Survey of Pathogens and Parasites in Fish from Devils Lake and Lake Ashtabula in North Dakota, and Lake Traverse, South Dakota.

2008 Survey Results

Technical Report 11-01
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minnow, walleye, and yellow perch from Devils Lake and in pumpkinseed sunfish from Lake Traverse (USFWS 2009).

We observed a greater diversity of parasitofauna in fish from Lake Traverse and Lake Ashtabula compared to fish from Devils Lake even though similar numbers of fish were examined from all survey sites. At Devils Lake, a total of sixteen different parasites were identified to the level of genus and of those, four parasites were identified to species. This included two protozoa, two myxosporeans, two monogeneans, two digenean trematodes, five cestodes, and four nematodes. At Lake Ashtabula, a total of thirty-four different parasites were identified to the level of genus, and of those, eleven parasites were identified to species. These included three protozoa, one microsporean, four myxosporeans, two monogeneans, ten trematodes, five cestodes, six nematodes, two acanthocephalans, one leech, and one parasitic crustacean. Fish from Lake Traverse had the greatest diversity of parasites. A total of fifty-four different parasites were identified to the level of genus, and of those, sixteen parasites were identified to species. These included four protozoa, one microsporean, seventeen myxosporeans, two monogeneans, four trematodes, eleven cestodes, ten nematodes, three acanthocephalans, one leech, and one parasitic crustacean.

One possibly unique parasite finding was a gryporhynchid metacestode from yellow perch at Devils Lake that may be from genus Paradilepis (T. Scholz, pers. com.). However, we could not place the worm in a taxonomic position closer than Family. The single specimen was preserved and then stained and mounted whole making measurement of rostellar hooks, a requirement for speciation, difficult if not impossible. We found other gryporhynchid metacestodes at Lake Traverse during this survey we suspect are metacestodes of *Paradilepis sp.* and *Valipora sp.* We also reported the observation of these worms from fish at Lake Traverse in 2007 (USFWS 2009). We could not find any previous records of larval gryprohynchid cestodes in fish from Lake Traverse or from fish in other bodies of water in the Red River basin or Hudson Bay drainage. It is likely that gryprohynchid metacestodes may be overlooked during routine parasite survey because of there small size and sites of infection. Final hosts for these worms are piscivorous birds. Further study is recommended of these unusual and poorly understood parasites in North America.

Histology provided another perspective on the observation of several parasites found during the classical parasite survey at Devils Lake. With the possible exception of the myxosporidians, both parasite search methods encountered similar protozoa, trematode, cestode, and nematode parasites. Neoplastic or viral lesions were not observed in any fish with histology. Many of the parasites found at Devils Lake were similar to those reported for a histology survey at Lake Winnipeg (Lumsden and Russel 2007). These findings included several meningial trematodes as well as myxosporeans in brachial and nervous tissues. At Devils Lake, myxosporeans were also commonly found throughout kidney tissue and in the urinary bladder. The widest diversity of myxosporidiosis was observed in fathead minnow where nine different types of infection have been documented.

Histopathology was a good tool for parasite screening when fish could be processed and examined in whole-fish sections. It was particularly valuable for screening small fish like fathead minnow and fingerlings of other species. Sections of whole fish allowed the

histopathologists to observe entire organs and systems; tissues that are collected in relatively small amounts when larger fish are sampled. Histology also allowed for high resolution observation of fine structures such as the brain, nerves, and other systems that are not easily screened with traditional parasite search methods such as tissue squashes. One limitation to histology however, was that many parasites could not be identified to taxonomic levels closer than class and order. Often times parasites observed in thin sections lacked sufficient morphological detail to permit their identification to genus and species. The best method for speciation of most parasites remains preservation of whole specimens which are then stained, mounted on glass slides, and examined in detail under the microscope.

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Chapter 1 — Introduction

Devils Lake is located in the northeastern corner of North Dakota in southern Ramsey and northern Benson counties. At approximately 67,000 ha, it is the largest natural body of water in the state. Devils Lake and neighboring Stump Lake receives most of the surface drainage in the Devils Lake Basin, an endorheic basin covering approximately 971,270 ha. Devils Lake basin lies within the Red River of the North (Red River) Basin, and the entire water shed is within the Hudson Bay drainage (Figure 1.1). Devils Lake does not have an outlet at surface elevations below 1458 ft-msl and lake levels are affected primarily by rainfall, snowmelt runoff, evaporation, and ground seepage. Surface runoff in the basin flows through many small coulees, wetlands, and lakes. Major inflows to Devils Lake include Big Coulee (Mauvais Coulee) and Channel A. Historically, Devils Lake Basin has experienced periods of climatic fluctuation which have caused significant changes in the lake's water surface elevation (Figure 1.2). The level of Devils Lake dropped significantly during periods of drought in the 1930s and reached a historic recorded low of about 1402 ft-msl (approximately 2 ft deep) in 1940. Since that time, the lake has been rising in a somewhat erratic fashion, with years of decline and increase. During the period from 1993 to 2001, Devils Lake surface area increased from 20,235 ha to about 50,600 ha. In 1999, the lake reached an elevation of 1446.6 ft-msl and water began to spill from Devils Lake into Stump Lake for the first time in several hundred years. On July 2, 2010, the National Weather Service monitoring station at Devils Lake reported a lake level of 1,451.98 ft (442.6 m). At an elevation of approximately 1458 ft-msl the combined lakes would overflow to the Sheyenne River, a major tributary to the Red River. Geologic records indicate Devils Lake has overflowed into the Sheyenne River twice in the last 4,000 years.

Figure 1.1— Map of Hudson Bay drainage basin showing geographic areas covered by the Red River basin (pink) and Devils Lake sub-basin (yellow). (Source: Manitoba Water Stewardship).

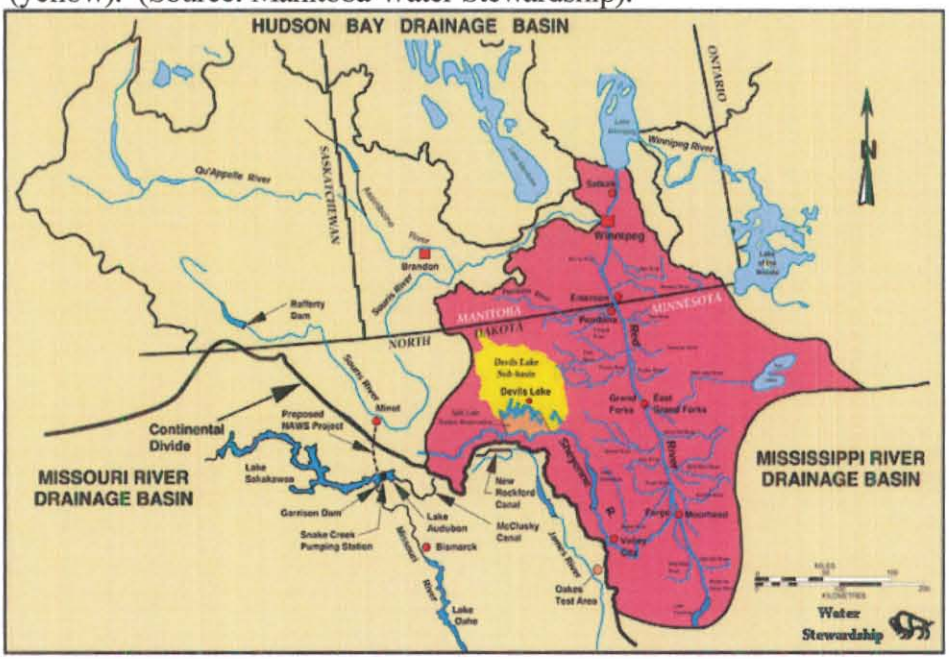
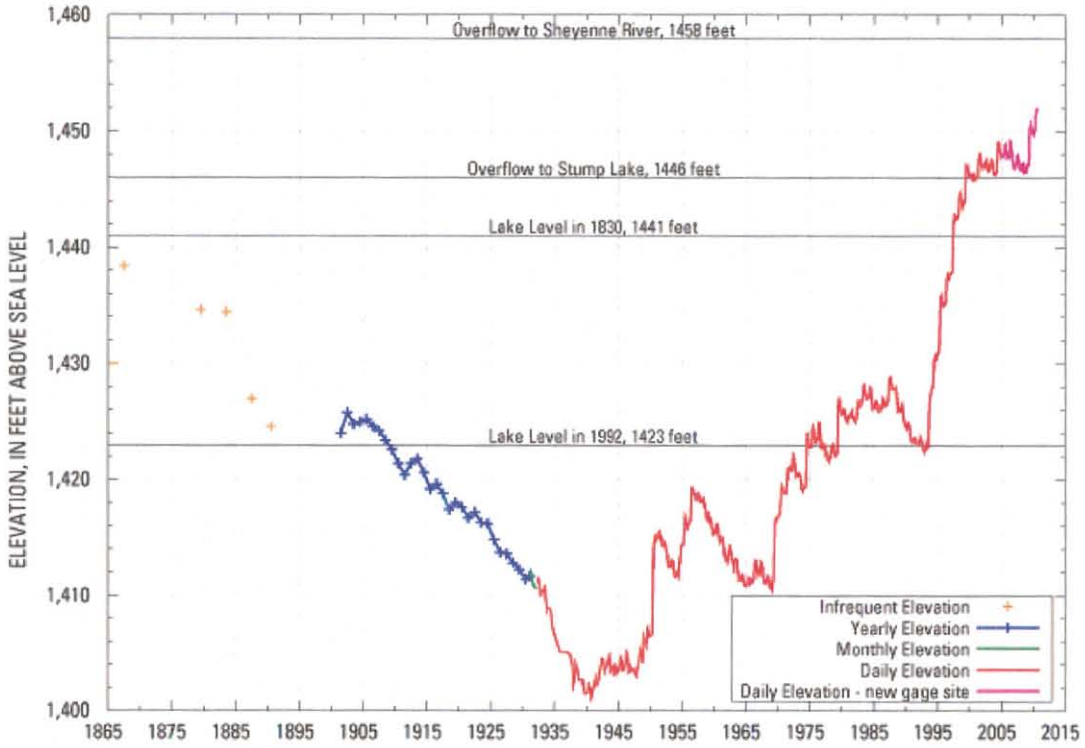


Figure 1.2— Water surface elevation for Devils Lake from 1867 through November 2010. (U. S. Geological Survey, North Dakota Water Science Center).



Stabilization of Devils Lake Basin lakes and wetlands has been a regional issue both in times of low and high water periods. In response to the steep rise in water during the last two decades, the State of North Dakota constructed an outlet on the southwest end of Devils Lake for the purposes of reducing flooding problems. Lake water is pumped into a canal which flows into the Sheyenne River. A second outlet on the east-end of Devils Lake designed to run at 250 cfs was recently authorized by North Dakota State Water Commission and is currently in phases of planning and design.

Diverting water from Devils Lake to the Sheyenne River has raised concerns about down stream water quality and the potential for biota transfer to receiving waters in the Hudson Bay drainage. Fish pathogens and parasites are one component of biota that has been cited as a potential serious threat. Until the last decade, few, if any, studies have been conducted in Devils Lake and the Red River basin that address the distribution and prevalence of specific bacterial and viral fish pathogens. There have been a number of surveys for fish parasites in regional prairie impoundments and lakes and in select streams in North Dakota (Mizelle and Kritsky 1967; Sutherland et al. 1979; Reinisch 1981; Forstie and Holloway 1984; Holloway 1986; Holloway and Hagstrom 1981). To address these concerns, the U.S. Army Corps of Engineers (ACE) first examined biota transfer as a component of an Environmental Impact Statement for

construction of an emergency outlet from Devils Lake to the Sheyenne River. During 2001-2002, the U. S. Fish and Wildlife Service, Bozeman Fish Health Center performed a fish pathogen survey under contract with the ACE (Peters 2002). Fish were collected from Devils Lake and the Sheyenne and Red rivers and tested for a specific list of bacterial and viral fish pathogens included in the U. S. Fish and Wildlife Service *National Wild Fish Health Survey* (2006) program. The survey did not include a fish parasite component. Beginning in 2005, the Council on Environmental Quality (CEQ) requested the U.S. Fish and Wildlife Service performed fish health survey work in Devils Lake and in the Sheyenne and Red rivers but not limited to the pathogens and parasites listed in the *National Wild Fish Health Survey* (Hudson and Peters 2005; Peters and Hudson 2007). In 2007, Lake Traverse, the southernmost body of water in the Hudson Bay drainage, was added to the list of sample sites (USFWS 2009).

In this report we provide results and discussion of fish pathogen and parasite surveys performed in June 2008. As in 2007, samples sites included Devils Lake and Lake Traverse but we did survey fish from the rivers. Lake Ashtabula, an impoundment of the Sheyenne River above Baldhill Dam, was surveyed in place of the Sheyenne River site sample in 2002, 2006 and 2007. Similar to previous years, study objectives were: 1) examine fish for the presence of fish pathogens; 2) perform comprehensive parasite surveys at Devils Lake, Lake Ashtabula, and Lake Traverse; 3) perform histological assessment of fish health at Devils Lake; 4) provide fish health specialists, fisheries managers, and other decision makers with a pathogen survey report that may be used in performing risk analysis, and 5) provide access to survey results through the U. S. Fish and Wildlife Service *National Wild Fish Health Survey* database on the worldwide web http://www.fws.gov/wildfishsurvey/database/page/intro

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Chapter 2 — Collection of Fish and Tissue Samples

Methods

We used several types of sampling gear to collect various sizes of fish from a variety of habitat types in lakes. Three types of multi-mesh gill nets were deployed as follows: 1) 125 ft X 6 ft with 5 panels incorporating ¾, 1, 1½, 1¾, and 2 inch mesh sizes; 2) 250 ft X 6 ft with panels of ¾, 1, 1½, 1¾, and 2 inch mesh sizes; and 3) 300 ft X 6 ft with 3 panels of 3, 4, and 5 inch mesh. Gill nets were typically set for 1-3 h intervals to minimize mortality and bycatch. We used modified fyke nets composed of a single lead and single throat that incorporated ⅛, ¼, and ½ inch mesh sizes. Fyke nets were typically deployed for 18 – 24 h intervals. Cylindrical hoop nets with 4 ft diameter and with 1½ mesh were used primarily in rivers and were set for 12 – 24 hr periods. A 30 ft X 6 ft beach seine was used to collect small cyprinids and young-of-year fish from shallow water along shoreline habitat. Additionally, electrofishing was used to capture fish at all three lakes. The electrofishing boat was equipped with a Smith and Root 5.0 GPP electrofishing system rated at 5,000 W of output power using pulsed DC at 7-9 A and 60 pulses/s.

We used a standard target sample size of 60 fish for each species to determine the presence or absence of bacterial and viral fish pathogens. This widely accepted sample size provided a 95% confidence level that an infected fish will be detected given a 5% presumed prevalence of infection and a population of 2,000 or more individuals (Ossiander and Wedemeyer 1973). For the histological survey at Devils Lake we set a maximum sample size of 60 fish per species. For comprehensive parasite surveys at Devils Lake and Lake Traverse we set a maximum sample size of 30 fish per species. Fish utilized for histology and comprehensive parasite exams were not used for other purposes unless species abundance or catch rates were significantly low. In cases where fish were used for multiple purposes the order of sample collection was 1) bacteriology, 2) virology, 3) histology, and 4) parasitology.

A temporary field station was set up at each body of water to provide workers shelter and an adequate laboratory environment for the aseptic examination of fish and collection of tissue samples. The field station consisted of a 30 ft long travel trailer to which we attached a 10 ft X 20 ft portable canopy with walls. In the trailer, we set up two work stations to perform comprehensive parasite surveys. Under the canopy we used ¾ inch plywood for flooring upon which six portable buffet tables were placed to provide space for fish necropsy and tissue collection. A portable 2000 W generator was used to provide electricity for laboratory equipment at sites without commercial power source. All gear in contact with fish was cleaned and disinfected between sample sites.

Upon collection, fish were transported alive to the temporary field laboratory for necropsy and tissue collection. Fish were held in large totes with aeration and overhead cover and in floating live-boxes in the lakes. Fish were euthanized with tricaine methanesulfonate (Finquel®) and then examined externally and internally for clinical signs of disease, parasites, and other abnormalities. Tissues samples for pathogen testing were collected using aseptic field techniques and packed in coolers with ice. Samples were transferred from the temporary field

stations to Bozeman Fish Health Center (USFWS, Bozeman, Montana) within 48 hours by commercial freight services. Upon arrival at the Fish Health Center, samples were logged-in and assigned case history numbers and then submitted to the appropriate laboratory sections where fish pathogen assays were performed. Samples were assayed for fish pathogens and parasites according to protocols and procedures for the *National Wild Fish Health Survey* (U.S. Fish and Wildlife Service 2006). Principle fish pathogens of the *National Wild Fish Health Survey* included specific organisms that are known to cause disease in cultured or wild fish and are considered prohibitive organisms in most state and federal fish health inspection programs. A summary of specific procedures used in this survey is explained in following chapters. Procedures may be examined in detail on the worldwide web following the Protocols and Procedures link on the *National Wild Fish Health Survey* website http://wildfishsurvey.fws.gov.

Description of Survey Sites

Devils Lake.— Devils Lake is located in northeastern North Dakota in southern Ramsey and northern Benson counties and is approximately 143 km west of Grand Forks and 155 km north of Jamestown. At its current surface elevation the lake covers approximately 67,000 ha and is the largest natural body of water in the state. The sampling area was located in a north-central section of the Devils Lake known as Six Mile Bay and extended north into the mouth of Channel A. The temporary field station was located in a parking area of the public access at Six Mile Bay landing.

Lake Ashtabula.— Lake Ashtabula is an impoundment of the Sheyenne River created by Baldhill Dam. The reservoir is located about 19 km northwest of Valley City, North Dakota. The reservoir covers approximately 2096 ha with a mean depth of 4.2 m. Our temporary field station was located in the parking area of the public access at Sibley Landing. Fish were sampled in an area 1 – 2 km north and south of the Highway 26 lake-crossing at Sibley, ND. Additional walleye were collected further south in and around the lake-crossing at County Highway 21.

Lake Traverse.— Lake Traverse is a long and shallow reservoir that lies along the border between the states of Minnesota and South Dakota about 20 km east of Sisseton, SD. The 4530 ha reservoir is controlled by Reservation Dam and is drained at its north end by the Bois de Sioux River a tributary to the Red River of the North. Lake Traverse is considered the southernmost body of water in the Hudson Bay watershed. A low continental divide separates the land at the southern shore of Lake Traverse from the Little Minnesota River which is tributary to the Mississippi River. Fish samples for this survey were collected from the southern end of the lake. The temporary field station was located approximately 6 km north of Browns Valley, Minnesota on private property with direct lake access.

Results

Fish were collected at Devils Lake on 12 – 14 June 2008. A total 583 fish representing seven species were collected and examined as a result of 258 h of netting and trapping effort and 4.13 h

of electrofishing (Table 2.1). The catch was composed of black crappie, fathead minnow, northern pike, walleye, white bass, white sucker, and yellow perch. The target sample size was obtained for fathead minnow, walleye, white bass, and yellow perch. Low catch rates for black crappie, northern pike, and white sucker were attributed to either relative low abundance or because seasonal distribution and occurrence in selected sample areas was low. Of the total catch, 292 fish were used for microbiology assays, 294 fish for histology, and 50 fish for the parasite survey. All black crappie, northern pike, and white sucker were processed for bacteriology, virology, and histology because the catch rate for these species was insufficient for separate sampling.

Fish from Lake Ashtabula were collected on 9 – 11 June 2008. Sampling operations were interrupted on 11 June as a result of severe thunderstorms with wind speeds reported at more than 140 km/h and nearly 4 cm rainfall. The temporary field station sustained considerable damage during the storm and tissue sampling activities were relocated to the maintenance shop at Valley City National Fish Hatchery which operated on electricity provided by the facility's emergency generator. Despite the weather related setback, a total 435 fish representing fourteen species were collected and examined as a result of 213 h of net and trap deployment and 3.25 h of electrofishing (Table 2.2). The catch was predominated by black bullhead, fathead minnow, spottail shiner, tadpole madtom, and walleye. Of the total catch, 390 fish representing 10 species were used in microbiology assays and 45 fish representing 14 species were examine for the parasite survey.

Fish from Lake Traverse were sampled on 16 – 18 June 2008. A total of 596 fish representing 16 species were collected as a result of approximately 8.0 h of electrofishing (Table 2.3). The target sample size was obtained for bluegill, bullheads, common carp, emerald shiner, freshwater drum, and white bass. Of the total catch, 522 fish representing 14 species were used for microbiology assays and 74 fish representing 16 species were examined for the parasite survey.

Table 2.1— Composition of fish collected from Devils Lake for microbial, histological, and parasite surveys. Tissues from fish marked with an asterisk were used for both microbial tests and for histology. Scientific names of fish are given in Appendix A.

	Number of fish sampled by test			Total number
Fish common name	Microbiology	Histology	Parasitology	sampled
Black crappie*	14	17	3	20
Fathead minnow	60	60	15	135
Northern pike*	34	35	2	37
Walleye	59	60	7	126
White bass	60	57	5	122
White sucker*	5	5	1	6
Yellow perch	60	60	17	137

Table 2.2— Composition of fish collected at Lake Ashtabula and tested for fish pathogens and parasites. Scientific names of fish are given in Appendix A.

	Number of fish sampled by test		
Fish common name	Microbiology	Parasitology	Total
Black bullhead	60	5	65
Black crappie	0	3	3
Blacknose dace	0	2	2
Bluegill	0	2	2
Fathead minnow	60	9	69
Northern pike	12	0	12
Smallmouth bass	9	2	11
Spottail shiner	60	2	62
Tadpole madtom	60	11	71
Trout perch	0	1	1
Walleye	55	3	58
White bass	32	3	35
White sucker	32	1	33
Yellow perch	10	1	11

Table 2.3.— Composition of fish collected at Lake Traverse and tested for fish pathogens and parasites. Scientific names are given in Appendix A.

	Number of fish sampled by test		
Fish common name	Microbiology	Parasitology	Total
Bigmouth buffalo	3	1	4
Black crappie	15	2	17
Bluegill	60	6	66
Bullhead (black and yellow)	60	2	62
Channel catfish	20	1	21
Common carp	60	0	60
Emerald shiner	60	12	72
Fathead minnow	0	20	20
Freshwater drum	60	3	63
Orangespotted sunfish	48	4	52
Rock bass	8	5	13
Shorthead redhorse	3	0	3
Walleye	58	11	69

Table 2.3.— continued.

White bass	60	2	62
White sucker	7	0	7
Yellow perch	0	5	5

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Chapter 3 — Bacterial Pathogens

Methods

Standard protocols and procedures for the USFWS National Wild Fish Health Survey (Heil 2009) were used for identification of bacteria cultured from fish at all survey sites. Briefly, isolation of bacterial pathogens was preformed by inserting a disposable sterile loop (1.0 or 10.0 µl.) into the kidney of the specimen. The loop was streaked across the surface of the tube containing brain-heart-infusion agar. Tubes were incubated at 22° C and monitored for bacterial growth at 24 – 72 h. Suspect bacterial growth was sub-cultured for isolation and then differentiated using biochemical profiling techniques and examined for motility by the hanging drop method. The API 20E (bioMerieux Vitek, Inc., Hazelwood, Mo.) test system was used to aid in identification of bacteria. Where appropriate, further confirmation of bacterial isolates was performed with either direct or indirect fluorescent antibody tests (DFAT, IFAT), serum agglutination tests, and with polymerase chain reaction (PCR) assay. Kidney tissue was also collected to quantify soluble antigen of *Renibacterium salmoninarum* by enzyme linked immunosorbent assay (ELISA; Pascho and Mulcahy 1987). When fish had insufficient kidney tissue for testing of individuals, pooling of tissue from two or more fish until a sufficient quantity of kidney was obtained for ELISA. Only kidney tissue from the same species was pooled. Samples were run in replicate and results of the ELISA were reported as the mean optical density (OD). Standardized negative reference tissue from fall Chinook salmon was used to determine the threshold of detection of *R. salmoninarum.* Whenever positive ELISA values were observed, we attempted to verify infection with *R. salmoninarum* in each species of fish using a nested PCR assay (Pascho et al. 1998). Generally, three samples having the highest ELISA OD values were selected for each species per sample site. DNA template was extracted using lysis methodology for gram-positive bacteria with Qiagen DNeasy (Valencia, Ca.) tissue kit and then amplified according to the PCR procedure. Amplified DNA was was compared to molecular weigh ladder in an eletrophoresis box containing a 1.5% agarose gel, and then stained with ethidium bromide and visualized with UV light.

Results

Devils Lake.— Fish species sampled at Devils Lake included: black crappie, northern pike, walleye, white bass, white sucker, and yellow perch. There was considerable growth of bacteria on the primary isolation medium. All primary cultures were sub-cultured for purity with presumed mixed isolates which resulted in 59 pure cultures. Upon screening with preliminary biochemical and motility tests, 6 pure cultures required further differentiation and identification with the API 20E commercial test system. There were no isolates of Gram-positive bacteria from fish sampled at Devils Lake. The Gram-negative bacteria identified were *Hafnia alvei* from two northern pike and two isolates of *Shewanella putrefaciens* from yellow perch (Table 3.1).

Table 3.1.— Gram-negative bacteria identified from fish sampled at Devils Lake.

