

Possibilities of *Manodistomum syntomentera* as a cause of deformities in *Rana pipiens*.

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ABSTRACT

Complete necropsies were performed during summer 1997 on forty-six northern leopard frogs (*Rana pipiens*) collected from central Minnesota (Stearns Scout Reservation, Wright County). Parasites found included *Megalodiscus* (rectum), *Protoopalina* (rectum), and *Gorgoderina* (urinary bladder). Four types of metacercariae were also recovered including *Manodistomum* (subdermal), *Diplostomulum* (muscles), Echinostomes (kidney) and Heterophyids (unencysted along femurs). On August 10, 1997, 200 *R. pipiens* were collected from the Clearwater River (Camp Stearns) and examined for morphological deformities. Of the 246 frogs examined throughout the summer, six *R. pipiens* had physical deformities (2.4%). Deformities included: missing digits, extra digits, partially formed extremities, incomplete limbs and misshapen leg bones. Since 1995, there have been many confirmed reports of frogs with physical deformities in Minnesota, Wisconsin and numerous other states. One current hypothesis proposes that metacercariae of digenetic trematodes encyst in developing limb buds of tadpoles, and the physical disruption results in supernumerary or missing limbs when frogs emerge at metamorphosis. Epidemiological evidence assembled during this study suggests that parasites do not play a role in frog deformities.

INTRODUCTION

Since 1995 there has been many confirmed reports of frogs with deformities in Minnesota, Wisconsin and several other states. Physical deformities include missing limbs, supernumerary limbs, incompletely formed digits, missing or supernumerary digits, missing eyes, and misplaced eyes. Frogs and other amphibians occupy a unique ecological niche inhabiting both water and land. Their unique place in nature renders them susceptible to a variety of environmental factors. Their undeniable dependence on water for breeding and early development makes frogs valuable biological indicators. They could be affected by a variety of environmental stimuli throughout development. The fact that deformed frogs are appearing is alarming in itself, but further implications apply to these important components of the food web. Declining populations can have repercussions that could affect fish, raccoon, heron, egret, and fox populations, in addition to reduced pressures on insect populations. The deformities may be indicators of an agent in the water that could have adverse effects on humans as well. The sharp increase in reports of deformed frogs has sparked wide public interest and concern and many theories currently exist regarding the nature of these deformities.

Laboratory work conducted by Sessions and Ruth (1990) indicates that physical obstructions may be the cause of limb malformations. While conducting research on another study in California, several deformed Pacific treefrogs (*Hyla regilla*) were found.

Inspection of these frogs in the laboratory identified a strong correlation between the presence of metacercarial cysts and limb malformations (Sessions and Ruth, 1990). Sessions and Ruth (1990) suggest metacercarial cysts (and the associated developing and regenerating tissues) of a digenetic trematode produce enough physical obstruction to interfere with limb development resulting in malformed, missing, or supernumerary limbs. In an attempt to simulate this physical disruption to the limb bud, Sessions and Ruth experimentally implanted resinous beads the same size as metacercarial cysts into the limb buds of *Xenopus laevis* tadpoles and deformities resulted. Metacercariae associated with frog deformities have been identified as *Manodistomum syntomentera* (Sessions and Ruth, 1990).

