 35 (62.9%) individuals were parasitized by the intestinal trematode, *Glypthelmins quieta*, and had a parasite load of 5.2 w/h. The same trematode was also found in seven of 56 (12.5%) *Bufo americanus*. The seven hosts involved in this case had a mean number of 5.1 worms per host. The second highest incidence, 10 of 24 (41.7%), was found in *Ambystoma maculatum*, which

harbored the trematode Brachycoelium elongatum. A mean of 10 w/h B. elongatum individuals were found in each Ambystoma maculatum, establishing this as the second highest parasite load in the present study. Nine of 23 (39.1%), the third highest incidence, was found in Rana catesbeiana parasitized by the amphistome, Megalodiscus temperatus. The parasite load was 2.4 w/h. Megalodiscus temperatus was also found in four of 13 (30.8%) Rana clamitans melanota and in one of 15 (6.7%) Rana sylvatica individuals. The latter two infestations had parasite loads of 3.0 w/h and 1.0 w/h, respectively.

The highest parasite load (11.2 w/h) was found in six of 24 (25.0%) Ambystoma maculatum, harboring Brachycoelium obesum. This trematode was also found in two of 12 (16.7%) Rana sylvatica with a parasite load of 9.0 w/h. Two of 15 (13.3%) Rana sylvatica individuals were also infested by B. elongatum with a parasite load of 2.5 w/h. The eleventh terrestro-aquatic species, Rana palustris, had a percent incidence of 23.8 (five of 21) and a parasite load of 6.2 w/h when it was parasitized by B. elongatum.

While trematodes were the predominating parasites recovered from terrestro-aquatic amphibians, one amphibian, Rana catesbeiana, was parasitized by a cestode, Cylindrotaenia americana. Of the 23 R. catesbeiana collected, two (8.7%) were infested by C. americana with a mean of 1.0 worms per host.

Four species of terrestro-aquatic amphibians; Eurycea bislineata, Gyrinophilus porphyriticus, Pseudacris brachyphona,

and Rana pipiens were found to be free of intestinal flatworms.

Aquatic Habitat

Thirty of 93 (32.3%) aquatic individuals harbored intestinal platyhelminths. This group of aquatic amphibia is represented by only one species, Notophthalmus viridescens. Twenty-three of 93 (24.7%) N. viridescens served as hosts for the trematode, Plagitura parva, while eight of 93 (8.6%) were parasitized by a cestode, Bothriocephalus rarus. The parasite loads were 4.8 Plagitura parva per host and 1.6 Bothriocephalus rarus per host, respectively.

Terrestrial Habitat

The terrestrial group included two species of amphibia, Plethodon glutinosus and Plethodon richmondi. Neither species were parasitized by intestinal flatworms.

Infestation Related to Sex of Amphibia

The total of 397 amphibians were also divided into two different groups; male and female. Most of the amphibians, a total of 297, were males, while 118 were females. Thirty-nine of 118 (33.8%) female amphibians were found to carry 5.6 intestinal flatworms per host, which was the lower parasite burden of these two groups. On the other hand, only 54 of 279 (19.4%) male amphibia were parasitized with intestinal flatworms and had a parasite load of 5.7 w/h.

Terrestro-aquatic Habitat

Of the 266 terrestro-aquatic amphibians, 185 were males and 81 were females. Thirty-three of 185 (17.8%) terrestro-aquatic males were found to harbor a mean number of 5.8 w/h. Terrestro-aquatic females were found to have a much higher incidence of flatworms. Twenty-eight of 81 (34.6%) were infested and had a greater parasite load of 6.0 w/h.

On an individual species basis, three of the 15 species of amphibian had a higher incidence and a higher parasite load in the females. Two of five (40.0%) female Rana palustris had a parasite load of 9.5 w/h, when infested with Brachycoelium elongatum, as compared to three of 16 (11.7%) males with a parasite load of 4.0 w/h, when infested with the same trematode (Fig. B). Three of 12 (25.0%) female Bufo americanus were found to harbor a mean parasite load of 10.0 Glypthelmins quieta, while only four of 44 (9.1%) males were found to carry a parasite load of 1.5 w/h with the same trematode (Fig. 2).