Name of bacteria		
Genus	Species	Species of fish
Hafnia	*alvei*	Northern pike
Shewanella	*putrefaciens*	White bass

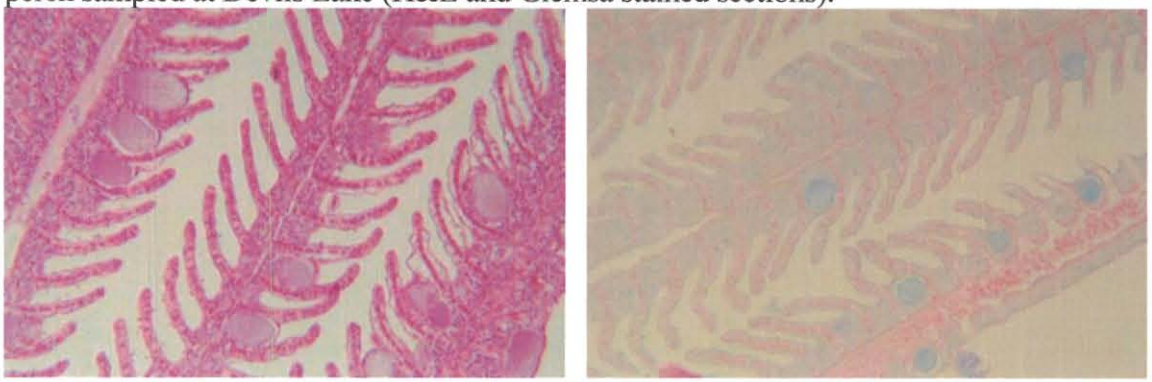
At Devils Lake, antigen of *R. salmoninarum* was detected by ELISA in kidney tissues of black crappie, northern pike, walleye, white bass, white sucker, and yellow perch (Table 3.2). Sixteen black crappie were tested resulting 20% of samples above the negative threshold value for detection. Positive ELISA samples assayed with nested PCR for *R. salmoninarum* were negative for the all species tested. These results suggest that ELISA samples with a high percentage of sample values above the negative cut-off value were likely false positive for *R. salmoninarum*. None of the fish sampled and tested had any clinical signs indicative of bacterial kidney disease.

Table 3.2.— Percent of samples with detectable levels of *R. salmoninarum* antigen and corroborative testing with a nested PCR assay for six species of fish from Devils Lake.

	ELISA		PCR Assay	
Fish species	Number tested	Percent positive (%)	Number tested	Percent positive
Black crappie	16	20.0	2	0
Northern pike	34	10.5	2	0
Walleye	60	89.4	3	0
White bass	60	64.0	3	0
White sucker	5	100.0	3	0
Yellow perch	60	100.0	3	0

We observed cellular pathology during histological evaluation of fathead minnow and yellow perch gill sections consistent with epitheliocystis. Examination of stained tissue sections revealed a hypertrophic response of gill epithelial cells (Figure 3.1). The disease is caused by bacteria in the order Chlamydiales. Members of Chlamydiales are obligate intracellular pathogens which do not grow on nutrient agar media used for general isolation of other bacteria.

Figure 3.1.— Photomicrographs of epitheliocystis in epithelium of gill lamellae from yellow perch sampled at Devils Lake (H&E and Giemsa stained sections).



Lake Traverse.— Fish species sampled at Lake Traverse included: black crappie, bluegill, bullhead, channel catfish, freshwater drum, orange spotted sunfish, rock bass, walleye, white bass, and white sucker. Bacteria were grown on primary isolation medium. Sub-cultures were taken for purity from all primary cultures with presumed mixed isolates of bacteria which resulted in 103 pure cultures. After screening with preliminary biochemical and motility testing, 7 pure cultures required further identification. Use of the commercial API 20E test system resulted in four species of Gram-negative bacteria. The most common bacterial species isolated was *Pantoea sp.* from bullhead and walleye. *Aeromonas hydrophila* was isolated from bluegill and *H. alvei* from freshwater drum and *Myroides sp.* from rock bass (Table 3.3). There were no isolates of Gram-positive bacteria from fish sampled at Lake Traverse.

Table 3.3.— Gram-negative bacteria identified from fish sampled at Lake Traverse.

Name of bacteria		
Genus	Species	Species of fish
Aeromonas	*hydrophila*	Bluegill
Hafnia	*alvei*	Freshwater drum
Myroides	*sp.*	Rock bass
Pantoea	*sp.*	Bullhead, walleye

At Lake Traverse, antigen of *R. salmoninarum* was detected by ELISA in kidney tissues of black crappie, bluegill, bullhead, channel catfish, common carp, freshwater drum, and white bass (Table 3.4). ELISA results for five species resulted in OD values below detection limits (BDL): bigmouth buffalo, rock bass, shorthead redhorse sucker, walleye, and white sucker. Positive ELISA samples assayed with nested PCR for *R .salmoninarum* were negative for all species tested. None of the fish sampled and tested at Lake Traverse had any clinical signs indicative of bacterial kidney disease.

Table 3.4.— Percent of samples with detectable levels of *R. salmoninarum* antigen and corroborative testing with a nested PCR assay for six species of fish from Devils Lake.

	ELISA		PCR Assay	
Fish species	Number tested	Percent positive (%)	Number tested	Percent positive
Black crappie	14	71.4	3	0
Bigmouth buffalo	3	0.0	0	0
Bluegill	60	61.5	3	0
Bullhead	60	100.0	3	0
Channel catfish	20	93.0	3	0
Common carp	54	100.0	3	0
Freshwater drum	60	80.0	3	0
Rock bass	8	0.0	0	0
Shorthead redhorse	3	0.0	0	0
Walleye	56	0.0	0	0
White bass	55	83.3	3	0
White sucker	7	0.0	0	0

Lake Ashtabula. — Fish species sampled at Lake Ashtabula included the following; walleye, yellow perch, white sucker, northern pike, white bass, smallmouth bass, black bullhead and tadpole madtom. Bacteria were grown on the primary isolation medium. Sub-cultures were taken for purity for from all primary cultures which resulted in 87 pure cultures. After screening with preliminary biochemical and motility test, 9 pure cultures required further identification. Biochemical profiling with the commercial API 20E test system resulted in two species of Gram-negative bacteria. The bacterial species isolated were *S. putrefaciens* from walleye, and *Myroides sp.* from yellow perch (Table 3.5). There were no isolates of Gram-positive bacteria from fish sampled at Lake Ashtabula.

Table 3.5.— Gram-negative bacteria identified from fish sampled at Lake Ashtabula.

Name of bacteria		
Genus	Species	Species of fish
Myroides	*sp.*	Yellow perch
Shewanella	*putrefaciens*	Walleye

At Lake Ashtabula, antigen of *R. salmoninarum* was detected by ELISA in kidney tissues of black bullhead, northern pike, tadpole madtom, walleye, white bass, white sucker, and yellow perch (Table 3.6). Mean OD values for nine smallmouth bass were below detection limits. Positive ELISA samples for all other species assayed with nested PCR for *R .salmoninarum* were negative. None of the fish sampled and tested at Lake Traverse had any clinical signs indicative of bacterial kidney disease.

Table 3.6.— Percent of samples with detectable levels of *R. salmoninarum* antigen and corroborative testing with a nested PCR assay for eight species of fish from Lake Ashtabula.

	ELISA		PCR Assay	
Fish species	Number tested	Percent positive (%)	Number tested	Percent positive
Black bullhead	60	26.0	3	0
Northern pike	12	10.0	1	0
Smallmouth bass	9	0.0	0	0
Tadpole madtom	55	23.3	3	0
Walleye	59	14.0	3	0
White bass	27	26.6	3	0
White sucker	31	95.8	3	0
Yellow perch	10	100.0	2	0

Discussion

Bacteriological assays did not detect any listed bacterial pathogens in fish from Devils Lake, Lake Traverse, or Lake Ashtabula. *S. putrefaciens* was the most common bacterial isolate found during this survey, followed by *H. alvei, Pantoea sp., A. hydrophila,* and *Myroides sp. S. putrefaciens* belongs in the family Shewanellaceae and is a Gram-negative non-fermentative facultative anaerobe. On solid nutrient media, *S. putrefaciens* colonies are round, fast-growing, and pink or reddish in color. It is also one of the main organisms associated with the odor of rotting fish. *S. putrefaciens* occurs commonly in saltwater and marine sediments and may be part of the normal microflora of marine fish (Austin and Austin 1987). Elevated salinity typical of closed basin waters such as Devils Lake may provide favorable environment conditions for adaption outside seawater. *S. putrefaciens* was detected in fish from Devils Lake and Lake Ashtabula.

Hafnia alvei belongs in the family Enterobacteriaceae and is a Gram-negative, facultatively anaerobic, rod-shaped bacterium. *H. alvei* was found at both Devils Lake and Lake Traverse. *Pantoea sp.* was found only at Lake Traverse. *Pantoea sp.* is a Gram-negative bacterium also

belonging to the family Enterobacteriaceae. Both *H. alvei* and *Pantoea sp.* are not generally considered primary fish pathogens. They are most often considered to be opportunistic bacterium.

A. hydrophila isolates were identified at Lake Traverse. *A. hydrophila* is a Gram-negative motile bacterium. The bacterium can be found in fresh and marine environments. Motile aeromonids are normal constituents of the aquatic environment given their wide distribution and high frequency of isolation from fish tissues. *A. hydrophila* can thrive in both warm and cool temperatures.
When infected with *A. hydrophila*, fish may develop internal clinical signs of hemorrhagic septicemia causing swollen spleen and kidney, and visceral hemorrhaging (Austin and Austin 1987). External clinical signs include; Hemorrhages of fins, gills, skin ulcerations, fin, tail rot, exophthalmia, and abdominal swelling.

Isolates of *Myroides sp.* was found at both Lake Traverse and Lake Ashtabula. *Myroides sp.* belonging to the family Flavobacteriaceae, is characterized by Gram-negative nonmotile rods that are light yellow in color on solid nutrient agar. Studies indicate that *Myroides sp.* is widely distributed in the environment especially water. (Shewan and McMeekin 1983).

R. salmoninarum is the cause of bacterial kidney disease which is a serious condition of wild and farm-raised trout and salmon worldwide. To the best of our knowledge the bacterium has not been implicated as an agent of disease in families other than Salmonidae. Inconsistencies between ELISA and PCR are apparent when examining assay results from all sample sites in this survey. Most kidney samples of the non-salmonids from the three survey sites had ELISA OD values positive for *R. salmoninarum* however, none of the ELISA positive samples that were selected for PCR were positive for the bacterium. Similar results were observed in earlier surveys of the study areas (Peters 2002; Hudson and Peters 2005; Peters and Hudson 2007; Peters 2009). PCR has higher sensitivity and specificity for detection of *R. salmoninarum* compared to ELISA (Pascho et al. 2002; Chase et al. 2006; Rhodes et al. 2006). This is one reason why we believe the ELISA is likely reporting false positive readings. There are a number of possible factors that may explain poor correlation between the test results. One, the standard reference tissue used to establish ELISA negative-positive thresholds may not be appropriate for all families of fish or geographical areas. The reference tissue used in the National Wild Fish Heath Survey is from fall Chinook salmon (Salmonidae) while all the samples collected in this survey were from fish in other taxonomic families. It is possible that certain protein elements or other background readings of non-salmonid kidneys may interfere with the ELISA resulting in higher background readings thus producing false-positive values based on the salmonid reference tissue. Another possible explanation is that polyclonal antibodies used in ELISA are cross-reacting with other species of bacteria (Brown et al. 1995).

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Chapter 4 — Histology Survey at Devils Lake

Methods

Fish were transported by boat to the temporary field station at Six Mile Bay, Devils Lake and kept alive until processed. Fish were euthanized with tricaine methanesulfonate (Finquel®) and processed according to protocols of the National Wild Fish Health Survey (USFWS 2009). In addition, specific teleost histological post mortem techniques were used as outlined in Systemic Pathology of Fish (Ferguson 2006). Fish less than 8cm total length were processed and examined whole. Fish larger than 8.0 cm were dissected and sections of major tissues and organs were processed and examined individually. These included spleen, heart, kidney, liver, skeletal muscle, gill, stomach, intestine and gonads. Tissues were preserved in Davidson's fixative for 48 hours and then transferred to 70% ETOH. Fixed samples were transported to Bozeman Fish Health Center, Montana. Samples were processed in a Leica ASP 300 tissue processor, embedded in paraffin, and then replicate 5.0 µm section were cut and mounted on glass slides. Tissue sections were stained with hematoxylin-eosin and with giemsa. Stained tissue sections were viewed with light microscopy at 40 – 100X magnification and photographs were captured with a digital imaging system. Histology interpretation was provided by two qualified histopathologists. Parasite findings for each species of fish were first summarized and then given in more detail according to class of parasite and affected tissues. Results were also summarized in table format for each species of fish. Figures are found at the end of the chapter.

Results

Fathead Minnow

Sixty fathead minnow were processed and examined in whole tissue sections. A total of 15 different parasites were observed (Table 4.1). Seven myxosporeans were observed and were the most common group of parasites in fathead minnows. Myxosporidians were most prevalent in the kidney tubules and were also found in kidney interstitium, skeletal muscle, peripheral nerves, cartilage, gills, and in connective tissue. Several protozoan parasites were observed in gills including *Trichodina* sp. and *Apiosoma* sp. Monogeneans and/or trematodes were also found in gill tissues. Trematodes were observed in skeletal muscle and the meninges of the brain. Cestodes were observed in the intestines of about 6% of fish.

Myxosporea

Renal. A large myxosporean parasite was observed in the kidney tubules in 88% of fish examined. Giemsa stained sections clearly demonstrated oval to ellipsoid spores with two pyriform polar capsules. Histology sectioning did not provide adequate morphological characteristics for accurate identification but most likely this is a species of *Myxobolus* (Figure 4-1). A smaller myxosporean parasite was found scattered in kidney interstitial tissue. These parasites were more elongate and elliptical in shape than the previous myxospores described in fathead minnows. The second myxosporean was found in 5 fathead minnow (Figure 4-2).

Table 4.1.— Summary of parasite findings from examination of 60 fathead minnow with histology.

Tissue	Parasite	Number positive	Figure number
Kidney - tubule	Myxosporean (lg)	53	4-1
Kidney - interstitium	Myxosporean	5	4-2
Skeletal muscle	Myxosporean	52	4-3
Skeletal muscle	Trematode - Digenetic (encysted)	18	4-8
Nerve - peripheral	Myxosporean	4	4-4
Brain - meninges	Trematode - Digenetic	29	4-9
GI - intestine	Cestode	4	4-15
Gill	Myxosporean – *Henneguya*-like.	3	4-5
Gill	Protozoa – *Trichodina* sp.	42	4-12
Gill	Protozoa – *Apiosoma* sp.	7	4-13
Skin-fin	Trematode – Monogenetic - *Gyrodactylus* sp.-like	1	4-11
Gill	Trematode - Monogenetic	1	4-10
GI – intestine	Sporozoan – coccidian	12	4-14
Cartilage/Bone	Myxosporean	4	4-6
Connective Tissue	Myxosporean	3	4-7

Intramuscular. Myxosporean parasites were observed in histology sections of skeletal muscle. Single to multiple plasmodia in muscle fibers contained both spore and trophozoite stages. Spores were ellipsoid to oval in shape with deeply staining polar capsules. These intra-muscular myxosporeans were observed in 52 fish (Figure 4-3). There was no host response to encysted parasites, however, ruptured cysts elicited a strong inflammatory response.

Peripheral nerve. Myxosporean parasites were also observed in peripheral nerve tissue. Numerous trophozoite stages with few mature spores were seen in sections. These parasites were observed in 4 fish (Figure 4-4).

Branchial. Histology sections of gill tissue provided observations of interlamellar cysts containing mature *Henneguya*-like myxospores. These branchial myxosporeans were oval shaped spores with elongated polar capsules.
Typical *Henneguya* caudal processes were not observed, but are difficult to retain in tissue sections. These parasites were observed in 3 fish (Figure 4-5).

Cartilage/Bone. Foci of myosporean spores surrounded by bone were observed in four fish out of 60 examined. No trophozoite stages were observed. Identification of myxospore was not possible due to poor morphological characteristics retained in tissue sections (Figure 4-6).

Connective Tissue. *Henneguya*-like myxospores were observed in three out of 60 fish examined. These parasites were similar in appearance to those observed in branchial tissue. These were highly suggestive of *Henneguya sp.* because the spores demonstrated thick caudal processes. (Figure 4-7).

Trematoda - Digenean

Intramuscular. An encysted digenetic trematode was confirmed in skeletal muscle sections. Sections did not contain intact parasites for accurate identification. Few of the trematode parasites were observed to have melanized capsules. These were encysted in sections of skeletal myofibers. Intramuscular trematodes were observed in 30% of fish (Figure 4-8).

Meningial. Sections of the brain showed digenetic trematodes in the meninges of 29 fish. These were observed within the endomeningial connective tissue. Lack of distinct organelle structures in sections precluded classification beyond Trematoda, however, the parasite morphology and location were presumptive for *Ornithodiplostomum sp.* (Figure 4-9).

Monogenea

Branchial. A monogenean trematode was seen in gill sections of one fish. Lack of reliable parasite morphology and organelle structures precluded identification to genus. The infected fish contained one or two parasites per section (Figure 4-10).

Integument. A monogenean trematode was identified on the skin and fins of one out of 60 fish examined. Morphology was suggestive of *Gyrodactylus sp.* although haptors were not seen (Figure 4-11).

Protozoa

Branchial. Histological sections of gill tissue showed an external protozoa identified as: *Trichodina sp.* Giemsa stained sections demonstrated the distinct morphology of these round ciliates. The disc-shaped organisms were observed on gill lamellar surfaces. The cytoskeletal denticles were identified in sections. *Trichodina sp.* was observed in 42 of 60 fish (Figure 4-12). Skin (oral cavity) sections also identified the external protozoan: *Apiosoma sp.* This conical shaped commensal ciliate is often non- pathogenic and feeds on organic debris. This external protozoan was observed in only 7 fish out of 60 (Figure 4-13).

Sporozoan - coccidian

Gastrointestinal. A coccidian parasite was observed in mucosal epithelium which was not seen in the previous survey. This observation may have been a function of seasonal sampling variation. These parasites were observed in of 20% of fish (Figure 4-14). There was considerable sloughing of the mucosal epithelium where the parasites were primarily observed. Presumptive identification: *Eimeria sp.*

Cestoda

Gastrointestinal. An intestinal cestode was identified in four of 60 fish. Tissue sections of intestine demonstrated a cestode infection in both the intestinal wall and the visceral cavity. Histology observation did not provide discriminating features necessary for accurate cestode species identification (Figure 4-15).

Walleye

A total of 8 different parasites were observed in tissue sections from 60 walleye (Table 4.2). The most common parasites found were cestodes. Even though most fish were heavily parasitized there was limited tissue response to the infestation. The protozoan parasite *Trichodina sp.*was also common in both large and small fish. A suspect nematode suggestive of *Contracecum sp.* was observed in sections of liver from 3 fish. A suspect microsporidian parasite was seen in ovarian tissue. There was a significant inflammatory response but spore morphology was not distinct.

Table 4.2.— Summary of parasite findings from examination of 60 walleye with histology.

Tissue	Parasite	Number positive	Figure number
GI - intestine	Cestode – (possibly *Proteocephalus sp.)*	46	4-16
Gill	Protozoa – *Trichodina sp.*	21	-
Heart	Myxosporean	1	-
Ovary	Microsporea- Suspect *Ovipleistophora ovariae*	1	4-21
Gill	Protozoa – *Ichthyophthirius sp.*	1	-
Skeletal Muscle	Myxosporean	1	4-19,20
Intestine	Nematode -*Contracecum sp.*	1	4-17,18
Liver	Larval Nematode	3	-

Cestoda

Gastrointestinal tract. There was a high prevalence of intestinal cestodes seen in histological sections (76.6%). Numerous cestode parasites were found throughout the lumen of the gastrointestinal tract in individual fish. Ovary and testes were observed in each proglottid. The scolex was difficult to discriminate, but other features were suggestive of a *Proteocephalus sp.* (Figure 4-16).

Nematoda

Hepatic. Enysted nematodes were found scattered through liver tissue from 3 fish. The lack of specific organelles prevented a complete parasite identification. Nematodes were also found

on the surface of intestinal tissue. Morphological structures were consistent with *Contracecum sp.* which were also seen routinely in field necropsies (Figures 4-17 and 18).

Myxosporea

Intramuscular. A myxosporean parasite was observed in skeletal muscle in only one fish. Sections contained mostly presporogonic spore stages. No caudal processes or significant identifiable features were observed for definitive identification (Figures 4-19 and 20).

Protozoa

Branchial. There were two protozoan parasites observed in walleye gill sections. *Trichodina sp.* was most numerous and observed in 21 of 60 fish examined. The early life stages of *Ichthyophthirius multifiliis* were observed in gill sections from 1 fish.

Microsporidean

Ovarian tissue. A suspect microsporidean parasite was seen in the ovary tissue of one fish. There was a massive granulamatous response in follicles and degenerative oocytes. Definitive spore morphology of a microsporidean was not observed in tissue sections. However, the location and staining characteristics were suggestive of *Ovipleistophora ovariae* (Figure 4-21).

White Bass

A total of 57 white bass were examined with histology. The most prevalent parasites observed were cestodes in the gastrointestinal tract and an external protozoan. A possible monogenean trematode and a nematode were also seen in tissue sections. (Table 4.3).

Cestoda

Gastrointestinal. Cestodes were observed in the gastrointestinal tract of 54 out of 57 fish (95%). The lack of identifying features of this organism precluded a definitive classification. However, the parasites were observed to have at least three suckers and were pseudo-segmented. Morphology was suggestive of *Proteocephalus* sp. (Figure 4-22-24).

Protozoa

Branchial. An external protozoan gill parasite was clearly identified as: *Trichodina sp.* Gill tissue showed mild to moderately severe damage (aneurysms, epithelial proliferation, and focal fusion of lamellae). *Trichodina sp.* was highly prevalent, observed in 55 out of 57 fish (96%). (Figure 4-25).

Monogenea

Branchial. A suspect monogenean trematode was observed in gill tissue sections in 18 of 57 fish examined. Hallmark monogenean structures such as haptors were absent in tissue sections. (Figure 4-26).

Nematoda

Gastrointestinal. Nematodes were found encysted mostly in muscularis or submucosa of the stomach and occasionally in pancreatic tissue. In one fish, nematodes were seen in liver tissue. These parasites were seen in 22 of 57 fish examined. (Figure 4-27).

Table 4.3.— Summary of parasite findings from examination of 57 white bass with histology.

Tissue	Parasite	Number positive	Figure number
GI - intestine	Cestode	54	4-22-24
Gill	Protozoa – *Trichodina* sp.	55	4-25
Gill	Monogenean Trematode	18	4-26
GI-muscle/liver	Nematode	22	4-27

Black Crappie

A total of 17 black crappie from Devils Lake were processed and examined with histology. We observed three major classes of parasites in these fish (Table 4.4). An external protozoan parasite was observed in gills of 6 fish. Myxosporeans were found in the kidney nephron. Nematodes were observed in the muscularis and viscera of the gastrointestinal tract.

Nematoda

Gastrointestinal. Large numbers of nematodes were found mostly in the visceral cavity. Encysted worms were primarily located in adipose/pancreatic tissue and occasionally in the muscularis of the stomach. This parasitic organism was detected in 12 fish (Figure 4-28).

Table 4.4.— Summary of parasite findings from examination of 17 black crappie by histology.

Tissue	Parasite	Number positive	Figure number
GI - viscera	Nematode	12	4-28
Gill	Protozoa – *Ichthyobodo* sp.	6	4-29
Kidney	Myxosporean – *Myxobolus sp.*	1	4-30,31

Protozoa

Branchial. A branchial protozoan parasite was observed on the surface of gills in 6 fish (35.2%). Identifying features of the organism provided a presumptive identification of *Icthyobodo sp.* Also known as "Costia", this ciliated protozoan parasite is considered to be a ubiquitous organism of freshwater fish (Figure 4-29).

Myxosporea

Renal. A myxosporean parasite was observed in kidney nephron sections. Spores were histomorphologically similar to a *Myxobolus sp* (Figures 4-30 and 31). This myxobolid parasite

was seen in 1 of 5 fish having posterior kidney tissue sections with tubules. No parasites were seen in hematopoietic tissue of head kidney. Trophozoite stages, were seen in kidney tubule lumens. Identification to species was not possible with histology observation.

Yellow Perch

Tissue samples were collected from a total of 60 yellow perch. A total of 10 parasites were seen in tissue section. Three external protozoans were observed with *Trichodina* sp. being the most prevalent of all parasites. Two myosporean parasites were seen in nerves and connective tissue. One cestode was observed in the gastrointestinal tract. A monogenean trematode was found in gill tissue and a nematode and coccidian parasite were seen in the GI tract. Finally, an encysted dignetic trematode was seen in skeletal muscle described in (Table 4.5). Histology analysis did not suggest significant pathology associated with the presence of parasitic organisms in the gastrointestinal tract or on gill surfaces.

Cestoda

Gastrointestinal. An unidentifiable cestode was detected in the gastrointestinal tract in 28 fish (46.6%) (Figures 4-32 and 33). Cestodes appeared to be in the initial stage of attachment to the gut lining. There was no host reponse to the cestodes. The characteristic two sucker attachment and smooth body wall were suggestive of *Ligula sp.*

Protozoa

Branchial. Two sizes of similar external protozoan parasites: *Trichodina sp.* and *Trichodinella sp.* were observed in gill sections in relatively high prevalence (48 out of 60 fish). These were the most abundant parasites overall. (Figures 4-34). A Costia-like protozoan was observed in only one fish out of 60.

The external protozoan was seen in the skin and oral cavity. These parasites were observed in three fish (Figure 4-35). Attachment and macronucleus was consistent with *Apiosoma sp.*

Trematoda - Digenean

Intramuscular. An encysted digenetic trematode was found in skeletal muscle. An oral sucker and hyaline cyst were observed. No presumptive identification was possible from tissue sections because there were no intact parasites. This parasite was found in 5 of 60 fish. (Figure 4-36).

