Survey of Pathogens and Parasites in Fish from Devils Lake, the Sheyenne and Red Rivers in North Dakota, and Lake Traverse, South Dakota.

2007 Survey Results

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U. S. Fish and Wildlife Service
Bozeman Fish Health Center
Bozeman, Montana

Survey of Pathogens and Parasites in Fish from Devils Lake, the Sheyenne and Red Rivers in North Dakota, and Lake Traverse, South Dakota.

2007 annual report of results and discussion of a multi-year survey designed to gather information on pathogens and parasites of fish from select waters in the Red River basin and Devils Lake sub-basin.

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putrefaciens or other opportunistic bacteria.. Epitheliocystis, a condition caused by members of Chlamydiales, was observed for the first time as cysts in gills from a small number of fish from both lakes. Epitheliocystis is usually a benign infection although there have been reports of proliferative lesions or hyperinfection leading to mortality mostly limited to aquaculture facilities. The disease was first reported in the U.S. by Hoffman et al. (1969) although now there are reports from more than 50 species of fish worldwide from both freshwater and marine environments.

No Gram-positive bacteria were found during the surveys although antigen of *Renibacterium salmoninarum* was detected by enzyme-linked immunosorbent assay (ELISA) in low levels in several species of fish from all four bodies of water. All ELISA-positive samples tested with the more sensitive and specific polymerase chain reaction assay were negative for the bacterium. A discussion of possible causal factors for false-positive ELISA reading is given in Chapter 3.

We observed a greater diversity of parasitofauna in fish from Lake Traverse compared to fish from Devils Lake. A similar number of fish were examined from both survey sites but more parasites from each taxonomic class were found in fish at Lake Traverse. Also, parasites from several genera found at Lake Traverse were not seen at Devils Lake. At Devils Lake, a total of fifteen different parasites were identified to the level of genus, and of those, five parasites were identified to species. At Lake Traverse, forty-one different parasites were identified to the level of genus or larval genus, and of those, twenty parasites were identified to species. Larvae of *Spiroxys sp.* was only parasite from Devils Lake that was not found in fish from Lake Traverse although the nematode has been reported previously from the Sheyenne River. We identified five parasites in fish from Devils Lake that were not observed in previous surveys conducted by this laboratory. These included *Onchocleidus chrysops*, *Dactylogyrus sp.*, metacercariae of the larval genus *Neascus*, *Spiroxys sp.*, and *Myzobdella lugubris*. In the only other known parasite survey of fish from Devils Lake, Reinisch (1981) found only eight different parasites. In the present survey, we found all parasites previously recorded by Reinisch with the possible exception of an unknown trematode and the acanthocephalan *Rhadinorhynchus sp.* It may be that *Rhadinorhynchus sp.* was not correctly identified as most records for this parasite are in marine fish from the Pacific coast.

To the best of our knowledge, the present parasite survey was the first of its kind to take place at Lake Traverse. One noteworthy finding at Lake Traverse was the collection of two gryprohynchid metacestodes. *Paradilepis sp.* was found individually in oval cysts in the liver of a rock bass and *Valipora sp.* (presumptive) was found in the gall bladder of a pumpkinseed sunfish. We could not find any previous records of larval gryprohynchid cestodes in fish from Lake Traverse or other bodies of water in the Red River basin.

Histology provided another perspective on the observation of several parasites found during the traditional parasite survey at Devils Lake. With the 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. 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 were documented. For white sucker, the only parasites observed were spores of *Myxobolus sp.* found in gills.

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; these 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 metazoan parasites could not be identified to taxonomic levels closer than class and order. Generally, metazoan parasites observed in thin sections lacked 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.

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We would like to express our appreciation to all project contributors for their diligent efforts in the field and laboratories. The survey would not have been possible without the tremendous sampling efforts by the staffs of the Missouri River Fish and Wildlife Conservation Office and the Great Plains Fish and Wildlife Conservation Office. We thank Ron Zitzow, manager of Valley City National Fish Hatchery, for coordinating the use of the sampling trailer with the regional wetlands office. We would like to extend our appreciation to Andrew Moran, Fish and Wildlife Director for the Spirit Lake Nation, for his continued cooperation and encouragement. We would also like to thank Rick Schwab of *Six Mile Bay Campground* and Todd Johnson of *Wing and Fin on Lake Traverse* for providing accommodations for our temporary field stations. Funding for the survey was provided by the U.S. Department of State.

Survey Cooperators

U. S. Fish and Wildlife Service
- Bozeman Fish Health Center
- Dexter Fish Health Unit
- Great Plains Fish and Wildlife Management Assistance Office
- Lacrosse Fish Health Center
- Missouri River Fish and Wildlife Management Assistance Office
- Valley City National Fish Hatchery

North Dakota Game and Fish Department
South Dakota Department of Game, Fish and Parks
Minnesota Department of Natural Resources, Division of Fish and Wildlife

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Chapter 1 — Introduction

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. Devils Lake and neighboring Stump Lake receives most of the surface drainage in the Devils Lake Basin which covers approximately 2.4 million acres. Devils Lake sub-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). Presently, the lakes have no perennial outlets and water levels are affected primarily by rainfall, snowmelt runoff, and evaporation. 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 1.1). 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 50,000 acres to about 125,000 acres. In 1999, the lake reached an elevation of 1446.6 ft-msl and water began to spill from East Devils Lake into Stump Lake for the first time in several hundred years. At an elevation of approximately 1459 ft-msl the combined lake would overflow to the Sheyenne River (a tributary of 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).

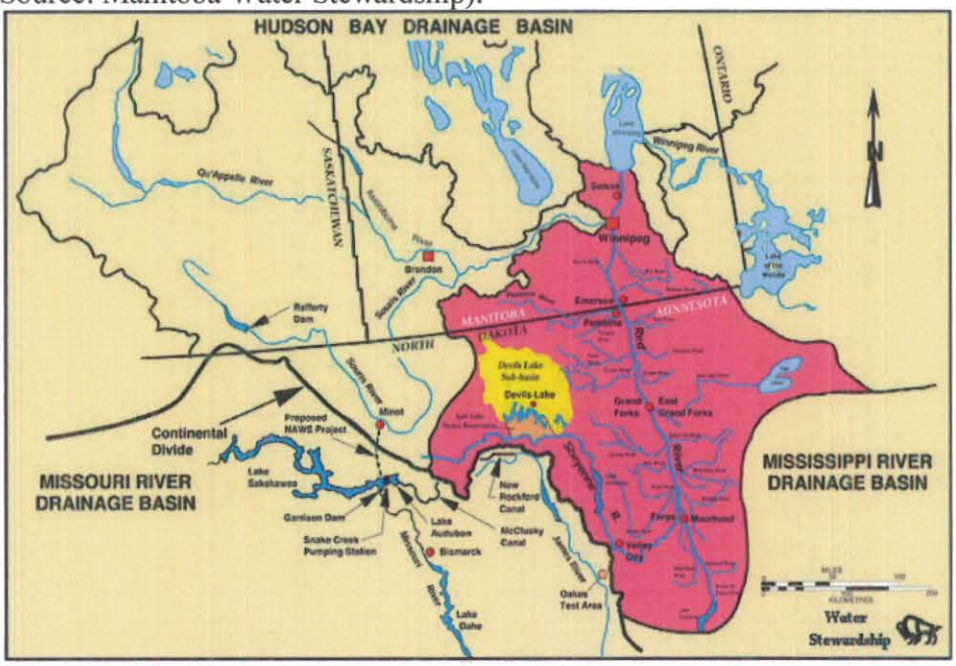
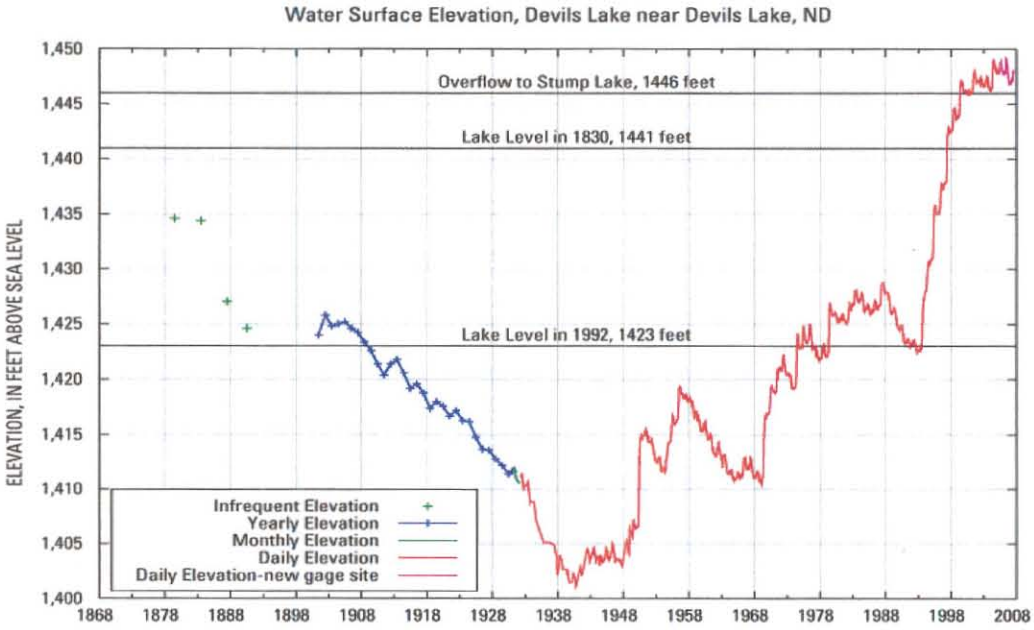


Figure 1.2— Water surface elevation for Devils Lake from 1867 through 2008. (Source: U. S. Geological Survey, North Dakota Water Science Center).



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 Devils Lake to carry water to the Sheyenne River for the purposes of reducing flooding problems. The Sheyenne River flows southeasterly to the Red River which flows north to Lake Winnipeg and Hudson Bay. Diverting water from Devils Lake to the Sheyenne River has raised concerns about 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 Red River basins 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. Antigen of *Renibacterium salmoninarum*, the regulated agent responsible for bacterial kidney disease in salmonids, was detected in low levels with an enzyme-linked immunosorbent assay (ELISA) screening test although active infection or DNA of the bacterium was not detected when samples were tested with the highly sensitive and

specific polymerase chain reaction (PCR) assay. No other pathogens were detected during the initial survey. 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 Red River basins but not limited to the pathogens listed in the *National Wild Fish Health Survey* (Hudson and Peters 2005, Peters and Hudson 2007). Results of these surveys included recovery of several parasites although most specimens had been described in earlier studies at Devils Lake or in the Red River basin. Parasites not previously described in earlier reports were found to be relatively common in North American and other parts of the world. Several species of bacteria were also isolated and identified although most were considered either normal residents of fish gastro-intestinal tracts or common constituents of soil and fresh water environments. Bacteria found most frequently included Gram-negative, motile species of the families Aeromonas and Pseudomonas. Two Gram-positive bacteria, *Corynebacterium renale* and *Streptococcus sobrinus* were also isolated but were not regarded as significant or unique. *C. renale* is not considered a fish pathogen and is often associated with aquatic habitats influenced by specific agricultural activities including livestock grazing. As with previous surveys, low levels of antigen of *Renibacterium salmoninarum* was detected with ELISA but not confirmed with the PCR assay. Investigators speculated that ELISA results were likely false-positive readings or may have been caused by cross-reacting bacteria or proteins. Regulated or prohibited bacterial and viral fish pathogens were not detected and investigators remarked that fish appeared healthy with no external or internal clinical signs of disease with the exception of a few common fish parasites observed grossly in the abdominal cavity and gastrointestinal tract.

In this report we provide results and discussion of fish pathogen and parasite surveys at Devils Lake, Lake Traverse, and the Red and Sheyenne rivers conducted between June and August 2007. Study objectives were to, 1) examine fish for the presence or absence of specific fish pathogens, 2) perform comprehensive parasite surveys at Lake Traverse and Devils Lake, 3) provide fish health specialists, fisheries managers, and other decision makers with a pathogen survey report that may be used in performing risk analysis, and 4) provide access to survey results through the U.S. Fish and Wildlife Service *National Wild Fish Health Survey* database on the worldwide web http://wildfishsurvey.fws.gov.

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Chapter 2 — Collection of Fish and Tissue Samples

Methods

Fish from Devils Lake, the Sheyenne River, and the Red River of the North were collected at the same sample sites used during previous pathogen surveys conducted by Bozeman Fish Health Center between 2001 and 2006. The sampling area at Devils Lake was located in a north-central section of the lake known as Six Mile Bay and extended north into the mouth of Channel A. Fish from the upper Sheyenne River were collected along a 0.5 km reach upstream and downstream from the bridge on State Highway 20. The Sheyenne River reach was located approximately 40 km south of Devils Lake extending along the southeastern border of the Spirit Lake Reservation. Fish from the Red River were collected along a 4.0 km reach upstream of the bridge at 52nd Avenue South in Fargo, North Dakota. The Red River sample reach was located approximately 27 km upstream of the confluence with the Sheyenne River.

For 2007, Lake Traverse was added to the list of survey sample sites. The lake is the southernmost body of water in the Hudson Bay watershed and is drained at its north end by the Bois de Sioux River which is tributary to the Red River of the North. Lake Traverse lies along the border between the states of Minnesota and South Dakota. 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 drainage. Fish for this survey were collected from the southern portion of the lake near the town of Browns Valley, Minnesota.

We used several types of sampling gear to collect various sizes of fish from a variety of habitat types in lakes and rivers. 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. In addition, electrofishing was used to capture fish at Lake Traverse and the Red River. The 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 (Figure 2.1). The field station consisted of a 30 ft long camp 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 or rivers. Fish were anesthetized 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.

Figure 2.1.— Lake-side temporary field sampling station.

Results

Fish were collected at Devils Lake on 11 – 14 June 2007. A total 646 fish representing seven species were collected and examined as a result of 511 hr of netting and trapping effort (Table 2.1). The catch rate for all gear types combined was 1.26 fish/hr. 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 black crappie, fathead minnow, walleye, and white bass. Low catch rates for northern pike, white sucker, and yellow perch were attributed to either relative low abundance or because seasonal distribution and occurrence in selected sample areas was low. Of the total catch, 289 fish were used to test for bacterial and viral pathogens, 287 fish for histology, and 118 fish for parasite survey. Most of the northern pike, white sucker, and yellow perch were processed for bacteriology, virology, and histology because the catch for these species was insufficient for separate sampling.

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 are given in Appendix A.

	Number of fish sampled by test			
Fish common name	Bacteriology and virology	Histology	Parasitology	Total number sampled
Black crappie	60	60	25	145
Fathead minnow	60	60	30	150
Northern pike*	24	23	10	34
Walleye	60	60	20	140
White bass	60	59	30	149
White sucker*	8	8	1	9
Yellow perch*	17	17	2	19

Fish from the Sheyenne River were collected on 20 – 21 August 2007. A total 100 fish representing thirteen species were collected and examined as a result of 357 hr of netting and trapping effort (Table 2.2). The combined catch rate for all gear types was 0.28 fish/hr. The catch was predominated by black bullhead and tadpole madtom. Fish from the Red River were collected on 22 – 23 August 2007. A total 392 fish representing twenty-one species were collected and examined as a result of 4.2 hr of electrofishing effort (Table 2.2). The catch per unit effort was 92.9 fish/hr. The most abundant species in the catch were channel catfish, common carp, emerald shiner, freshwater drum, and shorthead redhorse.

We collected fish at Lake Traverse on 26 – 28 June 2007 using a combination of nets, traps and electrofishing. A total of 674 fish representing 18 species were sampled as a result of 543 hr of net and trap deployment and 4.9 hr of electrofishing. The combined catch rate for all gear types was 1.2 fish/hr. The target sample size was obtained for black bullhead, crappie, bluegill sunfish, common carp, emerald shiner, freshwater drum, white bass, and yellow perch. Of the

total catch, 550 fish were tested for bacterial and viral pathogens and 124 fish were examined for the comprehensive parasite survey.

Table 2.2.— Composition of fish collected from the Red and Sheyenne rivers and tested for fish pathogens and parasites. Scientific names are given in Appendix A.

	Sample site	
Common name of fish	Red River	Sheyenne River
Bigmouth buffalo	5	0
Black bullhead	0	21
Black crappie	7	0
Bluegill	6	3
Channel catfish	60	0
Common carp	60	0
Common shiner	0	7
Creek chub	0	1
Emerald shiner	60	0
Fathead minnow	0	1
Freshwater drum	32	0
Goldeye	16	0
Iowa darter	0	3
Largemouth bass	1	0
Northern pike	3	3
Orangespotted sunfish	16	0
Quillback	21	0
Rock bass	5	0
Sauger	10	0
Shorthead redhorse	46	1
Smallmouth bass	2	0
Spottail shiner	0	23
Stonecat	2	0
Tadpole madtom	0	15
Trout perch	9	5
Walleye	9	9
White bass	18	0
White sucker	2	8
Yellow perch	2	0

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	Bacteriology and virology	Parasitology	Total
Bluegill sunfish	60	10	70
Bullhead (black and yellow)	60	7	67
Channel catfish	9	2	11
Common carp	60	4	64
Crappie (black and white)	60	7	67
Emerald shiner	60	6	66
Fathead minnow	0	21	21
Freshwater drum	60	10	70
Largemouth bass	1	1	2
Northern pike	5	3	8
Orangespotted sunfish	1	0	1
Pumpkinseed	18	5	23
Shorthead redhorse	3	0	3
Rock bass	11	11	22
Walleye	13	10	23
White bass	60	5	65
White sucker	9	2	11
Yellow perch	60	20	80

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Chapter 3 — Bacterial Pathogens

Methods

Isolation of aerobic bacterial pathogens was performed by inserting a disposable sterile loop (1.0 or 10.0 µL) into the kidney and streaked across the surface of tubes containing brain-heart infusion agar. Tubes were incubated at 22°C and monitored for bacterial growth at 24, 48, and 72 h. If no growth appeared after 10 d culture tubes were discarded. Suspect bacterial growth was sub-cultured for purity and then differentiated using a flow chart with standard biochemical profiling techniques and tests for motility by the hanging drop method. Commercial test systems were used to aid in identification of bacteria including the API 20E (bioMérieux Vitek, Inc., Hazelwood, Mo.) and Biolog Microbial ID/Characterization (Hayward, Ca.). Where appropriate, further confirmation of suspect 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 the enzyme-linked immunosorbent assay (ELISA; Pascho and Mulcahy 1987). When small fish had insufficient kidney for testing of individuals, we pooled 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* by the ELISA. The threshold of detection was calculated by adding the mean OD plus 2 SD of at least four negative controls. Kidney samples with mean ELISA OD values above the threshold were considered positive for soluble antigen of *R. salmoninarum* and were assigned to antigen level categories: OD values from the detection threshold to 0.199 were defined as low, 0.200 - 0.999 medium, and values of 1.00 or higher were considered high antigen levels (Pascho et al. 1991). 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. In cases where a species exhibited a broad range of positive ELISA values, we selected one sample each representing the upper, middle, and lower portions of the range. Kidney tissue remaining from the ELISA sample was used in the PCR. DNA template was extracted from samples with Qiagen DNeasy® (Valencia, Ca.) tissue kit and then amplified according to the PCR procedure. Amplified DNA was subjected to electrophoresis in a 1.5% agarose gel, and then stained with ethidium bromide and visualized with UV light.

Results

Devils Lake.— There was considerable growth of bacteria on the primary isolation medium. We sub-cultured for purity from all primary cultures with presumed mixed isolates which resulted in 59 pure cultures. Upon screening with preliminary biochemical and motility tests, we arrived at 12 pure cultures that required further differentiation and identification with commercial test systems listed in the preceding methods section. We did not isolate any Gram-positive bacteria from fish sampled at Devils Lake. The majority of the isolates were either aerobic or facultative anaerobic, Gram-negative motile rods from the Families Aeromonadaceae, Enterobacteriaceae, and Pseudomonadaceae (Table 3.1). *Aeromonas hydrophila* and *Hafnia*

alvei were the mostly commonly isolated species from these groups. Isolates of *Hafnia alvei* from northern pike had API-20E bio-chemical profiles similar to *Yersinia ruckeri* (Austin and Austin 1987). *Y. ruckeri* is the cause of enteric redmouth disease and is regulated fish pathogen listed in U. S. Fish and Wildlife Service policy. *H. alvei* isolates were tested against *Y. ruckeri* by IFAT and were negative. *Shewanella putrefaciens*, previously classified in the genus *Pseudomonas* or *Alteromonas*, was isolated from white bass. *S. putrefaciens* is a Gram-negative facultatively anaerobic rod-shaped bacterium whose chief phenotypic attribute is the production of hydrogen sulfide gas.

Table 3.1.— Bacteria found in fish from Devils Lake. Abbreviations of fish common names are explained in Appendix A.

Name of bacteria		
Genus	Species	Species of fish
Aeromonas hydrophila		BLC, FHM, NOP, WHB, WHS
Hafnia alvei		NOP
Shewanella putrefaciens		WHB

At Devils Lake, antigen of *R. salmoninarum* was detected by ELISA in kidney tissues of black crappie, northern pike, white sucker, and yellow perch (Table 3.2). The ELISA negative threshold OD value (cut-off) determined from standardized reference tissue was 0.097. The overall mean ELISA OD value for samples from Devils Lake was 0.092 (SD = 0.020). Antigen was detected in 29.3% (n = 75) of samples tested. All samples with OD values above the negative threshold were in the low antigen level category. Positive ELISA samples assayed with the nested-PCR for *R. salmoninarum* were negative for all species tested. These results suggest that ELISA samples in the low level category are likely false-positive for *R. salmoninarum*. None of the fish had any clinical signs indicative of bacterial kidney disease.

Table 3.2.— Percent of samples with detectable levels of *R. salmoninarum* antigen and mean antigen level category as measured by the ELISA, and corroborative testing with a nested PCR assay for six species of fish from Devils Lake. BDL = below detection limits.

	ELISA			PCR Assay	
Fish species	Number tested	Percent positive	Mean antigen level	Number tested	Percent positive
Black crappie	7	57.1	Low	3	0
Northern pike	21	47.6	Low	3	0
Walleye	7	0.0	BDL	0	0
White bass	30	0.0	BDL	0	0
White sucker	6	66.7	Low	3	0
Yellow perch	4	100.0	Low	3	0

Lake Traverse.— Similar to Devils Lake, numerous colonies of bacteria were grown on the primary isolation medium. We sub-cultured for purity from all primary cultures with presumed mixed isolates which resulted in 166 pure cultures. Upon screening with preliminary biochemical and motility tests, we arrived at about 23 pure cultures that required further differentiation and identification with commercial test systems which resulted in the identification of seven species of bacteria. The majority of the isolates were Gram-negative motile rods from the Families Aeromonadaceae and Enterobacteriaceae (Table 3.3). *Enterobacter sp.* and *A. hydrophila* were the most commonly isolated species from these groups. Other less commonly found bacteria were *Erwinia sp., Pantoea sp., and Pseudomonas sp.* The most notable findings were isolation of *Y. ruckeri* from black crappie and *Edwardsiella tarda* from channel catfish. *Y. ruckeri* is Gram-negative rod-shaped bacterium that is motile my means of seven or eight peritichously arranged flagella (Austin and Austin 1987) and is the organism response for enteric redmouth disease. It is a listed pathogen in the Fish Health Policy of U. S. Fish and Wildlife Service and Fish Health Protection Regulations of Canada. *E. tarda* is a Gram-negative, motile, rod-shaped bacterium that is known to cause disease (edwardsiellosis) in both marine and freshwater fish and is most frequently associated with channel catfish. *E. tarda* is not a listed pathogen in U. S. policy or Canadian fish health regulations.

Stenotrophomonas maltophilia, an aerobic, non-fermentative, Gram-negative bacterium was isolated from rock bass and walleye. *S. maltophilia* is ubiquitous in aqueous environments, soil and plants. Initially classified as *Pseudomonas maltophilia*, *S. maltophilia* was also grouped in the genus *Xanthomonas* before eventually becoming the type species of the genus *Stenotrophomonas*. They are motile due to polar flagella and grow well on MacConkey agar producing pigmented colonies. *S. maltophilia* are catalase-positive, oxidase-negative which distinguishes them from most other members of the genus. They are not known as primary pathogens of fish.