Two of 13 (15.4%) female Rana catesbeiana were found to harbor a parasite load of 1.0 w/h, when infested with the cestode, Cylindrotaenia americana, while 10 males were found to be cestode free (Fig. 6a).

Three species of amphibia had a higher incidence and a higher parasite load in the males. Two of 12 (16.7%) male Rana sylvatica were parasitized by the trematode, Brachycoelium elongatum, and had a parasite burden of 2.5 w/h, while their three female counterparts were not infested with this trematode (Fig. 5a). When infested with the trematode, Megalodiscus temperatus, one of 12 (8.3%) male R. sylvatica were found to harbor a parasite load of 1.0 w/h, while none of the females were infested with this parasite (Fib. 5c). Thirteen of 20 (65.0%) male Hyla crucifer were parasitized with a mean of 5.9 Glypthelmins quieta individuals per host, while only nine of 15 (60.0%) females had a parasite load of 4.1 w/h when infested with the same trematode (Fig. C).

Three species of terrestro-aquatic amphibia had a higher incidence in the females, but a higher parasite load in the males. One of three (33.3%) female Rana sylvatica, with a parasite load of 7.0 w/h, were infested with the trematode, Brachycoelium obesum, while only one of 12 (8.3%) males were carrying a parasite burden of 11.0 w/h (Fig. 5b). Six of 13 (46.2%) female Rana catesbeiana were found to harbor a parasite load of 2.2 Megalodiscus temperatus per host, while the same trematode infested three of 10 (30.0%) males with a parasite load of 3.0 w/h (Fig. 6b). Two of six (33.3%) female Rana

clamitans melanota were found with a mean of 4.0 Megalodiscus temperatus per host (Fig. 6).

One of the species of terrestro-aquatic amphibia had an equal male vs. female incidence with the females carrying the heavier infestation. Five of 12 (41.7%) female Ambystoma maculatum were infested with 12.2 Brachycoelium elongatum per host, while five of 12 (41.7%) male counterparts only averaged 7.8 B. elongatum per host (Fig. 1a).

One terrestro-aquatic species had an equal male vs. female incidence with the males harboring a higher parasite load. Three of 12 (25.0%) female A. maculatum harbored a parasite load of 6.7 B. obesum per host, while three of 12 (25.0%) males carried a mean of 15.7 w/h (Fig. 1b).

Four species of terrestro-aquatic amphibia; Eurycea bislineata, Gyrinophilus porphyriticus, Pseudacris brachyphona, and Rana pipiens, were found to be flatworm free.

Aquatic Habitat

In the aquatic amphibians, nine of 24 (27.5%) female individuals were found to carry 3.1 flatworms per host. Twenty-one of 69 (30.4%) males were infested with a parasite load of 4.3 platyhelminths per host. Generally, incidence was higher in the females and the parasite load was higher in the males. However, on a strict species basis, this does not hold true. Eight of 24 (33.3%) female Notophthalmus viridescens were infested with 3.8 Plagitura parva per host, while 15 of 69 (21.8%) males were harboring a parasite

load of 5.5 w/h when infested with the same trematode (Fig. 4a). One of 24 (4.2%) female N. viridescens were infested with a parasite load of 1.0 Bothriocephalus rarus per host, while the same cestode infested seven of 69 (10.1%) male individuals with a parasite load of 1.7 w/h (Fig. 4b).

Terrestrial Habitat

The two species of terrestrial amphibia, Plethodon glutinosus and Plethodon richmondi, were found to be free of intestinal flatworms.

Figure 1.