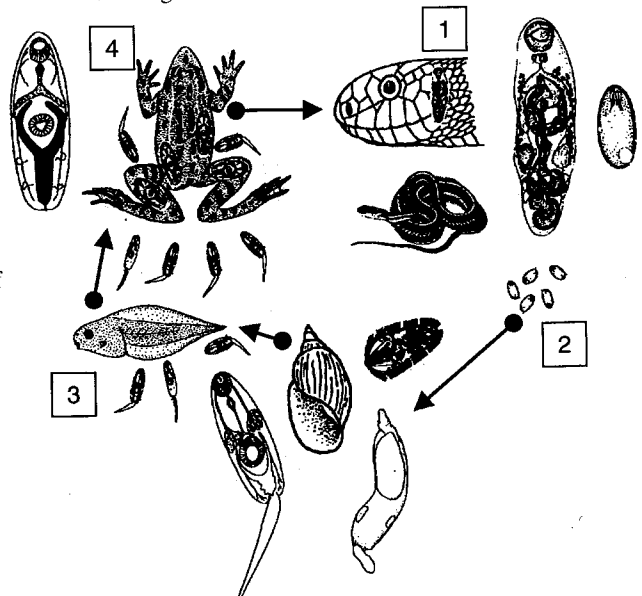
Life cycle of *M. syntomentera*

M. syntomentera uses the eastern garter snake (*Thamnophis sirtalis sirtalis*) as the definitive host and pond snails (*Physia gyrina*) and then frogs as the first intermediate and second intermediate hosts, respectively (Ingles, 1933) (Figure 1). The eggs of *M. syntomentera* are passed in the feces of the snake and then must be ingested by snails. The eggs hatch in the digestive tract of the snail and then bore through the wall of the snail's gut and become sporocysts. The sporocyst produce cercariae (a free swimming form) which emerge from the snail, seek out tadpoles, and preferentially attack the tail end. The cercariae burrow into the skeletal muscle tissue of the tadpole, form a protective cyst, and enter a dormant state (metacercaria). The metacercariae must survive until the frog (or tadpole) is eaten by a snake. The cyst wall is digested in the stomach of the snake, the young adults emerge, and migrate to the esophagus of the snake where they mature.

Laboratory work with parasites has not been attempted, to the best of my knowledge, and no known fieldwork has been previously conducted to determine if these parasites are indeed present in Minnesota frogs.

Figure 1.
Diagram showing the life cycle of the digenetic trematode *Manodistomum syntomentera*. 1. Adult in mouth and esophagus of garter snake. 2. Eggs shed in feces of snake and ingested by snail. 3. Cercariae burrow into tissue of tadpole and encyst. 4. Metacercarial cysts remain until the frog (or tadpole) is eaten by the snake.

(Courtesy of Dan Sutherland)



Study Area:

The site chosen for this study is a 1200-acre Boy-Scout camp located in Wright County in central Minnesota (Figure 2). This property contains shoreline on four different lakes, and is bisected by the Clearwater River. Several swamp and marsh areas are also present on the property, making it an ideal habitat for amphibians.

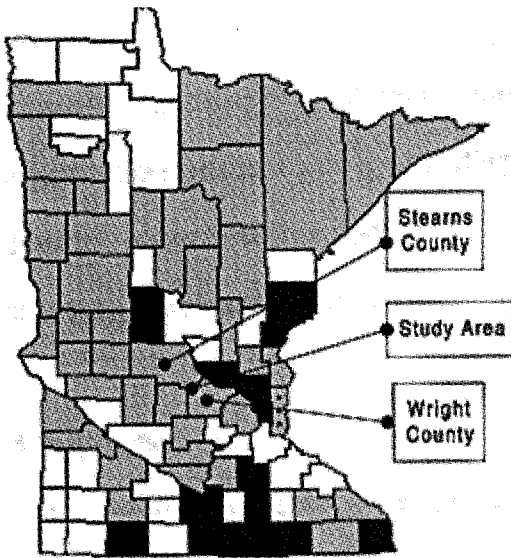


Figure 2. Minnesota county map showing study site. Degree of shading indicates reported cases of deformed frogs.

MATERIALS AND METHODS

Collection of Specimens

Forty-six northern leopard frogs (*R. pipens*) were collected and examined for parasites during June, July, and August 1997, and 200 additional frogs were collected and examined for deformities. Three eastern garter snakes were also collected and examined to confirm the presence of adult *M. syntomentera* at the study site. Frogs and snakes were collected using standard techniques (Conant, 1975). Collection of frogs was most easily accomplished at night, while snakes were collected during mid to late morning. Species were identified using an appropriate taxonomic key (Behler, 1996). Frog length was measured from the tip of the nose to the tip of the urostyle and snake length was measured from nose to tail.