Table 3.3.— Identification of Gram-negative bacteria and species of fish from which isolates were cultured for samples collected at Lake Traverse. Abbreviations of fish common names are explained in Appendix A.

Name of bacteria		
Genus	Species	Species of fish
Aeromonas hydrophila		CAP, WHB
Edwardsiella tarda		CCF
Enterobacter sp.		BLC, BLG, RKB, WAE, WHC, YEP
Erwinia sp.		BLG
Pantoea sp.		BLG
Pseudomonas sp.		BLC, WAE
Stenotrophomonas maltophilia		RKB, WAE
Yersinia ruckeri		BLC

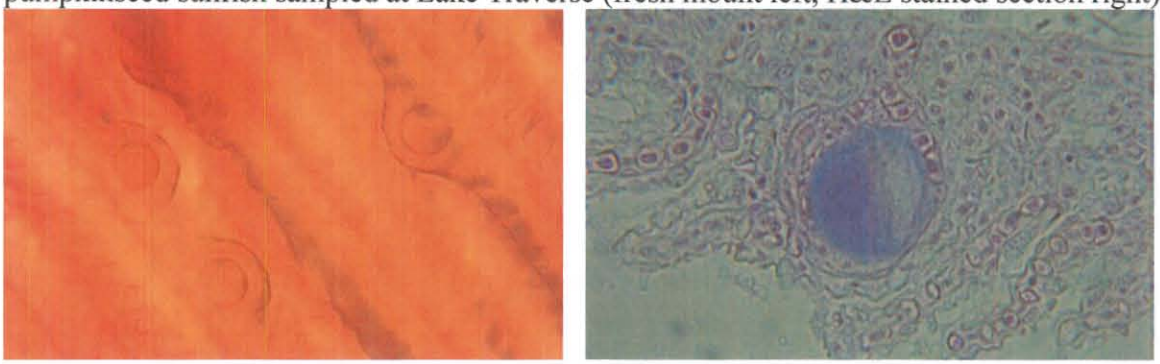
At Lake Traverse, antigen of *R. salmoninarum* was detected by ELISA in kidney tissues of black bullhead, black crappie, channel catfish, common carp, freshwater drum, rock bass, walleye, and white sucker (Table 3.4). All samples from bluegill, largemouth bass, northern pike, pumpkinseed, redhorse sucker, white bass, and white crappie had OD values below antigen detection limits. The ELISA negative threshold OD value (cut-off) ranged between 0.092 – 0.103. The overall mean ELISA OD value for samples from Lake Traverse was 0.096 (SD = 0.023). Antigen was detected in 26.8% (n = 138) of samples. Most samples (97.3%) with OD values above the negative threshold were in the low antigen level category. Only 1 sample, collected from freshwater drum, had an OD value in the medium antigen level category. We did not observe any high ELISA OD values in any species of fish. All ELISA-positive samples assayed with the nested-PCR were negative for *R. salmoninarum*. These results suggest ELISA samples in the low level category are likely false-positive for *R. salmoninarum*. None of the fish from 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 mean antigen level category as measured by the ELISA, and corroborative testing with a nested PCR assay for 15 species of fish from Lake Traverse. BDL = below detection limits.

	ELISA			PCR Assay	
Fish species	Number tested	Percent positive	Mean antigen level	Number tested	Percent positive
Black crappie	12	8.3	Low	1	0
Black bullhead	17	35.3	Low	3	0
Bluegill	5	0.0	BDL	0	0
Channel catfish	5	20.0	Low	1	0
Common carp	25	76.0	Low	3	0
Freshwater drum	16	31.3	Low	3	0
Largemouth bass	1	0.0	BDL	0	0
Northern pike	5	0.0	BDL	0	0
Pumpkinseed	1	0.0	BDL	0	0
Shorthead redhorse	3	0.0	BDL	0	0
Rock bass	8	12.5	Low	1	0
Walleye	12	16.7	Low	2	0
White bass	16	0.0	BDL	0	0
White crappie	6	0.0	BDL	0	0
White sucker	8	25.0	Low	2	0

During parasite survey at Lake Traverse, we observed small nodule-like lesions in wet mount preparations of gill lamellae from one pumpkinseed sunfish (Figure 3.1). Some cysts were excised and squashed between a microscope slide and cover slip then examined with light microscopy (1000X) revealing very small and uniform cocci-like cells. Other affected gill arches were fixed in Davidson's solution and processed with standard histology methods. Examination of stained tissue sections revealed a hypertrophic response of gill epithelial cells consistent with epitheliocystis. 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. Epitheliocystis was also observed in stained tissue sections of gills from small numbers of fathead minnow, walleye, and yellow perch from Devils Lake.

Figure 3.1.— Photomicrographs of epitheliocystis in epithelium of gill lamellae from pumpkinseed sunfish sampled at Lake Traverse (fresh mount left, H&E stained section right).



Red River.— Kidney tissue from 234 fish was inoculated on BHIA medium. We sub-cultured for purity from all primary cultures with presumed mixed isolates which resulted in 201 pure cultures. Upon screening with preliminary biochemical and motility tests, we arrived at about 39 pure cultures that required further differentiation and identification with commercial test systems which resulted in the identification of seven species of bacteria. The majority of the isolates were Gram-negative motile rods from the Families Aeromonadaceae and Enterobacteriaceae. No Gram-positive bacteria were isolated on BHIA medium. *A. hydrophila* was the most common bacterium being found in seven species of fish from the Red River (Table 3.5). Other frequently cultured bacteria included *Citrobacter sp.*, *Hafnia alvei*, *Pasturella sp.*, and *S. maltophilia*. With the possible exception of *A. hydrophila*, none of these bacteria are implicated as primary pathogens of fish. We did not isolate any species of reportable bacteria listed in either U.S. Fish and Wildlife Service policy or Canadian fish health regulations.

Antigen of *R. salmoninarum* was detected by ELISA in kidney tissues of black crappie, bigmouth buffalo, channel catfish, common carp, freshwater drum, goldeye, northern pike, quillback, shorthead redhorse, and stonecat (Table 3.6). All samples from bluegill, largemouth bass, rock bass, sauger, smallmouth bass, walleye, white bass, white sucker, and yellow perch had ELISA OD values below antigen detection limits. The ELISA negative threshold OD value (cut-off) ranged between 0.092 – 0.103. The overall mean ELISA OD value for samples from Lake Traverse was 0.097 (SD = 0.022). Of the 166 samples tested, antigen was detected 50.0%. All samples with OD values above the negative threshold were in the low antigen level category.

We did not observe any medium or high ELISA OD values in any species of fish. All ELISA-positive samples assayed with the nested-PCR were negative for *R. salmoninarum*. These results suggest ELISA samples in the low level category are likely false-positive for *R. salmoninarum*. None of the fish from the Red River had any clinical signs indicative of bacterial kidney disease.

Table 3.5.— Identification of Gram-negative bacteria and species of fish from which isolates were cultured for samples collected at the Red and Sheyenne rivers. Abbreviations of fish common names are explained in Appendix A.

	Name of bacteria		
Body of water	Genus	Species	Species of fish infected
Red River	*Aeromonas hydrophila*		BLC, BIB, CAP, CCF, OSS, RKB, WHB
	Citrobacter spp.		BLG, SNC
	Enterobacter cloacae		BLG
	Hafnia alvei		FWD, NOP
	Pasturella sp.		CAP, FWD, QBS
	Plesiomonas shigelloides		CCF
	Stenotrophomonas maltophilia		QBS, NOP, WAE
Sheyenne River	*Aeromonas hydrophila*		BLB, SSH, WHS
	Citrobacter freundii		NOP
	Citrobacter sp.		TPM
	Pleisomonas shigelloides		TPM, WAE
	Salmonella choleraesuis	- *arizonae*	BLB

Sheyenne River.— Kidney tissue from 54 fish were inoculated on BHIA medium. We sub-cultured for purity from all primary cultures with presumed mixed isolates which resulted in 101 pure cultures. Upon screening with preliminary biochemical and motility tests, we arrived at about 19 pure cultures that required further differentiation and identification with commercial test systems which resulted in the identification of five species of bacteria. The majority of the isolates were Gram-negative motile rods from the Families Aeromonadaceae and Enterobacteriaceae (Table 3.5). We did not isolate any species of reportable bacteria listed in U.S. and Canadian fish health policy or regulations. No Gram-positive bacteria were isolated on BHIA culture medium. Similar to other sample sites, *A. hydrophila* was one of the most commonly isolated bacterium in fish from the Sheyenne River. Other frequently cultured bacteria included *Citrobacter spp.* and *Pleisomonas shigelloides*. *Salmonella choleraesuis* subsp. *arizonae* was isolated from one bluegill. Synonyms include *Salmonella arizonae*, Arizona bacteria, and *Salmonella enterica* serovar *arizonae*. *S. choleraesuis arizonae* is not considered a primary pathogen of fish although it has been reported as widely distributed in lizards and snakes. The bacterium has been implicated in enteric and joint infections in humans. There are also reports of infection in human patients with a medical history of taking rattlesnake capsules prior to illness as well as infection acquired from reptilian pets.

Table 3.6.— Percent of samples with detectable levels of *R. salmoninarum* antigen and mean antigen level category as measured by the ELISA, and corroborative testing with a nested PCR assay for 19 species of fish from the Red River. BDL = below detection limits.

	ELISA			PCR Assay	
Fish species	Number tested	Percent positive	Mean antigen level	Number tested	Percent positive
Black crappie	2	100.0	Low	2	0
Bigmouth buffalo	5	20.0	Low	1	0
Bluegill	1	0.0	BDL	0	0
Channel catfish	30	3.3	Low	1	0
Common carp	27	88.9	Low	3	0
Freshwater drum	18	5.6	Low	1	0
Goldeye	14	71.4	Low	3	0
Largemouth bass	1	0.0	BDL	1	0
Northern pike	3	33.3	Low	1	0
Quillback	19	100.0	Low	3	0
Shorthead redhorse	24	95.8	Low	3	0
Rock bass	4	0.0	BDL	0	0
Sauger	7	0.0	BDL	0	0
Smallmouth bass	1	0.0	BDL	0	0
Stonecat	2	50.0	Low	1	0
Walleye	1	0.0	BDL	0	0
White bass	3	0.0	BDL	0	0
White sucker	3	0.0	BDL	0	0
Yellow perch	1	0.0	BDL	0	0

Antigen of *R. salmoninarum* was detected by ELISA in kidney tissues of black bullhead, northern pike, and walleye (Table 3.7). All samples (n = 8) from spottail shiner had ELISA OD values below antigen detection limits. The overall mean ELISA OD value for samples from Lake Traverse was 0.099 (SD = 0.024). Antigen was detected 24.0% of the 25 samples tested. All samples with OD values above the negative threshold were in the low antigen level category. We did not observe any medium or high ELISA OD values in any species of fish. All ELISA-positive samples assayed with the nested-PCR were negative for *R. salmoninarum*. As with

other survey sites, results suggest ELISA samples in the low level category are likely false-positive for *R. salmoninarum*. There were no clinical signs of bacterial kidney disease in any fish sampled from the Sheyenne River.

Table 3.7.— Percent of samples with detectable levels of *R. salmoninarum* antigen and mean antigen level category as measured by the ELISA, and corroborative testing with a nested PCR assay for four species of fish from the Sheyenne River. BDL = below detection limits.

	ELISA			PCR Assay	
Fish species	Number tested	Percent positive	Mean antigen level	Number tested	Percent positive
Black bullhead	8	25.0	Low	2	0
Northern pike	2	50.0	Low	1	0
Spottail shiner	8	0.0	BDL	0	0
Walleye	7	42.9	Low	3	0

Discussion

The two most notable findings in the survey for bacterial pathogens were isolation of *Y. ruckeri* from black crappie and isolation of *E. tarda* from channel catfish both from Lake Traverse. *Y. ruckeri*, a Gram-negative motile bacterium, causes enteric redmouth disease (ERM), an important disease of salmonid fish particularly for young rainbow trout in aquaculture. There are a few reports of the bacterium from non-salmonids including burbot, carp, goldfish, eel, emerald dace, perch, roach, and sturgeon (Austin and Austin 1987; Bergh 2008). The disease has been reported in the U. S., Canada, Europe, Australia, New Zealand, and South Africa. *Y. ruckeri* is a listed pathogen in the Fish Health Policy of U. S. Fish and Wildlife Service and Fish Health Protection Regulations of Canada. *E. tarda* is a Gram-negative, motile, rod-shaped bacterium that is known to cause the disease edwardsiellosis. While the bacterium has been found in both marine and freshwater fish, in the U. S. it is most frequently associated with channel catfish. The chief characteristic of edwardsiellosis is development of small cutaneous lesions in postero-lateral region of the body. The lesions may progress to large sized abscesses that when opened emit the unpleasant odor of hydrogen sulfide gas. *E. tarda* is not a listed pathogen in U. S. policy or Canadian fish health regulations. Fish from Lake Traverse harboring *Y. ruckeri* and *E. tarda* appeared to be asymptomatic carriers as no clinical signs of ERM or edwardsiellosis were observed during necropsy. No controlled or reportable bacterial pathogens were found in fish from Devils Lake or from the Red and Sheyenne rivers.

Aeromonas hydrophila and other closely associated motile aeromonids (*A. caviae, A. sobria, A. veronii*) are reported worldwide from several freshwater fish and some marine fish. During this survey, *A. hydrophila* was the most common occurring bacterium from all four sample sites. It was isolated from the kidneys of twelve different species of fish. Motile aeromonids are probably normal constituents of the aquatic environment given their wide distribution and high

frequency of isolation from fish tissues. Though often regarded as a secondary invader there are numerous reports implicating *A. hydrophila* as an opportunistic primary pathogen. A variety of different names have been given to diseases caused by the motile aeromonids including hemorrhagic septicemia, motile aeromonid septicemia, redsore disease, and red pest (Bergh 2008). *A. hydrophila* and other bacteria have been reported to cause post-spawning mortality in farm-raised fish. Clinical signs of disease include cutaneous hemorrhages of the fins, truck, and gills, skin ulcerations, fin and tail rot, exophthalmia, and distended abdomen. Internally, signs typical of septicemia are found including ascetic fluid, swollen spleen and kidney, and visceral hemorrhaging. Environmental variables, especially water temperature, and their impact on the physiological condition of fish are likely the most important factors affecting expression of motile aeromonid disease. In the present survey, *A. hydrophila* was often found on growth medium in mixed cultures with other species of bacteria especially representatives of the family Enterobacteriaceae. While this result is not uncommon, it does complicate interpretation of the role motile aeromonids play in fish diseases.

Shewanella putrefaciens (syn. *Alteromonas putrefaciens, Pseudomonas putrefaciens*) was isolated from white bass at Devils Lake but was not found at other survey sites. *S. putrefaciens* was isolated from fathead minnow from Devils Lake in 2005 but was reported under its original taxonomic name *Pseudomonas putrefaciens* (Hudson and Peters 2005). *Shewanella* is the sole genus in the family Shewanellaceae but was classified previously in Vibrionaceae. *S. putrefaciens* is a Gram-negative, oxidase-positive, non-fermentive facultative anaerobe. Colonies of the bacterium produce a reddish-orange or pink water-soluble pigment on nutrient agar. Another phenotypic attribute is the production of hydrogen sulfide gas. The bacterium also produces compounds that are a main cause of fish spoilage. *S. putrefaciens* occurs commonly in saltwater and marine sediments and has been isolated from marine fish (Lee et al. 1977; Gillespie 1981). The bacterium may be part of the normal microflora of marine fish (Austin and Austin 1999). Elevated salinity typical of closed basin waters such as Devils Lake may provide favorable environment conditions for adaptation outside of seawater. Koziñska and Pekala (2004) reported the first isolation of *S. putrefaciens* from freshwater fish in Poland. They showed the bacterium could grow without NaCl suggesting some strains can adapt to freshwater environments. Only two reports on pathogenicity of *S. putrefaciens* for fish were found. In marine fish, Saeed et al. (1987) observed disease and 80% mortality in rabbitfish *Siganus rivulatus* following intraperitoneal challenge of the bacterium. Koziñska and Pekala (2004) observed carp and rainbow trout *Oncorhynchus mykiss* developed disease with 33 – 50% mortality following intraperitoneal injection challenge with a high dose of two different isolates of *S. putrefaciens*. They went on to speculate that the bacterium is probably an opportunistic pathogen that could cause disease under specific conditions such as external stressors. *S. putrefaciens* has been infrequently implicated as a human pathogen with reports of bacteremia, soft tissue infections, and otis media (Brink et al. 1995; Chen et al. 1997) and in some cases may be considered a contaminant or saprophyte living as a secondary invader on previously damaged tissues (Jorens et al. 2004).

In addition to the preceding findings, several species of bacteria commonly associated with aquatic environments or in other animals and plants were found in fish during the surveys. The majority of these bacteria were species from families Enterobacteriaceae and Pseudomonadaceae. These families are characterized as Gram-negative, aerobic or facultative anaerobic, rod-shaped bacteria, which are usually motile. Examples found in this survey include

species from the genera *Enterobacter*, *Erwinia*, *Citrobacter*, *Hafnia*, *Pasturella*, *Pantoea*, and *Pleisomonas*. Many are saprophytes while others are common plant and animal parasites with worldwide distribution. With the possible exception of *A. hydrophila*, these bacteria are not generally considered primary fish pathogens although many are opportunistic and may cause disease if fish are subjected to sufficient stress. Members of Enterobacteriaceae are common in the environment and are frequently found in soil, water, animal waste and sewage, and on the surface of plants and seeds. They are found in animals from insects to humans and some are leading causes of nosocomial infections. Many are important disease agents of agriculture, poultry, cattle, and swine industries. We did not observe any clinical signs of bacterial diseases despite the isolation of one or more of these opportunistic bacteria from nearly all fish sampled.

We observed small almost transparent cysts in a wet mount preparation of gill lamellae from one pumpkinseed sunfish from Lake Traverse. The lesions were attributed to epitheliocystis a disease caused by bacteria in the order Chlamydiales. Epitheliocystis was also observed in stained tissue sections of gills from small numbers (n = 1 each) of fathead minnow, walleye, and yellow perch from Devils Lake. Epitheliocystis was described in the 1920s in Germany under the name mucophilosis. It was first report in the U. S. from bluegill (Hoffman et al. 1969). Since then, the condition has been observed in more than 50 species of fish worldwide in both freshwater and marine environments. Norwak and LaPatra (2006) recently reviewed the current understanding of epitheliocystis including characterization of the pathogen with immunohistochemical and molecular studies. Members of Chlamydiales are obligate intracellular pathogens which do not grow on nutrient agar media used for general isolation of other bacteria. The disease is characterized by a hypertrophic response of gill epithelium where each cyst remains contained within the cytoplasm of a single host cell (Rourke et al. 1984). Epitheliocystis is usually a benign infection although there have been reports of proliferative lesions or hyperinfection where much of the gill surface is covered with infected cells. Mortality has been mostly limited to fish reared in aquaculture facilities and is likely caused by respiratory distress. Although not applicable to disease control in free-ranging fish, a recent communication by Goodwin et al. (2005) showed successful treatment of epitheliocystis in largemouth bass by administration of oxytetracyline in baths.

R. salmoninarum is the cause of bacterial kidney disease (BKD) 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 (trout, salmon, and whitefish). Even so, *R. salmoninarum* has been reported in 53 non-salmonid species among 17 states in the National Wild Fish Health Survey Database (NWFHSD 2009). At least eight non-salmonid families including, Amiidae, Catostomidae, Centrarchidae, Clupeidae, Cottidae, Cyprinidae, Esocidae, and Ictaluridae are represented in those results. In the database, most cases of *R. salmoninarum* in non-salmonids were from two geographical areas, the Columbia River Basin in the Pacific Northwest and the Great Lakes, regions with established populations of Pacific salmon and other salmonids. Despite these interesting findings, analysis of the NWFHS database shows that a large proportion of ELISA-positive samples are not positive for *R. salmoninarum* when tested with the more sensitive and specific nested-PCR assay. At the time of this report, a query of the database showed only 26% of non-salmonid samples and 36% of salmonid samples that tested positive for *R. salmoninarum* by ELISA was confirmed positive by nested-PCR. Inconsistencies between results of ELISA and

PCR are also apparent when examining results of these tests for fish in the Red River basin. Many kidney samples of non-salmonids from the four survey sites had ELISA OD values judged positive for *R. salmoninarum* however, none of the ELISA-positive samples that were selected for testing with the nested-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). There are no sympatric populations of salmonids in the four survey sites.

PCR has a 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. Nearly all the ELISA-positive results fell into the low level antigen category with many OD values just slightly above the negative-positive threshold established with standard reference tissue. There are a number of possible factors that may help explain poor correlation between the tests results. For one, the standard reference tissue used to establish ELISA negative-positive thresholds may not be appropriate for all families of fish or all geographical areas. The reference tissue used in the National Wild Fish Health Survey is from fall chinook salmon (Salmonidae) while all samples collected in this survey were from fish in other taxonomic families. It is possible that certain proteinaceous elements or other constituents of non-salmonid kidneys may interfere with the ELISA and result in higher background readings thus producing false-positive values based on the salmonid reference tissue. Another possible explanation is that polyclonal antibodies used in the ELISA are cross-reacting with other species of bacteria (Dixon 1985; Turaga et al. 1987; Gudmundsdóttir et al. 1993; Brown et al. 1995; Jansson et al. 1996). We refer readers searching for more information on this topic to Pascho et al. (2002) who provide an excellent thorough review of *R. salmoninarum* including the history and present state of testing methods.

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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 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 are first summarized and then given in more detail according to class of parasite and affected tissues. Results are 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 16 different parasites were observed (Table 4.1). Nine 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 the thymus. 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 20% of fish. An annelid was observed on the gills of three fish.

Myxosporea

Renal. A large myxosporean parasite was observed in the kidney tubules. 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.* Intraluminal parasites were identified in kidney tubules of 8 fathead minnow (Figure 4-1).

A smaller myxosporean parasite was also seen in kidney tubules. Kidney tubules contained aggregates of immature multinucleate trophozoites and few spores. These smaller myxospores

had more spherical valves and polar capsules than the larger myxospores. Sections of distal tubule lumens in some fish were completely occluded with presporogonic parasites. These myxospores (probably *Myxobolus sp.)* were observed in 42 fish (Figure 4-2).

A third myosporean parasite was found scattered in kidney interstitial tissue. These parasites were more elongate and elliptical in shape than the previous two myxospores described in fathead minnows. These parasites were found in only 5 fish (Figure 4-3).

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)	8	4-1
Kidney - tubule	Myxosporean (sm)	42	4-2
Kidney - interstitium	Myxosporean	5	4-3
Skeletal muscle	Myxosporean	15	4-4
Skeletal muscle	Trematode - Digenetic (encysted)	12	4-10
Nerve - peripheral	Myxosporean	6	4-5
Brain - meninges	Trematode - Digenetic	5	4-11
GI - intestine	Cestode	12	4-15
Gill	Myxosporean	2	4-6
Gill	Myxosporean – *Henneguya*-like.	2	4-7
Gill	Protozoa – *Trichodina* sp.	12	4-13
Gill	Protozoa – *Apiosoma* sp.	1	4-14
Gill	Trematode – Monogenetic - *Gyrodactylus* sp.-like	13	4-12
Gill	Annelid (leech-like)	3	none
Cartilage/Bone	Myxosporean	1	4-9
Thymus	Myxosporean	2	4-8

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 15 fish (Figure 4-4).

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 6 fish (Figure 4-5).

Branchial. Histology sections of gill tissue provided observations of interlamellar cysts containing mature myxospores with distinct rounded shapes. These branchial myxosporeans exhibited stained polar capsules that were also round in appearance. These myxospores were observed in 2 fish (Figure 4-6).

Giemsa staining of gill sections also demonstrated interlamellar cysts containing oval shaped spores with elongated polar capsules that are typical of the myxosporean genus *Henneguya*. Typical *Henneguya* caudal processes were not observed, but are difficult to retain in tissue sections. These parasites were observed in 2 fish (Figure 4-7).

Thymus. Myxosporeans were observed in 2 fish. These were primarily trophozoites with few mature spores. These parasites were similar in appearance to those occurring in peripheral nerves (Figure 4-8).