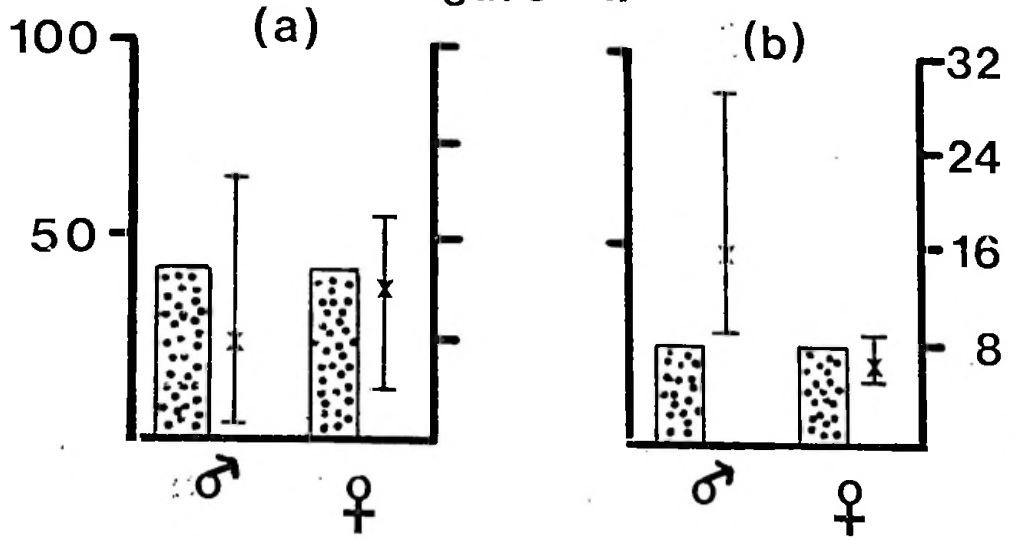
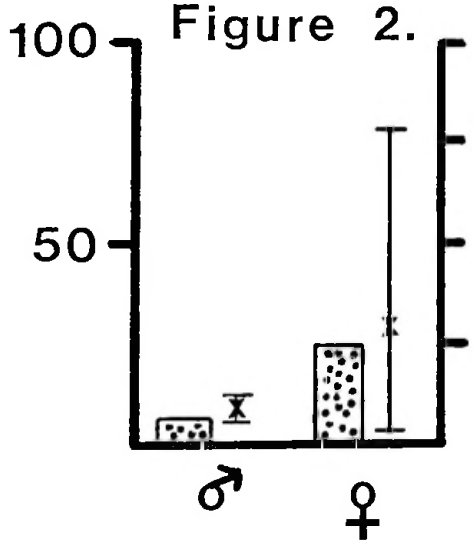


Figure 2.



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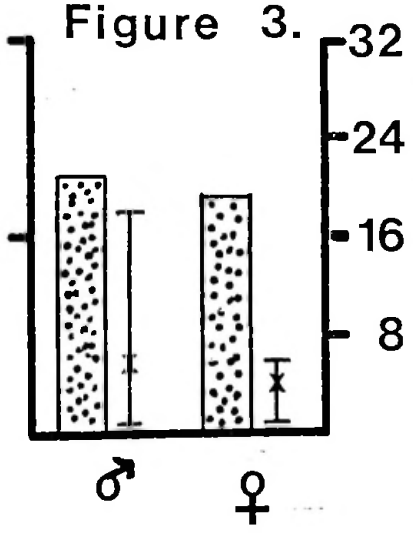

INCIDENCE (%)

MEAN NUMBER WORMS/HOST

Figure 4.