Monogenea

Branchial. A *Gyrodactylus*-like monogenean trematode was seen in gill tissue sections at relatively low prevalence: 5 out of 60 fish. (Figure 4- 37). Haptors were clearly seen in gill sections.

Nematoda

Gastrointestinal. A nematode was observed in the muscularis and viscera of the gastrointestinal tract. This parasite was found in relatively low prevalence (3 out of 60 fish). (Figure 4-38).

Sporozoan - coccidian

Gastrointestinal. A coccidian sporozoan was observed in the mucosal epithelium of the gastrointestinal tract in only one fish. (Figure 4-39). Spore morphology was typical for the genus *Eimeria.*

Myxosporea

Nervous and Connective Tissue. Two myxosporean parasites suggestive of *Henneguya sp.* detected in both nervous tissue (spinal cord) (2 fish) and also the connective tissue of the lower jaw (1 fish). It is possible these were the same parasite. Typical *Henneguya* spore morphology was seen with caudal processes in tact. (Figures 40-42). The parasite observed in nervous tissue may have been in a peripheral nerve that was completely occluded by a cyst.

Table 4.5.— Summary of parasite findings from examination of 60 yellow perch by histology.

Tissue	Parasite	Number positive	Figure number
Gill	Protozoa-*Trichodina sp.*	48	4-34
Gill	Trematode-Monogenean-*Gyrodactylus sp.*	5	4-37
Gill	Protozoa-Costia- like	1	-
Muscle-skeletal	Trematode-Digenetic	5	4-36
GI-intestine	Cestode	28	4-32,33
GI-muscle/vis.	Nematode	3	4-38
GI-mucosal epithelium	Sporozoan-coccidian	1	4-39
Skin – oral cavity	Protozoa-*Apiosoma sp.*	3	4-35
Nerve-sp cord	Myxosporean – *Henneguya?*	2	4-40,41
Connective tissue-lower jaw	Myxosporean – *Henneguya?*	1	4-42

Northern Pike

Five classes of parasites were observed in histology sections from a total of 35 northern pike (Table 4.6). We observed numerous cestodes in the gastrointestinal tracts of 20 fish. A myxosporean-*Myxidium sp.* was observed in the kidneys of 19 fish.

Table 4.6.— Summary of parasite findings from examination of 23 northern pike by histology.

Tissue	Parasite	Number positive	Figure number
GI	Cestode	20	4-44
Gill	Protozoa-*Ichthybodo sp.*	1	4-49
Gill	Protozoa-*Trichodina sp.*	1	4-50
Gill	Protozoa-*Apiosoma sp.*	2	4-51
Gill	Hirudinea: Leech	1	4-45,46
Kidney	Myxosporean – *Myxidium sp.*	19	4-47, 4-48
Gill	Monogenean – *Gyrodactylus sp.*	1	4-43

Monogenea

Branchial. *Gyrodactylus sp.* was observed in 1 fish. Identification of the monogenean to species was not possible due to the lack of identifying morphological characteristics. The corresponding figure shows this organism attached to the surfaces of gill lamellae (Figure 4-43).

Cestoda

Gastrointestinal. Sections of the gastrointestinal tract contained cestodes. Sections of the parasite were incomplete and did not provide key morphological characteristics needed for identification of the organism to genus or species. Minimal pathology and cellular damage was associated with cestode presence in the GI tract. Cestodes were only observed in 20 fish (57%) (Figure 4-44).

Hirudinea

Branchial. A suspect leech was observed on the gills of only one fish. Each parasite had numerous visible ova. The lack of distinct structure precluded exact identification (Figures 4-45 and 4-46).

Myxosporea

Renal. A myxosporean parasite was observed in kidney tubules in 19 fish. Histology evaluation provided a presumptive identification to genus: *Myxidium sp.* This parasite genus has been widely described in other freshwater fish species. Histology documentation provided identifying characteristics and location within kidney tissue with associated cellular changes (Figures 4-47 and 48).

Protozoa

Branchial. Gill sections contained three protozoan parasites in relatively low prevalence. *Ichthyoboda sp.* (Costia) and *Trichodina sp.* were observed on one fish each. *Apiosoma sp.* was seen on the gill sections of two fish (Figures 4-49-51).

White Sucker

Tissues from a total of 5 white suckers were collected from Devils Lake. Comprehensive observations of stained tissue sections found no parasites.

Discussion

Gill protozoa, myxosporeans, and gastrointestinal cestodes were among the most common parasite findings in the histology survey of fish from Devils Lake. Neoplastic or viral lesions were not observed in any fish. Lesions associated with bacteria were limited to very low prevalence of epitheliocystis caused by an intracellular Chlamydia-like organism (Chapter 3). Many of the parasites found at Devils Lake were similar to those reported for a histology survey at Lake Winnipeg (Lumsden and Russell 2007). These findings included several meningial trematodes as well as myxosporeans in branchial and nervous tissues. At Devils Lake, myxosporeans and trematodes were also commonly found in a variety of species. The widest diversity of myxosporidiosis was observed in fathead minnow where seven different types of infection were documented. Only five white sucker were examined from Devils Lake and no parasites were observed in histology sections. At Devils Lake, histozoic myxospores were found in a variety of tissues including kidney interstitium, skeletal muscle, peripheral nerves, gill lamellae, cartilage and bone.

Histology provided another perspective on the observation of several parasites found during the traditional systematic parasite survey at Devils Lake (Chapter 5). With the exception of the myxosporidians viewed with histology, both parasite search methods encountered similar protozoa, trematode, cestode, and nematode parasites.

Histology as a parasite/pathogen screening tool lacks sensitivity and specificity. In these investigations, many metazoan parasites could not be identified to taxonomic levels closer than class and order. Logistically, metazoan parasites presented in 5.0 μm thin sections lack sufficient morphological detail to permit their identification to genus and species. The best method for identification of most metazoan parasites remains preservation of whole specimens which are then stained, mounted on glass slides, and examined in detail under the microscope.

Histopathology was a valuable tool for parasite screening when fish could be processed and examined in whole-fish sections. In this survey, it was particularly valuable for screening small fish like fathead minnow and fingerlings of other species. Sections of whole fish allowed the histopathologists to observe entire organs and systems where as these tissues are collected in relatively small amounts from large fish. Histology also allows for high resolution observation of fine structures such as the brain, nerves, and other systems that are not easily screened with traditional parasite search methods such as tissue squashes. As the size of fish increased, the value of using histology for parasite screening was still useful but the likelihood that parasites could be missed probably increased. Processing of tissue imprints would be less costly and most micro-organisms would be observed whole permitting easier identification.

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Figures

Figure 4-1. Large myxosporean in fathead minnow kidney tubules.

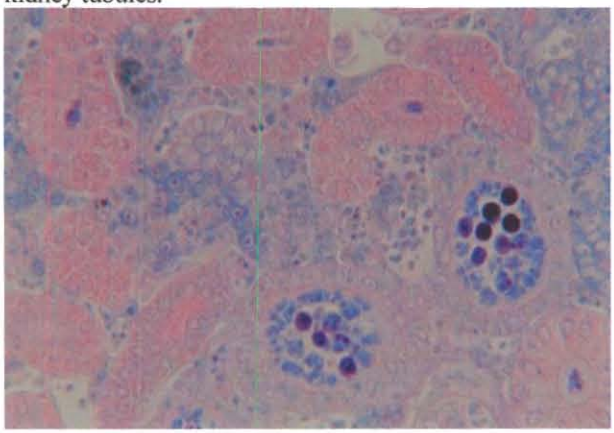


Figure 4-2. Small myxosporean observed in fathead minnow kidney tubules.

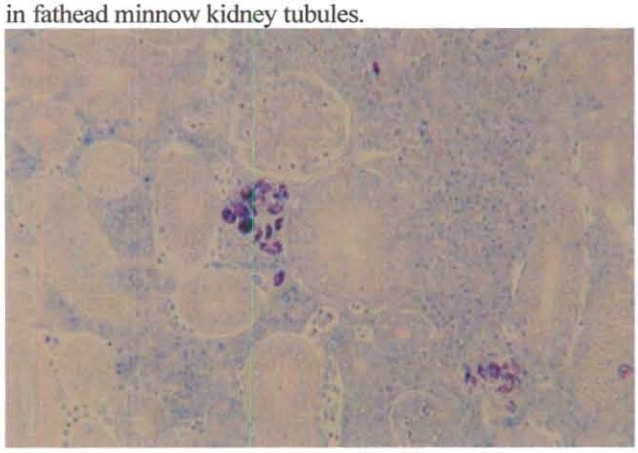


Figure 4-3. Myxosporean from skeletal muscle in fathead minnows.

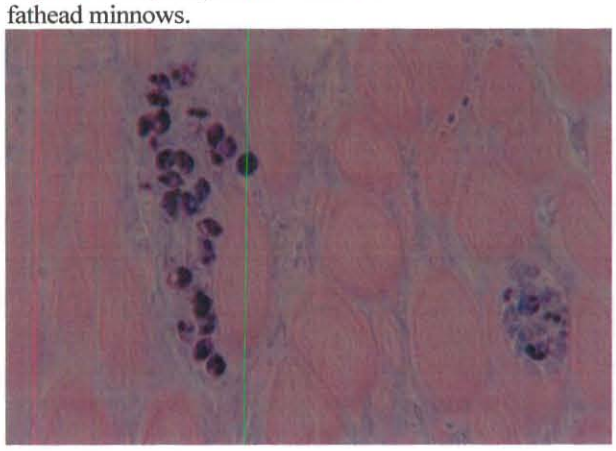


Figure 4-4. Myxosporean observed in fathead minnow peripheral nerve tissue.

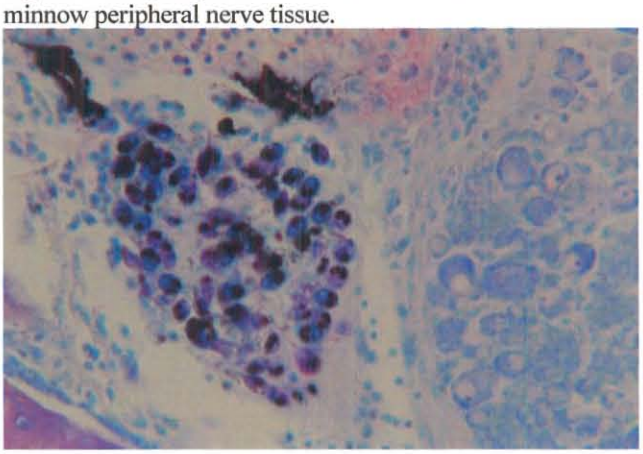


Figure 4-5. *Henneguya* – like myxospores encysted in branchial tissue of fathead minnows.

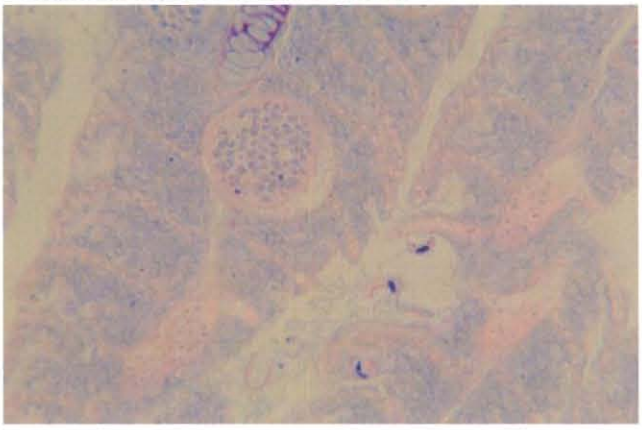


Figure 4-6. Mysospores observed in cartilage of fathead minnow.

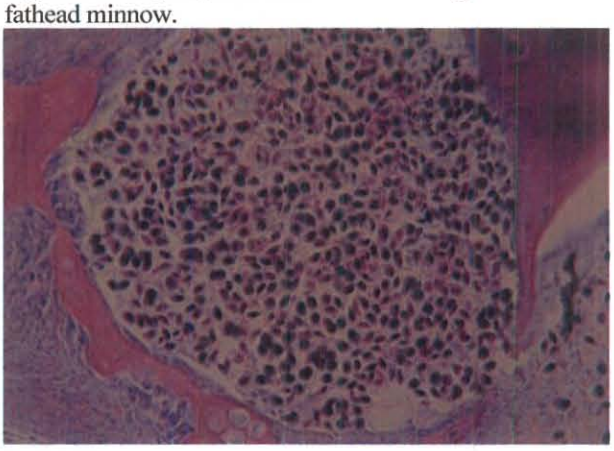


Figure 4-7. *Henneguya*-like myxospores in connective tissue of fathead minnows.

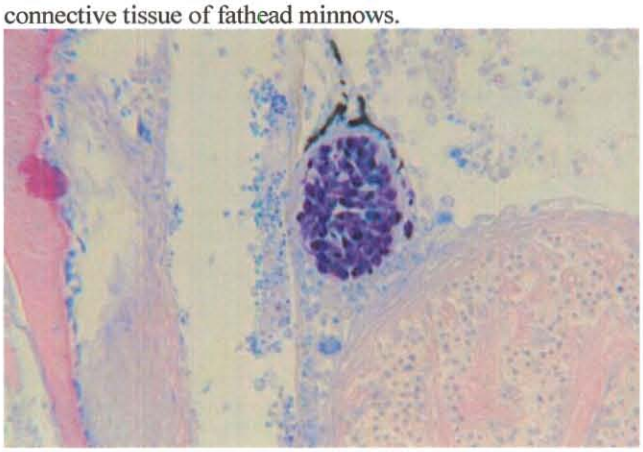


Figure 4-8. Fathead minnow –digenetic trematode in skeletal muscle.

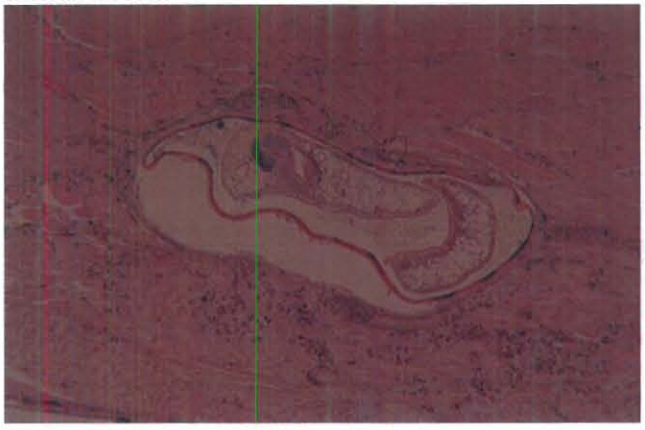


Figure 4-9. Digenetic trematode in fathead minnow meninges.

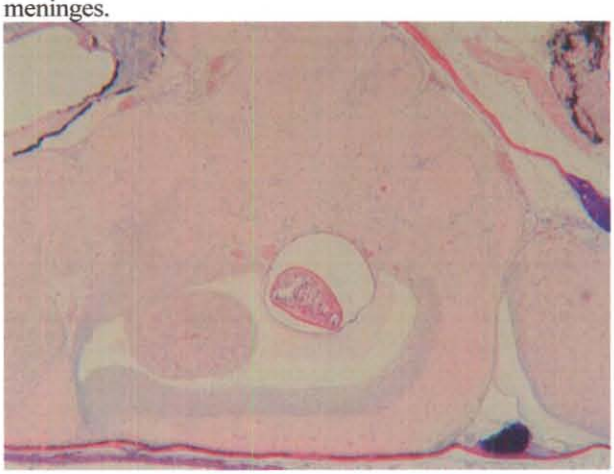


Figure 4-10. Fathead minnow branchial monogenean..

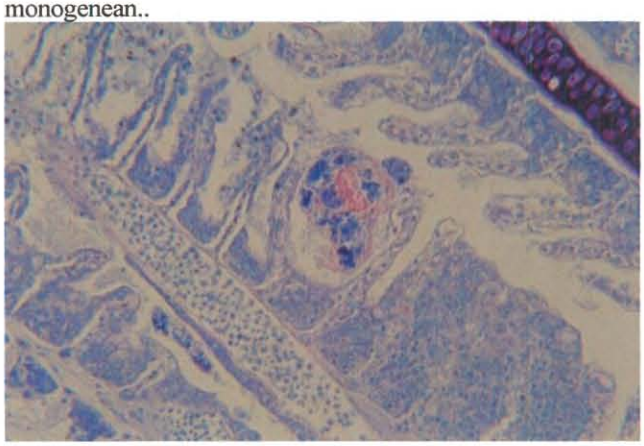


Figure 4-11. Fathead minnow suspect *Gyrodactylus sp.* in skin sections..

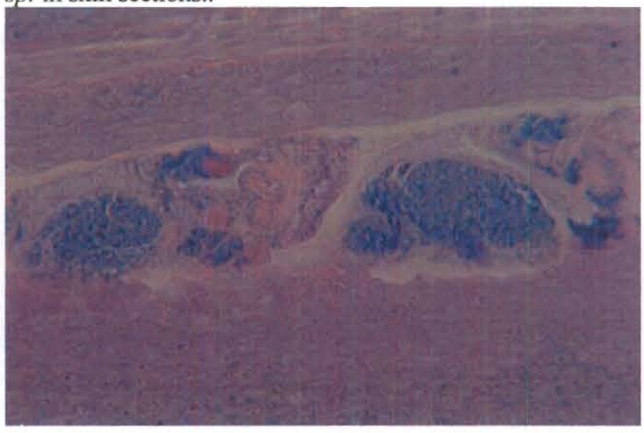


Figure 4-12. Fathead minnow branchial protozoan: *Trichodina sp.*.

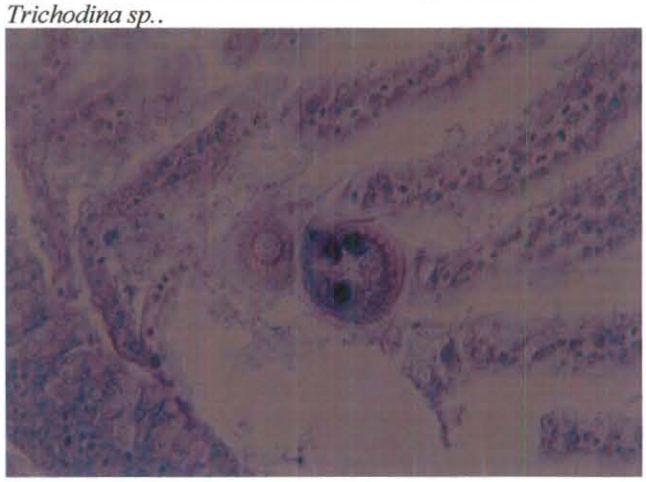


Figure 4-13. Branchial *Apiosoma sp.* observed in fathead minnow.

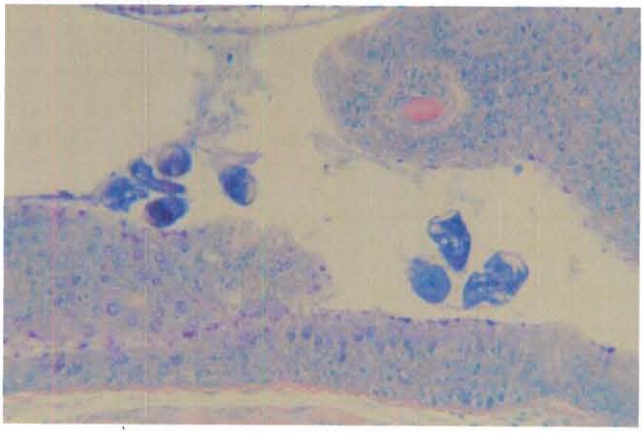


Figure 4-14. Coccidian parasite observed in fathead minnow gastrointestinal tract..

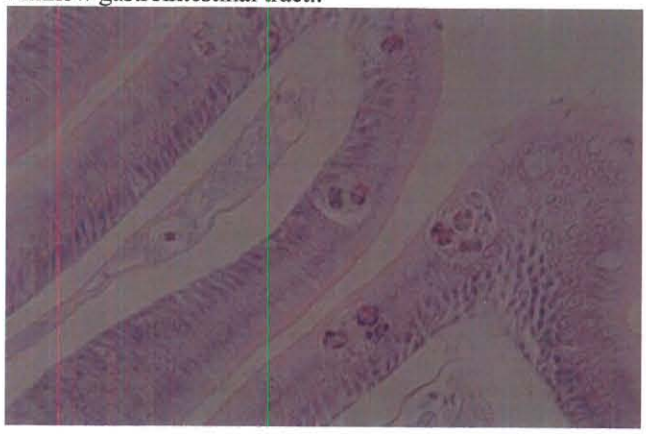


Figure 4-15. Fathead minnow intestinal cestode.

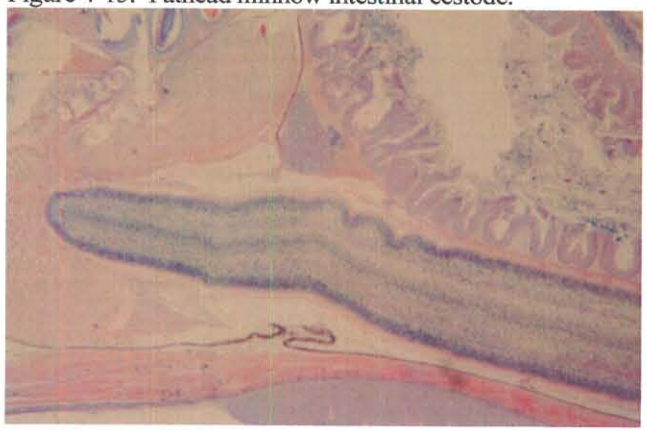


Figure 4-16. Walleye cestode.

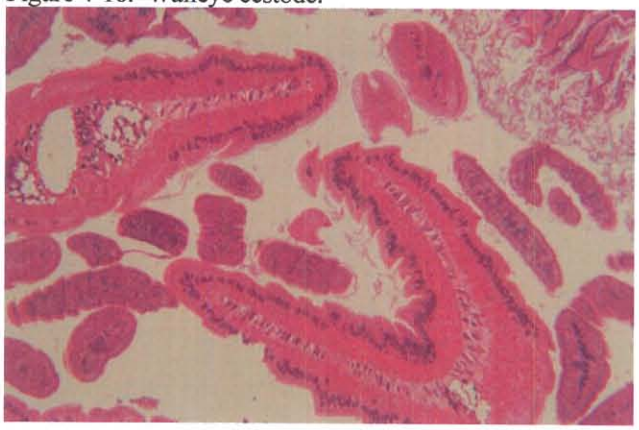


Figure 4-17. Nematodes observed in walleye GI/viscera.

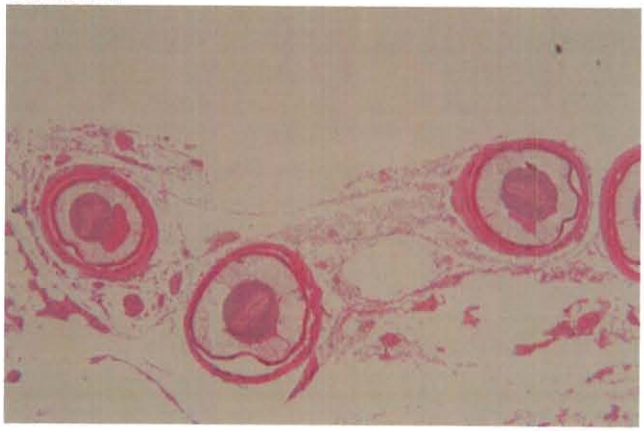


Figure 4-18. Nematodes observed in walleye GI/viscera.

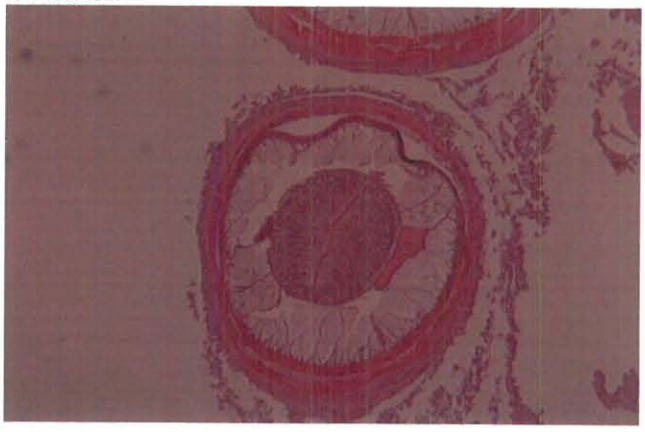


Figure 4-19. Myxosporean in walleye skeletal muscle.

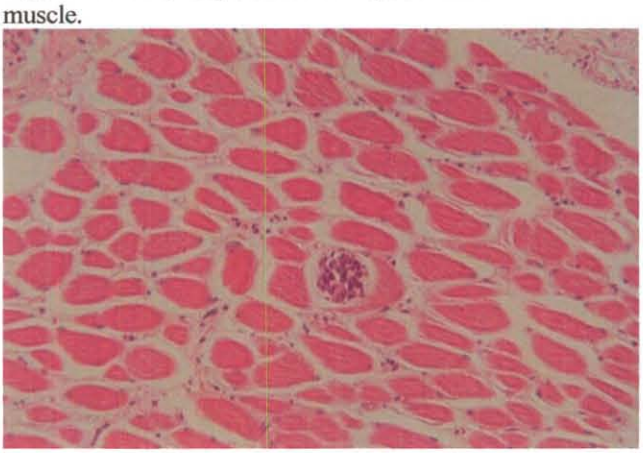


Figure 4-20. Giemsa stained myxosporean in skeletal muscle of walleye.