Cartilage/Bone. One single foci of myosporean spores surrounded by bone was observed in one fish out of 60 examined. No trophozoite stages were observed. Identification of myxospore was not possible due to poor morphological characteristics retained in tissue section (Figure 4-9).

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 20% of fish (Figure 4-10).

Meningial. Sections of the brain showed digenetic trematodes in the meninges of 5 fish. These were observed within the endomeningial connective tissue. Lack of distinct organelle structures in sections precluded classification beyond Trematoda. (Figure 4-11).

Monogenea
Branchial. A monogenean trematode was seen in gill sections of 13 fish. Based on parasite morphology and organelle structures, the parasite was tentatively identified as a *Gyrodactylus sp.*, however, no haptors or hooks were observed in the sections. Infected fish typically contained one or two parasites per section (Figure 4-12).

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 12 of 60 fish (Figure 4-13).

Gill 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 on the lamellar gill surface in only 1 fish out of 60 (Figure 4-14).

Annelida

Branchial. A leech-like annelid was observed in gill sections. This annelid was identified on the lamellar gill surface and was distinctly segmented. Identification to genus and species was not possible due to lack of intact coelom in the gastrointestinal tract. These segmented worms were observed in 3 of 60 fish examined.

Cestoda

Gastrointestinal. An intestinal cestode was identified in 12 of 60 fish. Tissue sections of intestine demonstrated a cestode infection. The morphology of the parasite was suggestive of a *Proteocephalus sp.* Histology observation did not provide discriminating features necessary for accurate cestode species identification (Figure 4-15).

Walleye

A total of 5 different parasites were observed in tissue sections from 60 walleye (Table 4.2). The most common parasite found was the protozoan *Trichodina sp.* on the gills. Even though most fish were heavily parasitized there was limited tissue response to the infestation. Cestodes were also common in both large and small fish but varied in abundance among individual walleye. A suspect nematode was observed in sections of liver from 3 fish.

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.)*	40	4-16
Gill	Protozoa – *Trichodina sp.*	58	4-17
Gill	Protozoa – *Apisoma sp.*	2	4-19
Gill	Protozoa – *Ichthyophthirius sp.*	11	4-18
Gill	Protozoa - flagellate	1	4-20
Liver	Suspect larval nematode	3	4-21

Cestoda

Gastrointestinal tract. There was a high prevalence, 40 of 60, of intestinal cestodes seen in histological sections. 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 are suggestive of a *Proteocephalus sp.*(Figure 4-16).

Protozoa

Branchial. There were 4 protozoan parasites observed in walleye gill sections. *Trichodina sp.* was most numerous. These parasites were very prevalent and observed on 58 of 60 fish (Figure

4-17). The early life stages of *Ichthyophthirius multifiliis* were observed in gill sections from 11 of 60 fish (Figure 4-18). A protozoan similar to *Apiosoma sp.* was observed in gill sections of 2 walleye (Figure 4-19). A flagellate protozoan parasite similar in appearance to *Ichthyoboda* sp. was observed on one small walleye (Figure 4-20). These parasites were numerous but appeared to cause little tissue reaction.

Nematoda (suspect)
Hepatic. Well circumscribed granulomas containing cellular debris were found scattered through liver tissue from 3 fish (Figure 4-21). The lack of specific organelles or infectious agents prevents a diagnosis of parasite or bacteria origin. These lesions could be the necrotic remains of encapsulated larval nematodes of *Spiroxys sp.* that were observed in white bass from Devils Lake.

White Bass

A total of 59 white bass were examined with histology. The most prevalent parasites observed were cestodes in the gastrointestinal tract. The only other parasite was an external protozoan identified as *Trichodina sp.* found in gill sections (Table 4.3).

Cestoda
Cestodes were observed in the gastrointestinal tract of 35 fish (59.2%). The lack of identifying features of this organism precluded a definitive classification. This cestode was observed in 35 of 59 fish (Figure 4-22).

Protozoa
An external protozoan gill parasite was clearly identified as: *Trichodina sp.* Although these parasites were numerous on gill surfaces, there appeared to be little pathology associated with their occurrence.

Table 4.3.— Summary of parasite findings from examination of 59 white bass with histology.

Tissue	Parasite	Number positive	Figure number
GI - intestine	Cestode	34	4-22
Gill	Protozoa – *Trichodina* sp.	28	none

Black Crappie

A total of 60 black crappie from Devils Lake were processed and examined with histology. We observed three major classes of parasite in these fish (Table 4.4). A very prevalent protozoan parasite was observed in gills of 58 fish. Myxosporeans were found in the kidney, urinary bladder, and in the gall bladder. Cestodes were observed in the viscera/peritoneal cavity.

Cestoda

A larval cestode was observed in sections of viscera. The presumptive identification of this organism was *Ligula sp.* This parasitic organism was detected in 37 fish (Figure 4-23).

Table 4.4.— Summary of parasite findings from examination of 60 black crappie by histology.

Tissue	Parasite	Number positive	Figure number
GI - viscera	Cestode – *Ligula sp.*	37	4-23
Gill	Protozoa – *Ichthyobodo* sp.	58	4-24
Kidney	Myxosporean – *Myxobolus sp.*	3	4-25
Urinary Bladder	Myxosporean – *Henneguya sp.*	3	4-26
Gall Bladder	Myxosporean – *Chloromyxum sp.*	2	4-27

Protozoa
A branchial protozoan parasite was observed on the surface of gill in 22 fish (36.7%). 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 4-24).

Myxosporea
A myxosporean parasite was observed in kidney sections. Spores were histomorphologically similar to a *Myxobolus sp* (Figure 4-25). This myxobolid parasite was seen in 3 of 60 fish. Identification to species was not possible with histology observation.

The myxosporean *Henneguya sp.* was identified in the urinary bladder of black crappie. This was a presumptive identification because the caudal processes were not intact in tissue sections (Figure 4-26). This *Henneguya*-like parasite was observed in 3 of 60 fish.

Histology sections of gall bladder suggested the presence of a *Chloromyxum sp.* This myxosporean genus has a two host life cycle and is commonly found in the gall bladders of freshwater fish species. Morphological features were consistent with the *Chloromyxum* genus and observed in 2 of 60 fish (Figure 4-27).

Yellow Perch

Tissue samples were collected from a total of 17 yellow perch. One cestode and one external protozoan parasite were described (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
The common freshwater cestode *Ligula sp.* was detected in the gastrointestinal tract in 5 fish (29.4%) (Figure 4-28).

Protozoa
The external protozoan parasite *Ichthyophthirius multifiliis* was observed in gill sections. This was an incidental finding in just one fish (Figures 4-29 and 4-30).

Table 4.5.— Summary of parasite findings from examination of 17 yellow perch by histology.

Tissue	Parasite	Number positive	Figure number
GI - intestine	Cestode – *Ligula sp.*	5	4-28
Gill	Protozoa – *Ichthyophthirius multifiliis*	1	4-29, 4-30

Northern Pike

Four classes of parasites were observed in histology sections from a total of 23 northern pike (Table 4.6). We observed monogenean trematodes in the gills of three fish and nematodes and cestodes were found in the gastrointestinal tracts of 2 fish. A myxosporean was observed in the kidneys of two fish.

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

Tissue	Parasite	Number positive	Figure number
GI	Cestode	2	4-32
GI	Nematode	2	4-33, 4-34
Kidney	Myxosporean – *Thelohanellus sp.*	2	4-35, 4-36
Gill	Trematode – Gyrodactylus *sp.*	3	4-31

Monogenea
Gyrodactylus sp. was observed in 3 fish (13%). 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 4-31).

Cestoda
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 2 fish (8.7%) (Figure 4-32).

Nematoda
Additional tissue sections of the gastrointestinal tract contained a nematode of unknown identification. The nematode was observed at a relatively low prevalence in 2 of 23 fish

examined. Photo documentation demonstrated the location and cellular orientation of the nematode parasite in northern pike (Figures 4-33 and 4-34).

Myxosporea
A myxosporean parasite was observed in kidney tissue in 2 fish. Histology evaluation provided a presumptive identification to genus: *Thelohanellus 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 (Figure 4-35 and 36).

White Sucker

Tissues from a total of 8 white suckers were collected from Devils Lake. Comprehensive observations of stained tissue sections found one major parasite class occurring in gill sections (Table 4.7). The parasite was detected in 3 fish (37.5%).

Myxosporea
A myxosporean – induced xenoma was observed in sections of gill tissue from white sucker (Figure 4-39). Several life stages were seen in sections including immature trophozoite stages and mature spores (Figures 4-37 and 4-38). Sections of spores contained within the xenoma are suggestive of a *Myxobolus sp.* Spores observed in tissues section were not of sufficient resolution to permit identification to species.

Table 4.7. — Summary of parasite findings from examination of 8 white suckers with histology.

Tissue	Parasite	Number positive	Figure number
Gill	Myxosporean - *Myxobolus sp.*	3	4-37, 4-38, 4-39

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 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 were documented. For white sucker, the only parasites observed were spores of *Myxobolus sp.* found in gills. At Devils Lake, histozoic myxospores were found in a variety of tissues including kidney interstitium, skeletal muscle, peripheral nerves, gill lamellae,

cartilage and bone, and the thymus. Coelozoic spores were found in kidney tubules, the gall bladder, and in the urinary bladder.

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. The obvious limitation to histology however, was that many metazoan parasites could not be identified to taxonomic levels closer than class and order. It was possible to identify some organisms to genus and a couple to species. More often though, metazoan parasites presented in 5.0 µm thin sections lack sufficient morphological detail to permit their identification to genus and species. In smaller scale surveys or when examining a specific condition, the histopathologist may choose to cut several additional sections from tissue blocks where the initial section revealed a partial organism. In this way morphological characteristic required for identification may be revealed. 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 an exceptional 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. When sampling large sized fish for histology, one generally selects a standard sample size from each target tissue, 1.0 cm^3 for example. Upon fixation, tissues may undergo further cutting and trimming so they fit the format of standard tissue processing equipment. Thus, the proportion of tissue sampled in relation to the actual size of the target tissue (organ) decreases as size of fish increases. Lastly, histology is an expensive process and requires considerable time to examine multiple tissues section from each fish. Examination of a series of tissue imprints from each host tissue could be used in place of histology. 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.

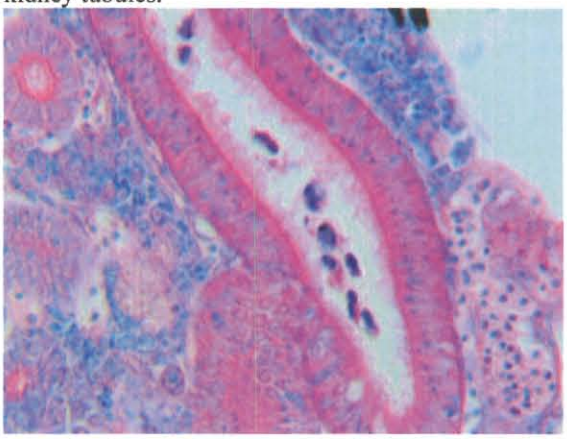


Figure 4-2. Small myxosporean observed in fathead minnow kidney tubules.

Figure 4-3. Myxosporean in fathead minnows kidney interstitium.

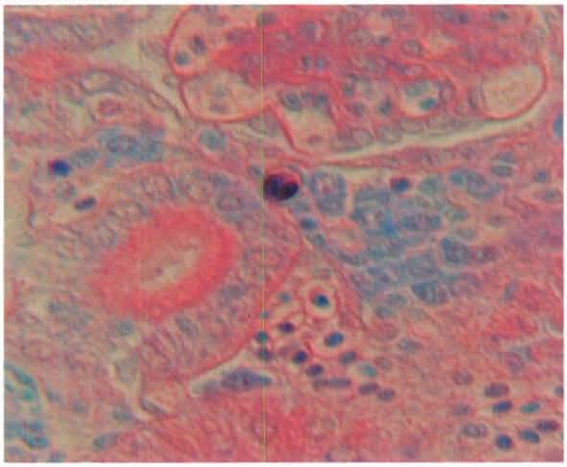


Figure 4-4. Myxosporean from skeletal muscle in Fathead Minnows.

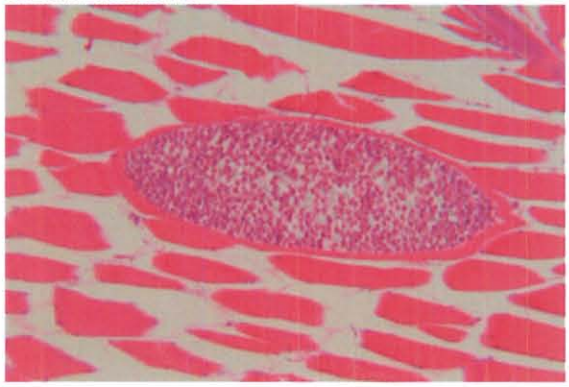


Figure 4-5. Myxosporean observed in fathead minnow peripheral nerve tissue.

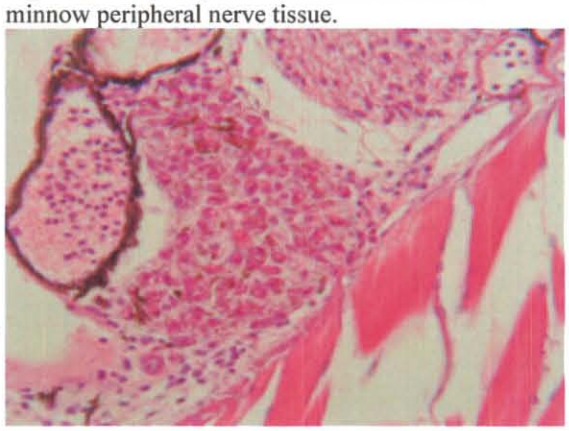


Figure 4-6. Fathead minnow branchial myxosporeans.

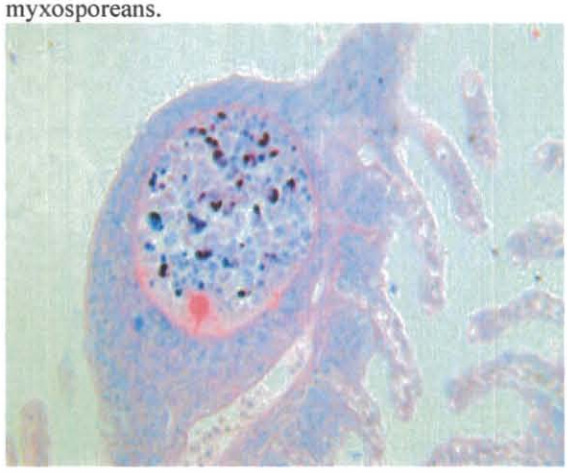


Figure 4-7. *Henneguya*-like myxospores in fathead minnows.

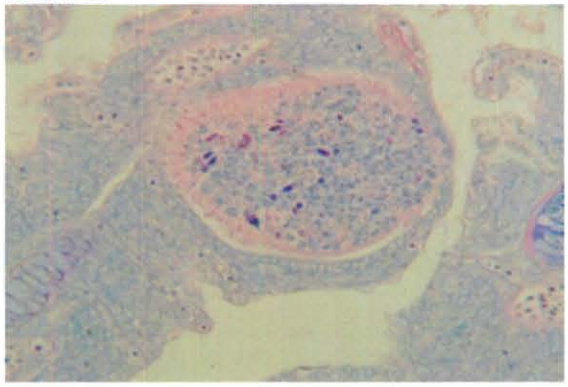


Figure 4-8. Fathead minnow thymic myxosporean.

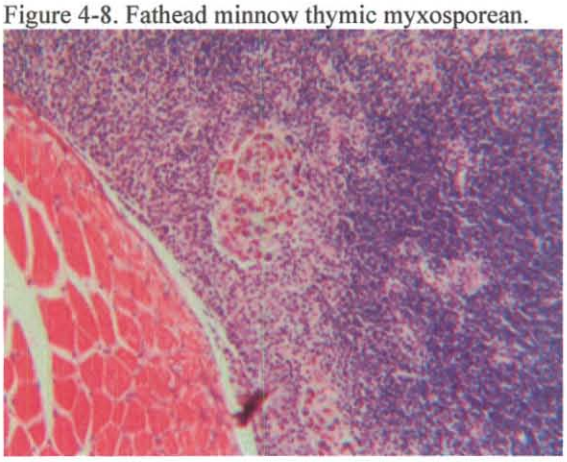


Figure 4-9. Myxosporean foci in fathead minnow cartilage/bone.

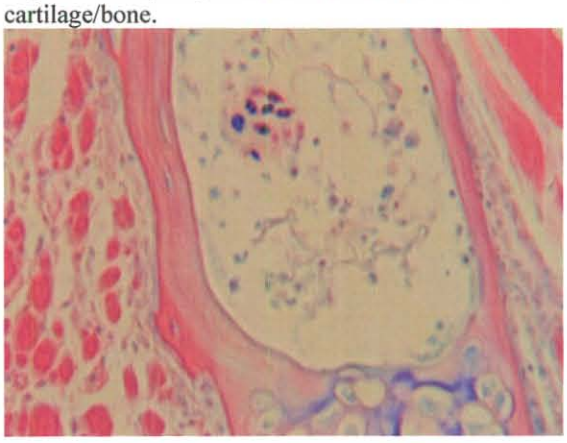


Figure 4-10. Fathead minnow intramuscular digenetic trematode.

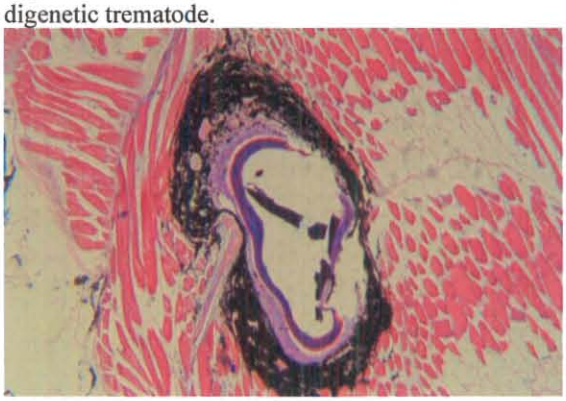


Figure 4-11. Fathead minnow meningial digenetic trematode.

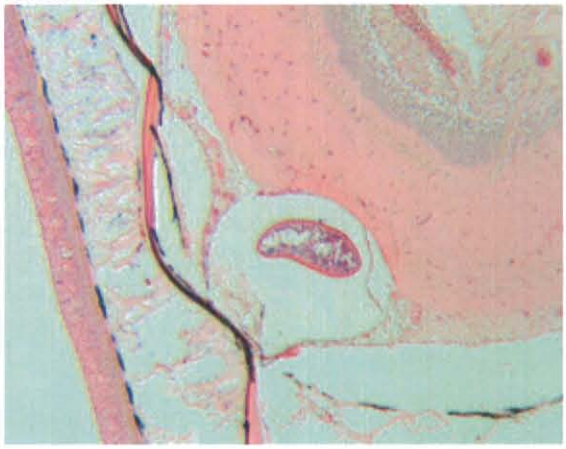


Figure 4-12. Fathead minnow branchial monogenetic trematode.

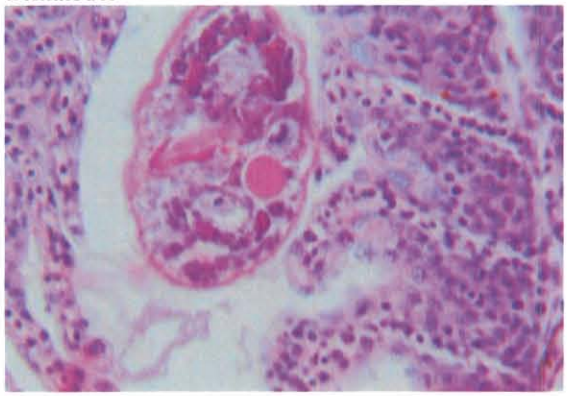


Figure 4-13. Branchial *Trichodina sp.* observed in fathead minnow.

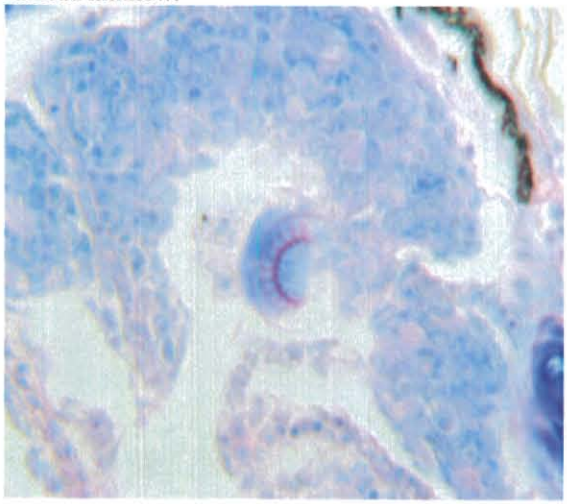


Figure 4-14. Branchial *Apiosoma sp.* observed in fathead minnow.

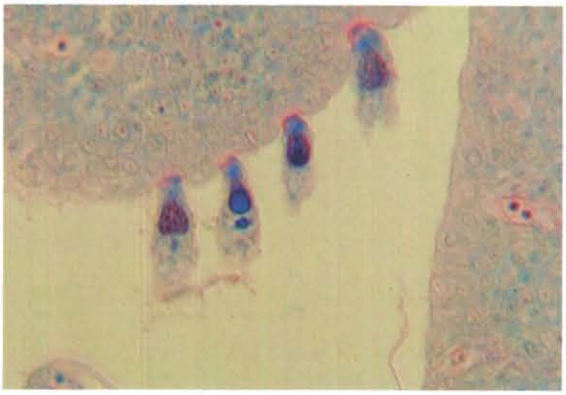


Figure 4-15. Fathead minnow intestinal cestode.

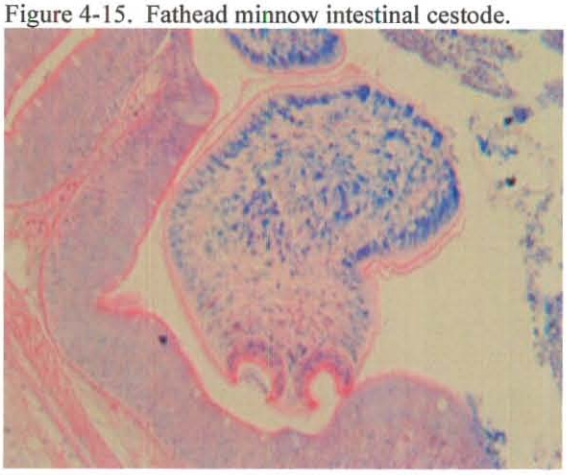


Figure 4-16. Walleye cestode.

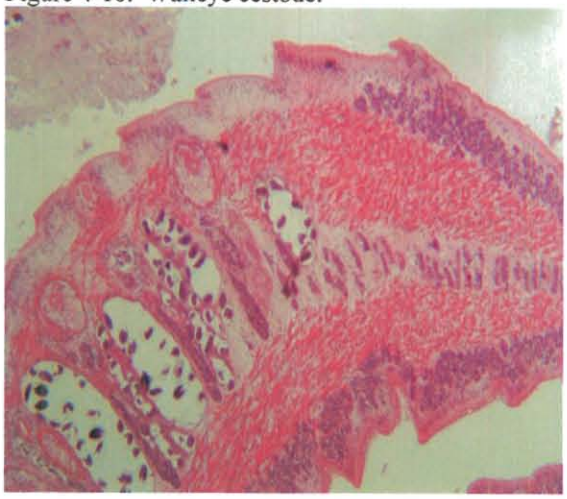


Figure 4-17. *Trichodina sp.* observed in walleye gills.

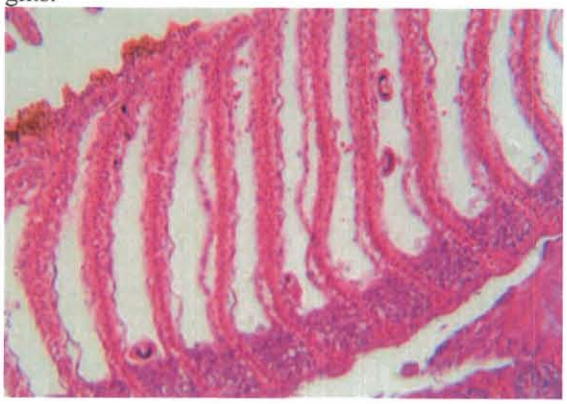


Figure 4-18. *Ichthyophthirius multifiliis* observed on gill lamellae of walleye.