Necropsies

Before dissection, I visually inspected the external surfaces of the frogs. All major tissues and viscera were inspected with a dissecting microscope (including all skeletal muscle, bladder, gastrointestinal tract, and lungs). Snake necropsies involved looking at only the gastrointestinal tract.

Sample Storage and Preparation

All trematodes and tapeworms collected were preserved in a ten-percent buffered formalin solution and nematodes were preserved in seventy-percent glycerin alcohol. All parasites were stored in glass vials labeled with a number corresponding to the parasites' host. Trematodes were stained with Semichon's Acetic Carmine using a standard staining scheme (Dailey, 1996). Nematodes were mounted using a glycerin jelly double coverslip method. Parasites were identified with the appropriate taxonomic keys (Ulmer, 1970)(Schell, 1970)(Yorke and Maplestone, 1969).

RESULTS

Necropsies performed on 46 leopard frogs (*Rana pipiens*) revealed large infestations of parasites in nearly all hosts. Six frogs of 246 studied (2.4%) had deformities including partial limbs, missing digits, extra digits, and misshapen leg bones. Prevalence and mean intensity were determined for the parasites found (Table 1).

Table 1. Prevalence and mean intensity of parasites found in normal and deformed *Rana pipiens*.

Parasite	Normal Frogs		Deformed Frogs	
	Prevalence ^a	Mean Intensity	Prevalence ^b	Mean Intensity
<i>Manodistomum</i>	2.5%	116	17.0%	1
<i>Diplostomulum</i>	52.5%	84	17.0%	6
Heterophyid	42.5%	102	50.0%	58
Strigeids	10.0%	185	33.0%	19
<i>Haematoloechus</i>	25.0%	4	0.0%	0
<i>Megalodiscus</i>	75.0%	7	17.0%	2
<i>Gorgoderina</i>	5.0%	1	17.0%	1
Proteocephalan	2.5%	1	17.0%	1
<i>Oswaldocruzia</i>	7.5%	1	0.0%	0
<i>Protoopalina</i>	32.5%	287	17.0%	150
<i>Nyctotherus</i>	2.5%	50	0.0%	0

Prevalence of infection expressed as percent of the whole. Mean intensity is average number of parasites per infected host.

^a n=40

^b n=6

Of the 46 total frogs examined, only 1 had no parasites. Of all parasites identified, *Megalodiscus* was the most prevalent. Adult *Manodistomum* were found in *T. sirtalis sirtalis*, indicating that this digenetic trematode is indeed geographically present at Camp Stearns. However, *Manodistomum* metacercariae were only found in two (one which was deformed) of the 46 frogs examined (prevalence 4.3%). Other metacercariae were much more prevalent such as *Diplostomulum* (47.8%) and Heterophyids (43.5%). Overall, all parasitic infections were more prevalent and more intense in the normal frogs than in the deformed frogs. Heterophyids were the most prevalent in the deformed frogs (50%), but not much more than in the normal frogs (42.5%).

CONCLUSION

Although adult *Manodistomum* were present in snakes in the area, the metacercariae were not common in the frogs examined. If physical obstruction is indeed the cause for these deformities, other parasite metacercariae may be the causative agent (e.g., *Diplostomulum* and Heterophyid), or there may be more than one parasite that is able to elicit such a developmental response in the tadpoles. However, the fact that these parasites are more prevalent in normal frogs than in deformed frogs would indicate that parasites are not the causative agent of abnormal limb formation in *R. pipiens*. Unfortunately, the sample size of the deformed frogs (n=6) is quite small. Further research in this area should include laboratory-controlled infections. The plastic beads that were inserted by Sessions and Ruth (1990) may have leached teratogens into the limb bud that produced the observed deformities. Infections with various parasites at various stages of tadpole development would show more conclusively if physical obstructions caused by metacercariae are one causative agent of these observed deformities.

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