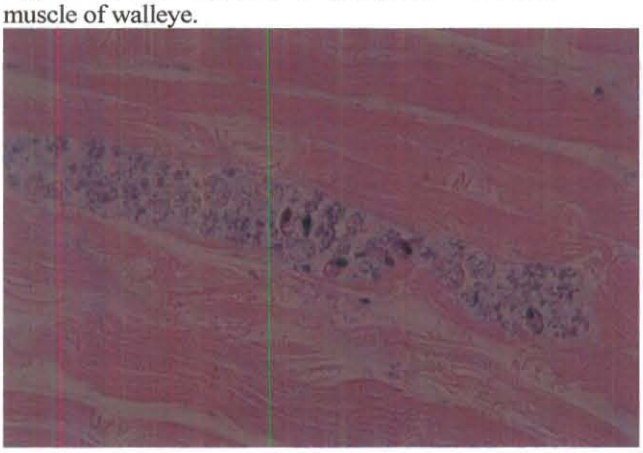


Figure 4-21. Microsporidian: suspect *Ovipleistophora o.* in walleye ovary.

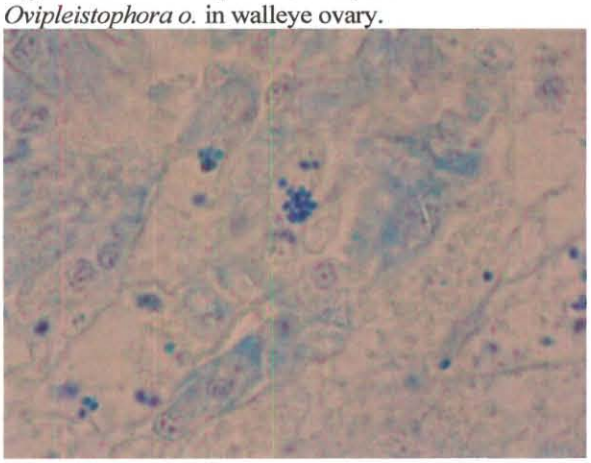


Figure 4-22. White bass cestode. Suspect *Proteocephalus sp.*

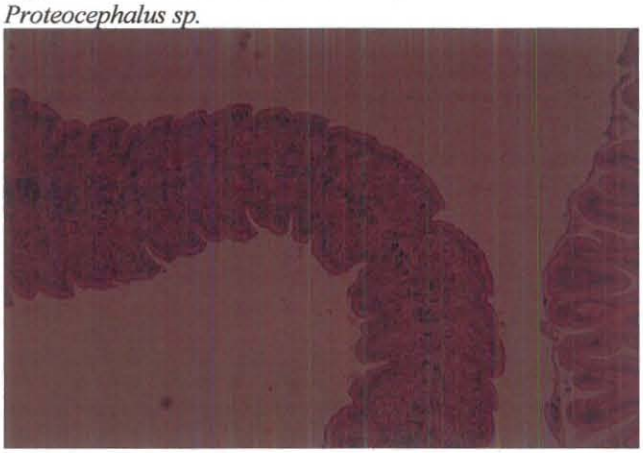


Figure 4-23. White bass cestode in GI tract.

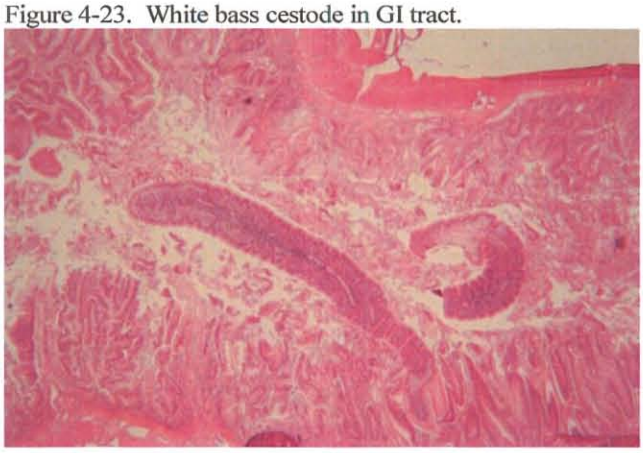


Figure 4-24. White bass cestode.

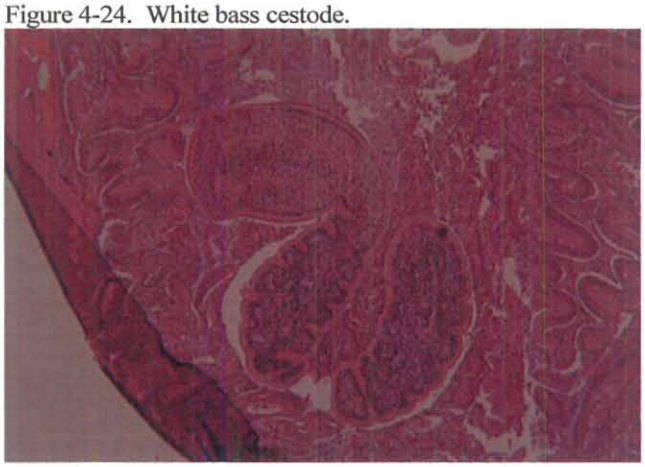


Figure 4-25. White bass *Trichodina sp.*-gill section.

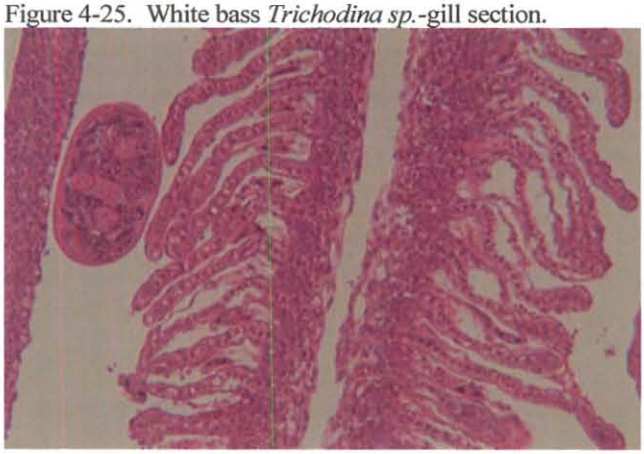


Figure 4-26. White bass monogenean trematode.

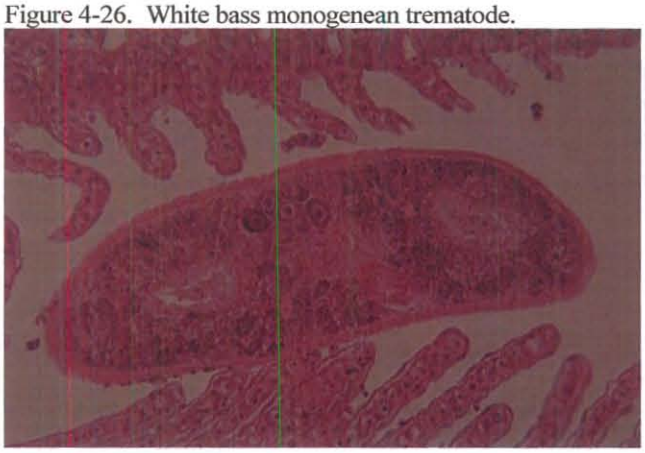


Figure 4-27. Nematode in white bass.

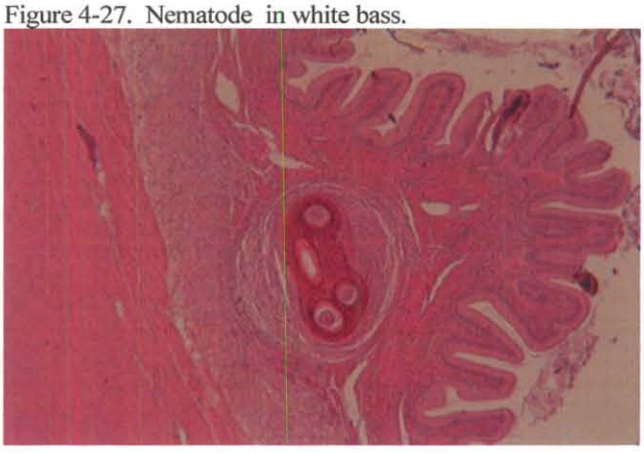


Figure 4-28. Black crappie nematode observed in visceral cavity.

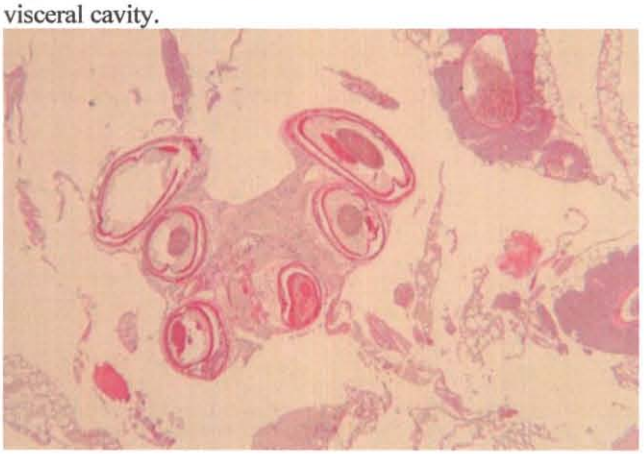


Figure 4-29. External protozoan: *Ichthybodo sp.* – black crappie.

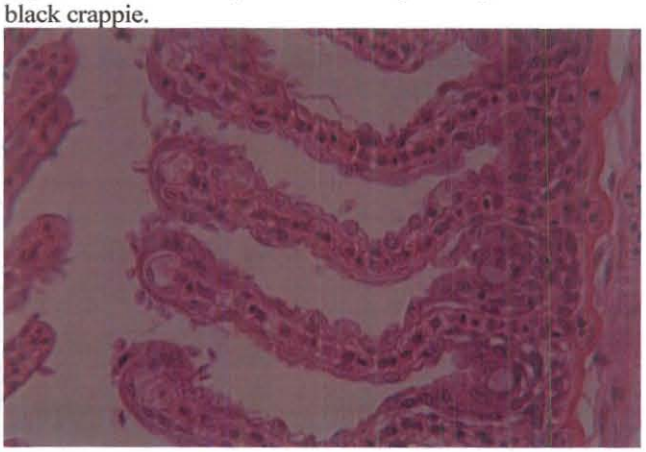


Figure 4-30. Black crappie myxosporean-kidney.

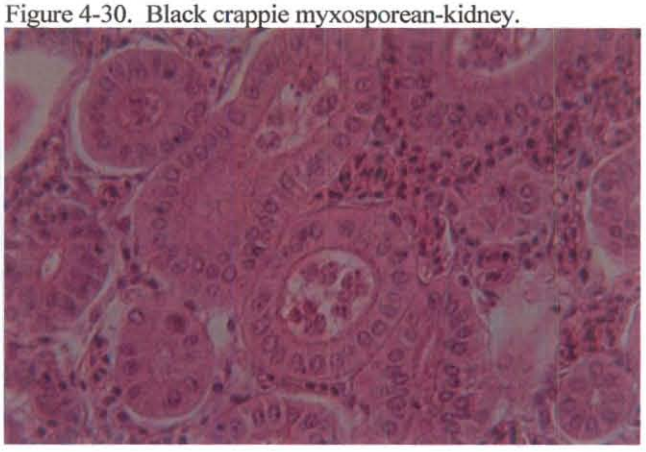


Figure 4-31. Black crappie. Suspect *Myxobolus sp.* in kidney.

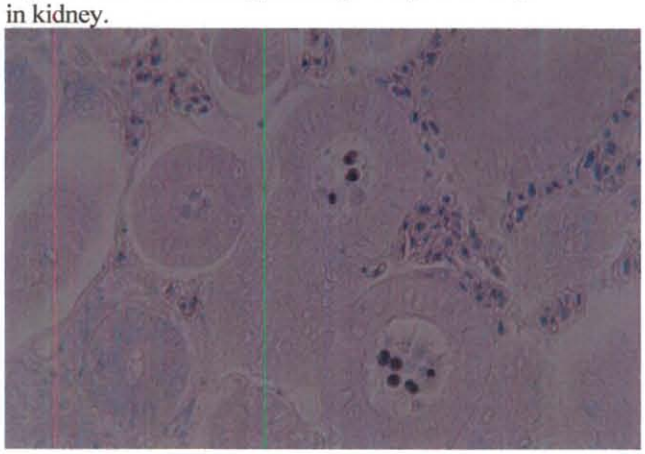


Figure 4-32. Yellow perch. cestode.

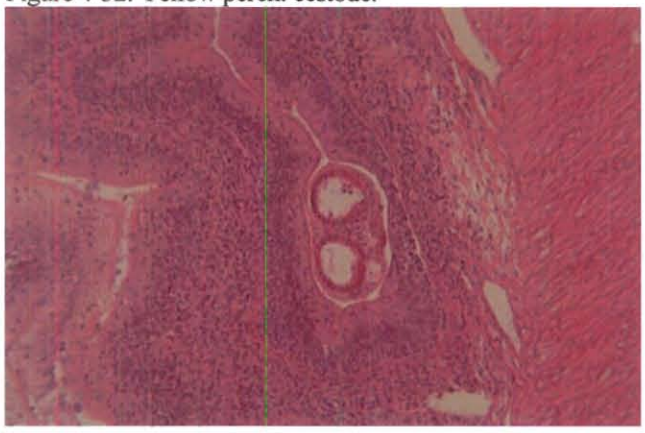


Figure 4-33. Yellow perch suspect *Ligula sp.*

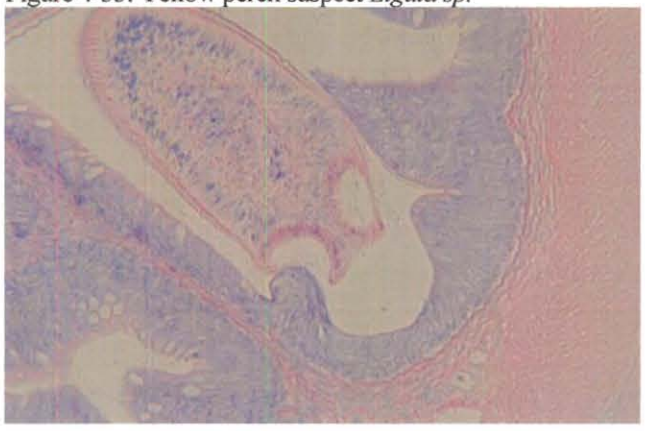


Figure 4-34. Yellow perch – *Trichodina sp.*

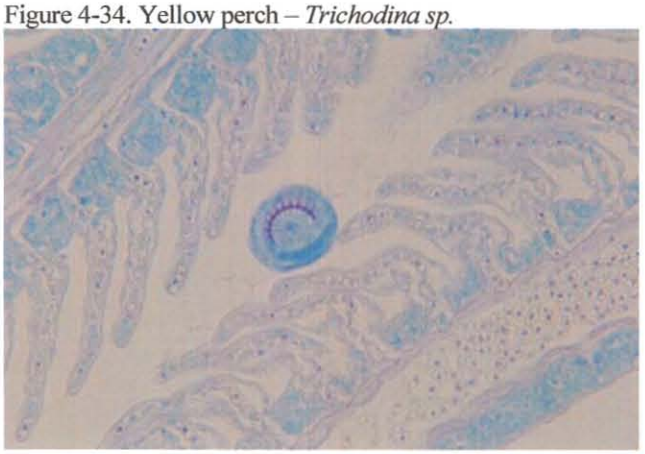


Figure. 4-35. Yellow perch – *Apiosoma sp.*

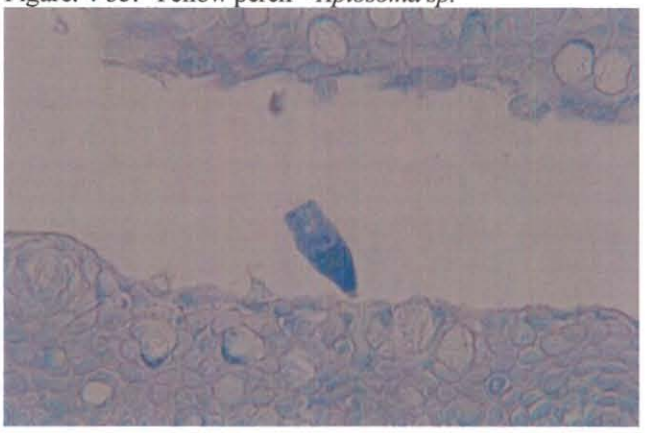


Figure 4-36. Yellow perch digenetic trematode-skeletal muscle.

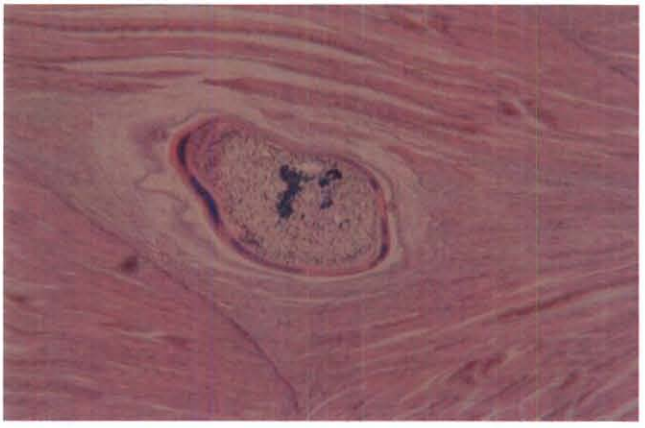


Figure 4-37. Yellow perch. *Gyrodactylus*-like monogenean.

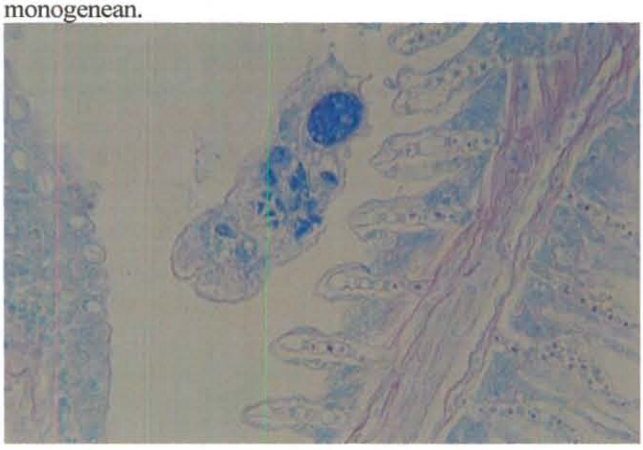


Figure 4-38. Yellow perch. Nematode in the muscularis and viscera of GI tract.

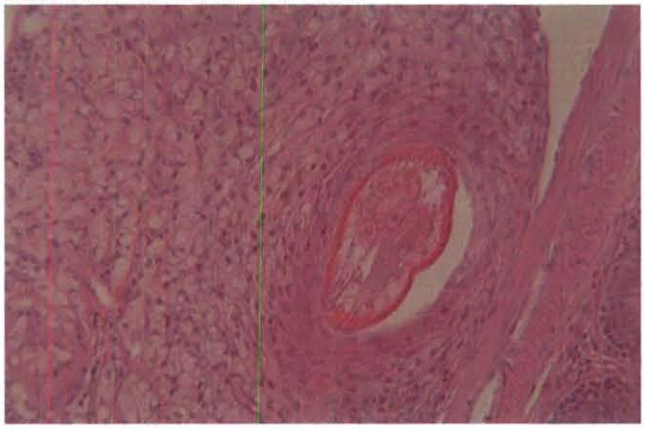


Figure 4-39. Yellow perch. Coccidian sporozoan in GI tract.

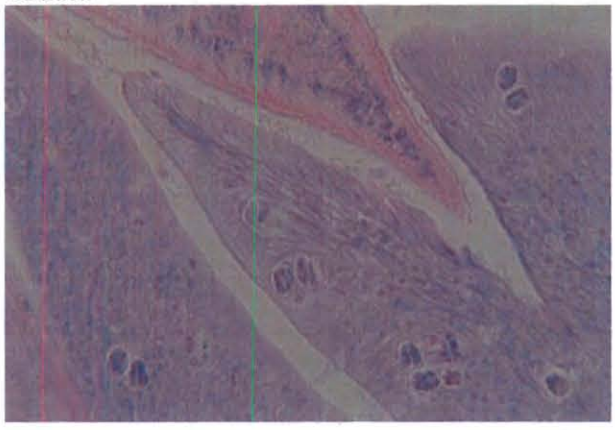


Figure 4-40. Yellow perch. Myxosporean in spinal cord.

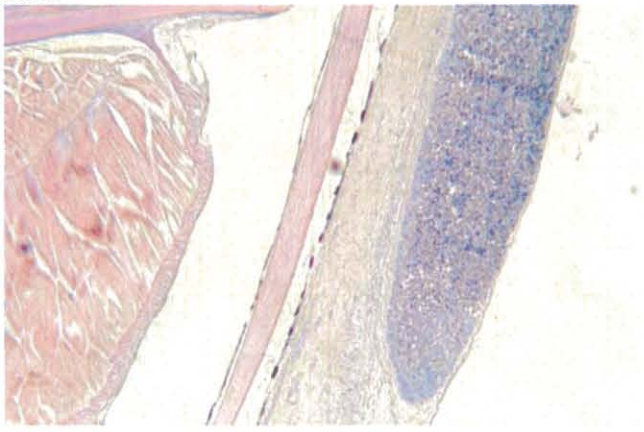


Figure 4-41. Yellow perch. *Henneguya*-like myxosporean in spinal cord.

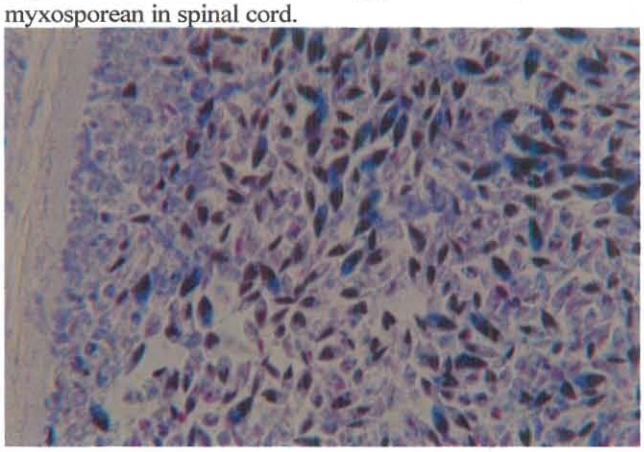


Figure 4-42. Yellow perch. Myxosporean observed in lower jaw connective tissue.

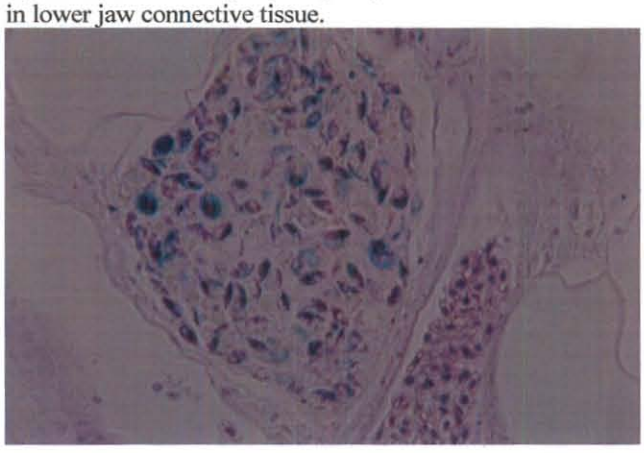


Figure 4-43. Northern pike. *Gyrodactylus*-like parasite on gill surface.

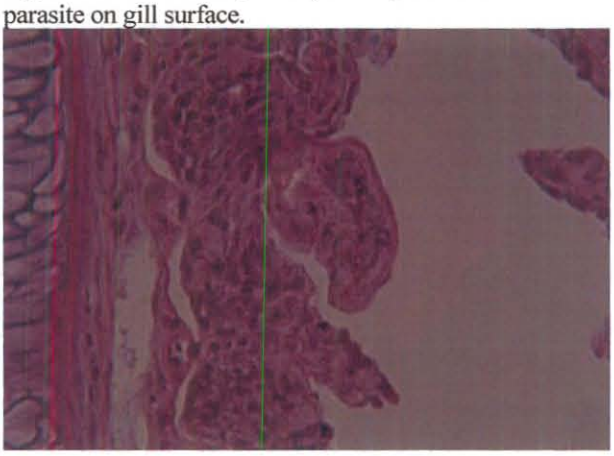


Figure 4-44. Northern pike. Mature cestode.

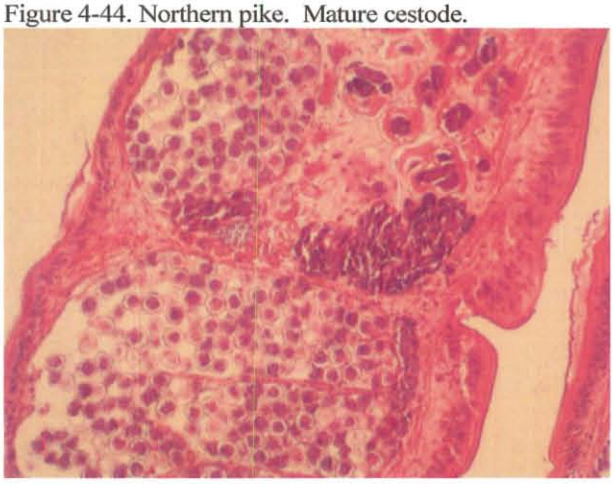


Figure 4-45. Northern pike. Suspect leech.

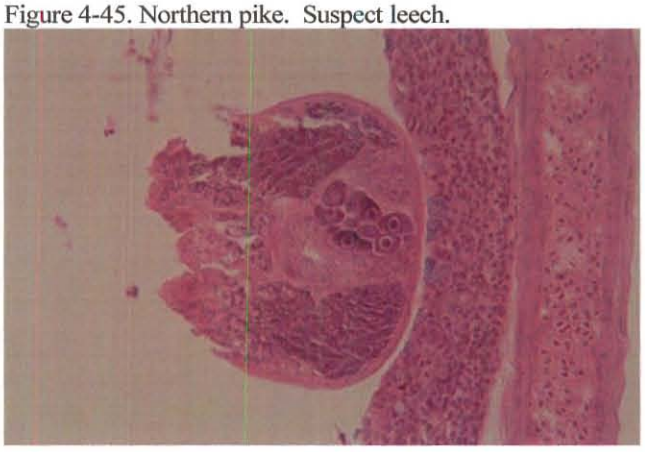


Figure 4-46. Northern pike. Leech-like parasite on gill surface.

Figure 4-47. Northern pike. *Myxidium sp.* in kidney tubule.

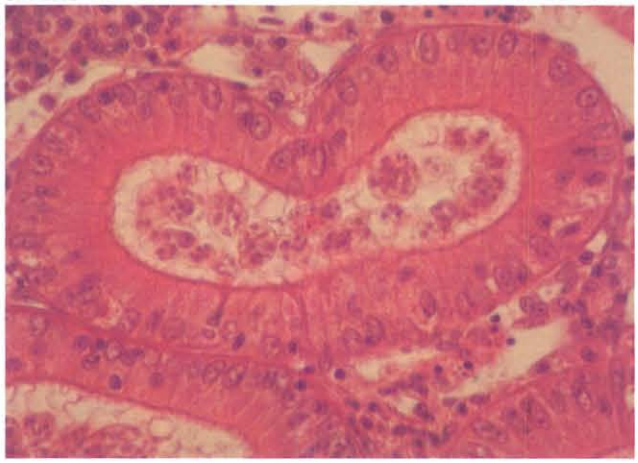


Figure 4-48. Northern pike. Giemsa stain of *Myxidium sp.* in kidney tubule.

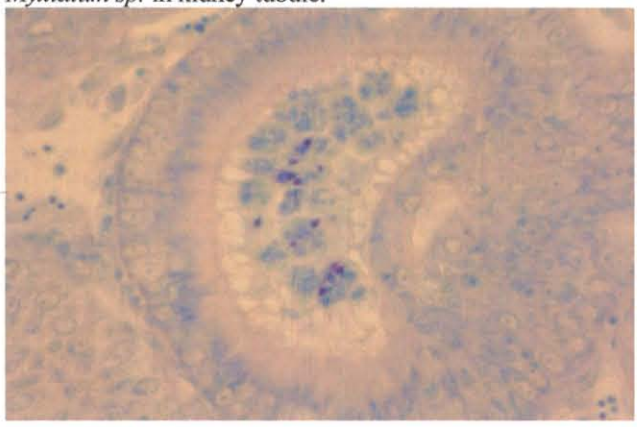


Figure 4-49. Northern pike. *Ichthyobodo sp.*

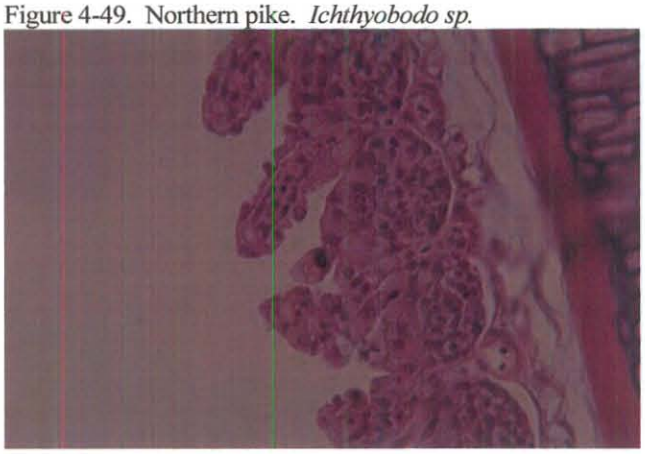


Figure 4-51. Northern pike. *Apiosoma sp.*

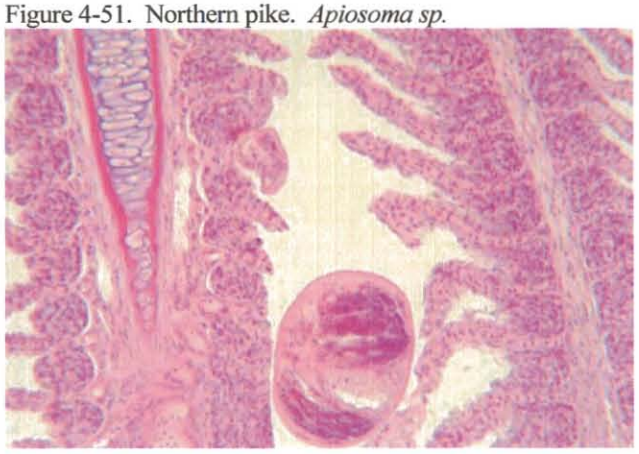
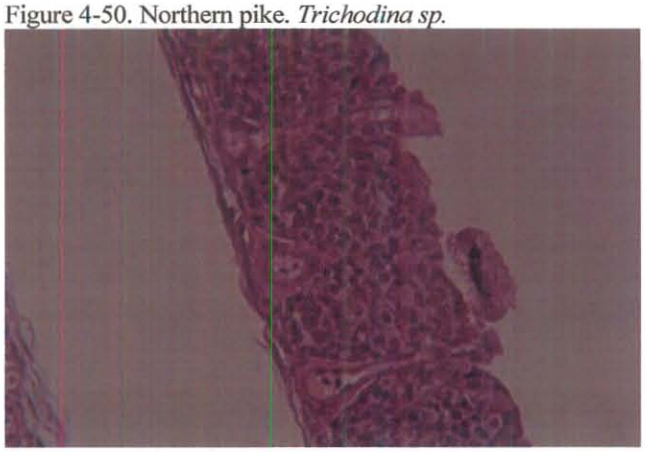


Figure 4-50. Northern pike. *Trichodina sp.*



Chapter 5 — Fish Parasites

Methods

All fish were monitored for macroparasites during necropsy and collection of tissue samples for bacteriology and virology assays. We performed comprehensive parasite surveys using additional randomly selected fish from each sample site. To help increase the number of fish examined we collected and froze gastrointestinal tracts of select species at Lake Ashtabula and Lake Traverse using fish previously sampled for bacteriology and virology. At the temporary field stations, work stations were equipped with dissecting microscopes with fiber optic lighting and compound microscopes with bright field and phase contrast lighting. A digital camera and lap top computer was used to capture and store images of parasites alive before they were placed in tissue fixatives. We attempted to examine a minimum of five freshly caught fish of each species at the temporary field stations. Additional fish not examined at the field station were flash-frozen in a bath with -70°C ETOH and then transferred to a 14.8 ft^3 chest freezer power by a 2000 W portable generator and transferred between sites in a utility trailer. Frozen fish were examined later at Bozeman Fish Health Center, Montana. Fish were examined externally and internally for parasites according to methods of the *National Wild Fish Health Survey* (2006). In brief, wet mounts were prepared from skin scrapings, fins and gill clips. The gastro intestinal tract was removed divided into three sections corresponding to the esophagus, stomach and pyloric caeca, and intestines. An incision was made along the length of each section and examined under a dissecting microscope. Sections were then scraped and contents were transferred to Petri dishes and suspended in normal physiological saline solution. We prepared tissue smears from major organs including brain, kidney, spleen, liver, gall bladder, heart. Eyes were removed and dissected. The skin was removed from one side of the fish and muscle groups were examined at regular intervals. We examined wet mounts, tissue smears, and gut contents with light microscopy at 20 – 400X magnification. Parasites recovered during the survey were photographed and then preserved in either alcohol-formalin-acetic acid (AFA) or glycerin-alcohol (nematodes) solutions. Staining, mounting, and identification of preserved specimens were performed by parasite specialists at the U. S. Fish and Wildlife Service Lacrosse Fish Health Center. In addition to findings presented here from the classical parasite survey, results and discussion of parasites observed in stained tissue sections of fish from Devils Lake are explained in the histology section (Chapter 4).

Results and Discussion

Devils Lake.— A total of 346 fish representing seven species were examined for parasites. For the comprehensive survey, 27 fish were examined fresh at the temporary field station during the three-day sampling operation at Six Mile Bay. Another 23 whole fish and 11 gastrointestinal tracts were flash frozen and examined later at the laboratory in Montana (Table 5.1). Nearly 300 fish were examined grossly for macroscopic parasites during necropsy and tissue collection for microbiological assays. A total of sixteen different parasites were identified to the level of genus and of those, four parasites were identified to species. One gryporhynchid metacestode from yellow perch could not be identified to a taxonomic position closer than Family (Table 5.2). The

parasite taxonomic community included two protozoa, two myxosporeans, two monogeneans, two digenean trematodes, five cestodes, and four nematodes. Parasites were recovered from all species of fish except white sucker. We identified eight different parasites from fathead minnow, six each from white bass and yellow perch, three from walleye, and two each from black crappie and northern pike.

Ciliated protozoan parasites were frequently observed in wet mounts of skin scrapings and/or gill lamellae of many fish (Figure 5.1). The mobile protozoan, *Trichodina sp.* was the most common ectoparasite of fish in Devils Lake. *Trichodina sp.* was observed in mucus from skin scrapings and in wet mounts of gill lamellae in four of the seven species examined. Additionally, the sessile ciliated protozoan *Apiosoma sp.* was observed on gills and in skin scrapings from walleye.

Spores of Myxosporea (Bivalvulida: Platysporina) from two different genera were observed in kidney tissue squash preparations from fathead minnow (Table 5.2). The first spore, a *Myxobolus sp.*, was oval-shaped and approximately 12.5μm L × 11.0μm W in frontal view (Figure 5.2). The spores had two even anterior polar capsules 5.0μm L × 3.0μm W. The second spore was slightly smaller in size and was 12.0μm L × 10.0μm W in frontal view (Figure 5.3). These spores had a long (23 – 38 μm) single caudal extension of the spore valve suggestive of genus *Unicauda*. We observed spores of both species extrude polar filaments in saline wet mount preparations of infected kidney tissue.

Fish from Devils Lake were host to two monogeneans, parasites that complete their life cycle on one host (Table 5.2). *Gyrodactylus hoffmani* was observed on fins of fathead minnow and *Onchocleidus chrysops* (syn. *Cleidodiscus chrysops*) was found on the gills of white bass. Additionally, two trematodes, parasites that require alternate hosts to complete their lifecycle, were found in fathead minnow from Devils Lake (Figure 5.4). Metacercariae of the larval genus *Neascus sp.* was found in hyaline cysts within melanized capsules in skeletal muscle. Metacercariae of larval genus *Diplostomulum* were observed in hyaline cysts in lateral musculature between the rib bones.

Cestodes from three taxonomic orders, Cyclophyllidea, Pseudophyllidea, and Proteocephalidea, were found in fish from Devils Lake (Table 5.2). Five of the seven species of fish sampled were infested with at least one species of cestode. Two cestodes were identified to the species level although many fish were infested with metacestodes (larvae) that could not be identified to a taxonomic position closer than Genus. *Bothriocephalus cuspidatus* and metacestodes of *Bothriocephalus sp.* (Pseudophyllidea) were the most common cestodes at Devils Lake and were found in the intestines of fathead minnow, northern pike, walleye, white bass, and yellow perch. Mature adult stages of *B. cuspidatus* were only observed in walleye. The mature adult stage of *Proteocephalus pinguis* (Proteocephalidea) was found in the intestine of northern pike while metacestodes of *Proteocephalus sp.* were recovered from intestines of fathead minnow, northern pike, white bass, and yellow perch. A metacestode of the Family Gryporhynchidae (Cyclophyllidea) was recovered from the intestine of yellow perch. The single specimen was preserved in AFA solution and then stained with acetocarmine (Figure 5.5). For this reason we could not make accurate measurements of the rostellar hooks which are necessary

for speciation of these poorly studied parasites in North America. This finding is believed to represents a new state, site, and host record.

Four species of nematodes (Nematoda) were identified from fish collected at Devils Lake (Table 5.2). The larval of *Contracaecum sp.* (Ascaridida: Anisakidae) was observed in characteristic cysts along the mesenteries of black crappie and white bass. Another ascarid worm, *Raphidascaris sp.*, was observed in the intestines of fathead minnow and yellow perch. As in other years, we found *Spiroxys sp.* (Spirurida: Gnathostomatidae) in small cysts (D < 1.0 mm) in viscera and mesenteries of white bass only. Lastly, larval forms of *Rhabdochona sp.* (Oxyuridea: Rhabdochonidae) were found in mesenteries of yellow perch.

Lake Ashtabula.— A total of 435 fish representing 14 species were examined for parasites (Table 5.3). Of the total, 390 fish were examined grossly for macroscopic parasites during necropsy and tissue collection for bacteriology and virology. For the comprehensive survey, 23 fish were examined fresh at the temporary field station at Sibley Landing before thunderstorms forced our retreat. Another 22 whole fish and gastrointestinal tracts from 57 fish were flash-frozen and examined later at the laboratory in Montana. A total of thirty-four different parasites were identified to the level of genus or larval genus and of those, eleven parasites were identified to species (Table 5.4). These included three protozoa, one microsporean, four myxosporeans, two monogeneans, ten trematodes, five cestodes, six nematodes, two acanthocephalans, one leech, and one parasitic crustacean. Parasites were recovered from all species of fish examined. We found thirteen different parasites in white bass, eight in black bullhead, seven each in blacknose dace and walleye, six each in spottail shiner and white sucker, five each in black crappie, smallmouth bass and tadpole madtom, four each in northern pike and trout perch, three in blue gill, and two each in fathead minnow and yellow perch.

Ciliated protozoan parasites were commonly observed in skin scrapings and in wet mount preparations of fins and gill lamellae (Figure 5.1). Mobile trichodinids were the most common ectoparasites at Lake Ashtabula and were observed on nine of fourteen species of fish examined (Table 5.4). Additionally, two sessile ciliated protozoa, *Apiosoma sp.* and *Epistylis sp.*, were found on either the gills fins or in skin scrapings from several species of fish.

We observed whitish-colored, spindle-shaped sporophorocysts in skeletal muscle of tadpole madtom similar to descriptions of the microsporidian *Heterosporis sp.* (Microsporea: Pleistophoridida) (Dyková and Lom 2007). Sporophorocysts were visible with stereo microscopy at 6X magnification or greater (Figure 5.6). Sporophorocysts appeared more concentrated in muscle above the lateral line and in the caudal peduncle. Sporophorocysts contained numerous sporophorous vesicles each with four developing sporoblasts (developing spores). Ovoid shaped spores were 7.3µm L × 4.2µm W. A posterior vacuole was observed when spores were viewed with dark field microscopy at 400 – 1000X magnification otherwise no other internal morphological features were evident in unstained fresh mount preparation. Differential staining with Grams stain was not successful. Histological sections of previously frozen tissue show distended muscle fibers with formation of a thick-walled case with proliferation of microsporidia within sporophorous vesicles.

Spores of four different *Myxobolus sp.* (Myxosporea: Platysporina) were found among three species of fish from Lake Ashtabula (Table 5.4). Blacknose dace were host to possibly two different myxospores the first of which was observed in wet mount preparations of skin scrapings (Figure 5.7). Ovoid spores had mean dimensions of 13.5μm L × 11.0μm W in frontal view and contain two even anterior polar capsules 6.6μm L × 4.2μm W (n = 10). The second myxosporean infecting blacknose dace was found in numerous oval-shaped cysts located in the soft connective tissue between principle fin rays (Figure 5.8). The cysts were occupied primarily with trophozoites in various stages of development and only a few mature myxospores were observed in stained histology sections. Spore morphology was similar to spores observed in skin scrapings however no direct comparisons were made because we were not able to accurately measure spores dimensions from histological sections. A third myxosporean was found in numerous elongate plasmodia in muscle of spottail shiner (Figure 5.9). In frontal view, oval-shaped spores were 11.6μm L × 9.8μm W (n = 10) and had two even anterior pyriform-shaped polar capsules 5.1μm L × 2.9μm W. Lastly, a fourth myxosporean was observed in whitish-colored elongate plasmodia in skeletal muscle of trout perch (Figure 5.10). In frontal view, pyriform spores were 12.5μm L × 6.3μm W (n = 5) with a knob-like projection of the anterior. Spores contained two mostly even pyriform-shaped polar capsules 5.5μm L × 2.0μm W. Several spores extruded polar filaments (mean length = 37.2 μm) in wet mount preparations. Spores were morphologically similar to those of *M. procerus* infecting trout perch in Duluth Harbor, Lake Superior (Cone et al. 1997).

Fish from Lake Ashtabula were host to two monogenean parasites (Table 5.4). We observed a *Gyrodactylus sp.* morphologically similar to *G. hoffmani* on the pectoral fin of one blacknose dace (Figure 5.11). *Onchocleidus chrysops* (syn. *Cleidodiscus chrysops*) was found on the gills of black bullhead and white bass.

Ten different trematodes were found among eleven of the fourteen species of fish examined from Lake Ashtabula (Table 5.4). Intestinal trematodes included *Alloglossidium corti* from tadpole madtom, *Crepidostomum cornutum* from yellow perch, and *Crepidostomum sp.* from white bass (Figure 5.12). Metacercariae of four different trematodes were found infecting muscle tissues including the yellow grub *Clinostomum marginatum* (syn. *C. complanatum*) in white bass, *Hysteromorpha triloba* in black bullhead and fathead minnow, larval genus *Neascus sp.* in fathead minnow and spottail shiner, and *Tylodelphys scheuringi* in trout perch (Figures 5.13-14). *Neascus sp.* was also found encysted in the liver of white bass. Other encysted metacercariae from the liver included *Postodiplostomum minimum* from blue gill and northern pike. Metacercariae of *Diplostomulum spathaceum* was observed in the lens of an eye of one walleye. Finally, metacercariae of *Prohemistomulum sp.* was found encysted in the peritoneal cavity of walleye.

Cestodes from three taxonomic orders, Caryophyllidea, Pseudophyllidea, and Proteocephalidea, were found in fish from Lake Ashtabula (Table 5.4). Eight of the 14 species of fish sampled were infested with at least one species of cestode. Two cestodes were identified to species and the others to genus because most specimens were immature metacestodes. *Bothriocephalus cuspidatus* and metacestodes of *Bothriocephalus sp.* (Pseudophyllidea: Bothriocephalida) were found in the intestines of northern pike, walleye, white bass. Mature adult stages of *B. cuspidatus* were observed in walleye and white bass. Adult forms of

Corallobothrium fimbriatum (Proteocephalidea: Proteocephalidae) were found in the intestines of black bullhead while metacestodes of *Corallobothrium sp.* were observed in the intestines of tadpole madtom (Figure 5.15). Metacestodes of *Proteocephalus sp.* (Proteocephalidea: Proteocephalidae) were found in the intestine of black crappie, northern pike, spottail shiner, and white bass. *Biacetabulum sp.* (Caryophyllidea: Caryophyllaeidae) was recovered from the intestine of white sucker.

Six species of nematodes (Nematoda) were found in fish from Lake Ashtabula (Table 5.4). The conspicuous encysted larval of *Contracaecum sp.* was quite common being observed in nine of fourteen species of fish examined (Figure 5.16). *Camallanus sp.* (Spirurida: Camallanidae) was the second most common nematode and was found in the intestines of black crappie, smallmouth bass, walleye, and white bass. Larval *Raphidascaris sp.* was observed encysted in mesenteries of white bass and white sucker. White sucker were also host to encysted larvae of *Spiroxys sp.* found along intestinal mesenteries as well as *Rhabdochona sp.* which was recovered from the intestine. Lastly, *Spinitectus sp.* (Oxyuridea: Cystidicolidae) was found in the intestine of white bass (Figure 5.16).

Two species of thorny-headed worms (Acanthocephala) were found in the intestines of fish from Lake Ashtabula (Table 5.4). *Neoechinorhynchus sp.* (Neoechinorhychidae) was observed in smallmouth bass and white bass (Figure 5.17) and *Pomphorhynchus bulbocolli* was found in black bullhead, tadpole madtom, white bass, and white sucker (Figure 5.18). Black bullheads were also hosts to the parasitic leech *Myzobdella lugubris* and the parasitic crustacean *Actheres pimelodi*.

Lake Traverse.— A total of 596 fish representing 17 species were examined for parasites (Table 5.5). Of the total, 522 fish were examined grossly for macroparasites during necropsy and tissue sample collection for bacteriology and virology. For the comprehensive survey, 36 fish were examined fresh at the temporary field station. Another 38 whole fish and gastrointestinal tracts from 137 fish were flash-frozen and examined later at the laboratory in Montana. A total of fifty-four different parasites were identified to the level of genus or larval genus, and of those, sixteen parasites were identified to species. These included four protozoa, one microsporean, seventeen myxosporeans, two monogeneans, four trematodes, eleven cestodes, ten nematodes, three acanthocephalans, one leech, and one parasitic crustacean (Table 5.6). Variable numbers of parasites were recovered from all species of fish examined. We found twelve species of parasites each in bluegill, rock bass and white bass, eleven in fathead minnow, ten in channel catfish, eight each in black bullhead, freshwater drum and yellow perch, seven in black crappie, six in orangespotted sunfish, five each in carp and walleye, four each in bigmouth buffalo, white sucker and yellow bullhead, two in emerald shiner, and one in shorthead redhorse.

The motile protozoan parasite *Trichodina sp.* was commonly observed on skin, fins and/or gills and pseudobranchs of ten different species of fish from Lake Traverse (Table 5.6). We also observed three sessile protozoan parasites including *Apiosoma sp.* on fins and gills of fathead minnow, white bass and yellow perch, *Capriniana piscium* on gills of walleye, and *Ambiphrya sp.* on fins of white bass (Figure 5.1).

A single whitish-colored cyst-like structure we suspect was a xenoma of unknown tissue type was found in mesenteries near the liver of a single bluegill. A wet mount squash preparation of the structure revealed numerous refractile nondescript ovoid spores 9.0μm L × 7.0μm W (n = 10). Spores took-up methylene blue stain but the procedure did not help distinguish internal morphology (Figure 5.19). We speculate the spores may belong to the microsporidian genus *Glugea* (Microspora: Pleistophoridida) however no other xenomas were found that could have been used for additional study.

Spores of Myxosporea (Bivalvulida) from two different suborders and comprising four genera were found in fish from Lake Traverse (Table 5.6). From suborder Variisporina we observed spores from two families. Spore of *Chloromyxum sp.* (Chloromyxidae) were found in the gall bladders of black crappie and bluegill (Figure 5.20). Ovoid shaped spores from the black crappie were 7.4μm L × 7.0μm W (n = 7) and contain four pyriform-shaped anterior polar capsules. Oval-shaped spores from bluegill were slightly larger measuring 8.0μm L × 8.0μm W (n = 5) with four characteristic polar capsules measuring 3.5μm L × 3.0μm W. The differences in spore size suggest *Chloromyxum* from black crappie and bluegill are different species. Spores of *Myxidium sp.* (Myxidiidae) were found in the either the gall bladder, liver or bile ducts of five species of fish including black bullhead, channel catfish, freshwater drum, white sucker, and yellow bullhead (Figure 5.21). Spores from all species of fish were fusiform-shaped with two oval-shaped polar capsules, one at each end of the spore. Spores had longitudinal ridges giving them a striated appearance. Spores from black and yellow bullheads and channel catfish were similar in size at 10.5 – 11.0μm L × 5.0 – 5.3μm W. However, spores from the gall bladder and hepatic bile ducts of freshwater drum were somewhat larger in size at 13.0μm L × 6.4μm W (n=6) suggesting a different species. No measurements were made on spores of *Myxidium sp.* from white sucker.

From suborder Platysporina, we observed myxospores from two genera within the family Myxobolidae. We observed several histozoic plasmodia in the epithelium of gill lamellae of channel catfish. The cyst-like structures contained numerous elongated fusiform spores with two anterior polar capsules and two long caudal extensions of the spore valves typical of *Henneguya sp.*, possibly *H. exilis* (Figure 5.22). Eight different species of *Myxobolus* were found in fish from Lake Traverse (Table 5.6). Beginning with bigmouth buffalo, numerous oval-shaped white colored plasmodia were observed in secondary lamellae of gills (Figure 5.23). Plasmodia contained ovoid spores with two even anterior pyriform polar capsules. Spores were 17.0μm L × 15.0μm W and polar capsules were 8.0μm L × 5.0μm W. We also found histozoic plasmodia in intestinal lamina epithelium of bigmouth buffalo. Plasmodia contained ovoid spores 15.1μm L × 11.9μm W with two even polar capsules 5.8μm L × 3.3μm W (Figure 5.24). In emerald shiner, we observed small oval plasmodia in gill lamellae that contained pyriform spores of *Myxobolus sp.*13.9μm L × 10.2μm W with pyriform polar capsules 4.8μm L × 3.0μm W (Figure 5.25). We also found pyriform-shaped spores 11.0μm L × 9.0μm W in the kidneys of emerald shiner (Figure 5.26). Fathead minnow were host to five different myxosporeans including four *Myxobolus sp.* and one *Unicauda sp.* Plasmodia and spores were found in various tissues including the fins, gills, muscle, kidney, and brain. From the pectoral fin a melanized plasmodia was excised and squashed revealing numerous pyriform spores 14.5μm L × 8.0μm W (Figure 5.27). We observed numerous plasmodia in gill lamellae which contained pyriform spores of *Myxobolus sp.* 16.1μm L × 9.8μm W with two even pyriform polar capsules 6.5μm L × 3.1μm

W (Figure 5.28). We found *Myxobolus sp.* infecting lateral skeletal muscle of fathead minnow. Spores were ovoid-shaped and 12.0μm L × 10.0μm W with two even pyriform polar capsules 6.0μm L × 4.0μm W (Figure 5.29). A squash preparation of fathead minnow brain tissue revealed ovoid spores of *Myxobolus sp.* 11.7μm L × 10.0μm W with two anterior even pyriform polar capsules (Figure 5.30). Lastly, we found ovoid spores 12.0μm L × 10.7μm W in a kidney squash preparation of fathead minnow. Spores had two even polar capsules 5.0μm L × 3.1μm W and a single posterior caudal extension of variable length (12 - 35μm) suggestive of *Unicauda sp.* (Figure 5.31).