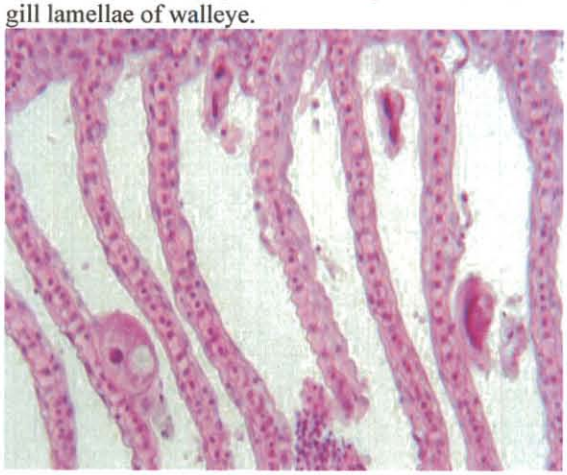


Figure 4-19. External branchial protozoan. *Apiosoma sp.*–like in walleye.

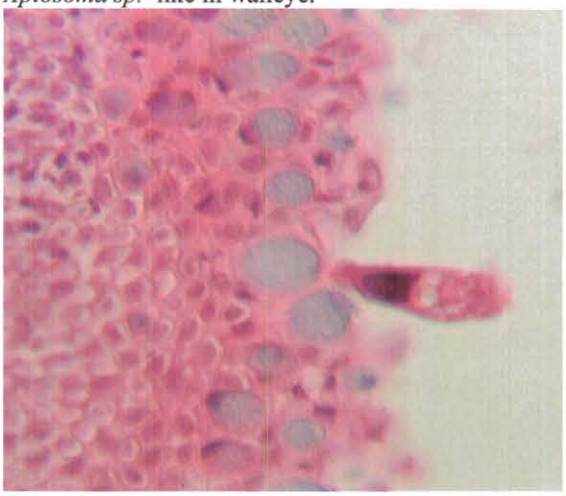


Figure 4-20. Flagellate protozoan similar to *Ichthyobodo sp.* on walleye gill.

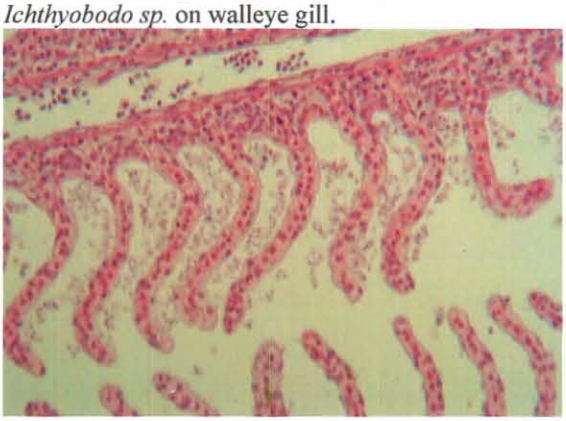


Figure 4-21. Hepatic granulomas observed in walleye. Possible nematode.

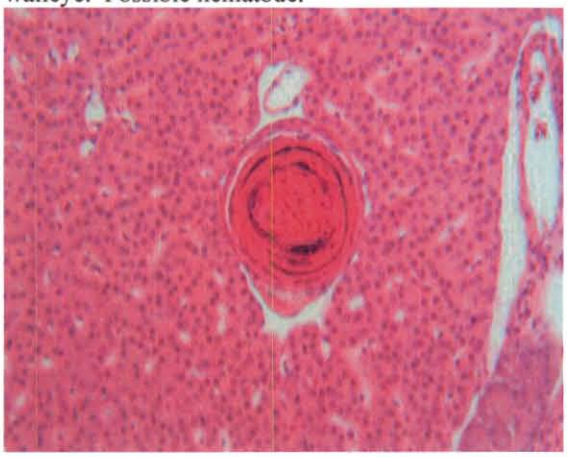


Figure 4-22. White bass cestode.

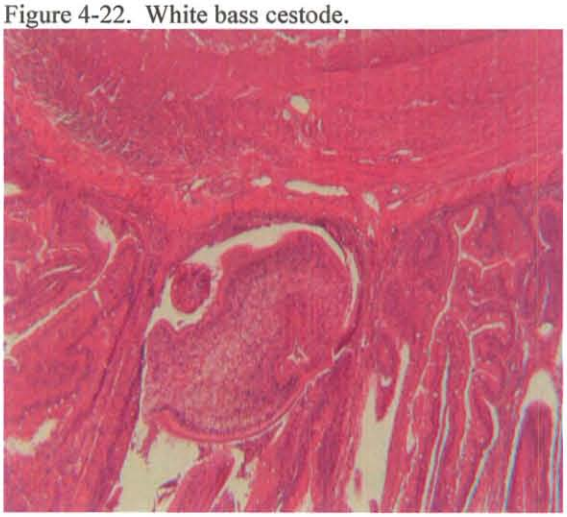


Figure 4-23. Black crappie. Presumptive identification: *Ligula sp.*

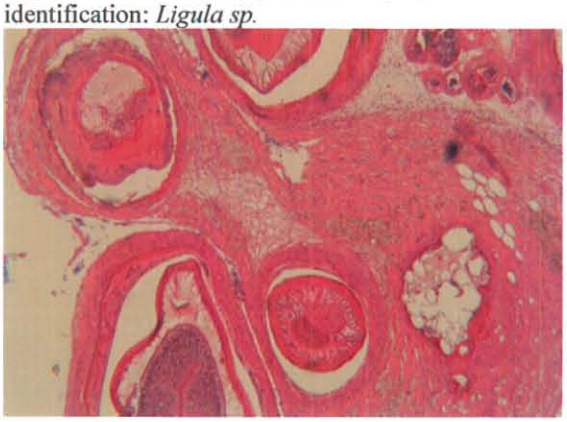


Figure 4-24. *Ichthyobodo sp.* (*Costia*) on gill lamellae of black crappie.

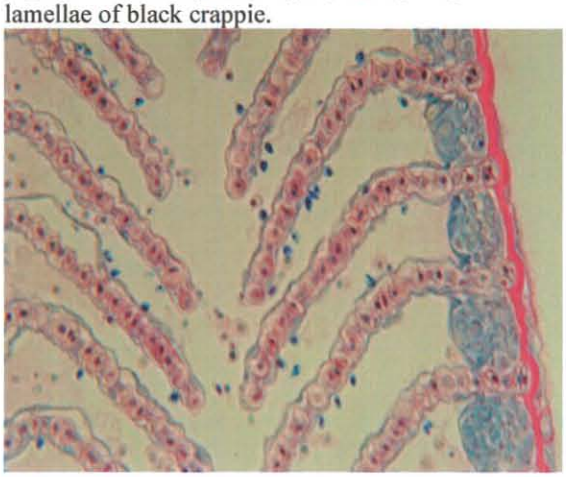


Figure 4-25. Black crappie kidney myxosporean.

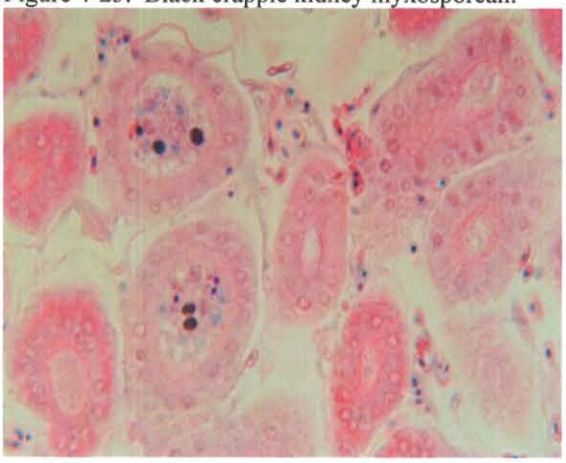


Figure 4-26. Black crappie. *Henneguya sp.* – in urinary bladder.

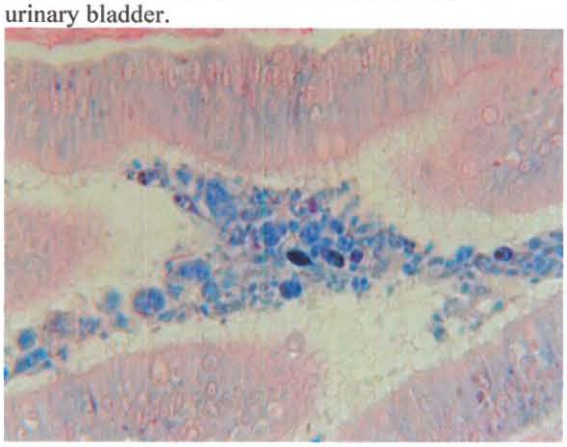


Figure 4-27. Black crappie *Chloromyxum sp.* in gall bladder.

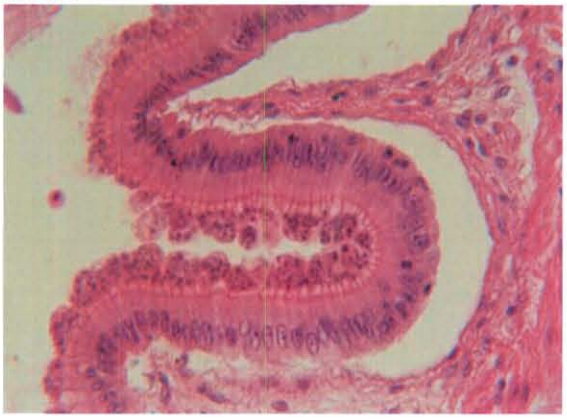


Figure 4-28. Yellow perch cestode observed in gastrointestinal tract.

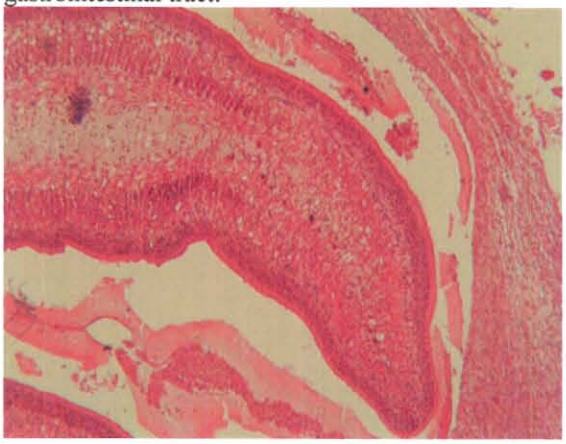


Figure 4-29. External protozoan: *Ichthyophthirius* trophont yellow perch.

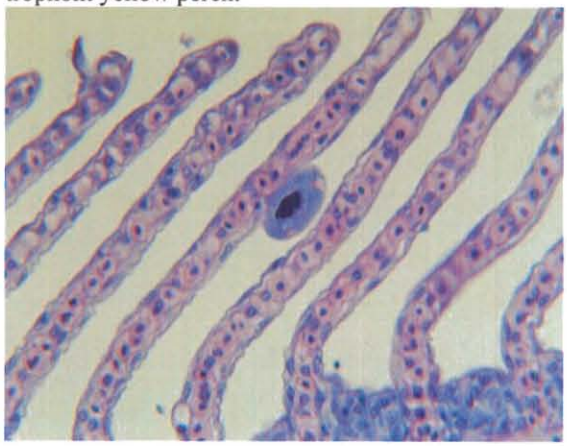


Figure 4-30. Yellow perch *Trichodina sp.* observed in gill sections.

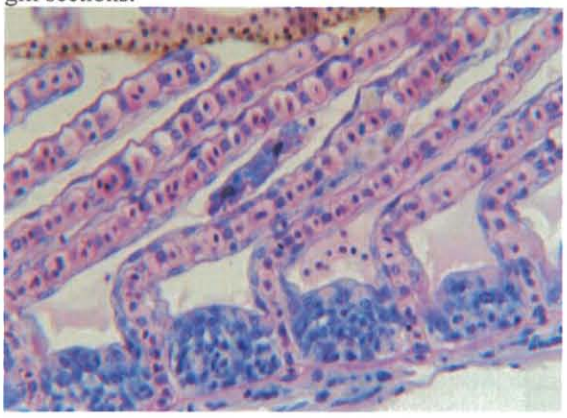


Figure 4-31. Northern pike. *Gyrodactylus sp.* observed in gill sections.

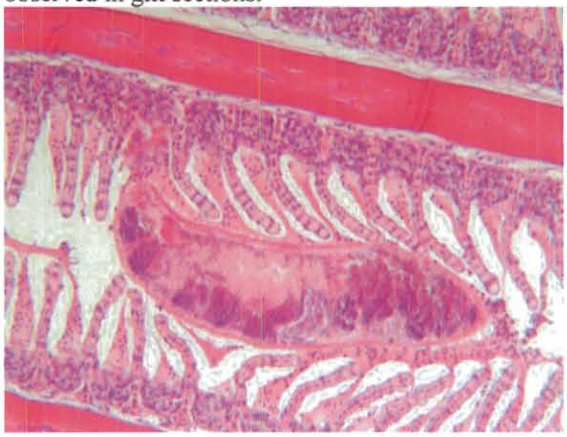


Figure 4-32. Northern pike cestode.

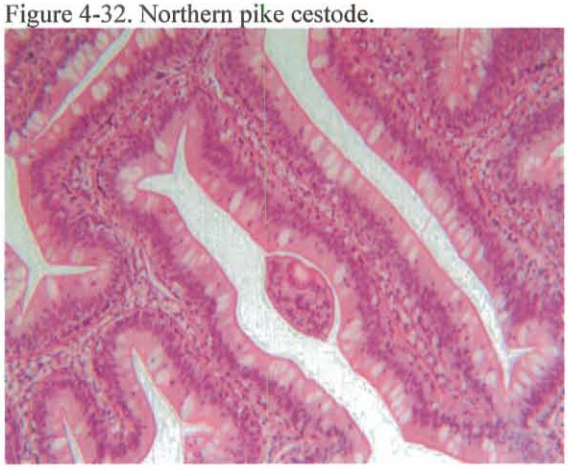


Figure 4-33. Northern pike gastrointestinal section. unknown nematode.

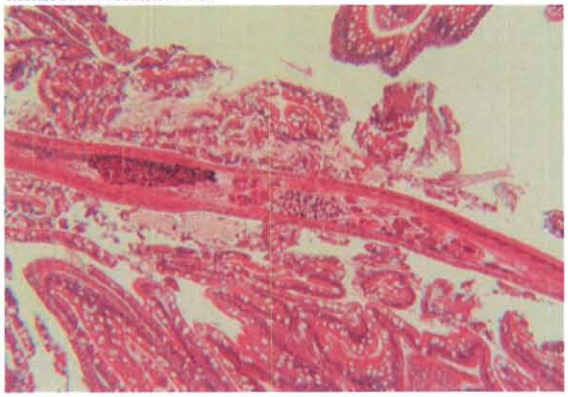


Figure 4-34. Northern pike nematode.

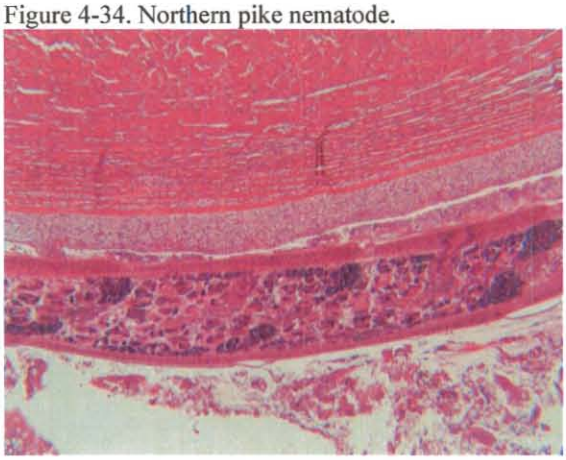


Figure. 4-35. Northern pike myxosporean parasite observed in kidney.

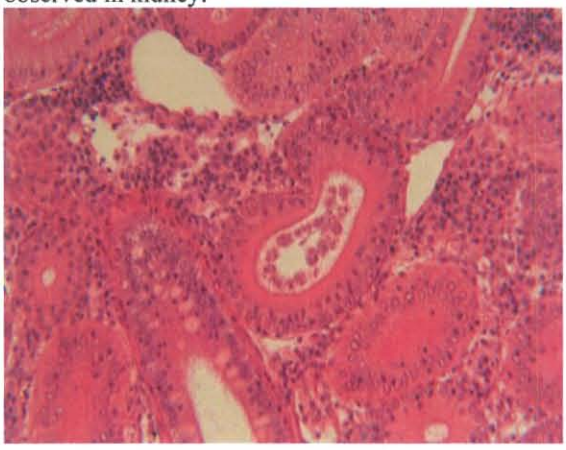


Figure 4-36. Northern pike kidney myxosporean.

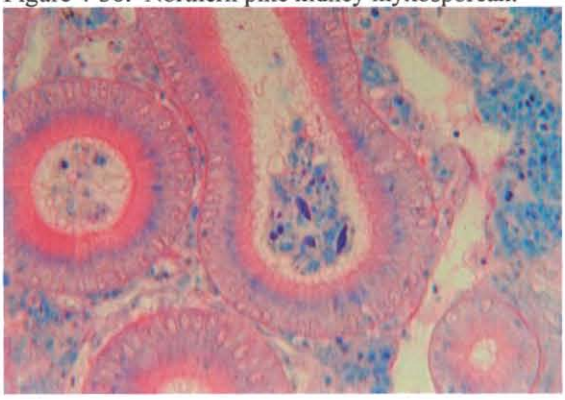


Figure 4-37. White sucker branchial *Myxobolus sp.* in lamellae.

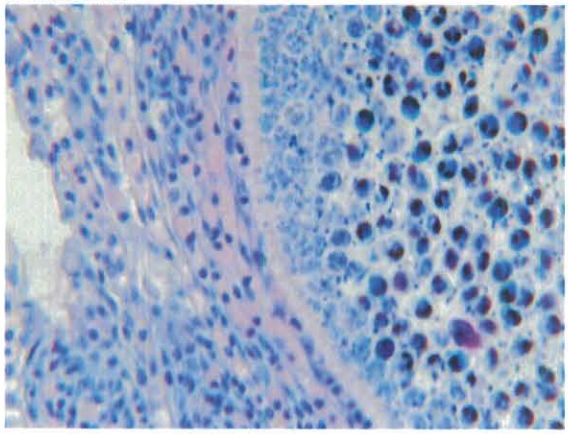


Figure 4-38. White sucker branchial *Myxobolus sp.* spore stage.

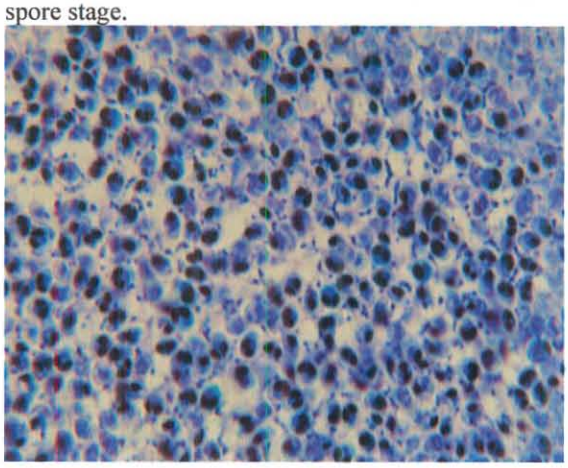
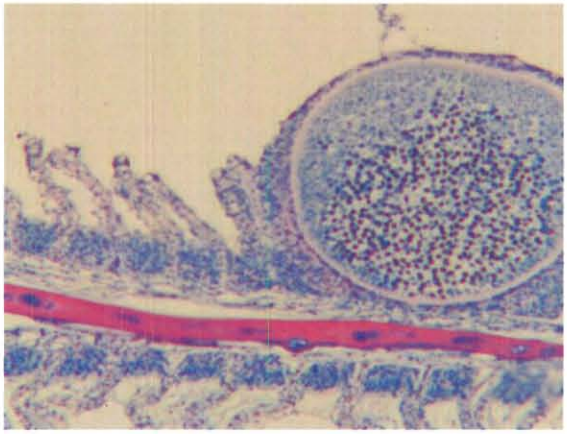


Figure 4-39. White sucker branchial *Myxobolus sp.* xenoma.



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 Devils Lake and Lake Traverse. At the temporary field stations, work stations were equipped with a dissecting microscopes with fiber optic lighting and a 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 a parasite specialist 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

Devils Lake.— A total of 407 fish representing seven species were examined for parasites. For the comprehensive survey, 47 fish were examined fresh at the temporary field station during a week-long sampling operation at Six Mile Bay. Another 71 fish 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 bacteriology and virology. A total of fifteen different parasites were identified to the level of genus and of those five parasites were identified to species (Table 5.2). These included three protozoa, four monogenean, one trematode, four cestodes, two nematodes, and one leech. Parasites were recovered from all species of fish except white sucker. We identified seven different parasites from fathead minnow, five from white bass, four from yellow perch, three from black crappie, and two each from northern pike and walleye.

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 spp.*, with their distinctive saucer-shaped body and aboral ciliated girdles, were the most common external parasites of fish at Devils Lake. *Trichodina spp.* was observed in mucus from skin scrapings, wet mounts of fins clips, and in wet mounts of gill lamellae. We observed *Trichodina spp.* on all species of fish examined with the exception of white sucker. Two sessile ciliated protozoan parasites were also observed on gills. The first, *Apiosoma sp.*, was found on the gills of black crappie and fathead minnow, and the second *Epistylis sp.*, was seen only on walleye.

Four different monogeneans, parasites that complete their life cycle on one host, were observed on fish from Devils Lake (Table 5.2). These occurred on three species of fish. *Dactylogyrus sp.* (Figure 5.2) and *Gyrodactylus hoffmani* (Figure 5.3) were observed on fathead minnow. *G. hoffmani* was observed on about 47% of the fish examined fresh at the field station and was observed most frequently on dorsal fins. Another species of *Gyrodactylus*, larger in size compared to *G. hoffmani*, was found on the skin of yellow perch but the single specimen was lost when attempting to recover it from the wet mount preparation. *Onchocleidus chrysops* (syn. *Cleidodiscus chrysops*) was found on the gills of white bass (Figure 5.4).

Only two trematode parasites were found in fish from Devils Lake (Table 5.2). Metacercariae of the larval genus *Neascus sp.* was found in melanized cysts in the caudal peduncle musculature of fathead minnow. One parasite presumptively identified as trematode metacercariae could not be placed in any taxonomic level below class because the specimen was in poor condition and did not stain well after fixation. The unidentified trematode was observed encysted in the lateral musculature between the rib bones of a single fathead minnow (Figure 5.5).

Cestodes from two taxonomic orders, 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 species although many fish were infested with metacestodes (larval forms) that could not be identified further than genus. *Bothriocephalus cuspidatus* and metacestodes of *Bothriocephalus sp.* were the most common cestodes at Devils Lake and were found in the intestines of fathead minnow, walleye, white bass, and yellow perch (Figure 5.6). All walleye we examined were infested with *B. cuspidatus*. *Proteocephalus pinguis* was found in the intestines of northern pike while metacestodes of *Proteocephalus sp.* were found in fathead minnow (Figure 5.7).

Two species of nematodes (Nematoda) were identified from fish collected at Devils Lake (Table 5.2). The larval nematode *Contracaecum sp.* (Ascaridida: Anisakidae) was observed in characteristic cysts along the mesenteries of three species of fish including black crappie, walleye, and white bass (Figure 5.8). Encysted larvae of *Contracaecum sp.* are macroscopic and conspicuous and there are numerous records of occurrence through out North America. They exhibit little if any fish host specificity. The second nematode was also a larval worm. *Spiroxys sp.* (Spirurida: Gnathostomatidae) was found in small cysts ($D < 1.0$ mm) in viscera and mesenteries of white bass only (Figure 5.9). There are several records of *Spiroxys* from North

American (Hoffman 1999) including one report from the Sheyenne River, North Dakota (Sutherland and Holloway 1979).

Lastly, the parasitic leech *Myzobdella lugubris* (Hirudinea: Pisciolidae) (synonym *M. moorei*) was found on the fins of yellow perch from Devils Lake (Figure 5.10). *M. lugubris* is a common fish parasite and is widely distributed in North America (Hoffman 1999).

Lake Traverse.— A total of 674 fish representing 19 species were examined for parasites (Table 5.3). For the comprehensive survey, 59 fish were examined fresh at the temporary field station and another 65 fish were frozen and examined later. Another 550 fish were monitored grossly for macroscopic parasites during necropsy and tissue collection for bacteriology and virology. A total of 41 different parasites were identified to the level of genus or larval genus, and of those, twenty parasites were identified to species. These included five protozoan, one microsporean, five myxosporeans, four monogeneans, eight trematodes, nine cestodes, six nematodes, one acanthocephalan, two leeches, and two parasitic crustaceans (Table 5.4). Parasites were recovered from all species of fish except orangespotted sunfish and shorthead redhorse although because of low catch rates neither species was examined comprehensively. We found twelve different parasites from fathead minnow, eleven from rock bass, seven from bluegill, and six each from channel catfish, freshwater drum, walleye, and yellow perch. We identified five parasites each from black crappie, pumpkinseed, and white bass and four or fewer from the remaining species of fish.

Motile protozoan parasites *Trichodina spp.* was commonly observed on the either skin, fins and/or gills and pseudobranchs of 10 different species of fish from Lake Traverse (Table 5.4). Two sessile ciliated protozoa *Apiosoma sp.* and *Epistylis sp.* were also observed but less frequently (Figure 5.1). Two flagellated protozoan parasites were occasionally found. *Hexamita sp.* was observed in the intestine of emerald shiner and *Ichthyobodo necator* (syn. *Costia*) was found on the skin and gills of emerald shiner and yellow perch.

We observed an irregular colored and slightly larger than normal oocyte in the ovary of one fathead minnow that was examined after being frozen. A wet mount of the excised oocyte revealed numerous oval shaped spores of the microsporidian *Ovipleistophora ovariae* (syn. *Pleistophora ovariae*, Pekkarinen et al. 2002). From an air dried smear fixed in methanol and stained with methylene blue, we estimated spores were about 7 – 7.5 µm in length and 3.5 – 4.5 µm wide (Figure 5.11). These measurements are within range of those given for spores of *O. ovariae* in previous studies (Summerfelt 1964, Nagel and Hoffman 1977, Canning and Lom 1986).

Spores of Myxosporea (Suborder Platysporina) from three different genera were found in fish from Lake Traverse: *Henneguya*, *Myxobolus*, and *Unicauda* (Table 5.4). From freshwater drum, numerous ovoid and irregular shaped cysts either singly or in aggregates were observed grossly in the kidney of one fish. Cysts contained ovoid spores with two anterior polar capsules and two caudal extensions of the spore valve characteristic of the genus *Henneguya* (Figure 5.12). Spores had a series of striations or ridges running parallel and between the sutural line similar to genus *Myxobilatus* although the suture did not appear to pass between the polar capsules. Spores had a total length of about 34 – 42 µm with spore valve length of 12 – 13 µm, spore width of 5 – 7 µm,

and caudal extension length of 22 – 30 µm. Spores of three different myxosporidians were observed in fathead minnow. The first, a *Myxobolus sp.*, was observed as numerous intralamellar cysts scattered throughout gill lamellae of affected minnows. Some of the larger cysts were visible grossly but under the microscope large numbers of smaller cysts were also evident (Figure 5.13). Spores were elongated pyriform shape and were approximately 12.5 – 13.5 µm long and 5.5 – 7 µm wide. Anterior polar capsules were also elongate and measured 7 – 8 µm long and 2 – 2.5 µm wide. Several spores extruded their polar filaments when viewed in wet mount under the microscope suggesting spores remained viable even though the specimen had been stored frozen at -70°C (Figure 5.14). Pre-spore trophozoites in various stages of development as well as mature spores were evident in stained tissue sections of affected gill lamellae (Figure 5.15). The second myxosporean of fathead minnow, also a *Myxobolus sp.*, was found in a relatively large cyst (D ~ 2.5 mm) grossly visible on the pectoral fin of one fish (Figure 5.16). Elongate pyriform spores were similar in shape to spores found in gill cysts but were slightly larger in size. Spores from the fin cyst measured in wet mount were 14.5 – 15 µm long and 7.5 – 8 µm wide in valvular view. Polar capsules were pyriform and of equal size being about 5.5 – 6 µm long and 2 – 2.5 µm wide. Several spores extruded one or both polar filaments when viewed in wet mounts under the microscope. The third myxosporean infecting fathead minnow was found in a small cyst (D < 1.0 mm) in connective tissue at the base of the caudal fin of one fish. The cyst contained mature ovoid spores with two divergent elliptical-shaped polar capsules at the anterior end (Figure 5.17). The posterior end of spores had a single caudal appendage that was not an extension of the spore valve. Caudal appendages were of variable length and were easily sheared from spore shells in the wet mount preparation. Unfortunately, the only fresh material available was mistakenly discarded before spore dimensions were examined with an ocular micrometer. Despite the lack of measurements, spore morphology was consistent with descriptions of the myxozoan genus *Unicauda* Davis, 1944. The final myxosporidian was found in a small creamy white-colored cyst (D < 1.0 mm) in mesenteric tissue between a fold of the intestine of one rock bass. A wet mount of cyst contents contained numerous ovoid shaped spores with morphology characteristic of the genus *Myxobolus* (Figure 5.18). The smooth spore body was biconvex in sutural view with both the anterior and posterior ends of the sutural ridge slightly projected. The anterior end of spores contained two pyriform polar capsules equal in size. Within moments of wet mount preparation nearly all spores began to extrude one of both polar filaments despite the host having been stored frozen (-70°C) for about 4 months prior to examination. In valvular view spores measured from wet mount preparation were 8 – 8.5 µm long and 7 – 7.5 µm wide. The polar capsules were 3 – 3.5 µm long and 2 – 2.5 µm wide.

Four different monogeneans were found on fish from Lake Traverse (Table 5.4). We observed *Dactylogyrus sp.* on the gills and *Gyrodactylus hoffmani* on the fins of fathead minnows (Figure 5.19). *Ligictaluridus sp.* was found on gills of black bullhead (Figure 5.19). *Microcotyle spinicirrus* was observed on the gills of most freshwater drum we examined. The parasite caused severe gill pathology in some of the fish (Figure 5.20). One freshwater drum infected with *M. spinicirrus* was co-infected with metacercariae of *Clinostomum marginatum* (syn. *C. complanatum*) a trematode (parasites requiring alternate hosts to complete their life cycles) commonly known as yellow grub (Figure 5.21). *C. marginatum* has been reported globally and shows little if any fish host specificity. Metacercariae from seven other trematodes were found in fish from Lake Traverse. These specimens were of the larval genus *Neascus* and

included *Postodiplostomum minimum* in walleye and *P. minimum centrarchi* in bluegill sunfish (Figure 5.22). Both specimens of *P. minimum* were observed in the liver of affected fish. Infections with three *Neascus sp.* were observed in rock bass. The first was observed grossly as melanized cysts near the base of the caudal fin in a condition commonly known as "black spot disease". The second was found in clear hyaline-like cysts associated with hemorrhaging in lateral muscle tissue (Figure 5.3). The third *Neascus sp.* was found in hyaline-like cysts in the liver. Lastly, trematode metacercariae were found encysted in lateral musculature of black crappie (Figure 5.24) and fathead minnow (Figure 5.25). The hyaline cysts of these *Neascus spp.* were grossly evident as a result of hemorrhaging in the surrounding tissues.

Cestodes from four taxonomic orders with representatives of one family within each order were found in fish from Lake Traverse. We identified five cestodes to species but also found metacestodes or plerocercoids of four additional cestodes that could not be identified closer than genus (Table 5.4). From the order Caryophyllidea (Caryophyllaeidae), *Hunterella nodulosa* was found in white sucker (Figure 5.26) and *Khawia iowensis* was found in common carp (Figure 5.27). 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 identified one tapeworm from the order Pseudophyllidae (Bothriocephalida). *Bothriocephalus cuspidatus* was found in one pumpkinseed sunfish and in all walleye examined (Figure 5.28). Additionally, metacestodes of *Bothriocephalus sp.*, presumably *B. cuspidatus*, were recovered from the intestine of a bluegill sunfish (Figure 5.29). Members of genus *Bothriocephalus* have a characteristic elongate scolex with two opposing bothria (dorsal and ventral sucking grooves) and lack a neck. *B. cuspidatus* is widely distributed in North America and has been reported from several species of fish most notably in walleye and sauger. We identified two tapeworm genera from order Proteocephalidea (Proteocephalidae). This group of cestodes has a scolex with four distinct simple suckers and sometimes a fifth or apical sucker at the anterior tip. *Corallobrothrium fimbriatum*, a common tapeworm of ictalurids, was found in channel catfish and yellow bullhead (Figure 5.30). Species of *Corallobothrium* have a well developed metascolex, a collar with many sucking grooves, which helps distinguish them from other genera in the order. *C. fimbriatum* has a comparatively large number of proglottids that are wider than long. The second representative of family Proteocephalidae and most common cestodes in fish at Lake Traverse were members of genus *Proteocephalus*. Mature specimens of *Proteocephalus pinguis* were found only in the intestines of northern pike often in great numbers (Figure 5.31). Specimen of *P. pinguis* had a characteristic spatulate scolex with five suckers (one apical) tapering into a neck lacking segmentation. Metacestodes of *Proteocephalus sp.* (Figure 5.32) were observed in five other species including bluegill and pumpkinseed sunfishes, rock bass, white sucker, and yellow perch. The final two cestodes found in fishes from Lake Traverse were metacestodes in the order Cyclophyllidea of the family Gryprohynchidae though previously placed in family Dilepididae. The first specimens were observed individually in oval cysts in the liver of one rock bass. The small hyaline-like cysts were grossly similar to cysts of digenean trematodes. Attempts to excise metacestodes from two cysts resulted in severely damaged specimens not suitable for preservation. A third larval cyst was preserved in AFA from which a stained whole-mount was made. Morphological features observed in wet mount (Hoffman 1999) and measurement of rostellar hooks (Scholz et al. 2004) from stained whole-mount preparation placed the specimen in the genus *Paradilepis*. We were not able to identify this metacestode to species. The second gryprohynchid metacestode was presumptively identified as *Valipora sp.*

based on similarity to a description by Hoffman (1999). The single *Valipora* specimen was found in the gall bladder of a pumpkinseed sunfish (Figure 5.34). The small (L < 1.0 mm) metacestode had an armed rostrum with a single row of hooks and four sucks resembling those of *Proteocephalus*. Photomicrographs of the parasite were taken from a wet mount preparation however no stained whole-mount was made because the small specimen was lost at some point during transfer to a collection vial.

Six species from class Nematoda with representatives of two orders were found in fish at Lake Traverse (Table 5.4). We observed two species of larval nematodes from the order Ascaridida (Anisakidae). *Contracaecum sp.* was the most common nematode observed in the survey and was found in nine species of fish including black crappie, bluegill, channel catfish, freshwater drum, northern pike, rock bass, walleye, white bass, and yellow bullhead. Larval forms of *Contracaecum sp.* were observed in large conspicuous cysts mostly in mesenteries and often in large numbers (Figure 5.8). Larvae of *Raphidascaris sp.* were found in small cysts (D < 1.0 mm) in mesenteries of black bullhead, on the intestine of one fathead minnow, and in the liver of walleye (Figure 5.35). *Contracaecum* and *Raphidascaris* have wide geographic distributions with several records from the U. S. and Canada. We identified nematodes from three families of the order Spirurida. *Camallanus oxycephalus* (Camallanidae) was found in the intestines of black crappie, rock bass, walleye, white bass, and yellow perch. *C. oxycephalus* had a distinctive buccal capsule with two lateral chitinous valves with longitudinal thickenings and most specimens were a deep red color upon removal from host intestines (Figure 5.36). Two species of *Spinitectus* (Cystidicolidae) were found. *S. carolina* was recovered from the intestines and pyloric caeca of bluegill sunfish (Figure 5.37) and *S. gracilis* was found in the intestine of channel catfish (Figure 5.38). An anterior cuticle with a series of transverse rings and associated spines was the most distinguishing morphological characteristic of *Spinitectus.* Lastly, larval forms of *Rhabdochona sp.* (Rhabdochonidae) were found in the intestines and or pyloric caeca of channel catfish, freshwater drum, rock bass, white bass, and yellow perch (Figure 5.39).

One acanthocephalan (Echinorhynchida: Pomphorhynchidae), commonly known as thorny-headed worms, was found in fish from Lake Traverse (Table 5.4). *Pomphorhynchus bulbocolli* was found imbedded in the intestines of six species of fish including black bullhead, black crappie, common carp, channel catfish, white sucker, and yellow perch (Figure 5.40). *P. bulbocolli* were easily recognized because of their long neck which supports a long cylindrical proboscis bearing several longitudinal rows of hooks. Numerous reports of this parasite indicate it is widely distribution across North America (Hoffman 1999). It has been observed in perch and white sucker from Lake Ashtabula, an impoundment of the Sheyenne River (Forstie and Holloway 1984).

Two parasitic leeches (Hirudinea: Pisciolidae) were found on fish from Lake Traverse (Table 5.4). *Myzobdella lugubris* (synonym *M. moorei*) was found anchored to the fins of four species of fish including black crappie, bluegill, pumpkinseed, and rock bass (Figure 5.41). *M. lugubris* was also found on the gills of one largemouth bass. *Piscicola punctata* was found attached to the skin just below the dorsal fin of one fathead minnow (Figure 5.42). Both leeches are widely distributed in North America and appear to lack any host specificity.

Two common parasitic copepods (Arthropoda: Crustacea: Copepoda) were observed on fish from Lake Traverse (Table 5.4). *Ergasilus cyprinaceus* (Ergasillidae) was found anchored to the gills of fathead minnow (Figure 5.43). *E. cyprinaceus* shows apparent host specificity for cyprinids and has been reported previously on white sucker from the Sheyenne River and fathead minnow from the James River in North Dakota (Sutherland and Holloway (1979). *Actheres pimelodi* (Lernaeopodidae) was found attached to the gill operculum of channel catfish (Figure 5.44). *A. pimelodi* is apparently host specific to species in the catfish family. *A. amploplitis*, a more frequently reported parasitic copepod is very similar to *A. pimelodi*. According to some accounts, *A. pimelodi* may be a smaller form of *A. amploplitis* (Hugghins 1972; Hoffman 1999). *A. amploplitis* is apparently less host specific and has been reported previously in black bullhead from North Dakota (Sutherland and Holloway 1979; Holloway and Hagstrom 1981) and South Dakota (Huggins 1972) and in lake whitefish *Coregonus clupeaformis* from Ontario (Dechtiar 1972).

Discussion

We observed a greater diversity of parasitofauna in fish from Lake Traverse compared to fish from Devils Lake. A similar number of fish were examined from both survey sites but, with the possible exception of Myxosporea, more parasites from each taxonomic class were found in fish at Lake Traverse. Also, parasites from several genera found at Lake Traverse were not found at Devils Lake. At Devils Lake, a total of fifteen different parasites were identified to the level of genus, and of those, five parasites were identified to species. These included three protozoa, four monogeneans, one trematode, four cestodes, two nematodes, and one leech. At Lake Traverse, forty-one different parasites were identified to the level of genus or larval genus, and of those, twenty parasites were identified to species. These findings included five protozoan, one microsporean, five myxosporeans, four monogeneans, eight trematodes, nine cestodes, six nematodes, one acanthocephalan, two leeches, and two parasitic crustaceans. In the only other known parasite survey of fish from Devils Lake, Reinisch (1981) found only eight different parasites. In the present survey, we found all parasites previously recorded by Reinisch with the possible exception of an unknown trematode and the acanthocephalan *Rhadinorhynchus sp.* It may be that *Rhadinorhynchus sp.* was not correctly identified as most records for this parasite are in marine fish from the Pacific coast. If acanthocephalan worms occur at Devils Lake their prevalence must be very low. We have yet to observe any species from this class of parasites even though hundreds of fish have been examined over the course of four different years.

We identified five parasites in fish from Devils Lake that were not observed in previous surveys conducted by this laboratory. These included the monogeneans *Onchocleidus chrysops* and *Dactylogyrus sp.*, metacercariae of the larval genus *Neascus*, immature forms of the nematode *Spiroxys sp.*, and the leech *Myzobdella lugubris*. All of these organisms are common parasites in North America and have been reported previously from fish in the Red River basin (Sutherland and Holloway 1979; Holloway and Hagstrom 1981; Forstie and Holloway 1984; Hoffman 1999). Larvae of *Spiroxys sp.* was only parasite from Devils Lake that was not found in fish from Lake Traverse although the nematode has been reported previously in black bullhead from the Sheyenne River (Sutherland and Holloway 1979). It is likely *Spiroxys sp.* occurs in fish at Lake Traverse but was missed during this survey.

To the best of our knowledge, the present parasite survey was the first of its kind to take place at Lake Traverse. Hugghins (1972) reported findings for thirteen parasites of fish from Big Stone Lake, South Dakota, which is located a short distance south of Lake Traverse. Big Stone Lake is headwaters for the Minnesota River which is tributary to the Mississippi River. At least eight of the parasites of fish from Big Stone Lake were also found in fish from Lake Traverse during this survey. Possibly other parasites observed by Hugghins (1972) at Big Stone Lake also occur at Lake Traverse but were missed during this initial investigation.

One noteworthy finding at Lake Traverse was the collection of two gryprohynchid metacestodes. *Paradilepis sp.* was found individually in oval cysts in the liver of a rock bass and *Valipora sp.* (presumptive) was found in the gall bladder of a pumpkinseed sunfish. To the best of our knowledge, there have been no previous records of larval gryprohynchid cestodes in fish from Lake Traverse or other bodies of water in the Red River basin. Metacestodes of gryprohynchid (formerly Dilepididae) cestodes are reported as parasites of fish in freshwater and brackish water environments while the adult tapeworms are found in piscivorous birds (Hoffman 1999; Scholz 2004). According to Scholz (2001) there is little information regarding the occurrence of metacestodes of this group of tapeworms in the U. S. The earliest report in America is a description by Chandler (1935) of *Glossocercus cyprinodontis* and *Cysticercoides menidiae* from fish collected in Galveston Bay, Texas. Scholz (2001) re-examined the holotype of *C. menidiae* and found it to be conspecific with *Ascodilepis transfuga*, a tapeworm of spoonbill that was previously only known in its adult form. Hoffman (1999) reported only three species of gryprohynchid cestodes from freshwater fish in North America. Later, Scholtz et al. (2002) reported on two gryprohynchid metacestodes, *Cyclustera ibisae* and *Glossocercus caribaensis*, from the mesenteries and liver of mummichog *Fundulus heteroclitus* and striped killifish *Fundulus majalis* from an estuary in South Carolina. Helminth surveys of fish in Mexico resulted in the finding of 13 species of gryprohynchids with most being new records (Scholz and Salgado-Maldonado 2001). More recently, Scholz and Harris (2006) reported the first occurrence of *Cyclustera ralli* (Cestoda: Cyclophyllidae) in the livers and mesenteries of mummichog from Virginia. We found four records of "dilepis" parasites from Canada. Cone and Anderson (1977) reported a *Dilepis sp.* in pumpkinseed from Ontario. Three records were from British Columbia including *Dilepis unilateralis* in largemouth bass (Molnár et al. 1974), *Dilepis sp.* in the liver of sockeye salmon (Bailey and Margolis 1987), and *Paradilepis simoni* (Ching 1982).

The histology survey at Devils Lake resulted in the detections of several parasites that were not observed with the traditional systematic parasite search methods reported in this chapter. These were composed primarily of microscopic organisms such as protozoa and myxosporean. Most of the larger metazoan and macroscopic parasites were found with both parasite search methods although it was difficult to identify organisms observed with histology to taxonomic levels closer than class. Surveys for microscopic parasites such as the microsporidia and myxosporidians can be difficult with traditional search methods unless the parasite is first detected in larger more obvious structures such as cysts or xenomas. We observed just one microsporidian parasite during the survey which was found within an off-colored and slightly enlarged oocyte in the ovary of a fathead minnow from Lake Traverse. Spores of the parasite could only be observed after the suspect oocyte was squashed between a glass slide and coverslip and examined at 400 – 1000X magnification with light microscopy. Five myxosporidan

parasites were also found in fish at Lake Traverse in part because they were contained in conspicuous cysts. No myxosporean cysts were found in fish from Devils Lake using traditional parasite search techniques. Several myxosporidians were observed during examination of stained tissue sections of fish from Devils Lake particularly from fathead minnow (Chapter 4). It is highly possible myxosporidian and microsporidian parasites occur in fish through out the study area but go largely undetected because these organisms can be difficult to find.

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			
Fish host	Grossly	Fresh	Frozen	Total	
Black crappie	60	10	15	85	3
Fathead minnow	60	15	15	90	7
Northern pike	24	3	7	34	2
Walleye	60	6	14	80	2
White bass	60	10	20	90	5
White sucker	8	1	0	9	0
Yellow perch	17	2	0	19	4

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, (l) liver, (m) musculature, (mt) mesenteries, (pc) pyloric caeca, (s) skin.

Parasite		
Class	Genus - species	Host and anatomical location
Protozoa	*Apiosoma sp.*	BLC(g), FHM(g)
	Epistylis sp.	WAE(g)
	Trichodina sp.	BLC(s), FHM(g), NOP(s), WAE(s), WHB(g,s), YEP(g,s)
Monogenea	*Dactylogyrus sp.*	FHM(g)
	Gyrodactylus hoffmani	FHM(f)
	Gyrodactylus sp.	YEP(s)
	Onchocleidus chrysops	WHB(g)
Trematoda	*Neascus sp.* (metacercariae)	FHM(m)
Cestoidea	*Bothriocephalus cuspidatus*	WAE(i), WHB(i,pc)
	Bothriocephalus sp. (metacestodes)	FHM(i), WAE(i,pc), YEP(i)
	Proteocephalus pinguis	NOP(i)
	Proteocephalus sp. (metacestodes)	FHM(i)
Nematoda	*Contracaecum sp.* (larvae)	BLC(mt), WAE(mt), WHB(mt)
	Spiroxys sp. (larvae)	WHB(mt,l)
Hirudinea	*Myzobdella lugubris*	YEP(f)

Table 5.3.— 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			
Fish host	Grossly	Fresh	Frozen	Total	
Black crappie	60	3	4	67	5
Bluegill sunfish	60	6	4	70	7
Bullheads	60			60	1
Black		0	5	5	4
Yellow		2	0	2	3
Channel catfish	9	1	1	11	6
Common carp	60	3	1	64	3
Emerald shiner	60	6	0	66	2
Fathead minnow	0	5	16	21	12
Freshwater drum	60	5	5	70	6
Largemouth bass	1	1	0	2	1
Northern pike	5	2	1	8	3
Orangespotted sunfish	1	0	0	1	0
Pumpkinseed	18	4	1	23	5
Shorthead redhorse	3	0	0	3	0
Rock bass	11	5	6	22	8
Walleye	13	5	5	23	6
White bass	60	4	1	65	5
White sucker	9	2	0	11	2
Yellow perch	60	5	15	80	6

Table 5.4.— 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: (f) fin, (g) gills, (gb) gall bladder, (i) intestine, (k) kidney, (l) liver, (m) musculature, (mt) mesenteries, (o) ovary, (op) operculum, (pc) pyloric caeca, (s) skin.

