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

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Executive Summary

We present results of a third fish pathogen and parasite survey at Devils Lake and a second survey of the Sheyenne and Red rivers in North Dakota. Surveys were performed to provide information to resource managers to assess the potential for biota transfer from operation of an outlet on Devils Lake to the Sheyenne River. Fish health biologists from Bozeman, Dexter, Idaho, and Lacrosse Fish Health Centers (FHC) worked cooperatively with the Missouri River Fish and Wildlife Management Assistance Office, Valley City National Fish Hatchery, North Dakota Game and Fish Department, and the Spirit Lake Nation to collect samples from the three bodies of water. In September 2006, 387 fish were collected from two sampling areas on Devils Lake. During October 2006, we collected 78 fish from the Sheyenne River near the southern boundary of the Spirit Lake Nation and 72 fish from the Red River south of Fargo, North Dakota. The catch on Devils Lake was composed of black crappie, fathead minnow, northern pike, walleye, white bass, white sucker, and yellow perch. We collected black bullhead, northern pike, tadpole madtom, walleye, and white sucker from the Sheyenne River, and channel catfish, freshwater drum, goldeye, sauger, stonecat, and walleye from the Red River. Fish were tested for the presence or absence of pathogens and parasites using protocols and procedures of the U.S. Fish and Wildlife Service *National Wild Fish Health Survey*. Five main components of the survey included: 1) record catch results and weigh and measure fish; 2) perform external and internal examination for gross signs of disease or other abnormalities, 3) aseptic collection of specific tissues samples; 4) external and internal parasites survey; and 5) application of standardized screening and confirmatory assays for specific fish pathogens.

Overall, fish appeared in good general health. We did not detect any fish virus in standard cell culture assays from the three bodies of water. Major microbial findings included the isolation several Gram-negative motile bacteria from the Families Aeromonadaceae, Enterobacteriaceae, and Pseudomonadaceae. Many of the bacteria within these families are normal constituents of aquatic ecosystems or are considered normal flora of animal gastro-intestinal tracts. *Aeromonas hydrophila, Hafnia alvei, Pseudomonas fluorescens* and *Pseudomonas sp*. were the most commonly isolated species from these groups. No Gram-positive bacteria were found during the surveys although antigen of *Renibacterium salmoninarum* was detected by enzyme-linked immunosorbent assay (ELISA) in very low levels from several species collected from all three bodies of water. Active infection with *R. salmoninarum* was not confirmed in these populations by the highly specific polymerase chain reaction (PCR) assay and there was reason to believe low ELISA optical density values may have represented false-positive readings. Other than *R. salmoninarum*, none of the fish pathogens listed in the *National Wild Fish Health Survey* were detected in fish from Devils Lake or the Red and Sheyenne rivers. Likewise, none of the regulated or prohibited fish pathogens indicated in federal fish health inspection policies were detected.

At Devils Lake, we observed or recovered parasites from all species of fish surveyed except white sucker. One ciliated protozoan parasite, *Trichodina sp*., was observed in wet mounts of gill filaments of yellow perch and skin scrapings from walleye and yellow perch. Five species of parasites from the Class Trematoda were found. At Devils Lake, *Gyrodactylus hoffmani* was observed on the fins of fathead minnow. *Neascus* of *Posthodiplotomum sp*. was found in fathead minnow and black crappie. *Diplostomum spathaceum* was observed in the lens of eyes from

fathead minnow. We found *Paurorhynchus hiodontis* encysted in mesenteric tissues of goldeye collected from the Red River. Three parasites of the Class Cestoidea were found including adult *Bothriocephalus cuspidatus* in walleye, metacestodes of *Bothriocephalus sp.* in black crappie, fathead minnow, and walleye. In addition, *Proteocephalus pinguis* was observed in northern pike, and *Ligula intestinalis* in fathead minnow. Larval forms of the parasitic nematode *Contracaecum sp.* were recovered from black crappie, white bass, and walleye at Devils Lake, and from black bullhead, tadpole madtom, and walleye from the Sheyenne River. A presumptive finding of a second or third larval stage of *Raphidascaris acus* was made from a nematode found in mesenteric tissues of yellow perch at Devils Lake.