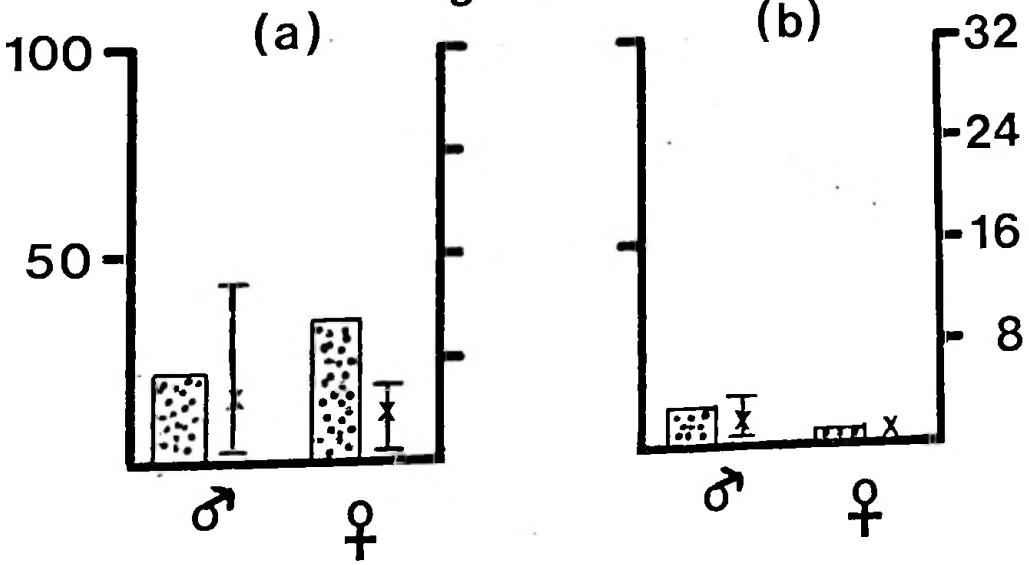

Figure 5a. Stippled bar represents incidence of *Brachycoelium elongatum* in *Rana sylvatica*. X represents the mean number of *B. elongatum* per host. Verticle lines represent the range.

Figure 5b. Stippled bar represents incidence of *Brachycoelium obesum* in *Rana sylvatica*. X represents the mean number of *B. obesum* per host. Verticle lines represent the range.

Figure 5c. Stippled bar represents incidence of *Megalodiscus temperatus* in *Rana sylvatica*. X represents the mean number of *M. temperatus* per host. Verticle lines represent the range.

Figure 6a. Stippled bar represents incidence of *Cylindrotaenia americana* in *Rana catesbeiana*. X represents the mean number of *C. americana* per host. Verticle lines represent the range.

Figure 6b. Stippled bar represents incidence of *Megalodiscus temperatus* in *Rana catesbeiana*. X represents the mean number of *M. temperatus* per host. Verticle lines represent the range.

Figure 5.