Parasite taxonomy		
Class	Genus - species	Host and anatomic location
Protozoa	*Apiosoma sp.*	YEP(g)
	Epistylis sp.	FHM(f,g,s)
	Ichthyobodo necator	EMS(g), YEP(g,s)
	Hexamita sp.	EMS(i)
	Trichodina spp.	BLB(g), BLC(g), CAP(s), FHM(f,g,s), FRD(s), PSS(g,s), RKB(g), WAE(g), WHB(g), YEP(s)
Microsporea	*Ovipleistophora ovariae*	FHM(o)
Myxosporea	*Henneguya sp.*	FRD(k)
	Myxobolus sp.	FHM(g), FHM(sk), RKB(mt)
	Unicauda sp.	FHM(m)
Monogenea	*Dactylogyrus sp.*	FHM(g)
	Gyrodactylus hoffmani	FHM(f)
	Ligictaluridus sp.	BLB(g)
	Microcotyle spinicirrus	FRD(g)
Trematoda	*Clinostomum marginatum*	FRD(g)
	Neascus sp. (metacercariae)	BLC(m), FHM(m), RKB(m), RKB(l)
	Neascus of Postodiplostomum minimum	WAE(l)
	Neascus of *Postodiplostomum minimum centrarchi*	BLG(l)
Cestoidea	*Bothriocephalus cuspidatus*	PSS(i), WAE(i)
	Bothriocephalus sp. (metacestodes)	BLG(i)
	Corallobothrium fimbriatum	CCF(i), YEB(i)
	Hunterella nodulosa	WHS(i)
	Khawia iowensis	CAP(i)
	Paradilepis sp.	RKB(l)
	Proteocephalus pinguis	NOP(i,pc)
	Proteocephalus sp. (metacestodes)	BLG(i), NOP(i), PSS(i), RKB(i), WHB(i), YEP(i)
	Valipora sp. (presumptive)	PSS(gb)

Table 5.4.— continued. Anatomical abbreviations: (f) fin, (g) gills, (gb) gall bladder, (i) intestine, (k) kidney, (l) liver, (m) musculature, (mt) mesenteries, (o) ovary, (op) operculum, (pc) pyloric caeca, (s) skin.

Parasite taxonomy		
Class	Genus - species	Host and anatomic location
Nematoda	*Camallanus oxycephalus*	BLC(i), RKB(i), WAE(i), WHB(i), YEB(i)
	Contracaecum sp. (larvae)	BLC(mt), BLG(mt), CCF(mt), FRD(mt), NOP(mt), RKB(mt), WAE(mt), WHB(mt), YEB(mt)
	Raphidascaris sp. (larvae)	BLB(mt), FHM(mt), WAE(l)
	Rhabdochona sp. (larvae)	CCF(i), FRD(i), RKB(pc), WHB(i), YEP(i)
	Spinitectus carolini	BLG(i, pc)
	Spinitectus gracilis	CCF(i)
Eoacanthocephala	*Pomphorhynchus bulbocolli*	BLB(i), BLG(i), CAP(i), CCF(i), WHS(i), YEP(i)
Hirudinea	*Myzobdella lugubris*	BLC(f), BLG(f), LMB(g), PSS(f), RKB(f)
	Piscicola punctata	FHM(s)
Crustacea	*Actheres pimelodi*	CCF(op)
	Ergasilus cyprinaceus	FHM(g)

Figures

Ken Peters, USFWS, captured images of parasites in fresh mounts and Dr. Becky Lasee and Kristen Dziubinski, USFWS, are credited with photomicrographs of stained whole-mount preparations.

Figure 5.1.— Photomicrographs of wet mount preparations showing ciliated protozoan parasites observed on gill lamellae or in skin scrapings of fish from Devils Lake. Specimens shown are *Trichodina sp.* (left), *Apiosoma sp.* (center), and *Epistylis sp* (right).

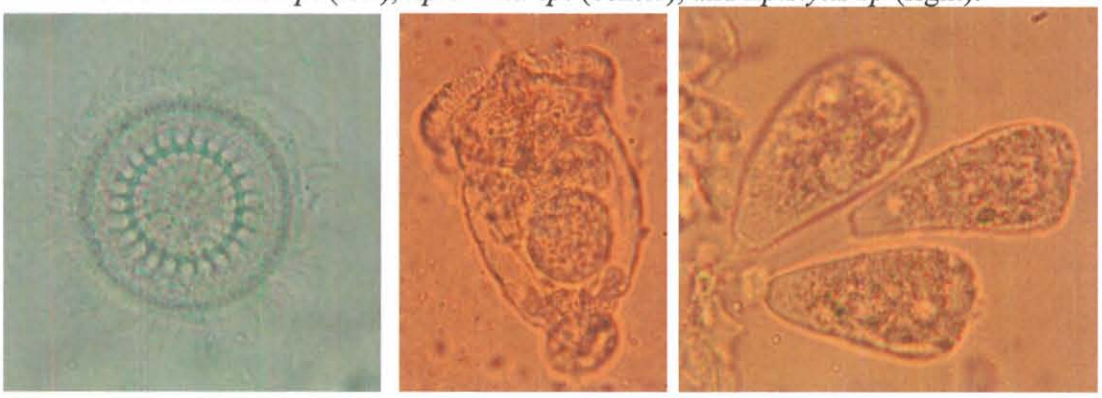


Figure 5.2.— Photomicrographs of *Dactylogyrus sp.* found on the gills of fathead minnow from Devils Lake (acetocarmine).

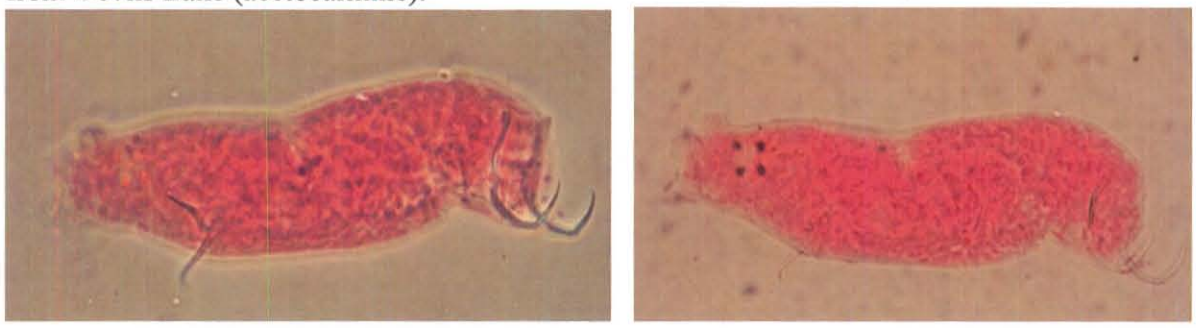


Figure 5.3.— Photomicrographs of wet mount preparation with *Gyrodactylus hoffmani* anchored near dorsal fin ray of fathead minnow from Devils Lake.

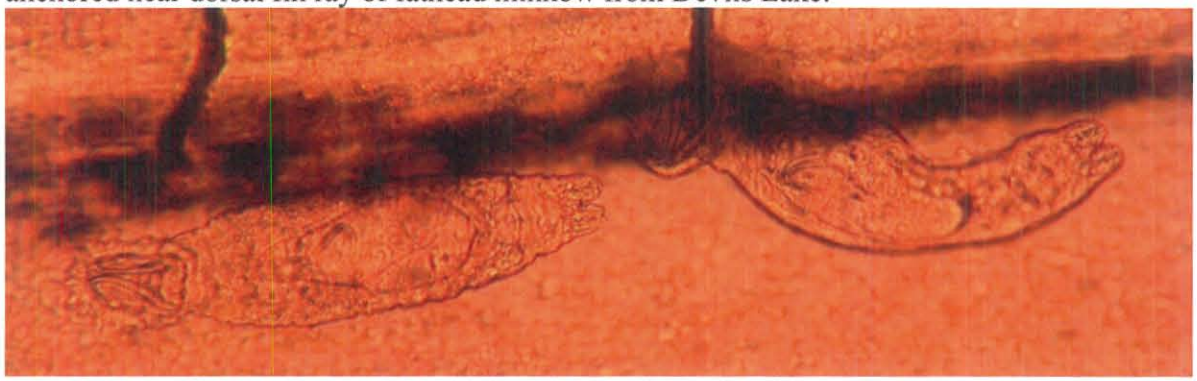


Figure 5.4.— Photomicrographs of wet mount preparation (left) and acetocarmine-stained specimen of the monogenean *Onchocleidus chrysops* from gill filament of white bass from Devils Lake.

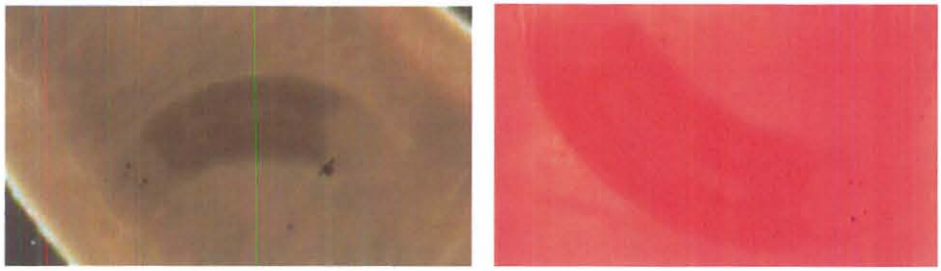


Figure 5.5.— Photomicrographs of unidentified trematode metacercariae (presumptive) observed in lateral muscle of fathead minnow from Devils Lake.

Figure 5.6.— Photomicrographs of *Bothriocephalus cuspidatus* with scolex (left), proglottids (center), and gravid posterior proglottid (right; acetocarmine). Specimen found in the intestine of walleye from Devils Lake.

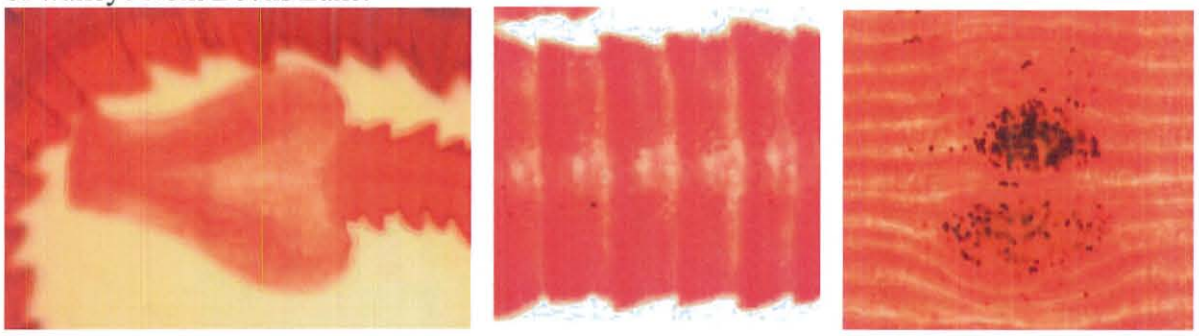


Figure 5.7.— Photomicrographs of *Proteocephalus pinguis* from the intestine of northern pike at Devils Lake. Acetocarmine-stained scolex with four characteristic suckers (center) and proglottids (right; acetocarmine).

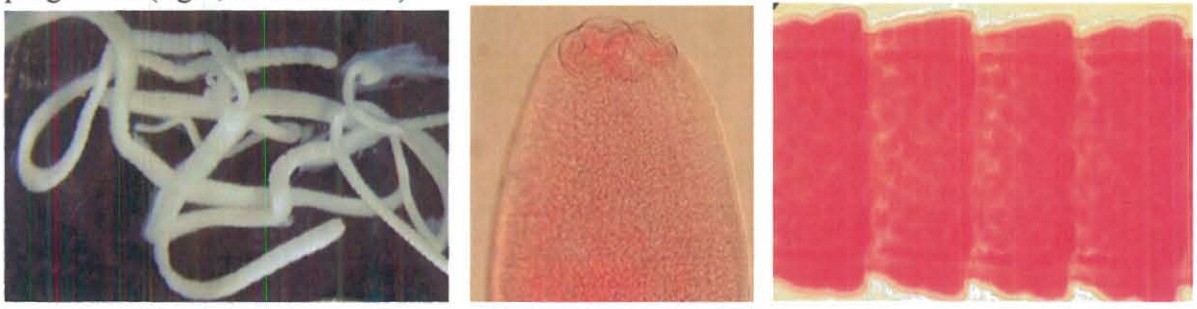


Figure 5.8.— Photomicrograph of larval *Contracaecum sp.* encysted in mesenteries (left) and cleared specimen mounted in glycerin-jelly and viewed with phase contrast (right).

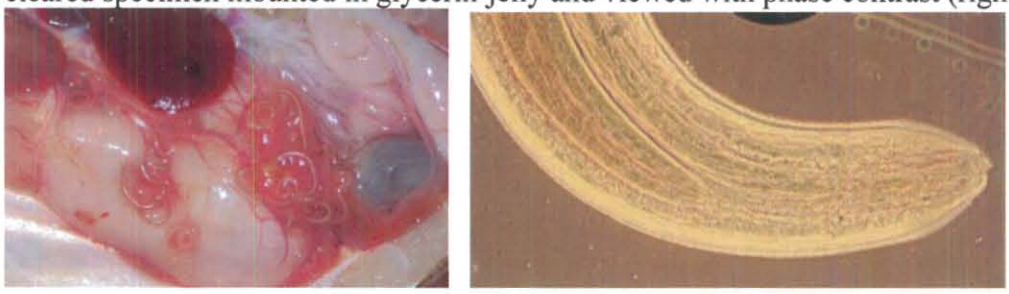


Figure 5.9.— Photomicrographs of encysted *Spiroxys sp.* in wet mount preparation (left) and cleared specimen mounted in glycerin-jelly and viewed with phase contrast (right).

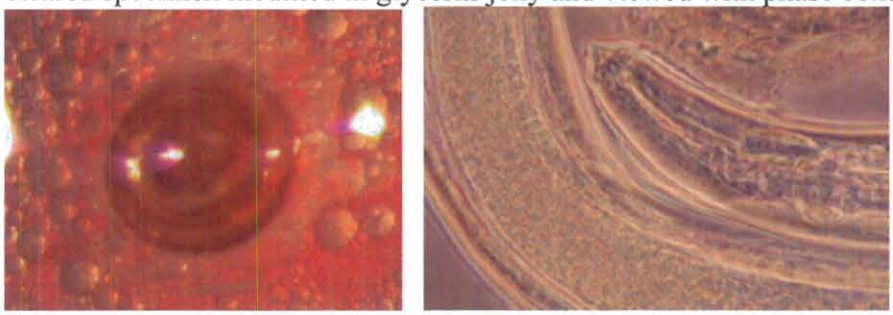


Figure 5.10.— Photomicrographs of the leech *Myzobdella lugubris* attached to anal fin of yellow perch (left) and anterior of stained specimen (right; acetocarmine).

Figure 5.11.— Photomicrographs of *Ovipleistophora ovariae* spores from an infected oocyte of fathead minnow from Lake Traverse (methylene blue).

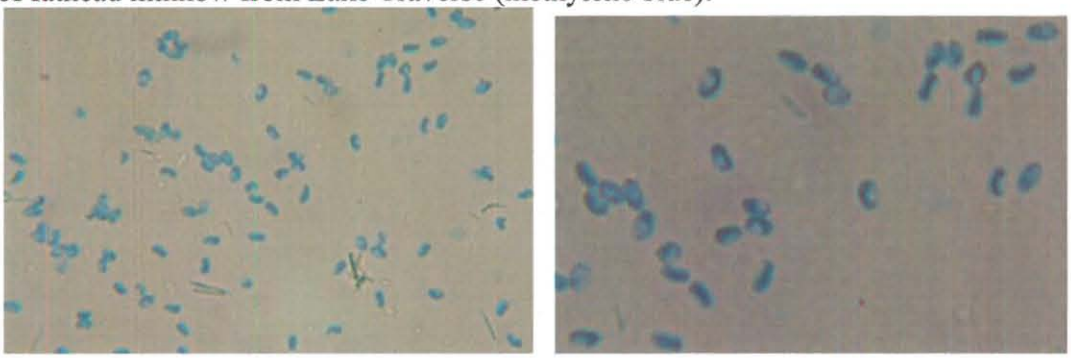


Figure 5.12.— Photomicrographs of *Henneguya sp.* (presumptive) cysts in the kidney of freshwater drum (left) and spore in wet mount preparation (right).

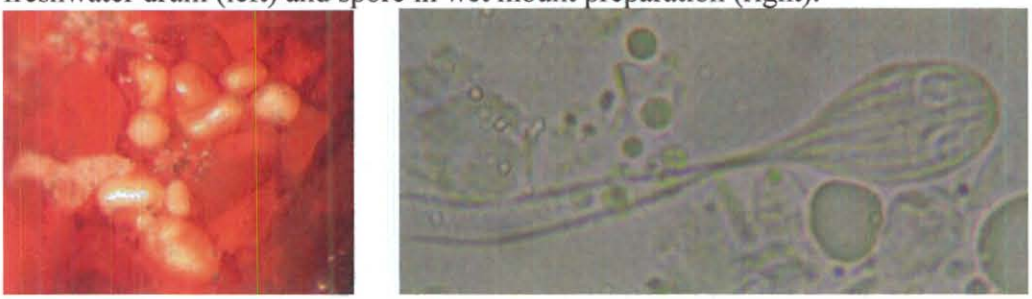


Figure 5.13.— Photomicrographs of *Myxobolus sp.* cysts in gill lamellae of fathead minnow. Grossly visible cyst in gill (left), wet mount revealing several smaller cysts (center), and stained tissue section of cysts (right, H&E).

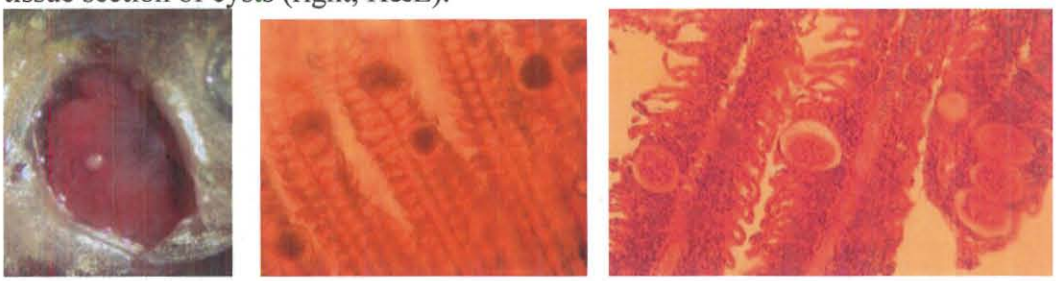


Figure 5.14.— Photomicrographs of *Myxobolus sp.* from gills of fathead minnow collected at Lake Traverse. Note uptake of methylene blue by polar capsules that have extruded polar filaments.

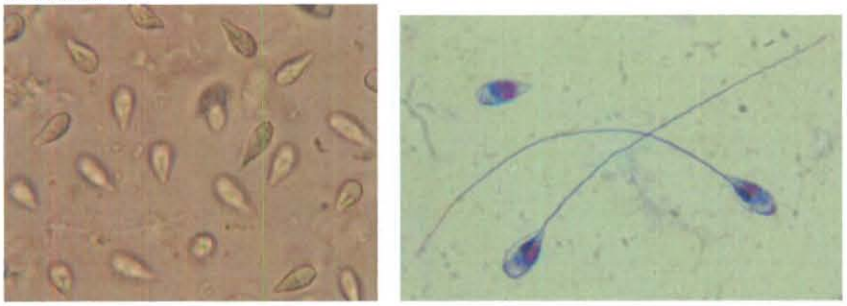


Figure 5.15.— Photomicrographs of stained tissue sections of fathead minnow gills infected with *Myxobolus sp.* Presporous trophozoites in various stages of development and a few mature spores are evident within intralamellar cysts (H&E and Giemsa).

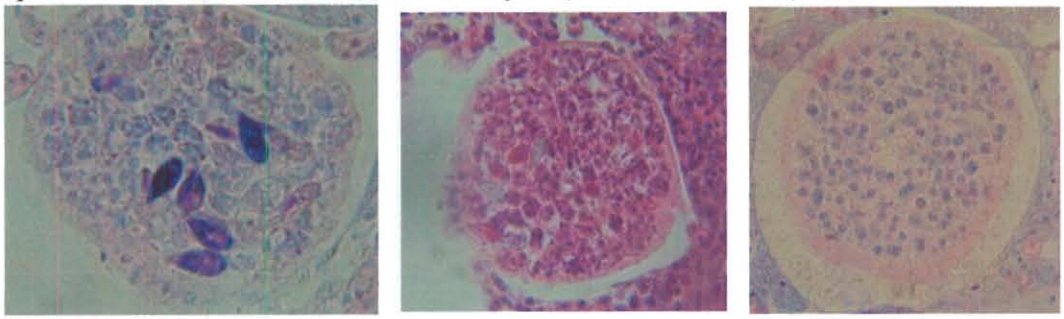


Figure 5.16.— Photomicrographs of *Myxobolus sp.* cyst on pectoral fin of fathead minnow (left) and wet mount of spores observed with bright field (center) and phase contrast (right).

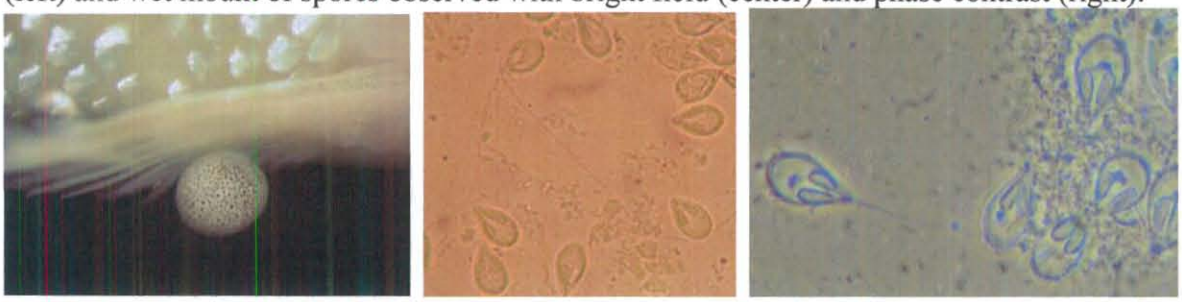


Figure 5.17.— Photomicrographs of spores of *Unicauda sp.* in wet mount from cyst found in connective tissue of fathead minnow from Lake Traverse.

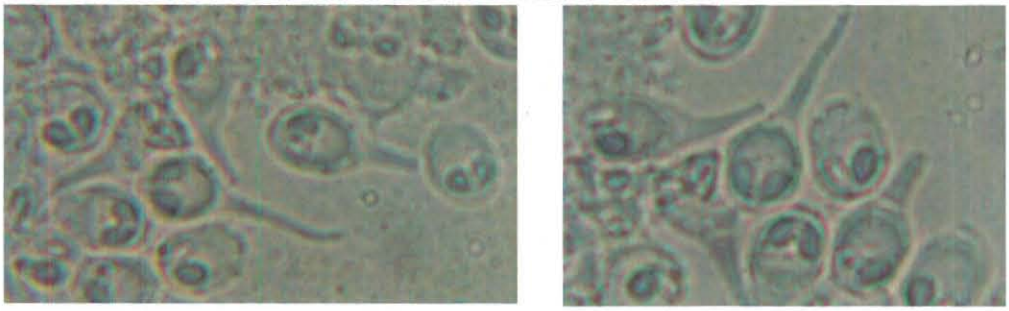


Figure 5.18.— Photomicrographs of *Myxobolus sp.* found in rock bass from Lake Traverse. Spores are shown with extruded polar filaments in valvular view (left and center) and sutural view (right).

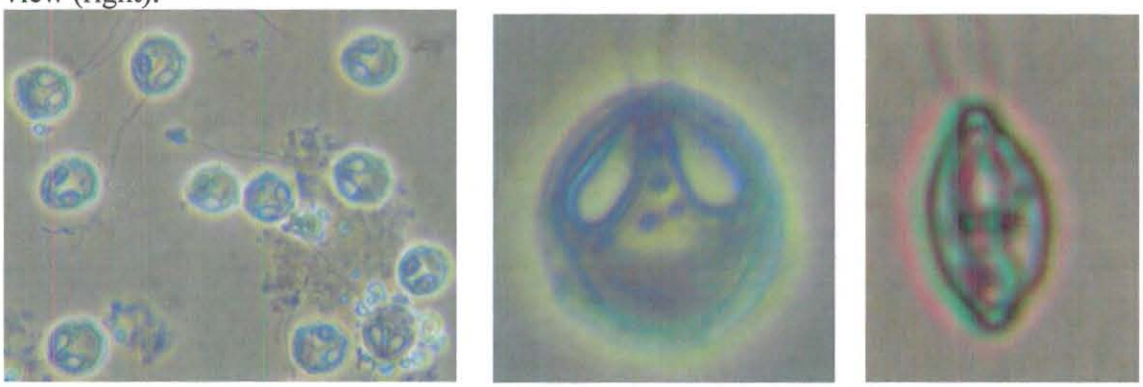


Figure 5.19.— Photomicrographs of *Dactylogyrus sp.* (wet mount, left), *Gyrodactylus hoffmani* (acetocarmine, center), and *Ligictaluridus sp.* (wet mount, right) found on fish from Lake Traverse.