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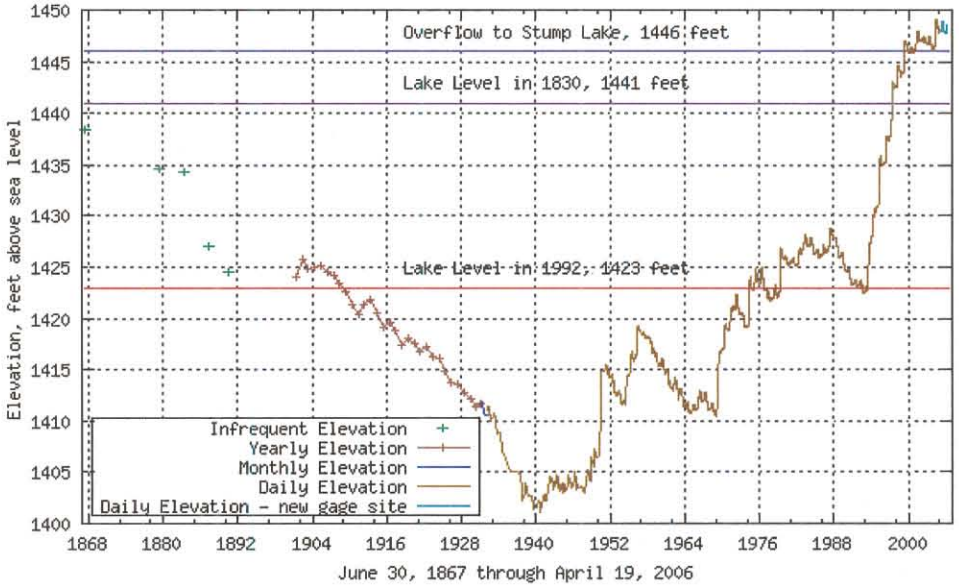
Introduction

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 sub-basin which covers approximately 2.4 million acres. The Devils Lake sub-basin lies within the much larger Red River-Hudson Bay drainage. 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, the Devils Lake basin has experienced periods of climatic fluctuation which have caused extreme changes in the lake's water surface elevation (Figure 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. If the elevation of Devils Lake remains above 1446 ft-msl it will continue to spill into Stump Lake until they reach equilibrium. Should the present wet conditions continue beyond that point, Stump Lake will overflow to the Sheyenne River at approximately 1459 ft-msl. Geologic records indicate Devils Lake has overflowed into the Sheyenne River twice in the last 4,000 years.

Figure 1.— Water surface elevation for Devils Lake from June, 1867 through April, 2006. (Source: U. S. Geological Survey, North Dakota Water Science Center).



Stabilization of Devils Lake sub-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 decade, an outlet has been constructed to carry water from Devils Lake 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 studies have been conducted in the Devils Lake sub-basin or in the Red River drainage 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). To address these concerns, the U.S. Army Corps of Engineers 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* (2005). Antigen of *Renibacterium salmoninarum*, the regulated agent responsible for bacterial kidney disease in salmonids, was detected in low levels with an ELISA screening test although active infections or DNA of the bacterium was not confirmed with the highly sensitive and specific polymerase chain reaction (PCR) assay. No other pathogens were detected. In 2005, the Council on Environmental Quality (CEQ) requested the U.S. Fish and Wildlife Service performed a second fish pathogen survey at Devils Lake but not limited to the pathogens listed in the *National Wild Fish Health Survey* (Hudson and Peters 2005). Results of the second survey at Devils Lake included recovery of several parasites although most specimens had been described in earlier studies in the Devils Lake sub-basin or the Red River drainage. Parasites not previously described in earlier reports where found to be relatively common in North American and other parts of the world. Results of the second surveys at Devils Lake also included the isolation of several species of bacteria though most were considered either normal residents of fish gastro-intestinal tracts or common constituents of soil and fresh water environments. Bacteria most frequently isolated 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 the 2002 survey, low levels of antigen of *Renibacterium salmoninarum* was detected with ELISA but not confirmed with the PCR assay. Investigators speculated that ELISA results may have represented false positive readings or have been caused by cross-reacting bacteria or proteins. Regulated or prohibited bacterial and viral fish pathogens were not detected in either previous survey and investigators remarked that fish appeared healthy with no external or internal clinical signs of disease.