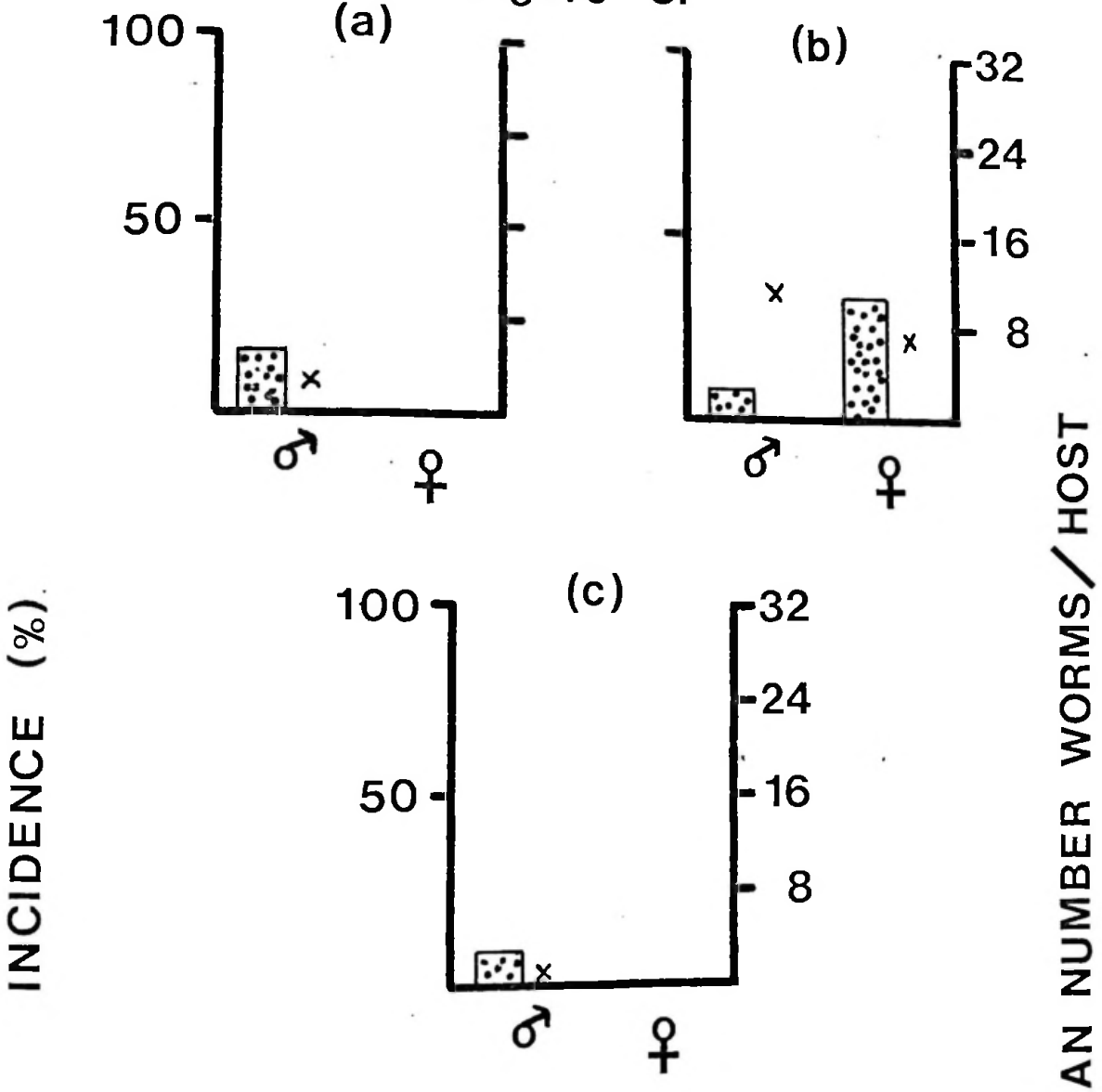

Figure 6.

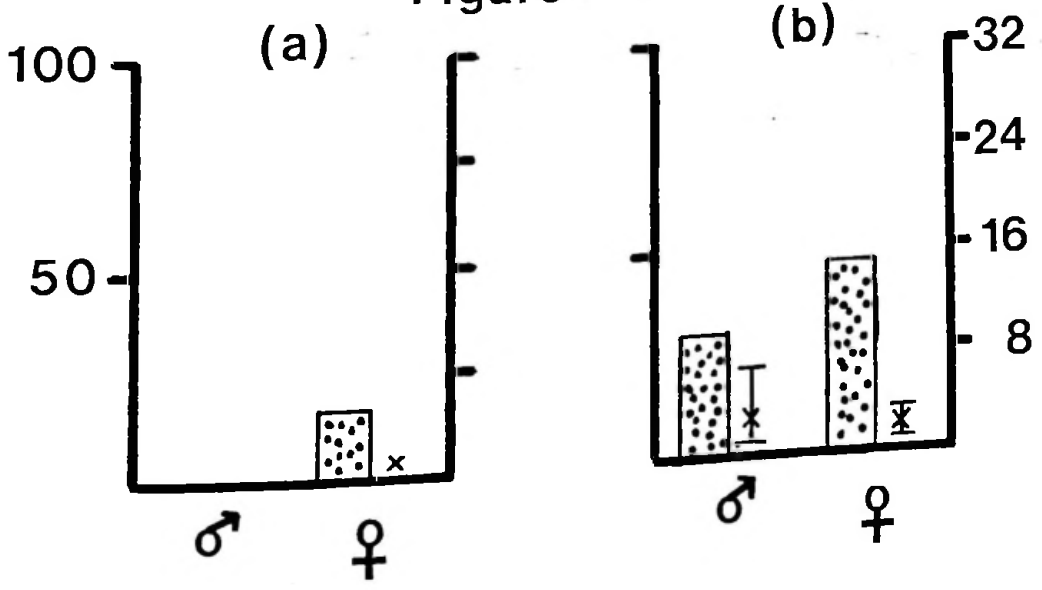

Figure 7. Stippled bar represents incidence of *Megalodiscus temperatus* in *Rana clamitans melanota*. X represents the mean number of *M. temperatus* per host. Verticle lines represent the range.

Figure 8. Stippled bar represents incidence of *Brachycoelium elongatum* in *Rana palustris*. X represents the mean number of *B. elongatum* per host. Verticle lines represent the range.

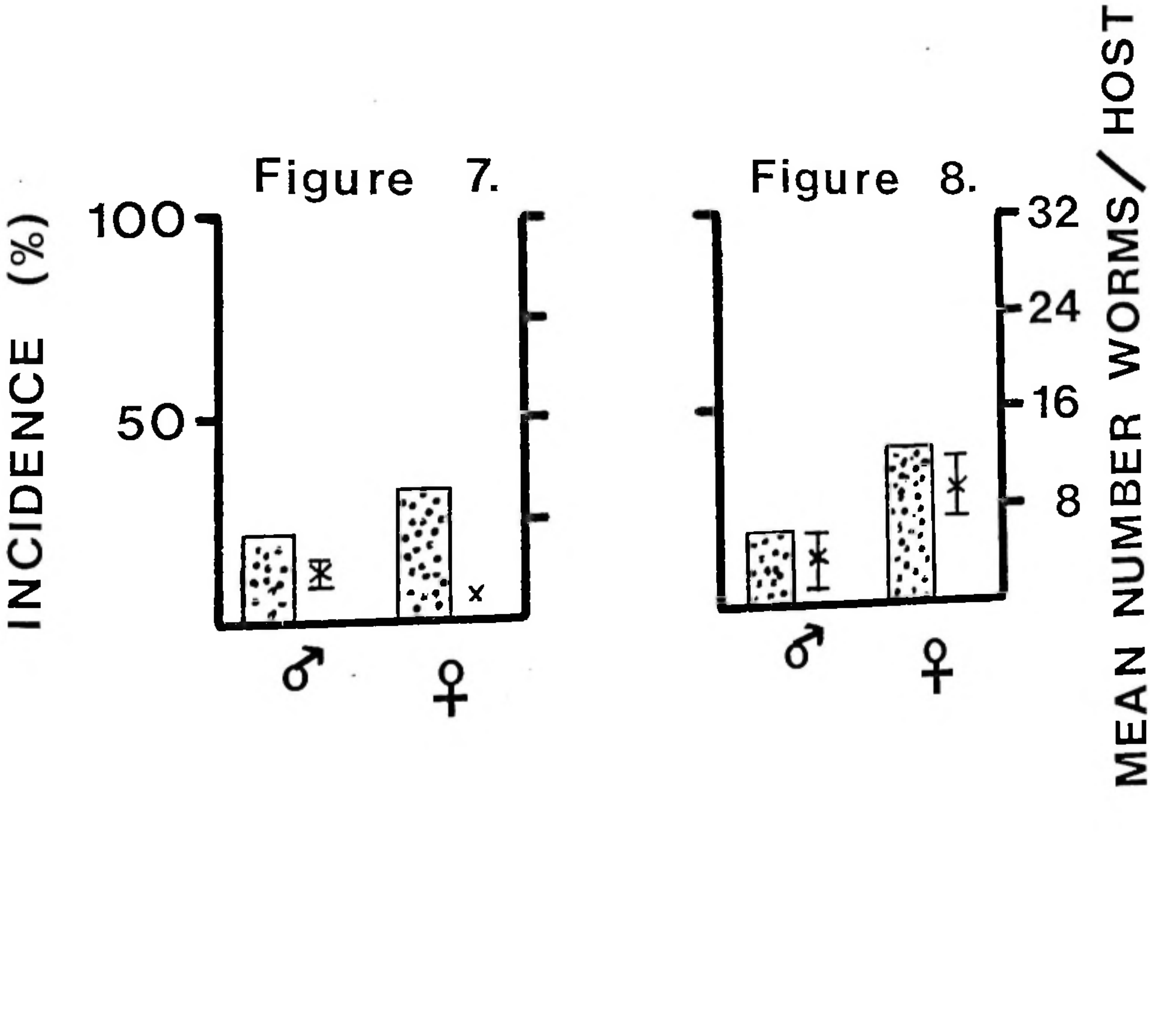

Figure 7.
Figure 8.

DISCUSSION

There appears to be a variation in the infestation rates (incidence of parasitism) and the intensity of the parasite load depending on the sex and habitat of the amphibian.

In the present study, incidence of infestation was higher in the female amphibians, while the parasite load was smaller than in male amphibians. While this varied slightly from one amphibian species to another, it generally held true (Figs. 1 thru 8). This was not altogether surprising, however, since Joy and Mills (1975), working in West Virginia, found 78.6% Ambystoma opacum males infested by Brachycoelium as compared to 74.3% of the females carrying this trematode parasite. Information of a similar nature (i.e. higher incidences of parasitism in females) is available, not from work on amphibians, but rather investigations in fish populations. For example, in the three subspecies of blacknose dace, Rhinichthysi atratulus, in West Virginia, females consistently showed higher incidences of infestation by Neascus rhinichthysi than males, (Tarter and Joy, 1976).