Three different monogeneans were found on fish from Lake Traverse (Table 5.6). We found *Gyrodactylus sp.* in the nares of fathead minnows (Figure 5.32) and *Microcotyle spinicirrus* anchored to the gills of freshwater drum (Figure 5.33). An unidentified monogenean with four eye spots was observed on the gills of rock bass although the specimen was lost during an attempt to transfer the parasite to the fixative (Figure 5.34).

We found four different trematodes during examination of fish from Lake Traverse (Table 5.6). Metacercariae of *Clinostomum marginatum* were found in skeletal muscle of rock bass. Metacercariae of larval genus *Neascus* of *Postodiplostomum minimum centrarchi* were found encysted in the liver of bluegill and skeletal muscle of orangespotted sunfish. *Neascus* of *Ornithodiplostomum ptychocheilus* were found in the cranium of fathead minnow (Figure 5.35). Encysted metacercariae of *Neascus sp.* were also found in skeletal muscle of bluegill, fathead minnow, and rock bass. Hyaline cysts of *Neascus sp.* were typically found in subcutaneous melanized capsules resulting in a condition commonly referred to as black spot disease. One adult trematode *Megalogonia ictaluri* was found in the intestine of channel catfish (Figure 5.35).

Cestodes from four taxonomic orders with representatives of one family within each order were found in fish from Lake Traverse. We identified four cestodes to species but also found metacestodes or plerocercoids of at least six additional cestodes that could not be identified to a taxonomic level closer than genus (Table 5.6). From the order Caryophyllidea (Caryophyllaeidae), *Hunterella nodulosa* was found in white sucker and *Khawia iowensis* was found in common carp. Photomicrographs of both these caryophyllid cestodes can be found in survey results from 2007 (Peters 2009). Species in Caryophyllaeidae are distinct from most other cestodes in that they lack proglottids and thus each worm contains only one set of reproductive organs. We found tapeworms from one genus in the order Pseudophyllidae (Bothriocephalida). *Bothriocephalus cuspidatus* and metacestodes of *Bothriocephalus sp.* were commonly observed in the intestines of several fish including orangespotted sunfish, walleye, white bass, and yellow perch. *B. cuspidatus* is widely distributed in North America and has been reported from several species of fish most notably in walleye and sauger. Tapeworms from two genera of the order Proteocephalidea (Proteocephalidae) were identified during the survey. *Corallobrothrium fimbriatum*, a common tapeworm of ictalurids, was found in black bullhead (Figure 5.38) while metacestodes of *Corallobrothrium sp.* were recovered from channel catfish. Metacestodes of *Proteocephalus sp.* were found in black crappie, rock bass, white sucker, and yellow perch. Finally, we found metacestodes representing order Cyclophyllidea (Gryprohynchidae) in four species of fish from Lake Traverse. We found suspect metacestodes of *Paradilepis sp.* in the liver of black bullhead (Figure 5.39) and *Valipora sp.* in the gall bladder

of orangespotted sunfish (Figure 5.40). Other unidentified suspect Gryporhynchid metacestodes were observed in the liver of bigmouth buffalo and gall bladder of bluegill (Figure 5.41).

Eight species or roundworms from class Nematoda with representatives of three orders were found in fish from Lake Traverse (Table 5.6). The most common nematodes encountered during the survey were from order Ascaridida (Anisakidae) with representatives from three genera. We found numerous larvae of *Contracaecum sp.* encysted in mesenteries of black bullhead, black crappie, bluegill, channel catfish, common carp, freshwater drum, rock bass, walleye, white bass, and yellow bullhead. Larvae of *Raphidascaris sp.* were found in small cysts (D < 1.0 mm) on the intestine of bigmouth buffalo, mesenteries of black bullhead and white sucker, and on the liver of rock bass (Figure 5.42). *Hysterothlacium brachyrum* was recovered from the intestine of rock bass (Figure 5.43). Nematodes representing three families of the order Spirurida were found during the survey. *Camallanus oxycephalus* (Camallanidae) was found in the intestines of black crappie and white bass while specimens of *Camallanus.* sp were found in the intestines of channel catfish, freshwater drum, rock bass, and yellow perch (Figure 5.44). Two species of *Spinitectus* (Cystidicolidae) were found. *S. carolina* was recovered from the intestines of white bass and yellow perch (Figure 5.45) and *S. gracilis* was found in the intestine of black crappie, bluegill, white bass, and yellow perch (Figure 5.46). Larval forms of *Rhabdochona sp.* (Rhabdochonidae) were found in the intestines of ten species of fish including black bullhead, black crappie, common carp, channel catfish, freshwater drum, orangespotted sunfish, rock bass, walleye, white bass, and yellow perch (Figure 5.47). Lastly, larvae of *Spiroxys sp.* (Oxyuridea: Gnathostomatidae) were found in small cysts in the mesenteries of rock bass.

Two species of thorny-headed worms (Eoacanthocephala) were found in the intestines of fish from Lake Traverse (Table 5.6). *Pomphorhynchus bulbocolli* (Pomphorhynchidae) was the most commonly observed species and was found in common carp, channel catfish, freshwater drum, orangespotted sunfish, shorthead redhorse, white sucker, and yellow bullhead (Figure 5.48). *Neoechinorhynchus sp.* (Neoechinorhychidae) was found in the intestine of white bass (Figure 5.49). Additionally, Polymorphidae cystacanths from bluegill (Figure 5.50) and cystacanths of *Pomphorhynchus sp.* were found encysted in the mesenteries and intestines of channel catfish, freshwater drum, and white bass (Figure 5.51).

One parasitic leech (Hirudinea: Pisciolidae) was found on fish from Lake Traverse (Table 5.6). *Myzobdella lugubris* (synonym *M. moorei*) was found anchored to the fins of black bullhead and bluegill (Figure 5.51). Finally, one parasitic copepods (Arthropoda: Crustacea: Copepoda) *Ergasilus cyprinaceus* (Ergasillidae) was found anchored to the gills of fathead minnow.

Tables

Table 5.1— Number of fish collected from Devils Lake for parasite survey and examined either fresh at a temporary field station or after being frozen and thawed.

	Number of fish examined					Number of parasites identified
		Comprehensive exam				
			Frozen		Total	
Fish host	Grossly	Fresh	Whole	GI tract		
Black crappie	14	3	0	0	17	2
Fathead minnow	60	5	10	0	75	8
Northern pike	34	2	0	0	36	2
Walleye	59	6	1	6	66	3
White bass	60	3	2	5	65	6
White sucker	5	1	0	0	6	0
Yellow perch	60	7	10	0	77	6

Table 5.2— Piscine hosts and anatomical location of parasites recovered from fish collected at Devils Lake. Fish common name abbreviations are explained in Appendix A. Anatomical abbreviations: (f) fin, (g) gills, (i) intestine, (k) kidney, (l) liver, (m) musculature, (mt) mesenteries, (s) skin.

Parasite taxonomic classification		
Class	Family or *Genus – species*	Host and anatomical location
Protozoa	*Apiosoma sp.*	WAE(g,s)
	Trichodina sp.	BLC(g,s), WAE(g), WHB(g), YEP(g)
Myxosporea	*Myxobolus sp.*	FHM(k)
	Unicauda sp.	FHM(k)
Monogenea	*Gyrodactylus hoffmani*	FHM(f)
	Onchocleidus chrysops	WHB(g)
Trematoda	*Diplostomulum sp.*	FHM(m)
	Neascus sp.	FHM(m)
Cestoidea	*Bothriocephalus cuspidatus*	WAE(i)
	Bothriocephalus sp.	FHM(i), NOP(i), WHB(i), YEP(i)
	Gryporhynchidae	YEP(i)
	Proteocephalus pinguis	NOP(i)
	Proteocephalus sp.	FHM(i), NOP(i), WHB(i), YEP(i)
Nematoda	*Contracaecum sp.*	BLC(mt), WHB(mt)
	Rhabdochona sp.	YEP(mt)
	Raphidascaris sp.	FHM(i), YEP(i)
	Spiroxys sp.	WHB(mt)

Table 5.3— Number of fish collected from Lake Ashtabula for parasite survey and examined either fresh at a temporary field station or after being frozen and thawed.

	Number of fish examined					Number of parasites identified
		Comprehensive exam				
			Frozen			
Fish host	Grossly	Fresh	Whole	GI tract	Total	
Black Bullhead	60	5	0	0	65	8
Black crappie	0	1	2	0	3	5
Blacknose dace	0	0	2	0	2	7
Bluegill	0	0	2	0	2	3
Fathead minnow	60	9	0	0	69	2
Northern pike	12	0	0	4	12	4
Smallmouth bass	9	2	0	0	11	5
Spottail shiner	60	2	0	0	62	6
Tadpole madtom	60	1	10	18	71	5
Trout perch	0	0	1	0	1	4
Walleye	55	1	2	15	58	7
White bass	32	1	2	10	35	13
White sucker	32	1	0	10	33	6
Yellow perch	10	0	1	0	11	2

Table 5.4— Piscine hosts and anatomical location of parasites recovered from fish collected at Lake Ashtabula. Fish common name abbreviations are explained in Appendix A. Anatomical abbreviations: (e) eye, (f) fin, (g) gills, (i) intestine, (k) kidney, (l) liver, (m) musculature, (mt) mesenteries, (pc) peritoneal cavity, (py) pyloric caeca, (s) skin.

Parasite taxonomic classification		
Class	*Genus – species*	Host and anatomical location
Protozoa	*Apiosoma sp.*	BLC(g), BLD(f), SMB(g,s), WHB(g)
	Epistylis sp.	BLD(g), SSH(f,g,s), TRP(f,g,s)
	Trichodina sp.	BLC(g,s), BLD(s), BLG(g,s), SMB(g), SSH(f,g,s), TRP(s), WAE(f,g,s), WHB(g) YEP(f,g,s)
Microsporea	*Heterosporis sp.* (unconfirmed)	TPM(m)
Myxosporea	*Myxobolus sp.*	BLD(s,f), SSH(m), TRP(m)
Monogenea	*Gyrodactylus sp.*	BLD(f)
	Onchocleidus sp.	BLB(g), WHB(g)
Trematoda	*Alloglossidium corti*	TPM(i)
	Clinostomum marginatum	WHS(m)
	Crepidostomum cornutum	YEP(i)
	Crepidostomum sp.	WHB(i)
	*Diplostomulum sp*athaceum	WAE(e)
	Hysteromorpha triloba	BLB(m), FHM(m)
	Neascus sp.	FHM(m), SSH(m), WHB(l)
	Posthodiplostomum minimum	BLG(l), NOP(l)
	Prohemistomulum sp.	WAE(pc)
	Tylodelphys scheuringi	TRP(m)
Cestoidea	*Biacetabulum sp.*	WHS(i)
	Bothriocephalus cuspidatus	WAE(i), WHB(i)
	Bothriocephalus sp.	NOP(i)
	Corallobothrium sp.	BLB(i), TPM(i)
	Proteocephalus sp.	BLC(i), NOP(i), SSH(i), WHB(i)
Nematoda	*Contracaecum sp.*	BLB(mt), BLC(mt), BLD(mt), BLG(mt) NOP(mt), SMB(mt), SSH(mt), WAE(mt) WHB(mt)
	Camallanus sp.	BLC(i), SMB(i), WAE(i), WHB(i)
	Rhabdochona sp.	WHS(i)
	Raphidascaris sp.	WHB(mt), WHS(mt)
	Spinitectus sp.	WHB(i)

Table 5.4— continued. Anatomical abbreviations: (e) eye, (f) fin, (g) gills, (i) intestine, (k) kidney, (l) liver, (m) musculature, (mt) mesenteries, (pc) peritoneal cavity, (py) pyloric caeca, (s) skin.

Nematoda	*Spiroxys sp.*	WHS(i)
Eoacanthocephala	*Neoechinorhynchus sp.*	SMB(i), WHB(i)
	Pomphorhynchus bulbocolli	BLB(mt), TPM(i), WHB(i), WHS(i)
Hirudinea	*Myzobdella lugubris*	BLB(f)
Crustacea	*Actheres pimelodi*	BLB(s)

Table 5.5— Number of fish collected from Lake Traverse for parasite survey and examined either fresh at a temporary field station or after being frozen and thawed.

	Number of fish examined					Number of parasites identified
		Comprehensive exam				
			Frozen			
Fish host	Grossly	Fresh	Whole	GI tract	Total	
Bigmouth buffalo	3	1	0	3	4	4
Black crappie	15	1	1	10	17	7
Bluegill	60	2	4	10	66	12
Bullheads	60				60	
Black		2	0	12	2	8
Yellow		0	0	1	0	4
Channel catfish	20	1	0	11	21	10
Common carp	60	0	0	31	60	5
Emerald shiner	60	0	12	0	72	2
Fathead minnow	0	5	15	0	20	11
Freshwater drum	60	2	1	8	63	8
Orangespotted sunfish	48	2	2	9	52	6
Rock bass	8	5	0	8	13	12
Shorthead redhorse	3	0	0	3	3	1
Walleye	58	11	0	12	69	5
White bass	60	2	0	12	62	12
White sucker	7	0	0	7	7	4
Yellow perch	0	2	3	0	5	8

Table 5.6.— Piscine hosts and anatomical location of parasites recovered from fish collected at Lake Traverse. Fish common name abbreviations are explained in Appendix A. Anatomical abbreviations: (b) brain, (bd) bile duct, (f) fin, (g) gills, (gb) gall bladder, (i) intestine, (k) kidney, (l) liver, (m) musculature, (mt) mesenteries, (n) nares, (o) ovary, (op) operculum, (py) pyloric caeca, (s) skin.

Parasite taxonomic classification		
Class	Family or *Genus – species*	Host and anatomic location
Protozoa	*Apiosoma sp.*	FHM(f), WHB(g), YEP(f)
	Capriniana piscium	WAE(g)
	Ambiphrya sp.	WHB(f)
	Trichodina spp.	BLC(g,s), BLG(s), CCF(g), FHM(n), FRD(f,s), OSS(g,s), RKB(g), WAE(g) WHB(f,g,s), YEP(s)
Microsporea	*Glugea sp.* (suspect)	BLG(mt)
Myxosporea	*Chloromyxum sp.*	BLC(gb), BLG(gb),
	Henneguya sp.	CCF(g)
	Myxidium sp.	BLB(gb), CCF(bd,l), FRD(bd,gb), WHS(gb), YEB(gb)
	Myxobolus sp.	BIB(g), BIB(i), EMS(g), EMS(k), FHM(g) FHM(m), FHM(f), FHM(b)
	Unicauda sp.	FHM(k)
Monogenea	*Gyrodactylus sp.*	FHM(n)
	Microcotyle spinicirrus	FRD(g)
	Unidentified monogenean	RKB(g)
Trematoda	*Clinostomum marginatum*	RKB(m)
	Megalogonia ictaluri	CCF(i)
	Neascus sp.	BLG(m), FHM(m), RBK(m)
	Neascus of *Ornithodiplostomum ptychocheilus*	FHM(b)
	Neascus of *Postodiplostomum minimum centrarchi*	BLG(l), OSS(m)
Cestoidea	*Bothriocephalus cuspidatus*	WAE(i)
	Bothriocephalus sp.	OSS(i), WHB(i), YEP(i)
	Corallobothrium fimbriatum	BLB(i)
	Corallobothrium sp.	CCF(i)
	Gryporhynchidae	BIB(l), BLG(gb)
	Hunterella nodulosa	WHS(i)
	Khawia iowensis	CAP(i)
	Paradilepis sp. (suspect)	BLB(l)

Table 5.6.— continued. Anatomical abbreviations: (b) brain, (bd) bile duct, (f) fin, (g) gills, (gb) gall bladder, (i) intestine, (k) kidney, (l) liver, (m) musculature, (mt) mesenteries, (n) nares, (o) ovary, (op) operculum, (py) pyloric caeca, (s) skin.

Cestoidea	*Proteocephalus sp.*	BLC(i), RKB(i), WHB(i), YEP(i)
	Valipora sp. (suspect)	OSS(gb)
Nematoda	*Camallanus oxycephalus*	BLC(i), WHB(i)
	Camallanus sp.	CCF(i), FRD(i), RKB(i), YEP(i)
	Contracaecum sp.	BLB(mt), BLC(mt), BLG(mt), CAP(mt) CCF(mt), FRD(mt), RKB(mt), WAE(mt) WHB(mt), YEB(mt)
	Hysterothylacium brachyurum	RKB(i)
	Raphidascaris sp.	BIB(i), BLB(mt), RBK(l), WHS(mt)
	Rhabdochona sp.	BLB(i), BLC(i), CAP(i), CCF(i), FRD(i) OSS(i), RKB(i), WAE(i), WHB(i), YEP(i)
	Spinitectus carolini	WHB(i), YEP(i)
	Spinitectus gracilis	BLC(i), BLG(i), WHB(i), YEP(i)
	Spinitectus sp.	RKB(i), YEB(i)
	Spiroxys sp.	RKB(mt)
Eoacanthocephala	*Neoechinorhynchus sp.*	WHB(i)
	Polymorphidae cystacanth	BLG(i), CCF(l,mt), FRD(mt), WHB(mt)
	Pomphorhynchus bulbocolli	CAP(i), CCF(i), FRD(i), OSS(i), SHR(i), WHS(i), YEB(i)
	P. bulbocolli cystacanth	BLB(mt), BLG(mt), CAP(mt), OSS(mt), WHS(i,mt)
Hirudinea	*Myzobdella lugubris*	BLB(f), BLG(f)
Crustacea	*Ergasilus cyprinaceus*	FHM(g)

Figures

Kenneth Peters, USFWS Bozeman FHC, captured images of parasites in fresh mounts and stained specimens. Eric Leis and Sarah Bauer, USFWS Lacrosse FHC are credited with additional photomicrographs of stained specimens.

Figure 5.1.— Examples of protozoan parasites observed on various species of fish in the Red River basin. From left to right, *Apiosoma sp.*, *Trichodina sp.*, *Capriniana piscium*, and *Ambiphrya sp.*

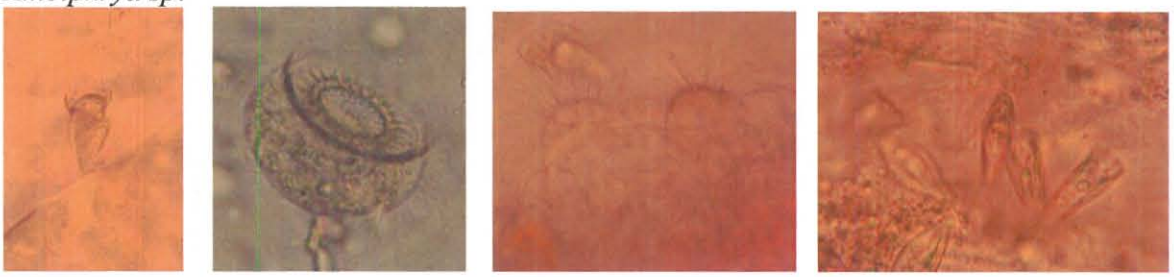


Figure 5.2.— Photomicrographs of *Myxobolus sp.* from kidney of fathead minnow from Devils Lake. Photo at right shows spore in sutural view with polar filaments extruded.

Figure 5.3.— Photomicrographs of a myxosporean found in kidney tissue of fathead minnow from Devils Lake. Spores had a long single caudal extension of spore valve and several spores extruded polar filaments in wet mount preparations (center, right).

Figure 5.4.— Photomicrographs of metacercariae of larval genus *Neascus* (left, center) and *Diplostomulum* (right) found encysted in the musculature of fathead minnow from Devils Lake.

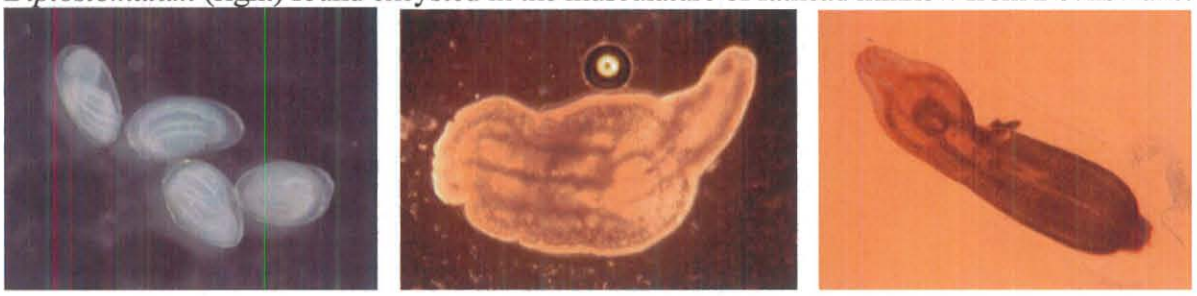


Figure 5.5.— Photomicrographs of acetocarmine-stained specimen of a gryporhynchid metacestode in yellow perch from Devils Lake.

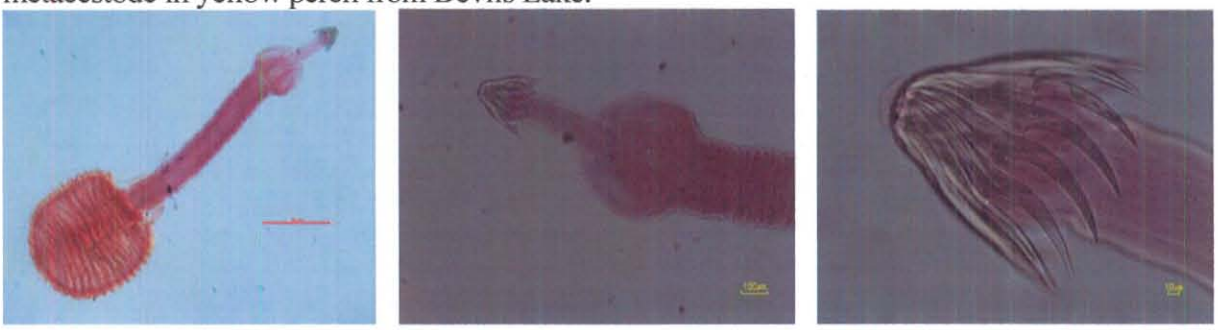


Figure 5.6.— Photomicrographs of suspect *Heterosporis sp.* in skeletal muscle of tadpole madtom from Lake Ashtabula.

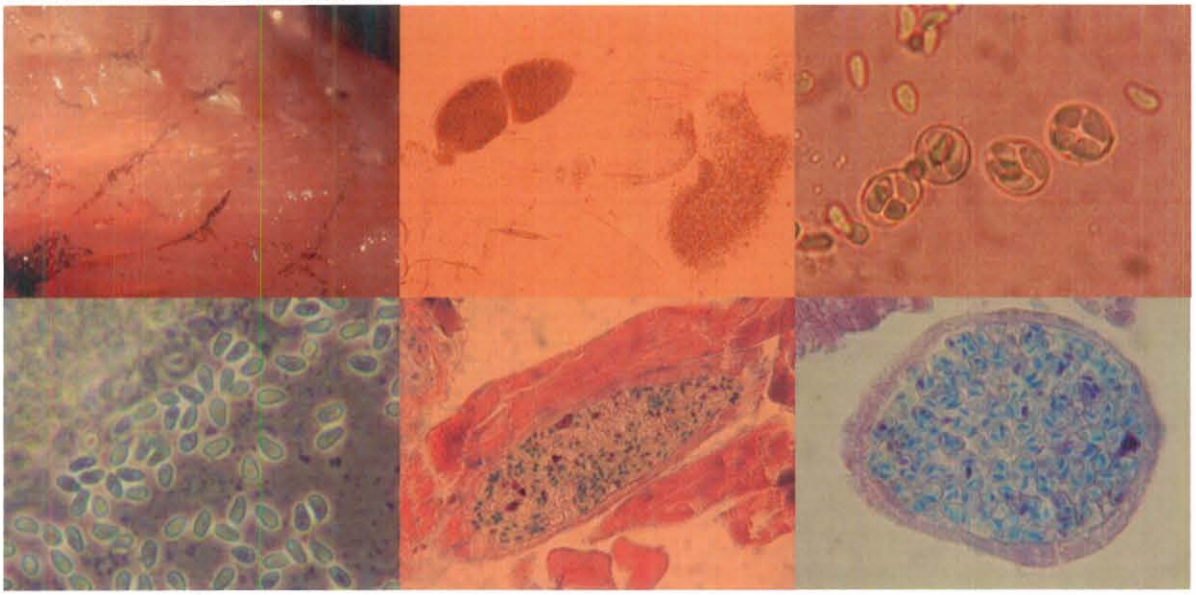


Figure 5.7.— Photomicrographs of *Myxobolus sp.* found in skin scrapings of blacknose dace from Lake Ashtabula.

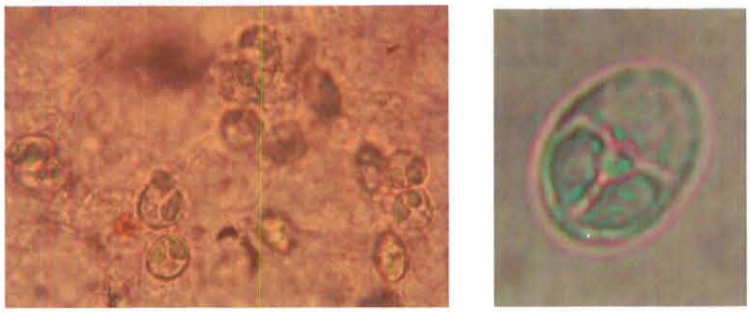


Figure 5.8.— Photomicrographs of *Myxobolus sp.* found encysted in tissue between fin rays of blacknose dace from Lake Ashtabula. Cysts were occupied primarily with developing trophozoite and relatively few mature spores.