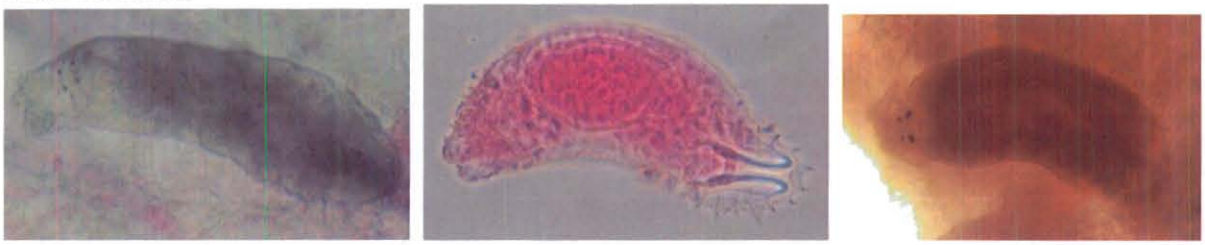


Figure 5.20.— Photomicrographs of *Microcotyle spinicirrus* found on gill lamellae of freshwater drum from Lake Traverse (acetocarmine).

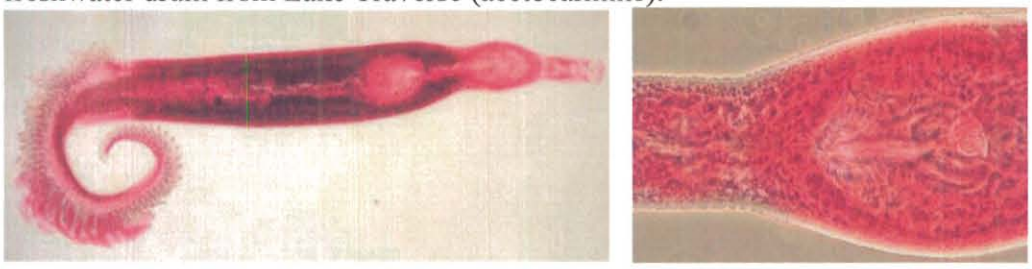


Figure 5.21.— Photomicrographs of the yellow grub *Clinostomum marginatum* on the gills of freshwater drum. Note extensive pathology of gill lamellae caused by *M. spinicirrus* (see Figure 5.20) on either side of *C. marginatum* in photo to the left.

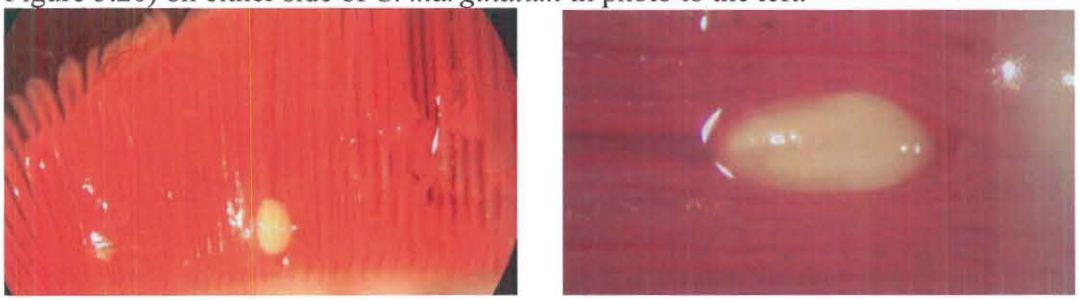


Figure 5.22.— Photomicrographs of specimens from the larval genus *Neascus* including *Postodiplostomum minimum* from walleye (left) and *P. minimum centrarchi* from bluegill sunfish (right).

Figure 5.23.— Photomicrographs of *Neascus spp.* in caudal fin ("black spot", left) and lateral muscle (center) from rock bass. Metacercariae of *Neascus sp.* excised from muscle cyst (right).

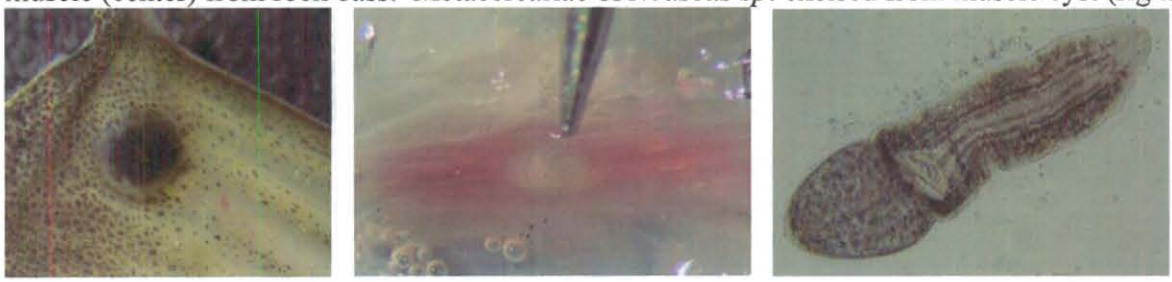


Figure 5.24.— Photomicrographs of *Neascus sp.* (presumptive) in lateral muscle of black crappie (left), cyst removed from muscle (center), and metacercariae removed from cyst (right).

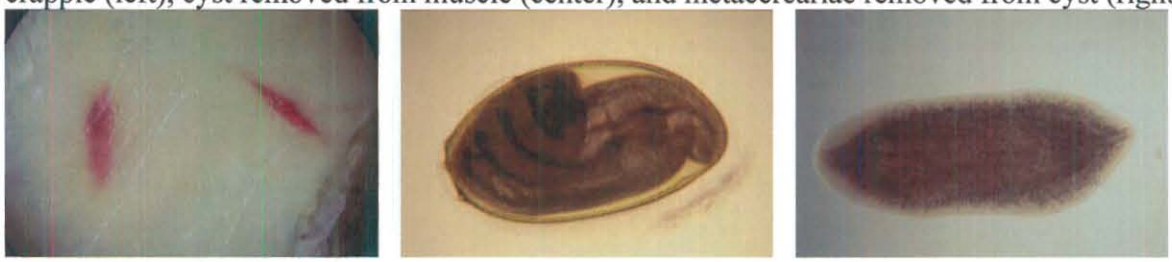


Figure 5.25.— Photomicrographs of *Neascus sp.* (presumptive) from muscle of fathead minnow. Hyaline cyst (left) recovered from muscle tissue and metacercariae removed from cysts (center = bright field; right = phase contrast).

Figure 5.26.— Photomicrographs of *Hunterella nodulosa* scolex (left) and mid-section (right) from intestine of white sucker collected at Lake Traverse (acetocarmine).

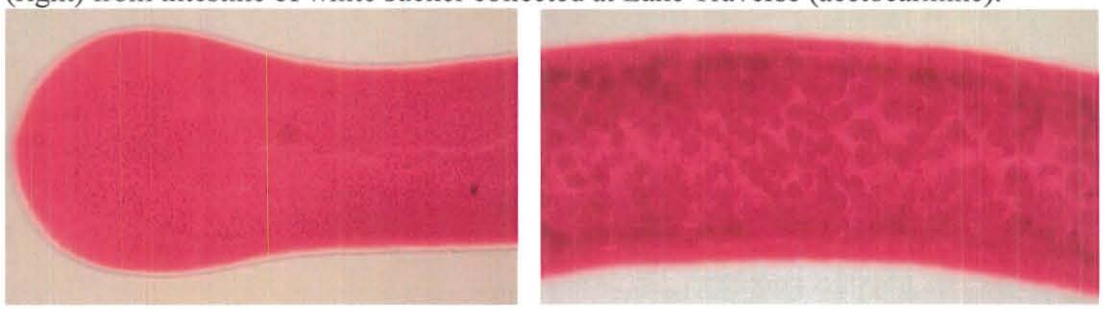


Figure 5.27.— Photomicrographs of *Khawia iowensis* from the intestine of common carp collected at Lake Traverse. Folded worm with anterior end on top and posterior end below (left), scolex with suctorial grooves (center), and posterior with portion of ovary and vitellaria (rt).

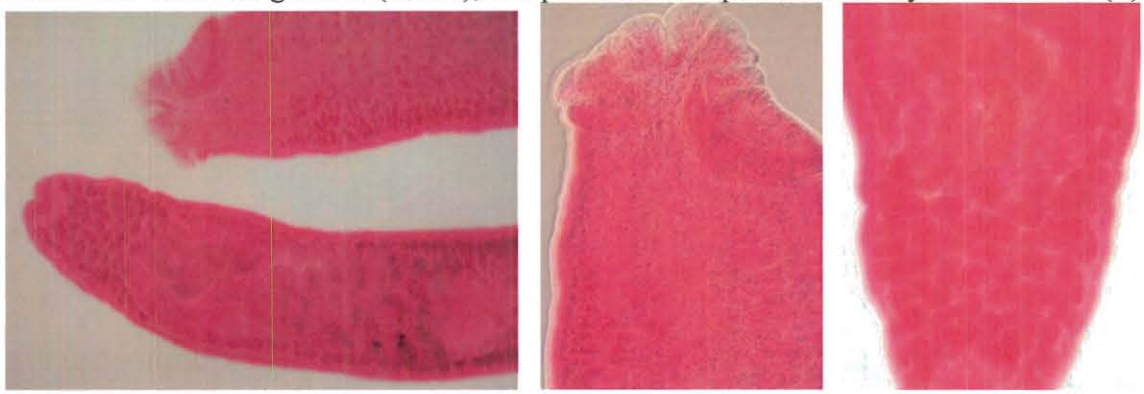


Figure 5.28.— Photomicrographs of *Bothriocephalus cuspidatus* scolex (left) and proglottids (center and right; acetocarmine).

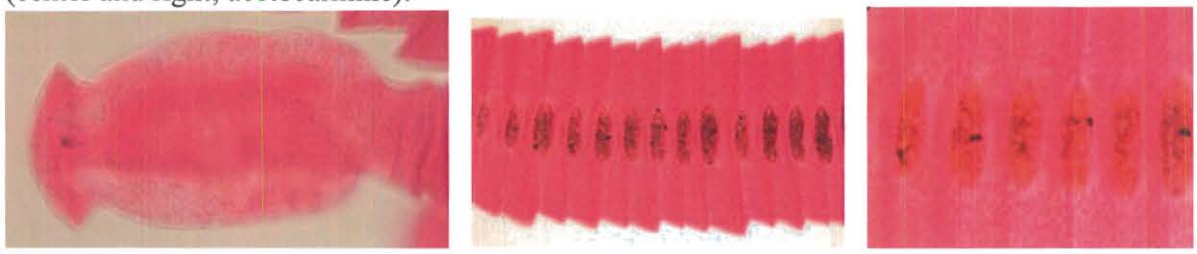


Figure 5.29.— Photomicrographs of wet mount with *Bothriocephalus cuspidatus* metacestode from the intestine of bluegill sunfish collected at Lake Traverse.

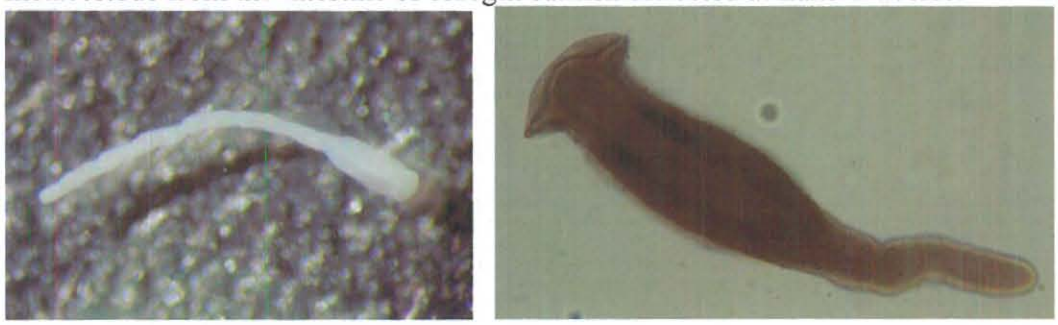


Figure 5.30.— Photomicrographs of *Corallobrothrium fimbriatum* from intestine of channel catfish and yellow bullhead collected at Lake Traverse. Fresh mount (left), acetocarmine-stained scolex (center), and mature proglottids (right).

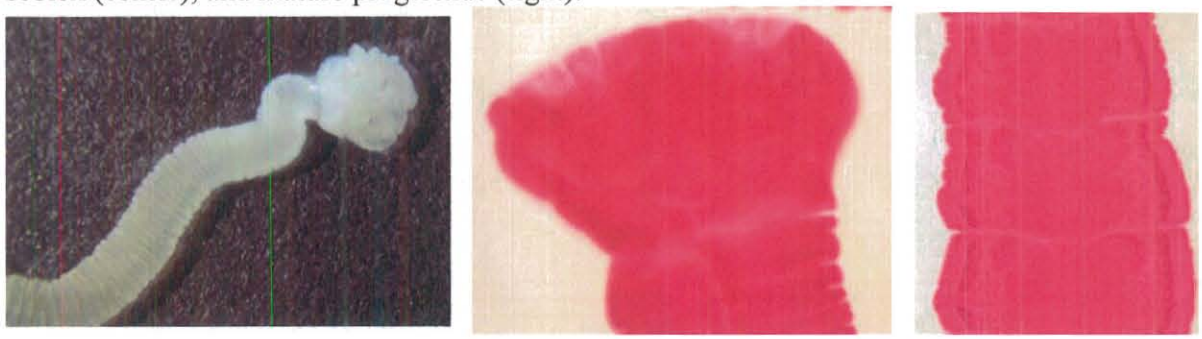


Figure 5.31.— Photomicrographs of *Proteocephalus pinguis* fresh mount (left), scolex with five suckers (right), and acetocarmine-stained view of scolex (center).

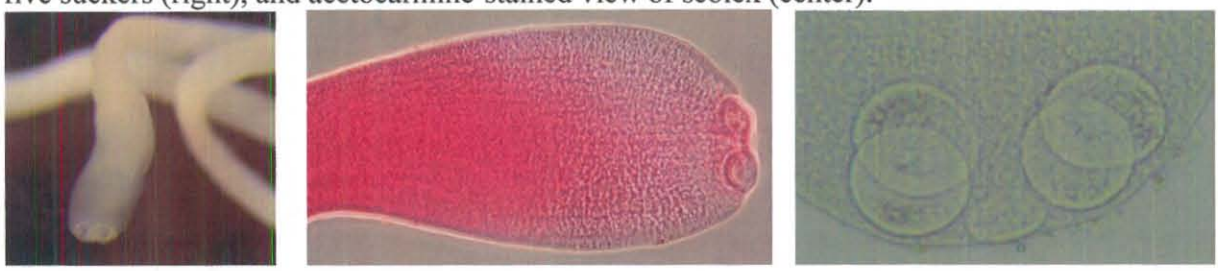


Figure 5.32.— Photomicrographs of *Proteocephalus sp.* metacestodes in fresh mounts.

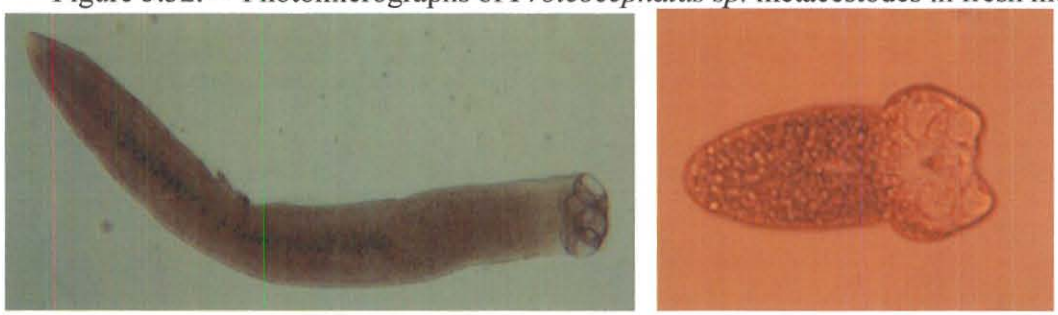


Figure 5.33.— Photomicrographs of gryporhynchid metacestode *Paradilepis sp.*, found encysted in the liver of a rock bass from Lake Traverse (top, fresh mount; lower, acetocarmine).

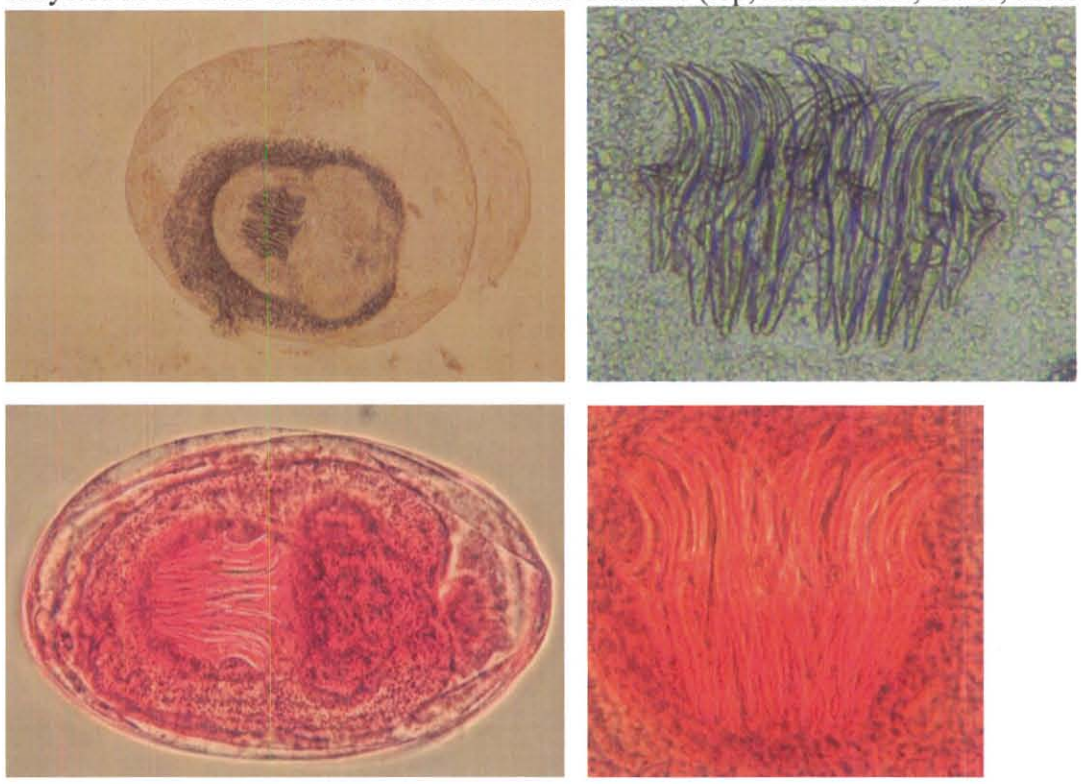


Figure 5.34.— Photomicrographs of gryporhynchid metacestodes, presumptively identified as *Valipora sp.*, in wet mount preparation of gall bladder of pumpkinseed sunfish from Lake Traverse.

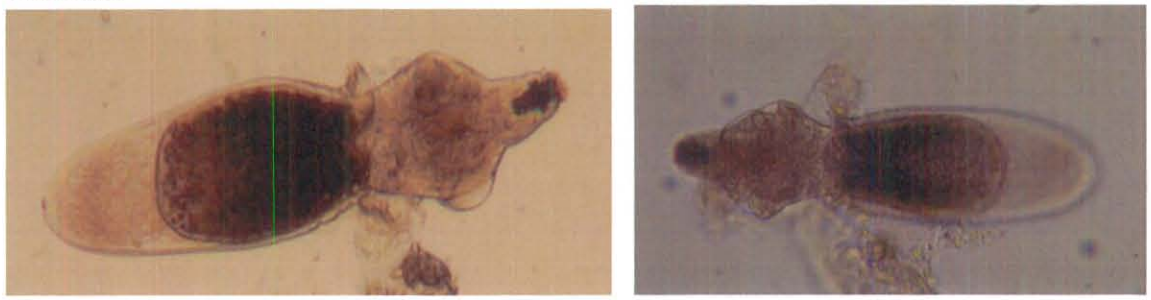


Figure 5.35.— Photomicrographs of *Raphidascaris sp.* larvae found in black bullhead, fathead minnow, and walleye from Lake Traverse.

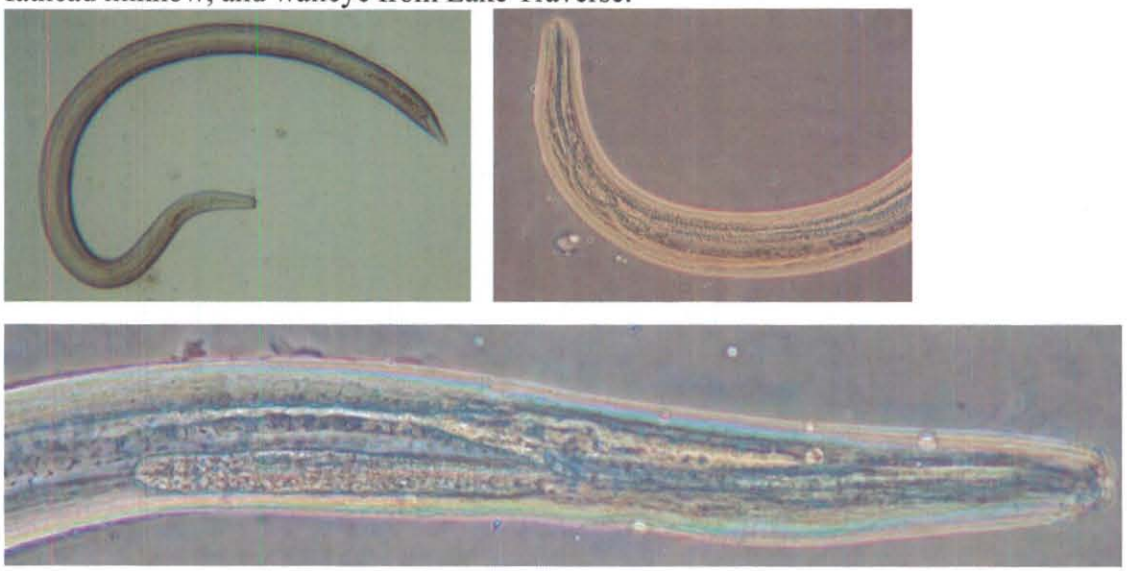


Figure 5.36.— Photomicrographs of *Camallanus oxycephalus* in fresh mount (left), anterior end showing chitinous valves (center), and viviparous female with larvae (right).

Figure 5.37.— Photomicrographs of *Spinitectus carolina* found in the pyloric caeca and intestines of bluegill from Lake Traverse.

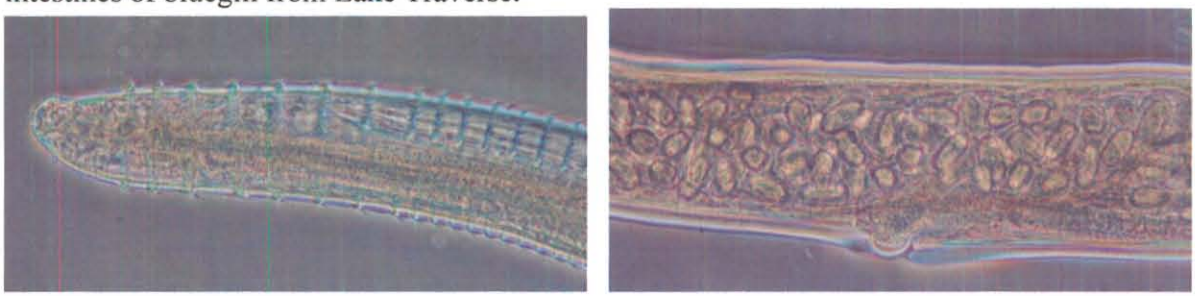


Figure 5.38.— Photomicrographs of *Spinitectus gracilis* found in the intestines of channel catfish from Lake Traverse.