However, host sex does not always determine relative incidence levels. Lawrence (1970) indicated no clear pattern of fish sex relationship for Trigonodistomum attentuatum, a digenetic trematode, in the white sucker, Catostomus commersoni. Mackiewicz (1972), in his review of the Caryophyllidae, an order of monozoic cestodes, found that

relationships between host sex and incidence were difficult to determine. Mackiewicz felt that variations of spawning seasons and feeding habitats were the most likely reasons for any variation in incidence levels.

It was found in the present investigation, that the terrestro-aquatic amphibians harbor more species of platyhelminths have a greater mean number of worms per host, have a greater number of multiple infestations, and harbor a greater number of species in these multiple infestations than aquatic or terrestrial amphibians. The aquatic species had a greater parasite load than the terrestro-aquatic or terrestrial species. The terrestrial amphibians were found to be free of intestinal flatworms.

Pearse (1924) concluded that fishes with a most restricted habitat have a greater infestation, but carry fewer species of parasites and have a smaller mean number of worms per host, where as (1924a) those with a variety of habitats are correlated with a large number of species, buy a smaller incidence. Rankin (1937) concluded from his studies in North Carolina, that terrestro-aquatic salamanders have a variety of habitats, and carry more species of parasites than do other salamanders. He also found that the aquatic salamanders were more heavily parasitized than terrestrial ones. Little (1928) found aquatic amphibians more heavily infested than terrestrial ones. Rumbold (1928) and Brandt (1936), dealing with turtles and salientia respectively, came to this same general conclusions.

Rankin (1937) also concluded that the absence of

platyhelminths from terrestrial salamanders suggests that a reduction of time spent in water has minimized the chance of infestation with various parasites. Platyhelminths are probably confined to aquatic habitats by their dependence on aquatic invertebrates as intermediate hosts, corroborated by Cheng's General Parasitology. Bouchard (1951) concluded that since most parasites depend on aquatic invertebrates as intermediate hosts, these parasites would be expected to occur most frequently in aquatic salamanders, less so in those which only occasionally frequent water, and uncommon in terrestrial hosts, whose life, following transformation from the larva, is spent entirely on land. He also found that among cestodes, the greatest incidence is among aquatic, just as was found in this study (Figs. 4b and 6a).

On the other hand, Mann (1932) and Pearse (1932) found that terrestrial species of salamanders have about as many parasites as aquatic species. Rankin (1945) in Massachusetts, indicated that the aquatic newt, Notophthalmus viridescens, harbored more species of parasites than did the terrestro-aquatic and terrestrial species of salamanders.

Host specificity of the platyhelminths for the species of amphibians in this study, would, in part, account for the presence of a platyhelminth in one species and not in the other, confirmed by Cheng (1973).

The author concludes that the cestodes found, Bothriocephalus rarus and Cylindrotaenia americana, have their greatest incidence in aquatics as both use aquatic invertebrates

as intermediate hosts (Schmidt, 1970). Three of the trematodes, Glypthelmins quieta, Megalodiscus temperatus, and Plagitura parva, would also be restricted to water as they all use aquatic snails as intermediate hosts (Schell, 1970).

Five of the platyhelminths in this study, Bothriocephalus rarus, Cylindrotaenia americana, Glypthelmins quieta, Megalodiscus temperatus, and Plagitura parva, are, for the first time, being reported from West Virginia. This is not surprising, however, as all five have been reported from various states across the United States. Four new host records are also reported: Brachycoelium elongatum, B. obesum, and Megalodiscus temperatus from the woodfrog, Rana sylvatica, and B. elongatum from the pickerel frog, Rana palustris.

In summation, female amphibians have a slightly higher incidence of infestation and a slightly lower parasite load than male amphibians. Aquatic amphibians are usually restricted, more or less, to one specific habitat and would be expected to have a higher incidence of infestation from the parasites present in that habitat. Terrestrial amphibians, though also restricted to a single habitat, are in one which the number and species of parasites are usually low. Absence of infestation in this study, may be due to the low numbers of terrestrial amphibians collected. The terrestro-aquatic amphibians migrate frequently, have a variety of habitats, and consequently harbor numerous species of parasites.

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