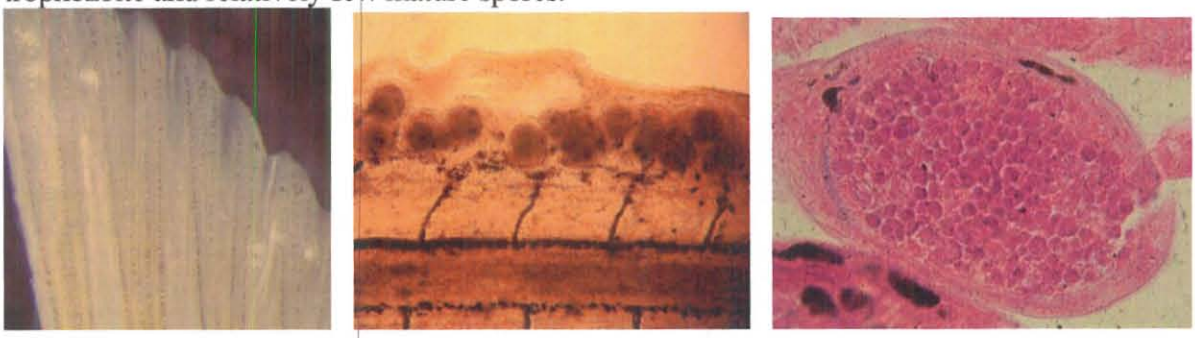


Figure 5.9.— Photomicrographs of *Myxobolus sp.* infecting muscle of spottail shiner from Lake Ashtabula.

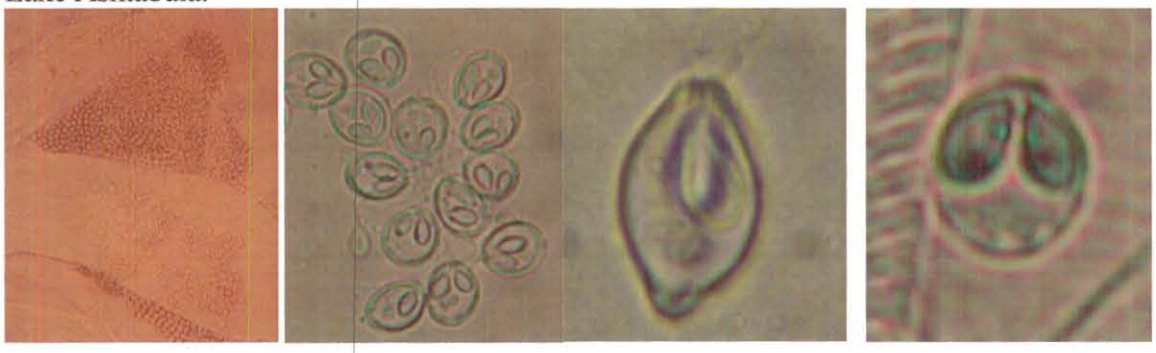


Figure 5.10.— Photomicrographs of *Myxobolus sp.* infecting muscle of trout perch from Lake Ashtabula.

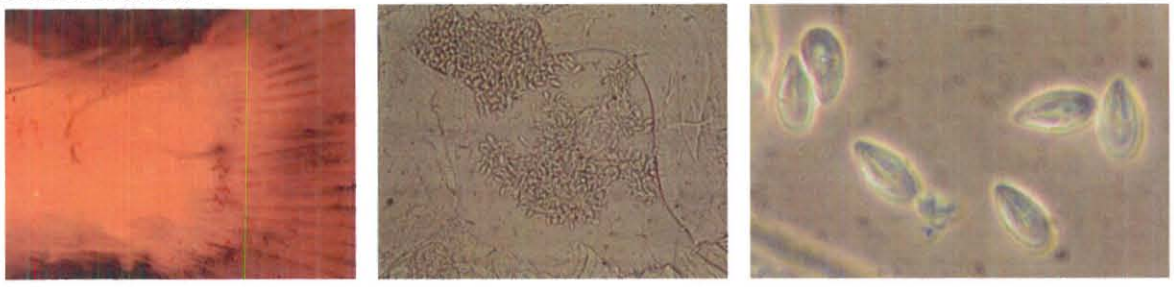


Figure 5.11.— Photomicrographs of *Gyrodactylus sp.* on the pectoral fin of a blacknose dace from Lake Ashtabula.

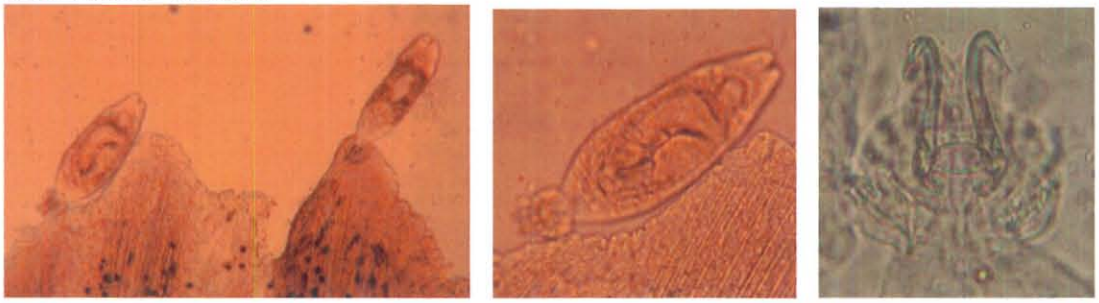


Figure 5.12.— Photomicrographs of intestinal trematodes found in fish from Lake Ashtabula. Fresh mounts of *Alloglossidium corti* (left) and *Crepidostomum cornutum* (right).

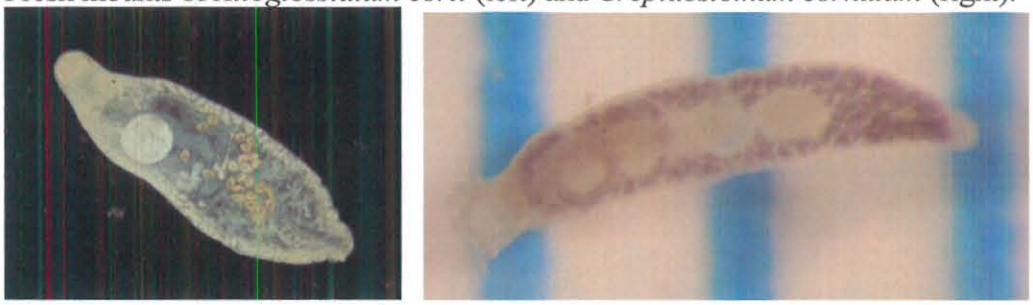


Figure 5.13.— Photomicrographs of trematodes infecting muscle tissue of fish from Lake Ashtabula. *Clinostomum complanatum* from white sucker (left), *Hysteromorpha triloba* in black bullhead (center), and *Tylodelphys scheuringi* from trout perch (right).

Figure 5.14.— Photomicrographs of encysted and freed forms of larval.genus *Neascus sp.* (black spot disease) from muscle tissue of fathead minnow collected at Lake Ashtabula. Cyst with melanized capsule near pelvic fin (left), melanized capsule, encysted and freed metacercariae (center), and fresh mount of metacercariae (right).

Figure 5.15.— Examples of proteocephalid cestodes in fish from Lake Ashtabula included *Corallobothrium fimbriatum* from black bullhead (left) and metacestodes of *Proteocephalus sp.* from white bass (right).

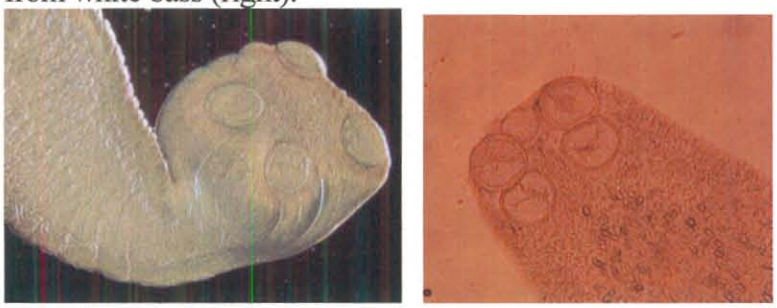


Figure 5.16.— Examples of nematodes found in fish from Lake Ashtabula. *Contraceacum sp.* encysted in mesenteries and *Camallanus sp.* spilling from the intestine of black crappie (left), *Raphidascaris sp.* from white sucker (center), and *Spinitectus sp.* from white bass (right).

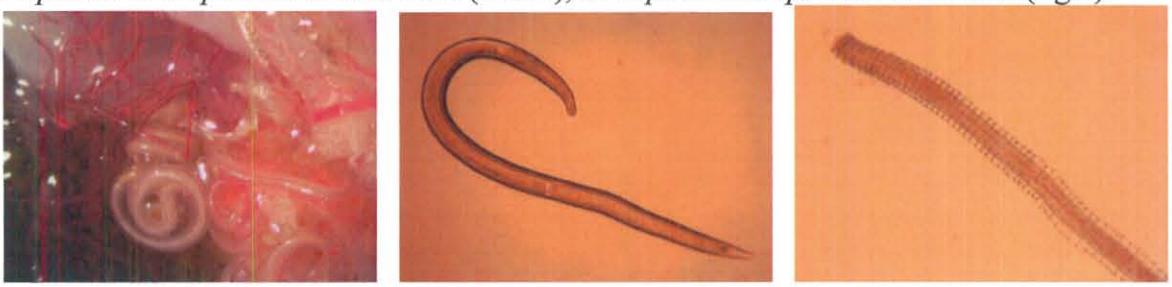


Figure 5.17.— Photomicrographs of *Neoechinorhyncus sp.* from the intestines of smallmouth bass and white bass from Lake Ashtabula.

Figure 5.18.— Photomicrographs of *Pomphorhynchus bulbocolli* in the intestine of white sucker (left) and in tadpole madtom (center, right).

Figure 5.19.— Photomicrographs of unstained microsporidian spores (left) and spores stained with methylene blue (center, right) from a xenoma found in mesenteries of bluegill from Lake Traverse.

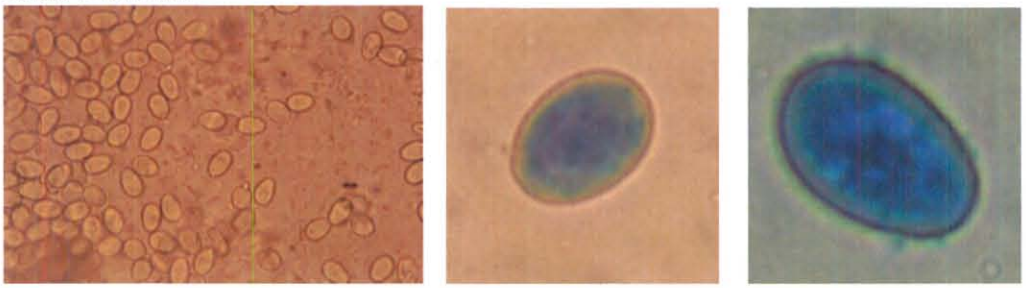


Figure 5.20.— Photomicrographs of remnant plasmodia with spores of *Chloromyxum sp.* from the gall bladder of black crappie at Lake Traverse (left). *Chloromyxum sp.* found in gall bladder of bluegill with four polar capsules distinctive of the family Chloromyxidae.

Figure 5.21.— Examples of *Myxidium sp.* found in fish from Lake Traverse. Plasmodium (left) and mass of spores (center) from the gall bladder of black bullhead. Spore of *Myxidium sp.* shown with opposing polar filaments extruded in a wet mount preparation (right).

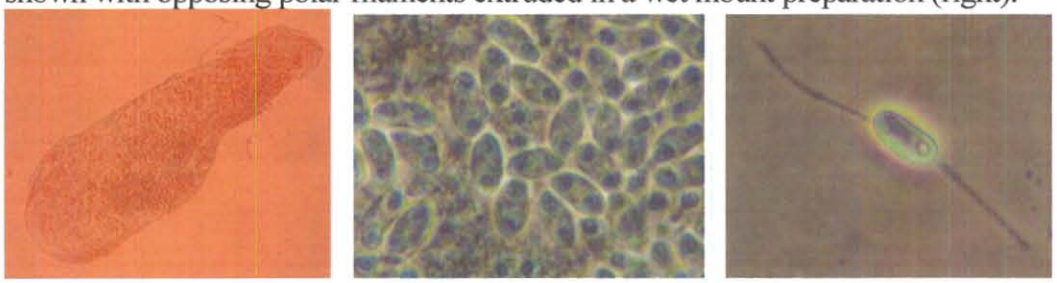


Figure 5.22.— Photomicrographs of *Henneguya sp.* found in gills of channel catfish from Lake Traverse. Two long caudal extensions of spore valves are characteristic of the genus. Several spores extruded polar filaments in wet mount (left). The mobile ciliated protozoan *Trichodina sp.* appears in wet mount with spores of *Henneguya sp.* (right).

Figure 5.23.— Photomicrographs of *Myxobolus sp.* in gill lamellae of bigmouth buffalo from Lake Traverse. Wet mount of plasmodia in gill lamellae (far left), spores in wet mount (left-center), and plasmodia in stained tissue sections (H&E right-center, Giemsa far right).

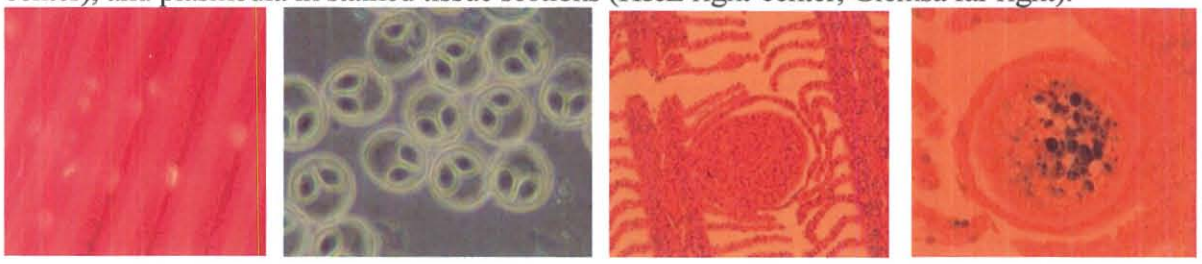


Figure 5.24.— Photomicrographs of *Myxobolus sp.* plasmodia (left) and spores (center, right) from the intestinal lamina epithelium of bigmouth buffalo from Lake Traverse.

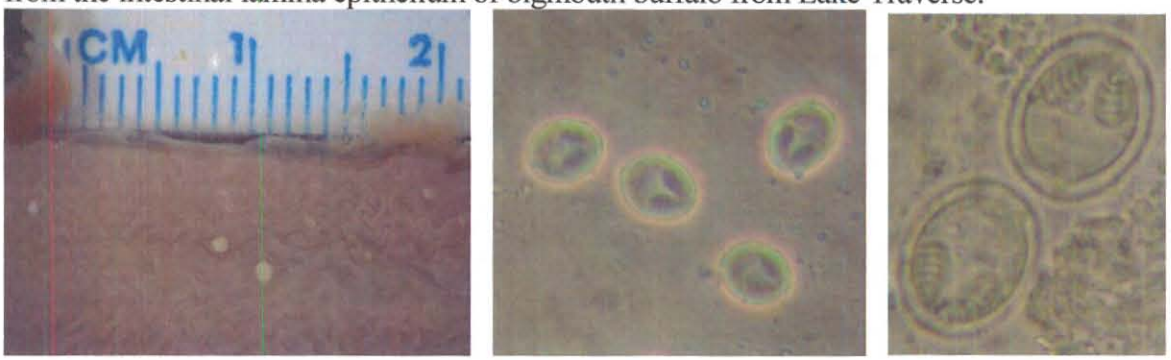


Figure 5.25.— Photomicrographs of *Myxobolus sp.* extruding polar filaments in wet mounts. From the gills of emerald shiner sampled at Lake Traverse.

Figure 5.26.— Photomicrographs of ovoid spores of *Myxobolus sp.* observed in kidney tissue squash preparation of emerald shiner from Lake Traverse.

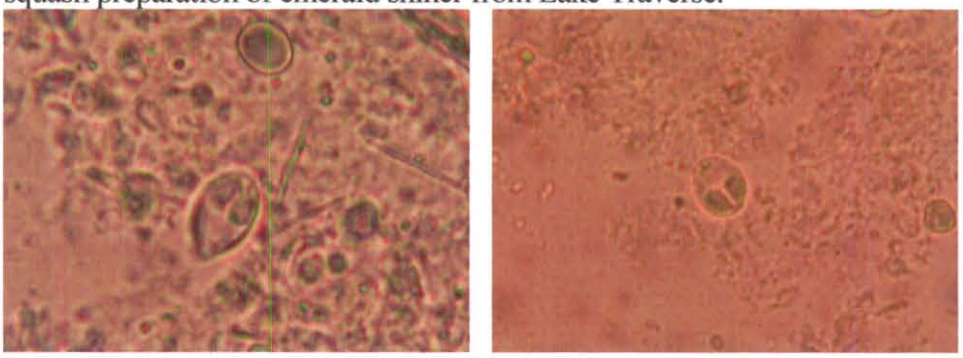


Figure 5.27.— Photomicrographs of *Myxobolus sp.* plasmodium on pectoral fin of fathead minnow from Lake Traverse. Plasmodia contained pyriform spores some of which extruded polar filaments during examination of wet mount preparation.

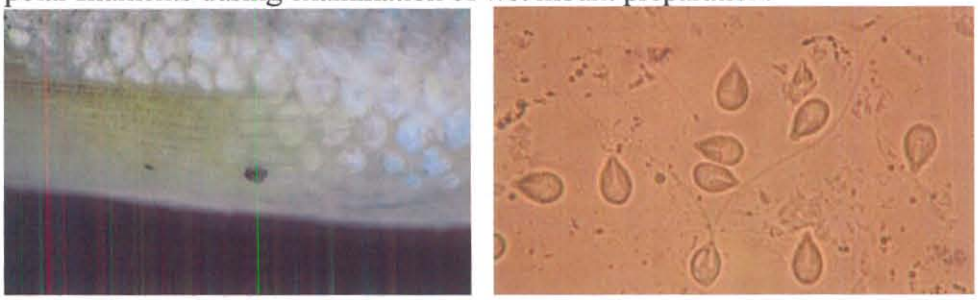


Figure 5.28.— Photomicrographs of *Myxobolus sp.* infecting gill epithelium of fathead minnow from Lake Traverse.

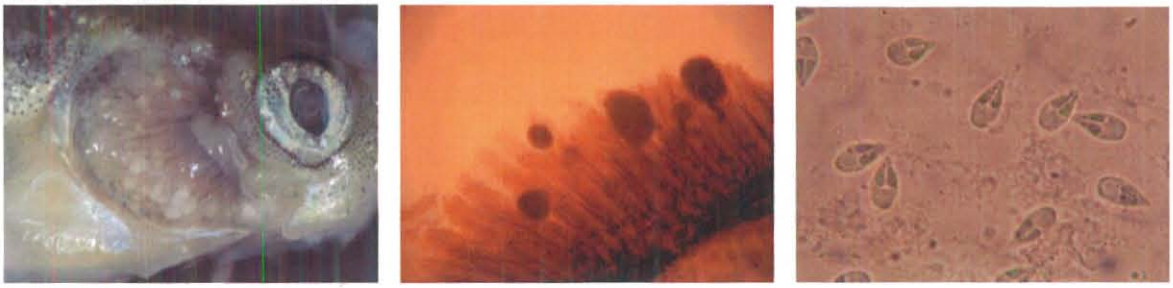


Figure 5.29.— Histozoic plasmodia in lateral skeletal muscle of fathead minnow from Lake Traverse contained numerous oval shaped spores of *Myxobolus sp.*

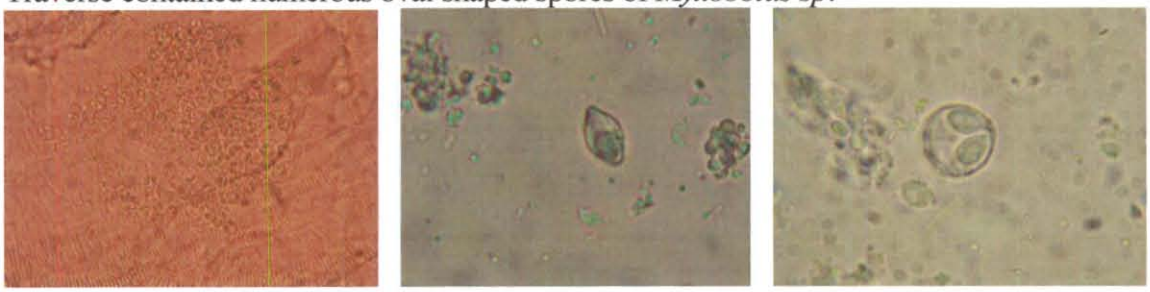


Figure 5.30.— Photomicrographs of histozoic plasmodia of *Myxobolus sp.* in nervous tissue of the cranium (forebrain) containing oval spores with 2 even anterior polar capsules.

Figure 5.31.— Photomicrograph of fathead minnow kidney tissue squash revealing myxosporeans with what appears to be a single caudal extension of the spore valve suggestive of *Unicauda sp.* From Lake Traverse.

Figure 5.32.— Photomicrographs of *Gyrodactylus sp.* found in the nares of fathead minnow from Lake Traverse.

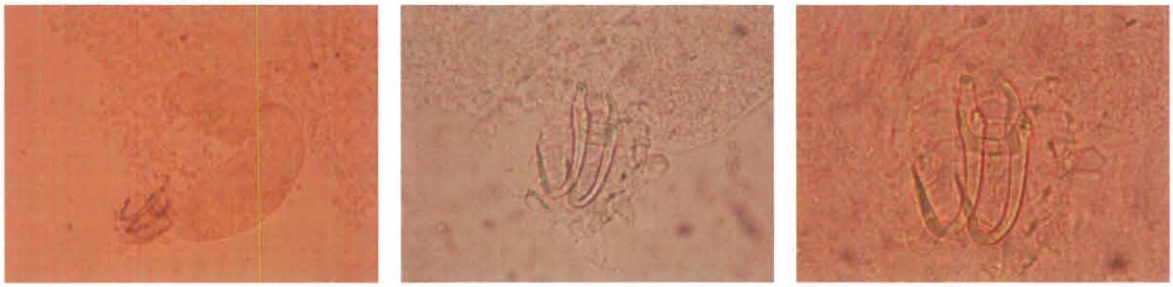


Figure 5.33.— Photomicrograph of the monogenean parasite *Microcotyle spinicirrus* anchored to gill lamellae of freshwater drum from Lake Traverse.

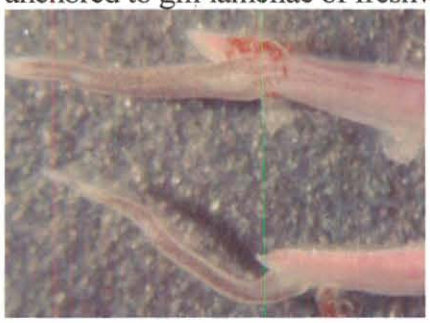


Figure 5.34.— Photomicrograph of unidentified monogenean parasite with four eye spots found on the gill of rock bass from Lake Traverse.

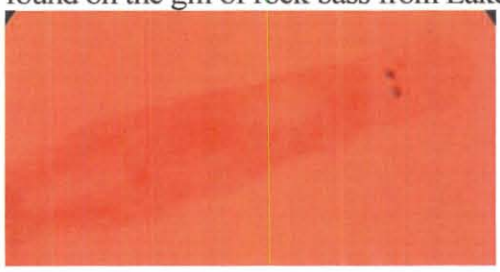


Figure 5.35.— Photomicrographs of metacercariae of *Neascus* of *Ornithodiplostomum ptychocheilus* found in cysts in the cranium of fathead minnow from Lake Traverse.

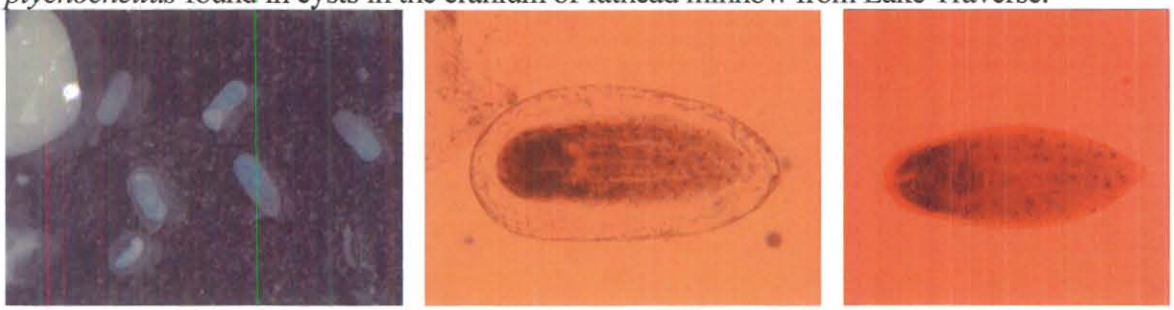


Figure 5.36.— Photomicrographs of trematode metacercariae of larval genus *Neascus sp.* in subcutaneous melanized capsules (left) and freed from cyst (right) found in fathead minnow from Lake Traverse.

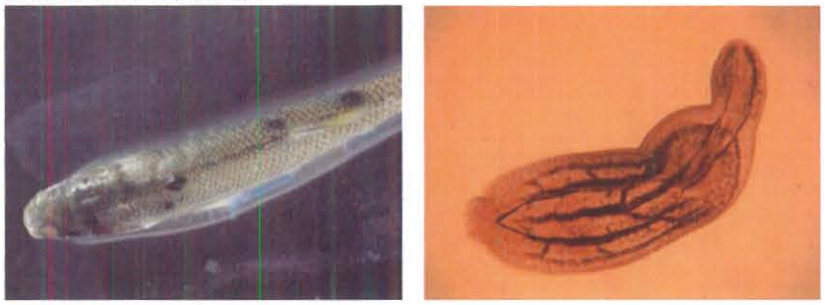


Figure 5.37.— Photomicrographs of the trematode *Megalogonia ictaluri* found in the intestine of channel catfish from Lake Traverse.

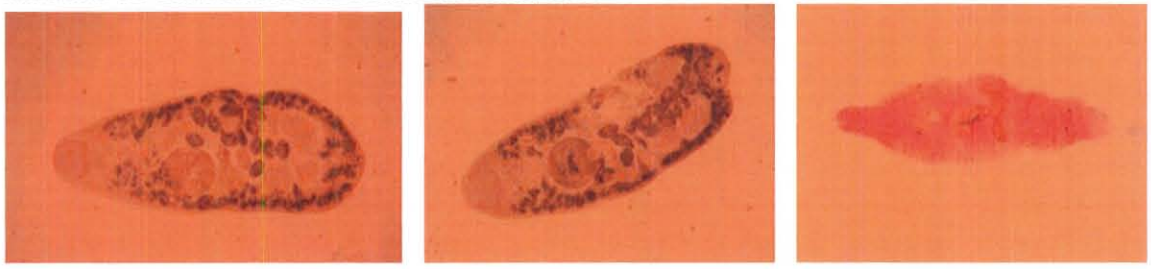


Figure 5.38.— Photomicrographs of the proteocephalid cestode *Corallobothrium fimbriatum* from the intestine of a black bullhead from Lake Traverse.

Figure 5.39.— Photomicrographs of Gryporhynchid metacestodes found in the liver of black bullhead from Lake Traverse. Suspect *Paradilepis sp.*

Figure 5.40.— Photomicrographs of Gryporhynchid metacestodes found in gall bladder of orangespotted sunfish from Lake Traverse. Suspect *Valipora sp.*

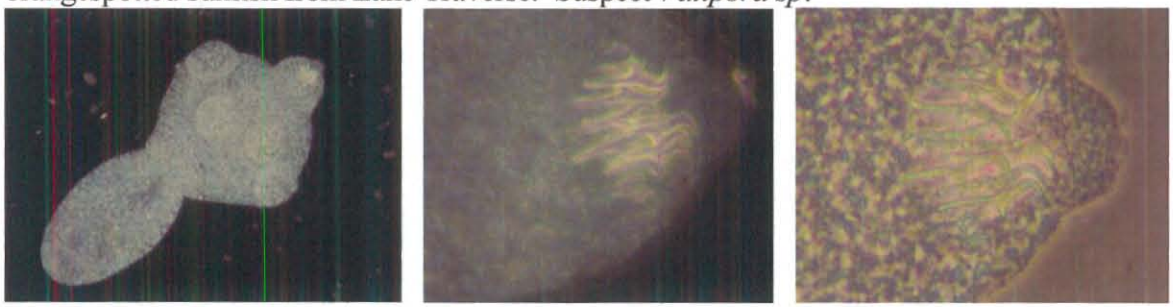


Figure 5.41.— Photomicrographs of unidentified suspect Gryporhynchid metacestodes found in liver of bigmouth buffalo (left) and gall bladder of bluegill (right).

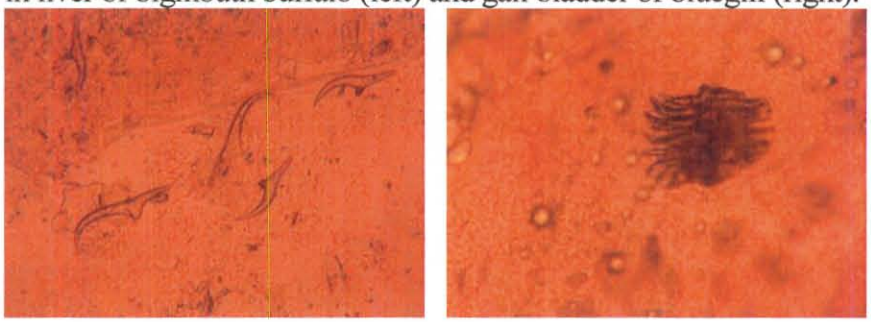


Figure 5.42.— Photomicrographs of examples of larval *Raphidascaris sp.* collected from fish at Lake Traverse.

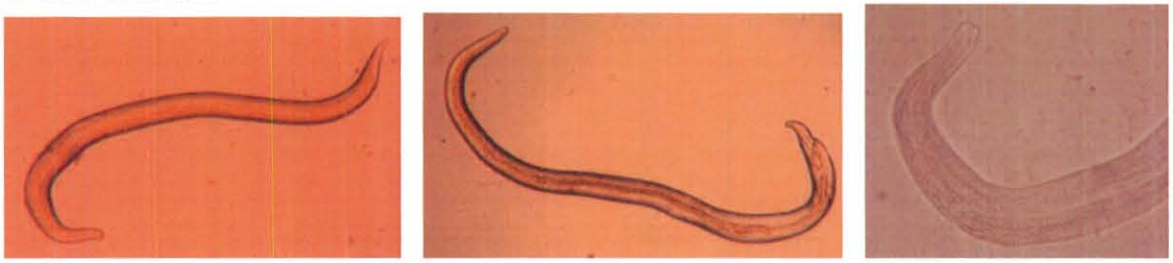


Figure 5.43.— Photomicrographs of *Hysterothlacium brachyrum* found in the intestine of rock bass from Lake Traverse.

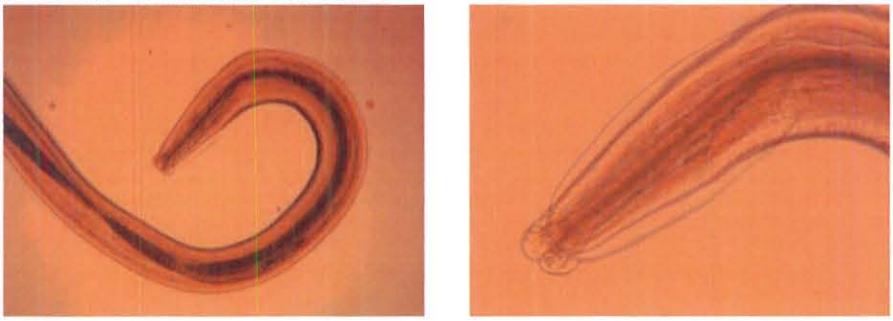


Figure 5.44.— Photomicrographs of *Camallanus oxycephalus* and *Camallanus sp.* found in the intestines of several species of fish from Lake Traverse.

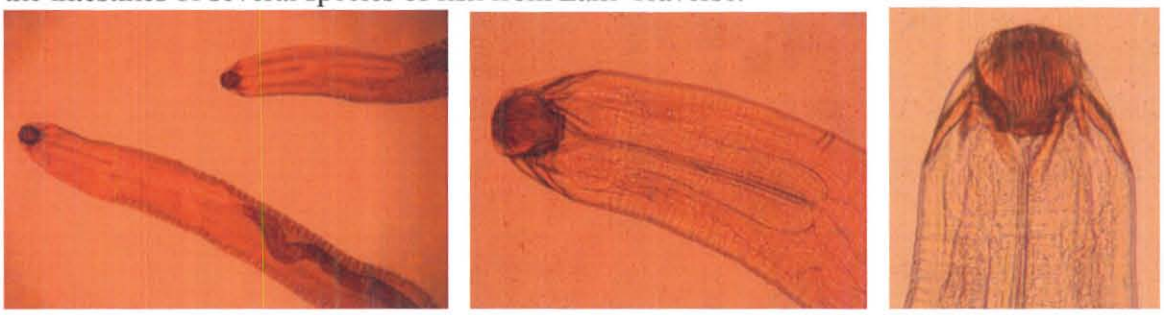


Figure 5.45.— Photomicrographs of *Spinitectus carolina* found in the intestines of white bass and yellow perch from Lake Traverse.

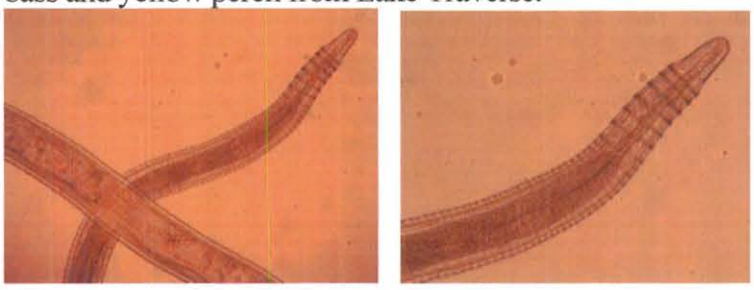


Figure 5.46.— Photomicrographs of *Spinitectus gracilis* found in the intestines of black crappie, bluegill, and yellow perch from Lake Traverse.

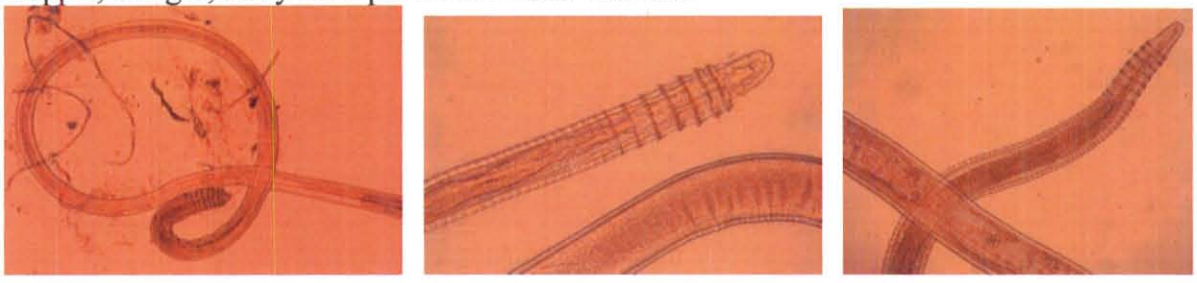


Figure 5.47.— Photomicrographs of examples of larval *Rhabdochona sp.* found in the intestines of ten species of fish from Lake Traverse.

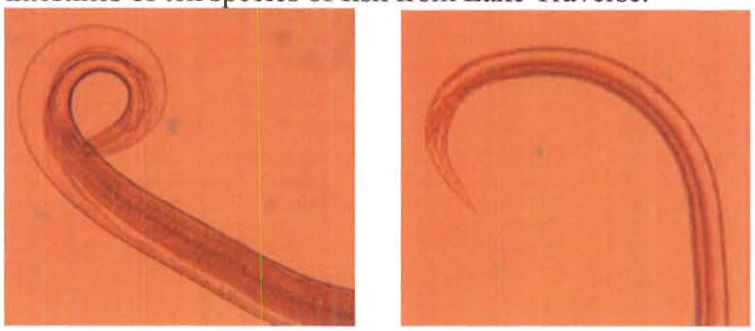


Figure 5.48.— Photomicrographs of *Pomphorhynchus bulbocolli* found in intestines of several fish from Lake Traverse.

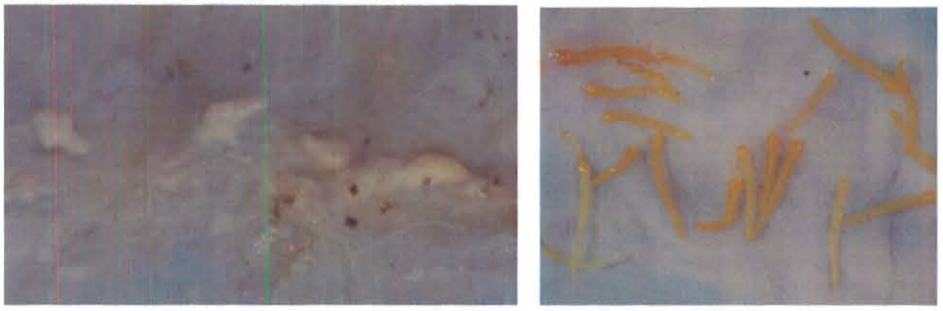


Figure 5.49.— Photomicrographs of *Neoechinorhynchus sp.* in fresh mount (left) and stained with acetocarmine (right) found in the intestine of white bass from Lake Traverse.

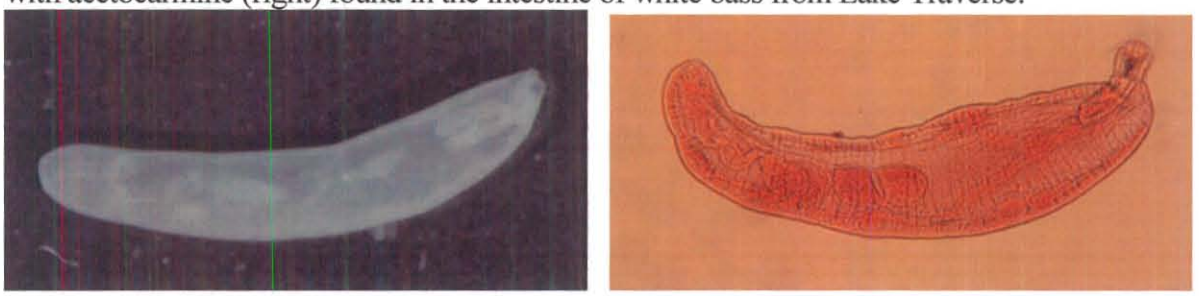


Figure 5.50.— Photomicrographs of Acanthocephalan Polymorphidae cystacanth found in the intestine of bluegill from Lake Traverse. Rostrum and proboscis inverted (left) and rostrum and proboscis everted (center) and stained with acetocarmine (right).

Figure 5.51.— Photomicrographs of Acanthocephalan larvae (cystacanths) found in the mesenteries and intestine of channel catfish, freshwater drum, and white bass from Lake Traverse.

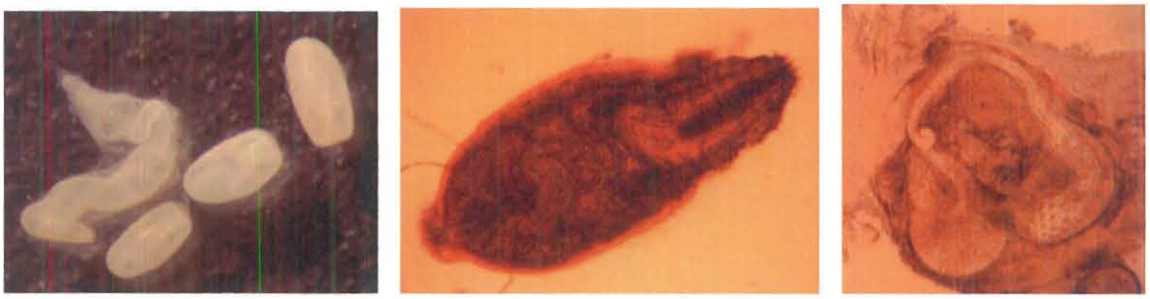
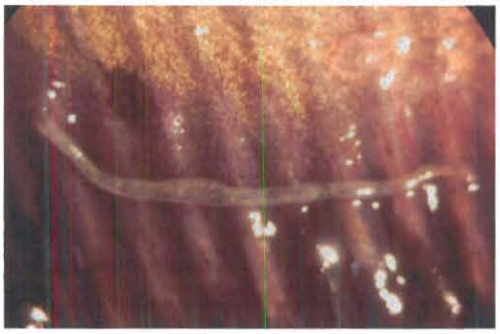


Figure 5.52.— Photomicrographs of the parasitic leech *Myzobdella lugubris* anchored to the fin of a black bullhead from Lake Traverse.



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Chapter 6 — Fish Viruses

Methods

Cell culture protocols and techniques used to test fish tissues for viral pathogens were taken from the *National Wild Fish Health Survey - Laboratory Procedures Manual* (USFWS 2009). In brief, tissue samples were processed according to standard methods within 48 h of collection. All viral assays were started (i.e. cells were inoculated with tissue samples) within 72 h of tissue collections. Samples of kidney and spleen (fingerling and adult fish) or whole viscera (fry) were pooled from a maximum of five fish. To target largemouth bass virus (LMBV), samples from the family Centrarchidae (basses and sunfishes) also included swim bladder tissue. Pooled tissue samples were placed in transport medium containing Hank's balanced salt solution (HBSS) with antibiotics and an antimycotic and held at 4°C. Prior to processing, the HBSS transport medium was decanted and tissues were weighed (0.0 g) to calculate a 1:10 dilution with fresh HBSS medium. The samples were homogenized and centrifuged at 4°C for 15 minutes at 2500 times gravity relative centrifugal force (X g). An aliquot of the supernatant was transferred to a new tube containing an equal amount of antibiotic incubation medium (anti-inc) so the final dilution was 1:20 volume/volume. The samples were incubated for 24 hours at 4°C. After incubation, samples were re-centrifuged at 4°C for 15 minutes at 2500 X g and inoculated in replicate onto confluent monolayers of *epithelioma papulosum cyprini* (EPC) and chinook salmon embryo-214 (CHSE-214) cell lines in 24-well tissue culture plates and incubated at 15°C. To test for viruses that replicate at warmer temperatures, such as largemouth bass virus (LMBV) and spring viremia of carp virus (SVCV), tissue homogenates were inoculated in replicate onto *epithelioma papulosum cyprini* (EPC) and bluegill fry (BF-2) cell lines and incubated at 22°C. Tissue samples of fish taken from the family Ictaluridae (catfishes) were also screened using the brown bullhead (BB) cell line incubated at 22°C. Fathead minnow tissue samples were also inoculated in replicate on the fathead minnow epithelial (FHM) cell line and incubated at 22°C. Finally, tissue samples from common carp were inoculated on the koi fin (KF-1) cell line and incubated at 22°C to screen for koi herpes virus (KHV) (Hedrick *et al.* 2000). All viral assays were re-inoculated onto new 24-well test plates in duplicate (blind passed) after 10-14 days of incubation, and monitored for cytopathic effect (CPE) using inverted light microscopy for a total incubation period of 28 days. Blind passes are useful in detecting potential subclinical carriers of virus (OIE 2006).

Results

A total of 59 pooled tissue samples representing 292 fish were collected and tested for virus from among seven species of fish sampled at Devil's Lake (Table 6.1). A total of 80 pooled tissue samples representing 390 fish from 10 species were collected and tested for virus from Lake Ashtabula (Table 6.2). Finally, a total of 107 pooled tissue samples representing 514 fish were tested for virus from among 14 species of fish collected at Lake Traverse (Table 6.3). Overall, 246 replicate tissue samples, representing 1,196 fish, were collected from the three bodies of water in the Red River Basin in June of 2008. All samples were tested for viral pathogens on multiple cell lines at two different incubation temperatures. We did not observe

any cytopathic effect (CPE) indicative of replicating viral agents in any of the cell culture assays from any body of water. None of the fish examined during necropsy and tissue collection demonstrated any clinical signs to suggest a viral infection.

Discussion

During this comprehensive survey, no viruses were detected by cell culture assays of fish tissue samples taken from Devils Lake, Lake Ashtabula, or from Lake Traverse. No clinical signs typical of viral disease were noted during necropsies. Fish tissues collected from Devils Lake were also examined with histopathology methods, and no cellular anomalies attributed to viral disease were observed. Between 2001 and 2007, the U.S. Fish and Wildlife Service completed four thorough fish pathogen surveys at Devils Lake (Peters 2002; Hudson and Peters 2005; Peters and Hudson 2007; USFWS 2009). In the course of those surveys, 240 pooled tissue samples representing 1,169 fish and seven different species were tested for the presence of viral pathogens. In the present survey, another 292 fish in 59 pooled samples from seven species were tested. This brings the total number of fish tested to date for viral pathogens from Devils Lake to 1,461. Fish were sampled during four different months including: June, July, September and October to discount the potential for seasonal variation in pathogen sampling. The methods for virus isolation and detection used during the surveys are widely accepted as highly efficient and sensitive procedures for screening large numbers of samples. Replicate samples have been tested on several cells lines with demonstrated susceptibility to reportable and regulated viruses listed in fish health policies of Canada, Europe, and the United States.

Tables

Table 6.1.— Number of pooled tissue samples tested for virus with multiple cell lines at two incubation temperatures for seven species of fish from Devils Lake. Abbreviations for cell lines are explained in the methods section. CPE = cytopathic effect, ND = not detected.

Species sampled	Number of pools tested	Number of fish tested	Cell line and incubation temperature		CPE
			15°C	22°C	
Black crappie	3	14	EPC, CHSE-214	BF-2, EPC, FHM	ND
Fathead minnow	12	60	EPC, CHSE-214	BF-2, EPC	ND
Northern pike	7	34	EPC, CHSE-214	BF-2, EPC	ND
Walleye	12	59	EPC, CHSE-214	BF-2, EPC	ND
White bass	12	60	EPC, CHSE-214	BF-2, EPC	ND
White sucker	1	5	EPC, CHSE-214	BF-2, EPC	ND
Yellow perch	12	60	EPC, CHSE-214	BF-2, EPC	ND

Table 6.2.— Number of pooled tissue samples tested for virus with multiple cell lines at two incubation temperatures for thirteen species of fish from the Lake Ashtabula. Abbreviations for cell lines are explained in the methods section. CPE = cytopathic effect, ND = not detected.

Species sampled	Number of pools tested	Number of fish tested	Cell line and incubation temperature		CPE
			15°C	22°C	
Black bullhead	12	60	EPC, CHSE-214	BB, BF-2, EPC	ND
Fathead minnow	12	60	EPC, CHSE-214	BF-2, EPC, FHM	ND
Northern pike	3	12	EPC, CHSE-214	BF-2, EPC	ND
Smallmouth bass	2	9	EPC, CHSE-214	BF-2, EPC	ND
Spottail shiner	12	60	EPC, CHSE-214	BF-2, EPC	ND
Tadpole madtom	12	60	EPC, CHSE-214	BB, BF-2, EPC	ND
Walleye	11	55	EPC, CHSE-214	BF-2, EPC	ND
White bass	7	32	EPC, CHSE-214	BF-2, EPC	ND
White sucker	7	32	EPC, CHSE-214	BF-2, EPC	ND
Yellow perch	2	10	EPC, CHSE-214	BF-2, EPC	ND

Table 6.3.— Number of pooled tissue samples tested for virus with multiple cell lines at two incubation temperatures for twenty-two species of fish from the Red River. Abbreviations for cell lines are explained in the methods section. CPE = cytopathic effect, ND = not detected.

Species sampled	Number of pools tested	Number of fish tested	Cell lines and incubation temperature		CPE
			15°C	22°C	
Bigmouth buffalo	1	3	EPC, CHSE-214	BF-2, EPC	ND
Black crappie	3	15	EPC, CHSE-214	BF-2, EPC	ND
Bluegill	12	60	EPC, CHSE-214	BF-2, EPC	ND
Bullhead	12	60	EPC, CHSE-214	BF-2, BB, EPC	ND
Channel catfish	4	20	EPC, CHSE-214	BF-2, BB, EPC	ND
Common carp	12	60	EPC, CHSE-214	BF-2, EPC, KF-1	ND
Emerald shiner	12	60	EPC, CHSE-214	BF-2, EPC	ND
Freshwater drum	12	60	EPC, CHSE-214	BF-2, EPC	ND
Orangespotted sunfish	10	48	EPC, CHSE-214	BF-2, EPC	ND
Rock bass	2	8	EPC, CHSE-214	BF-2, EPC	ND
Shorthead redhorse	1	3	EPC, CHSE-214	BF-2, EPC	ND
Walleye	12	58	EPC, CHSE-214	BF-2, EPC	ND
White bass	12	60	EPC, CHSE-214	BF-2, EPC	ND
White sucker	2	7	EPC, CHSE-214	BF-2, EPC	ND

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Appendix A

Table A.1.— Scientific name, common name and three letter abbreviation of fish sampled throughout the survey study areas. Abbreviations for bodies of water are DL = Devils Lake, LT = Lake Traverse, and LA = Lake Ashtabula.

Abbreviation	Common name	Scientific name	Body of water sampled
BLB	Black bullhead	*Ameiurus melas*	LA, LT
BLC	Black crappie	*Promoxis nigromaculatus*	DL, LA, LT
BLD	Blacknose dace	*Rhinichthys atratulus*	LA
BIB	Bigmouth buffalo	*Ictiobus cyprinellus*	LT
BLG	Bluegill	*Lepomis macrochirus*	LA, LT
CAP	Common carp	*Cyprinus carpio*	LT
CCF	Channel catfish	*Ictalurus punctatus*	LT
EMS	Emerald shiner	*Notropis atherinoides*	LT
FHM	Fathead minnow	*Pimephales promelas*	DL, LA, LT
FRD	Freshwater drum	*Aplodinotus grunniens*	LT
NOP	Northern pike	*Esox lucius*	DL, LA
OSS	Orangespotted sunfish	*Lepomis humilis*	LT
RKB	Rock bass	*Ambloplites rupestris*	LT
SHR	Shorthead redhorse	*Moxostoma macrolepidotum*	LT
SMB	Smallmouth bass	*Micropterus dolomieu*	LA
SSH	Spottail shiner	*Notropis hudsonius*	LA
TPM	Tadpole madtom	*Noturus gyrinus*	LA
TRP	Trout-perch	*Percopsis omiscomaycus*	LA
WAE	Walleye	*Sander vitreus*	DL, LA, LT
WHB	White bass	*Morone chrysops*	DL, LA, LT
WHS	White sucker	*Catostomus commersoni*	DL, LA, LT
YEB	Yellow bullhead	*Ameiurus natalis*	LT
YEP	Yellow perch	*Perca flavescens*	DL, LT