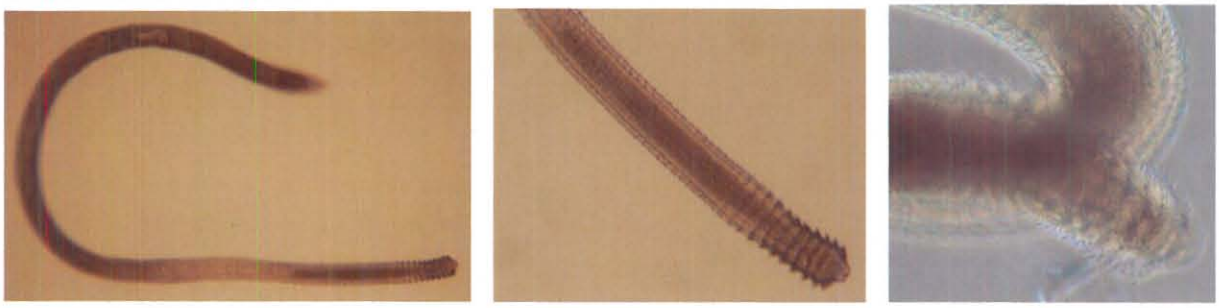


Figure 5.39.— Photomicrographs of *Rhabdochona sp.* in fresh mount (top-left), posterior end of male showing anal papillae and spicules (top-center), posterior end of female showing eggs in uterus (top-right), anterior end of female (lower-left), and egg released from vulva (lower-right).

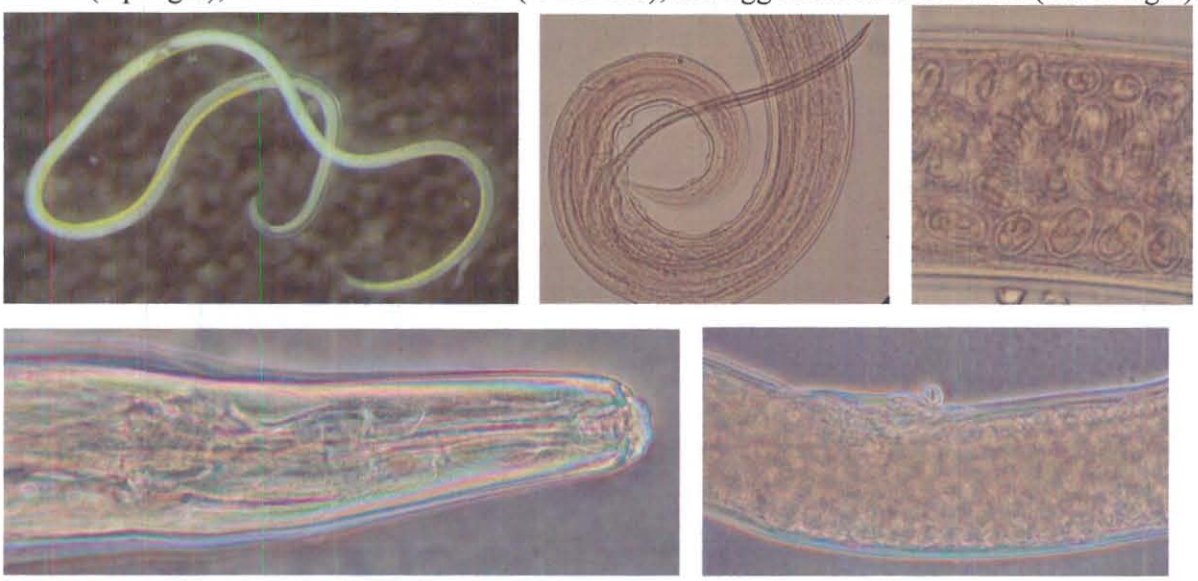


Figure 5.40.— Photomicrographs of *Pomphorhynchus bulbocolli* embedded in the intestinal wall of white sucker (left) and long neck supporting cylindrical proboscis (right). proboscis bearing several longitudinal rows of hooks (lower left), and posterior end of male showing cement gland (lower right).

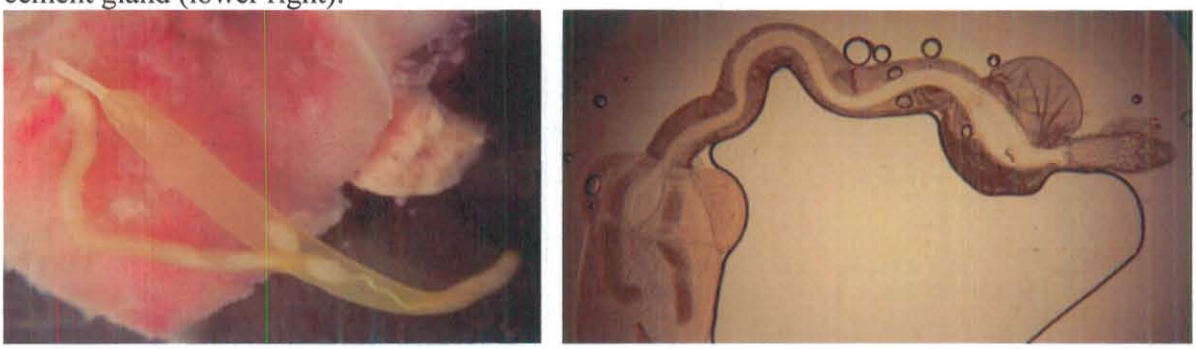


Figure 5.40.— continued. Photomicrographs of *P. bulbocolli* proboscis bearing several longitudinal rows of hooks (left) and posterior end of male showing cement gland (right).

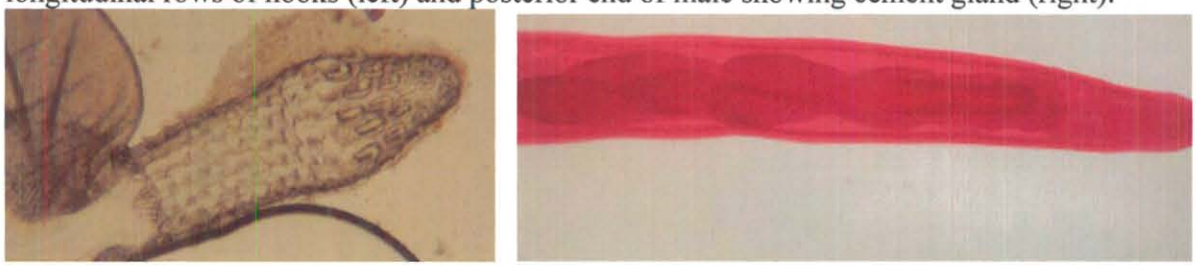


Figure 5.41.— Photomicrographs of the parasitic leech *Myzobdella lugubris* attached to fin of rock bass (left) and anterior end stained with acetocarmine (right).

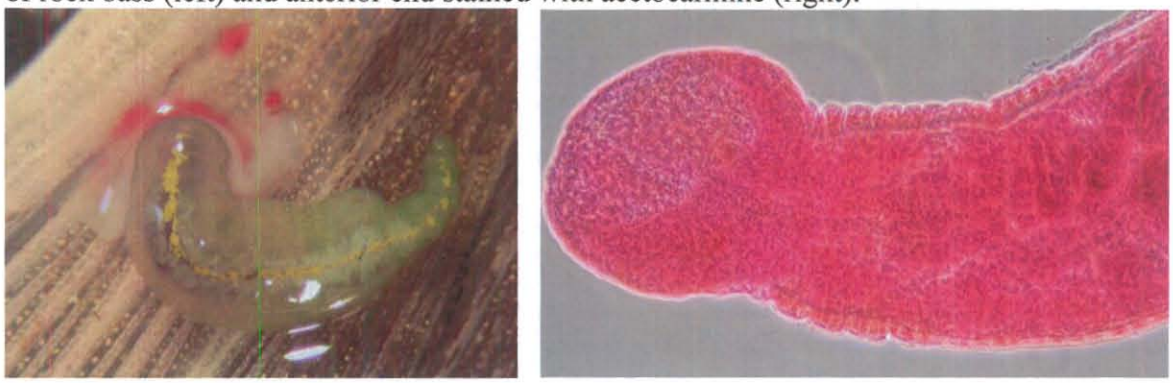


Figure 5.42.— Photomicrographs of the parasitic leech *Piscicola punctata* from skin of fathead minnow collected at Lake Traverse (fresh mount and acetocarmine).

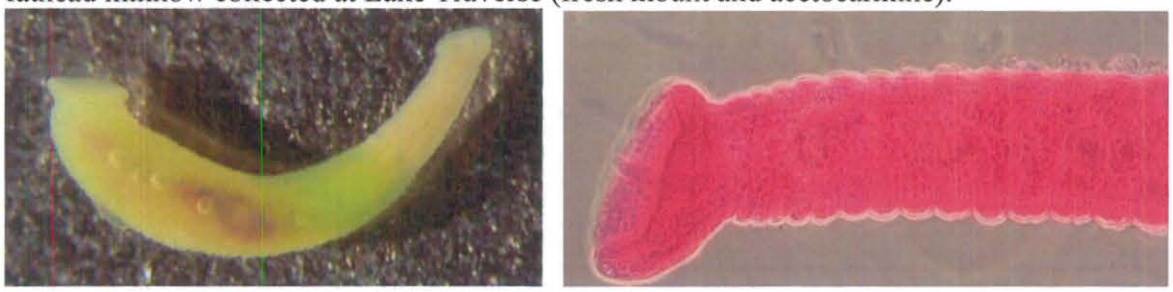


Figure 5.43.— Photomicrographs of the parasitic copepod *Ergasilus cyprinaceus* anchored to gill lamellae of fathead minnow from Lake Traverse.

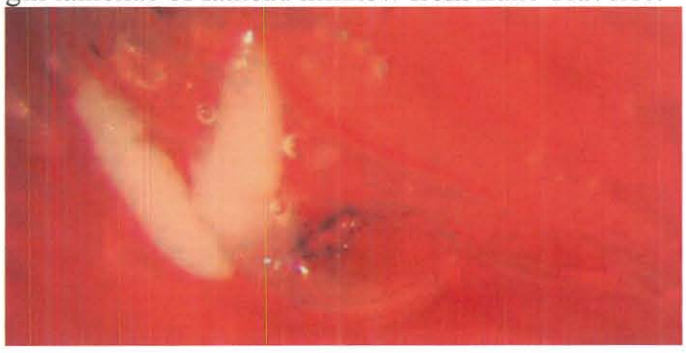
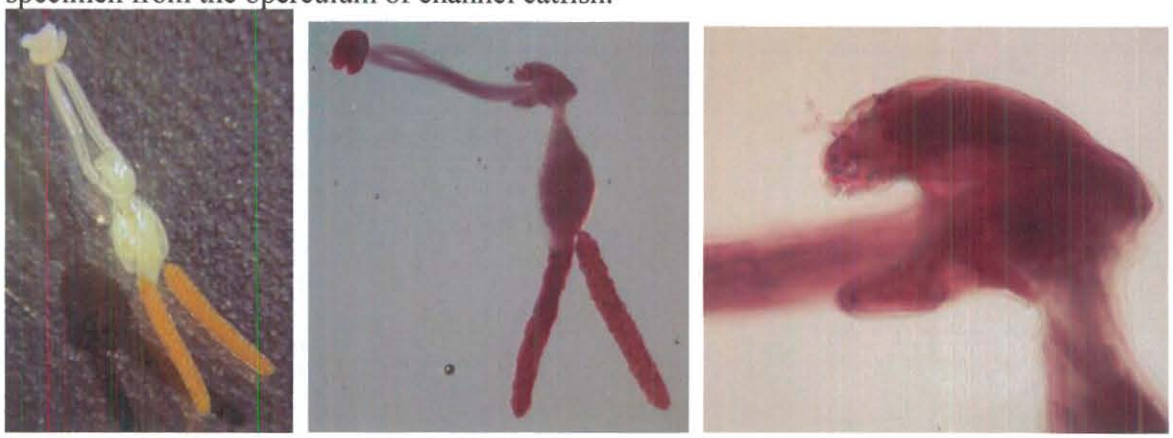


Figure 5.44.— Photomicrographs of Actheres fresh mount (left) and acetocarmine-stained specimen from the operculum of channel catfish.



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Chapter 6 — Fish Viruses

Methods

Details of cell culture techniques used to test fish tissues for viruses were from *National Wild Fish Health Survey - Laboratory Procedures Manual* (USFWS 2006). In brief, tissue samples were processed according to standard methods within 48 h of collection. All viral assays were begun 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, samples from cetrarchids (family including bass and sunfishes) also included swim bladder tissue. Pooled tissue samples were placed in transport medium composed of Hank's balanced salt solution (HBSS) with antibiotics and held at 4°C. Prior to processing, the HBSS medium was decanted and tissues were weighed (0.0 g) to calculate an appropriate dilution with fresh HBSS. After dilution and maceration, tissue homogenates were inoculated in replicate onto confluent monolayers of *Epithelioma papulosum cyprini* (EPC) and/or chinook salmon embryo-214 (CHSE-214) cell lines in 24-well tissue culture plates and incubated at 15°C. To test for viruses that prefer warmer temperatures such as largemouth bass virus and spring viremia of carp virus, tissue homogenates were inoculated onto bluegill fry (BF-2) and/or fathead minnow (FHM) cell lines and incubated at 22°C. Tissue samples of fish from the family Ictaluridae (catfishes) were also screened using channel catfish ovary (CCO) and brown bullhead (BB) cell lines incubated at 22°C. Finally, tissue samples from common carp were inoculated on koi fin (FK-1; Hedrick et al. 2000) cell line and incubated at 22°C to screen for Koi Herpes Virus. Viral assays were monitored for cytopathic effect (CPE) using inverted light microscopy for 28 d.

Results

A total of 59 pooled tissue samples representing 289 fish were collected and tested from among seven species of fish sampled at Devils Lake (Table 6.1). We collected a total of 27 pooled tissue samples representing 100 fish and thirteen species from the Sheyenne River (Table 6.2). At the Red River, 88 pooled tissue samples collected from 392 fish and 22 species were tested (Table 6.3). Finally, a total of 113 pooled tissue samples representing 550 fish were tested from among seventeen species of fish collected at Lake Traverse. Overall, nearly 290 replicate tissue samples collected from fish from the four bodies of water were tested on multiple cell lines at two different incubation temperatures. We did not observe any cytopathic effect 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 had any clinical signs of suggest infection by virus.

Discussion

During this survey, no viruses were detected in cell culture assays of fish tissue samples from Devils Lake, the Red and Sheyenne rivers, or from Lake Traverse. No clinical signs typical of viral disease were noted during necropsies. At Devils Lake, where fish tissues were also

examined with histology, no cellular anomalies attributed to viral disease were observed. Between 2001 and 2006, the U. S. Fish and Wildlife Service completed three fish pathogens surveys at Devils Lake (Peters 2002; Hudson and Peters 2005; Peters and Hudson 2007). In the course of that work, 880 fish were tested in about 180 pooled tissue samples for the presence of fish viruses. In the present survey, another 289 fish in 59 pooled samples were tested. This brings the total number of fish from Devils Lake tested for viruses to 1169. Fish have been collected during four different months including June, July, September, and October. 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.

References

Hudson, C. and K. Peters. 2005. Survey of specific fish pathogens in free-ranging fish from Devils Lake, North Dakota. U. S. Fish and Wildlife Service, Bozeman Fish Health Center Technical Report 05-02.

Peters, K. 2002. Survey of specific fish pathogens in free-ranging fish from Devils Lake and the Sheyenne and Red rivers in North Dakota. U. S. Fish and Wildlife Service, Bozeman Fish Health Center Technical Report.

Peters, K. and C. Hudson. 2007. Survey of specific fish pathogens and parasites in free-ranging fish from Devils Lake and the Sheyenne and Red rivers in North Dakota; 2006 survey results. U. S. Fish and Wildlife Service, Bozeman Fish Health Center Technical Report 07-01.

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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	Cell line and incubation temperature		CPE
		15°C	22°C	
Black crappie	12	EPC, CHSE-214	BF-2, EPC, FHM	ND
Fathead minnow	12	EPC, CHSE-214	BF-2, EPC, FHM	ND
Northern pike	5	EPC, CHSE-214	BF-2, EPC, FHM	ND
Walleye	12	EPC, CHSE-214	BF-2, EPC, FHM	ND
White bass	12	EPC, CHSE-214	BF-2, EPC, FHM	ND
White sucker	2	EPC, CHSE-214	BF-2, EPC, FHM	ND
Yellow perch	4	EPC, CHSE-214	BF-2, EPC, FHM	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 Sheyenne River. Abbreviations for cell lines are explained in the methods section. CPE = cytopathic effect, ND = not detected.

Species sampled	Number of pools tested	Cell line and incubation temperature		CPE
		15°C	22°C	
Black bullhead	5	EPC, CHSE-214	BB, CCO, BF-2, FHM	ND
Bluegill	1	EPC, CHSE-214	BF-2, FHM	ND
Common shiner	2	EPC, CHSE-214	BF-2, FHM	ND
Creek chub	1	EPC, CHSE-214	BF-2, FHM	ND
Fathead minnow	1	EPC, CHSE-214	BF-2, FHM	ND
Iowa darter	2	EPC, CHSE-214	BF-2, FHM	ND
Northern pike	1	EPC, CHSE-214	BF-2, FHM	ND
Shorthead redhorse	1	EPC, CHSE-214	BF-2, FHM	ND
Spottail shiner	5	EPC, CHSE-214	BF-2, FHM	ND
Tadpole madtom	3	EPC, CHSE-214	BB, CCO, BF-2, FHM	ND
Trout perch	1	EPC, CHSE-214	BF-2, FHM	ND
Walleye	2	EPC, CHSE-214	BF-2, FHM	ND
White sucker	2	EPC, CHSE-214	BF-2, FHM	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	Cell lines and incubation temperature		CPE
		15°C	22°C	
Bigmouth buffalo	1	EPC, CHSE-214	BF-2, EPC, FHM	ND
Black crappie	2	EPC, CHSE-214	BF-2, EPC, FHM	ND
Bluegill	2	EPC, CHSE-214	BF-2, EPC, FHM	ND
Channel catfish	12	EPC, CHSE-214	BB, CCO, BF-2	ND
Common carp	12	EPC, CHSE-214	BF-2, EPC, FHM, KF-1	ND
Emerald shiner	12	EPC, CHSE-214	BF-2, EPC, FHM	ND
Freshwater drum	7	EPC, CHSE-214	BF-2, EPC, FHM	ND
Goldeye	4	EPC, CHSE-214	BF-2, EPC	ND
Largemouth bass	1	EPC, CHSE-214	BF-2, EPC, FHM	ND
Northern pike	1	EPC, CHSE-214	BF-2, EPC	ND
Orangespotted sunfish	4	EPC, CHSE-214	BF-2, EPC, FHM	ND
Quillback	5	EPC, CHSE-214	BF-2, EPC, FHM	ND
Rock bass	1	EPC, CHSE-214	BF-2, EPC, FHM	ND
Sauger	2	EPC, CHSE-214	BF-2, EPC, FHM	ND
Shorthead redhorse	10	EPC, CHSE-214	BF-2, EPC, FHM	ND
Smallmouth bass	1	EPC, CHSE-214	BF-2, EPC, FHM	ND
Stonecat	1	EPC, CHSE-214	BB, CCO, BF-2, EPC	ND
Trout perch	2	EPC, CHSE-214	BF-2, FHM	ND
Walleye	2	EPC, CHSE-214	BF-2, EPC, FHM	ND
White bass	4	EPC, CHSE-214	BF-2, EPC, FHM	ND
White sucker	1	EPC, CHSE-214	BF-2, EPC, FHM	ND
Yellow perch	1	EPC, CHSE-214	BF-2, EPC, FHM	ND

Table 6.4.— Number of pooled tissue samples tested for virus with multiple cell lines at two incubation temperatures for sixteen species of fish from the Lake Traverse. Abbreviations for cell lines are explained in the methods section. CPE = cytopathic effect, ND = not detected.

Species sampled	Number of pools tested	Cell lines and incubation temperature		CPE
		15°C	22°C	
Bullheads	12	EPC, CHSE-214	BB, CCO, BF-2, EPC	ND
Black crappie	12	EPC, CHSE-214	BF-2, EPC, FHM	ND
Bluegill	12	EPC, CHSE-214	BF-2, EPC, FHM	ND
Channel catfish	2	EPC, CHSE-214	BB, CCO, BF-2, EPC	ND
Common carp	12	EPC, CHSE-214	BF-2, EPC, FHM, KF-1	ND
Emerald shiner	12	EPC, CHSE-214	BF-2, EPC, FHM	ND
Freshwater drum	12	EPC, CHSE-214	BF-2, EPC, FHM	ND
Largemouth bass	1	EPC, CHSE-214	BF-2, EPC, FHM	ND
Northern pike	1	EPC, CHSE-214	BF-2, EPC, FHM	ND
Orangespotted sunfish	1	EPC, CHSE-214	BF-2, EPC, FHM	ND
Pumpkinseed	4	EPC, CHSE-214	BF-2, EPC, FHM	ND
Shorthead redhorse	1	EPC, CHSE-214	BF-2, EPC, FHM	ND
Rock bass	2	EPC, CHSE-214	BF-2, EPC, FHM	ND
Walleye	3	EPC, CHSE-214	BF-2, EPC, FHM	ND
White bass	12	EPC, CHSE-214	BF-2, EPC, FHM	ND
White sucker	2	EPC, CHSE-214	BF-2, EPC, FHM	ND
Yellow perch	12	EPC, CHSE-214	BF-2, EPC, FHM	ND

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, RR = Red River, and SR = Sheyenne River.

Abbreviation	Common name	Scientific name	Body of water sampled
BLB	Black bullhead	*Ameiurus melas*	SR, LT
BLC	Black crappie	*Promoxis nigromaculatus*	DL, RR, LT
BIB	Bigmouth buffalo	*Ictiobus cyprinellus*	RR
BLG	Bluegill	*Lepomis macrochirus*	RR, SR, LT
CAP	Common carp	*Cyprinus carpio*	RR, LT
CCF	Channel catfish	*Ictalurus punctatus*	RR, LT
CKC	Creek chub	*Semotilus atromaculatus*	SR
CMS	Common shiner	*Notropis cornutus*	SR
EMS	Emerald shiner	*Notropis atherinoides*	RR, LT
FHM	Fathead minnow	*Pimephales promelas*	DL, SR, LT
FRD	Freshwater drum	*Aplodinotus grunniens*	RR, LT
GDE	Goldeye	*Hiodon alosoides*	RR
IWD	Iowa darter	*Etheostoma exile*	SR
LMB	Largemouth bass	*Micropterus salmoides*	RR, LT
NOP	Northern pike	*Esox lucius*	DL, RR, SR, LT
OSS	Orangespotted sunfish	*Lepomis humilis*	RR, LT
PSS	Pumpkinseed	*Lepomis gibbosus*	LT
QBS	Quillback	*Carpiodes cyprinus*	RR
RKB	Rock bass	*Ambloplites rupestris*	RR, LT
SAR	Sauger	*Stizostedion canadense*	RR
SHR	Shorthead redhorse	*Moxostoma macrolepidotum*	RR, SR, LT
SMB	Smallmouth bass	*Micropterus dolomieu*	RR
SNC	Stonecat	*Noturus flavus*	RR
SSH	Spottail shiner	*Notropis hudsonius*	SR
TPM	Tadpole madtom	*Noturus gyrinus*	SR
TRP	Trout-perch	*Percopsis omiscomaycus*	RR, SR
WAE	Walleye	*Sander vitreus*	DL, RR, SR, LT
WHB	White bass	*Morone chrysops*	DL, RR, LT
WHC	White crappie	*Promoxis annularis*	LT

Table A.1.— continued.

Abbreviation	Common name	Scientific name	Body of water sampled
WHS	White sucker	*Catostomus commersoni*	DL, RR. SR, LT
YEB	Yellow bullhead	*Ameiurus natalis*	LT
YEP	Yellow perch	*Perca flavescens*	DL, RR, LT