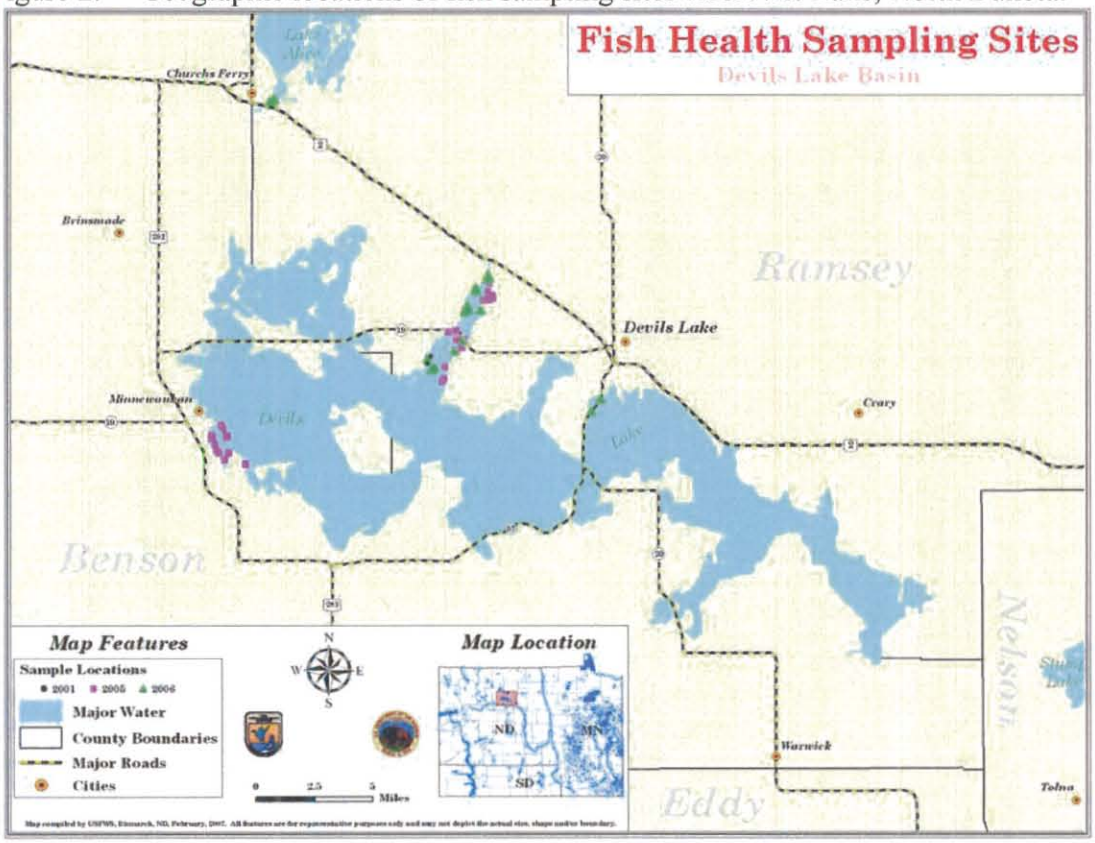
In this report we provide results and discussion of the latest fish pathogen and parasite survey from Devils Lake and the Sheyenne and Red rivers completed in 2006. Study objectives were to,

1) examine fish from Devils Lake and the Red and Sheyenne rivers for the presence of specific fish pathogens and parasites, 2) provide fish health specialists, fisheries managers, and other decision makers with a pathogen survey report that may be used in performing risk analysis, and 3) 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.

Methods

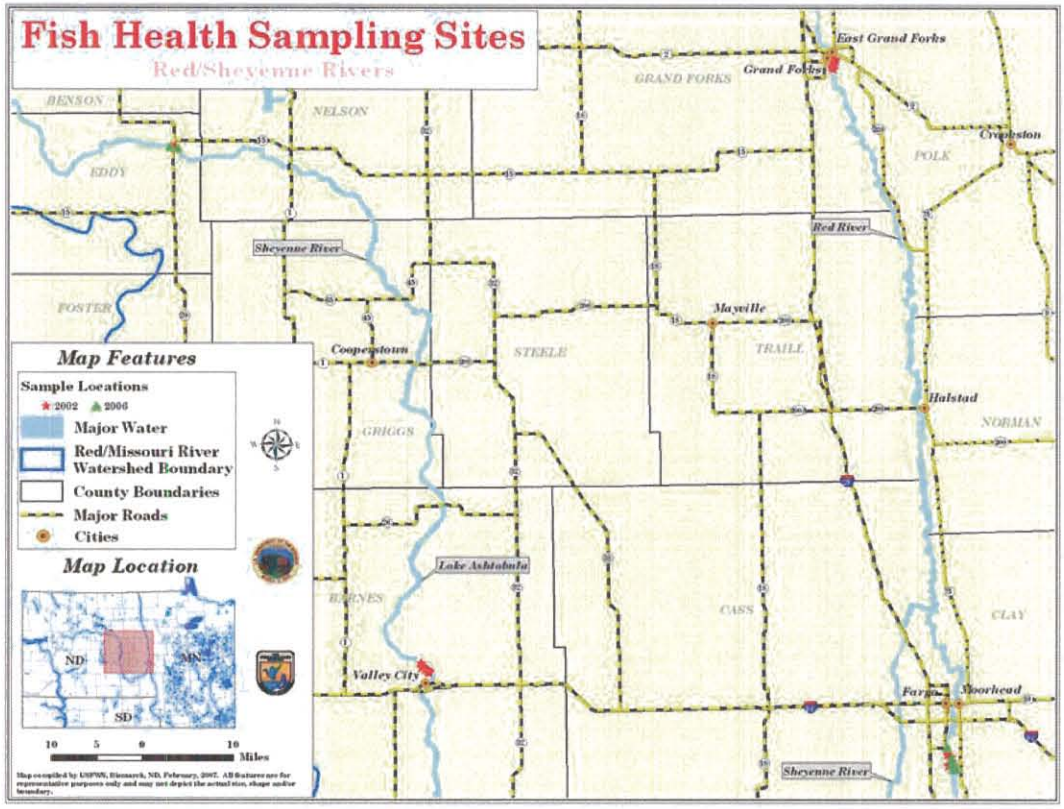
Collection of fish and tissue sampling.— We used a standard target sample size of 60 fish for each species to determine the presence or absence of bacterial and viral fish pathogens. This widely accepted sample size provides a 95% confidence level that an infected fish will be detected given a 5% presumed prevalence of infection and a population of 2,000 or more individuals (Ossiander and Wedemeyer 1973). At Devils Lake, fish were sampled from two areas between 25 September and 29 September 2006 (Figure 2). The primary sample area was in Six Mile Bay located in the north-central section of the lake. Sampling in Six Mile Bay extended north into the mouth of Channel A. A small number of fish were collected from a disconnect bay separated from Devils Lake by North Dakota Highway 57. Fish were collected using experimental gill nets and modified fyke nets designed for shoreline sets. Two types of multi-mesh gill nets were deployed: 1) 125 ft X 6 ft with 5 panels incorporating ¾, 1, 1½, 1¾, and 2 inch mesh sizes; 2) 300 ft X 6 ft with 3 panels of 3, 4, and 5 inch mesh. Gill nets were checked in 1-3 h intervals to minimize fish mortality. Modified fyke nets were composed of a single lead and single throat and incorporated both ¼ and ½ inch mesh. Nets with ¼ inch mesh were used primarily to capture fathead minnow. Fyke nets were typically deployed as overnight sets. Upon collection, fish were transported alive to a temporary field laboratory near the Devils Lake public access at Six Mile Bay. Fish were held in large totes with lake water or live boxes until examined.

Figure 2.— Geographic locations of fish sampling sites on Devils Lake, North Dakota.



On 11 October 2006, fish were collected from the Sheyenne River along a 0.5 km reach up- and downstream from the bridge on State Highway 20 (Figure 2). The reach extended along the southeastern border of the Spirit Lake Nation. Fish were collected from the Red River on 12 – 13 October 2006 in a 2.0 km reach upstream of the bridge at 52nd Avenue south in Fargo, North Dakota. Sampling gear was composed of 125 ft. multi-mesh gill nets similar to those used on Devils Lake. In addition, we deployed modified fyke nets with ⅛ inch mesh and hoop nets with 1½ mesh. Nets and traps were set for 18 – 24 h intervals. Inclement weather including high winds, freezing temperatures and snow prevented the use of the temporary field station at the Red and Sheyenne River sampling sites. Instead fish collected from the rivers were transported in coolers to a U. S. Fish and Wildlife Service maintenance shop in Valley City, North Dakota. Fish were held on ice until processing was completed during the same day as capture.

Figure 3.— Geographic locations of sampling sites on the Sheyenne and Red rivers.



For necropsy, fish were anesthetized with tricaine methanesulfonate (Finquel®), weighed (g) and measured (L_t, mm), and then examined externally and internally for clinical signs of disease or other abnormalities. Tissues samples for pathogen testing were collected using aseptic field techniques and packed in coolers with ice for transfer to either the Bozeman Fish Health Center (USFWS, Bozeman, Montana) or the LaCrosse Fish Health Center (USFWS, Onalaska, Wisconsin). Upon arrival at the Health Centers, 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 2005). 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 procedures used in this survey is provided below. Details of these procedures may be examined on the worldwide web following the Protocols and Procedures link on the *National Wild Fish Health Survey* website at http://wildfishsurvey.fws.gov.

Virology.— Standard cell culture techniques were used to test fish for viruses. 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 black crappie and white bass 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 was decanted and tissues were weighed for 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 25°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 25°C. Finally, samples from five species collected at Devils Lake were inoculated onto recently developed koi fin (FK-1; Hedrick et al. 2000) cell line and incubated at 25°C. Viral assays were monitored for cytopathic effect (CPE) using inverted light microscopy for 28 d.

Bacteriology.— 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. Several commercial systems were used to identify bacteria including the API 20E (bioMérieux Vitek, Inc., Hazelwood, Mo.), Crystal Enteric/Nonfermenter (Becton Dickinson, Inc., Cockeysville, Md.), and Biolog Microbial ID/Characterization (Hayward, Ca.) for Gram positive isolates. Where appropriate, further confirmation of suspect bacterial isolates was performed with either direct or indirect fluorescent antibody tests, serum agglutination tests, or with polymerase chain reaction (PCR) assays. 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 a 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.

Parasitology.— We randomly selected 30 fish of each species at Devils Lake to perform a comprehensive parasite survey. The goal was to examine a minimum of five freshly caught fish of each species at the temporary field station. Fish not examined at the field station were frozen and examined later at Bozeman Fish Health Center. Fish were examined externally and internally for parasites according to methods of the *National Wild Fish Health Survey* (2005; Section 8.1). 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, 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; cestodes and trematodes) 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.

Results

Sampling.— On Devils Lake, a total of 387 fish representing seven species were collected as a result of nearly 400 hours of netting effort (Table 1). The catch was composed of black crappie, fathead minnow, northern pike, walleye, white bass, white sucker, and yellow perch. From the total, seven northern pike and forty nine yellow perch were collected from a small disconnect bay of Devils Lake separated by State Highway 57. The target sample size of 60 fish was obtained for all species except white sucker (n=27). Low catch rates for white sucker were attributed to either relative low abundance or because seasonal distribution and occurrence in selected sample areas was low.

A total of 78 fish representing five species were collected on the Sheyenne River from approximately 144 hours of netting effort (Table 1). The catch was predominately composed of black bullhead, tadpole madtom, and walleye. Smaller numbers of northern pike and white sucker were also collected. On the Red River, we collected a total of 72 fish from as a result of 330 hours of netting effort. Red River catch was composed primarily of channel catfish and goldeye with smaller numbers of sauger, stonecat, and walleye. