

# Parasites of the Bullfrog in Indiana<sup>1</sup>

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## Abstract

Parasitological examinations were made of 72 bullfrogs from four Indiana areas during May through September 1970, of which 91.7 per cent contained parasites. Parasites found included: (protozoans) *Hexamita intestinalis*, *Nyctotherus cordiformis*, *Trypanosoma rotatorum*, and an unidentified species of *Trichomonas*; (trematodes) *Gorgoderia amplicava*, *Gorgoderina attenuata*, *Haematoloechus breviplextus*, *Haematoloechus longiplextus*, and *Glypthelmins quieta*; and (nematodes) *Cxysomatium longicaudata* and one unidentified species. Two species of Hirundinae, *Placobdella rugosa* and *Glossiphonia stagnalis*, were found apparently as parasites.

## Introduction

Brandt (2), in a comprehensive study of the parasites of several species of North Carolina frogs, has presented the only extensive review of the parasites of bullfrogs, *Rana catesbeiana* Shaw. He found the bullfrog to harbor a number of helminth and protozoan parasites and one arthropod. Reports of parasites of bullfrogs in Indiana are few. Cort (5) observed the lung flukes *Haematoloechus longiplextus* and *H. breviplextus* from a North Judson, Indiana, bullfrog, and Walton (21) found cysts of the nematode *Physaloptera ranae* in the stomach and intestinal walls in bullfrogs of northern Indiana. Krull (11) also found *H. longiplextus* in an Indiana bullfrog. More recently, Myer (17) reported metacercariae of the fluke *Mesostephanus kentuckiensis* in Ohio bullfrogs. Since Cable (3) reported cercariae of *M. kentuckiensis* in snails from McCormick's Creek and Clifty Creek, Indiana, this trematode may occur in Indiana bullfrogs, although none has yet been reported.

## Materials and Methods

The bullfrogs examined in this study were collected from four Indiana Fish and Game Areas: Glendale, in southwestern Indiana (Daviss County), Crosley Lake in the southeast (Jennings County), Willow Slough in the northwest (Newton County), and Tri-County in the northeast (Kosciusko and Noble Counties). Frogs were collected at night by hand or by a dip net.

Five monthly collections were made at each of the four study areas. The frogs were taken to the laboratory at Ball State University and placed in a refrigerator until examined. Some frogs were dissected and searched for parasites within 24 hours after collection, but others were held for as long as 10 days before examination.

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Before examination the bullfrogs were either pithed or beheaded. The heart, lungs, stomach, large and small intestines, cloaca, liver, gall bladder, kidneys, and urinary bladder were examined. Before removing the organs, the host was searched for any coelomic parasites, using a dissecting microscope. Upon removal, each organ was placed individually in frog Ringer's Solution in a watch glass in order to retain any parasites which might leave the organ.

Each organ was then opened or teased apart under a binocular dissecting microscope. Except for protozoans, all parasites found were counted and representatives preserved for permanent mounting. Smears were made of the contents of the intestines and of peripheral, renal, and cardiac blood for examination under a compound microscope.

Blood smears were made by using Wright's stain or by fixing in 95% alcohol and staining with Giemsa's stain. Intestinal smears were fixed in Schaudinn's fluid and stained with Heidenhein's iron haematoxylin.

All worms were washed thoroughly in physiological saline before fixing. Trematodes were relaxed in distilled water and then fixed in either 10% formalin, formol-acetic-alcohol (F.A.A.), or Bouin's solution which had been heated to bubbling but not vigorous boiling. Nematodes were dropped into warm F.A.A., 10% formalin, or 70% acid-alcohol for fixation. Leeches were fixed in 70% alcohol using the method described by Mann (14). For permanent storage, all worms were transferred to either 70% alcohol plus 5% glycerine or 5% formalin plus enough glycerin to make a 5% solution, depending upon the method of fixation used.

Stains used for trematodes and nematodes included Grenacher's Alcoholic Borax-Carmine, Mayer's MCl Carmine, either with or without Fast Green counterstaining, and Harris' or Delafield's Haematoxylin. After staining, the specimens were destained, dehydrated, cleared, and mounted in Permount.

Cort (4, 5), Harwood (8), Higgins (9), Kudo (12, 13), Mann (14), Miller (16), Miller (15), Najarian (18), and Yamaguti (23) were used for making identifications.

## Results

Seventy-two bullfrogs were examined for parasites between May and September of 1970, 20 each from Glendale and Crosley Lake and 16 each from Willow Slough and Tri-County. Those frogs 100 mm or more in snout-vent length were classed as adult, and those below 100 mm were considered juveniles. According to Wright and Wright (22), 100 mm is approximately the minimum breeding size. The range of length of the 49 adult frogs examined was 100 mm to 142 mm, with an average length of 115.6 mm. The 23 juveniles ranged from 47 mm to 77 mm, the average being 62 mm.

Of the 72 bullfrogs examined, 91.7% were found to be infested by one or more species of parasites (Table 1). Four species of protozoans

TABLE 1. *Per cent of bullfrogs infested, by size, collection areas, and month of collection.*

Number of frogs examined	Size		Collection Areas				Month of Collection					
	Adult (49)	Juv. (23)	Total (72)	C.L. (20)	Glen. (20)	T.C. (16)	W.S. (16)	May (9)	June (19)	July (17)	August (13)	Sept. (14)
<i>Hexamita intestinalis</i>	69.4	87.0	75.0	85.0	95.0	62.5	50.0	55.6	68.4	82.4	76.9	85.7
<i>Trichomonas sp.</i>	85.7	87.0	86.1	85.0	90.0	68.75	93.75	66.7	84.2	100.0	92.3	78.6
<i>Nyctiotherus cordiformis</i>	34.7	30.4	33.3	45.0	20.0	25.0	43.75	44.4	42.1	29.4	30.8	21.4
<i>Trypanosoma rotatorum</i>	6.12	4.35	5.56	0.0	0.0	18.75	6.25	0.0	5.26	17.65	0.0	0.0
<i>Gorgodera amplicava</i>	28.6	13.0	23.6	5.0	30.0	18.75	43.75	11.1	10.53	41.2	30.8	21.4
<i>Gorgoderina attenuata</i>	53.1	13.0	40.3	45.0	55.0	43.75	12.5	33.3	47.4	29.4	46.2	28.6
<i>Haematoloechas breviplexus</i>	22.5	13.0	19.4	10.0	0.0	37.5	37.5	0.0	15.8	23.5	15.4	21.4
<i>Haematoloechas longiplexus</i>	14.3	17.4	15.3	5.0	50.0	0.0	0.0	0.0	10.53	23.5	0.0	7.14
<i>Glyphelmims quita</i>	8.16	8.7	8.33	0.0	10.0	18.75	6.25	11.1	15.8	11.8	0.0	0.0
<i>Ozysomatium longicaudata</i>	10.2	4.35	8.33	10.0	15.0	6.25	0.0	0.0	10.53	17.65	7.69	0.0
Nematode (unidentified)	2.04	0.0	1.39	0.0	0.0	0.0	6.25	0.0	0.0	5.88	0.0	0.0
Leeches <sup>2</sup>	0	0	0	0	0	4	24	6	11	10	1	0

<sup>2</sup>Actual number of leeches found.

were found: *Hexamita intestinalis*, *Nyctotherus cordiformis*, *Trypanosoma rotatorum*, and an unidentified *Trichomonas* sp. Trematodes included the bladder flukes *Gorgodera amplicava* (3.817)<sup>2</sup> and *Gorgoderina attenuata* (0.772), the lung flukes *Haematoloechus breviplexus* (0.780) and *H. longiplexus* (0.793), and the intestinal fluke *Glypthelmins quieta* (1.913). The nematodes found were *Oxysomatium longicaudata* (2.822) and one unidentified species. No cestodes or acanthocephalans were encountered.

Two species of leeches, *Placobdella rugosa* and *Glossiphonia stagnalis*, were found in the collecting jars, but were never seen attached to the frogs. Probably the leeches were on the frogs at the time of collection.

### Discussion

In addition to the primary species-specific limitations, many factors influence the kinds and numbers of parasites in a host: age, geographic distribution, diet, the presence of suitable intermediate hosts, seasonal aspects of the life cycle, etc. The operation of some of these factors as they apply to parasitism in the bullfrog has been reported by other authors, and data from the present study suggest the operation of others. For example, bullfrogs were introduced into the Rocky Mountain region relatively recently, but their parasites do not yet seem to be well established there (20). This may well be due to the absence of suitable intermediate hosts.

Although the samples are small, the data in Table 1 suggest some seasonal variation in the abundance of parasites, most species showing peaks of occurrence in June, July, or August. Leeches were most abundant in June and July, and the recording of trypanosomes only in these 2 months may well be related to the abundance of the leeches, their possible intermediate hosts.

Although most parasites were found in all four collecting areas (Table 1), there were two possibly significant exceptions. The blood protozoan, *Trypanosoma rotatorum*, was found in only the two northern collecting areas. This may again be due to the presence in these areas of large numbers of leeches. Leeches have been reported as intermediate hosts of trypanosomes in certain other amphibians: *Trypanosoma diemyctyli* in the Red-spotted Newt, *Notophthalmus viridescens* (1), and in various European frogs (6).

The other exception is the lung fluke *Haematoloechus longiplexus*, which was found only in the two southern areas.

With respect to adult frogs *versus* juveniles, only in two species of parasites is there a notable difference in the overall amount of infestation. *Hexamita intestinalis* was found more often in juvenile bullfrogs than in adults (86.9% incidence compared to 69.5%). The

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<sup>2</sup>Average number of parasites per individual bullfrog.

bladder fluke, *Gorgoderina attenuata*, was found in over half (53.1%) of the adult frogs, but in very few of the smaller frogs (13%).

Multiple infestation in bullfrogs is a common occurrence and has been reported by Cort (5), Miller (15), and Najarian (18). In this study, some individual frogs contained six different species of parasites: three of the four protozoans and three of the five trematodes, although a typical frog would harbor one or two species of protozoans and a species of fluke.

Two species of leeches, *Placobdella rugosa* and *Glossiphonia stagnalis*, are here reported for the first time as parasites of bullfrogs.

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### Literature Cited

1. BARROW, J. H. 1953. The biology of *Trypanosoma diemyctyli* (Tobey). I. *Trypanosoma diemyctyli* in the leech *Batrachobdella picta* (Verrill). Trans. Amer. Microbiol. Soc. 72:197-216.
2. BRANDT, B. B. 1936. Parasites of certain North Carolina Salientia. Ecol. Monogr. 6:491-532.
3. CABLE, R. M. 1937. Cercaria of Indiana. A preliminary note on larval trematodes from McCormick's Creek with a description of three new species. Proc. Indiana Acad. Sci. 47:227-228.
4. CORT, W. W. 1912. North American frog bladder flukes. Trans. Amer. Microbiol. Soc. 31:151-166.
5. ————. 1915. North American frog lung flukes. Trans. Amer. Microbiol. Soc. 34:203-240.
6. FRANCA, C. 1908. Le cycle evolutif des trypanosomes de la grenouille (*T. costatum*, *T. rotatorium*, and *T. inopinatum*). Arch. do R. Inst. Bact., Camara Pestana. 2:89-93.
7. GOODCHILD, C. G. 1950. Establishment and pathology of gorgoderid infections in anuran kidneys. J. Parasitol. 36:439-446.
8. HARWOOD, P. D. 1932. The helminths parasitic in the Amphibia and Reptilia of Houston, Texas, and vicinity. Proc. U.S. Nat. Mus. 81:1-71.
9. HIGGINS, H. 1929. Variations in the *Nyctotherus* (Protozoa, Ciliata) found in frog and toad tadpoles and adults. Trans. Amer. Microbiol. Soc. 48:141-157.
10. JAHN, T. L., and F. F. JAHN. 1949. How to know the Protozoa. Wm. C. Brown Co. Publ. Dubuque, Ia. 234 p.
11. KRULL, W. H. 1931. Life-history studies on two frog lung flukes, *Pneumoneccs medioplexus* and *Pneumobites parviplexus*. Trans. Amer. Microbiol. Soc. 50:215-277.

12. KUDO, R. R. 1922. On the Protozoa parasitic in frogs. Trans. Amer. Microbiol. Soc. 41:59-76.
13. ————. 1966. Protozoology. 5th Ed. Charles C. Thomas. Springfield, Ill. 1174 p.
14. MANN, K. H. 1962. Leeches (Hirudinea). Their structure, physiology, ecology, and embryology. Vol. 2. Pergamon Press. New York, N. Y. 201 p.
15. MILLER, E. L. 1930. Studies on *Glythelmins quieta* Stafford. J. Parasitol. 16:237-243.
16. MILLER, J. A. 1937. A study of the leeches of Michigan with keys to orders, suborders, and species. Ohio J. Sci. 37:85-90.
17. MYER, D. G. 1960. On the life history of *Mesostephanus kentuckiensis* (Cable, 1935) n. comb. (Trematoda: Cyathocotylidae). J. Parasitol. 46:819-832.
18. NAJARIAN, H. H. 1955. Trematodes parasitic in the Salientia in the vicinity of Ann Arbor, Michigan. Amer. Midland Natur. 53:195-197.
19. SWEZY, O. 1915. Binary and multiple fission in *Hexamitus*. Univ. Calif. Pub. Zool. 16:71-88.
20. WAITZ, J. A. 1962. Parasitic helminths as aids in studying the distribution of species of *Rana* in Idaho. Trans. Ill. State Acad. Sci. 54:152-156.
21. WALTON, A. C. 1931. Notes on some larval nematodes found in frogs. J Parasitol. 17:228-229.
22. WRIGHT, A. H., and A. A. WRIGHT. 1949. Handbook of frogs and toads of the United States and Canada. Comstock Publishing Company, Inc. Ithaca, N. Y. 640 p.
23. YAMAGUTI, S. 1961. Systema helminthum. Interscience Publishers, Inc., New York. N. Y. (5 volumes). 4818 p.
24. ZEBROWSKI, G. 1922. The occurrence of secondary parasitism in the frog. Proc. Indiana Acad. Sci. 32:205-208.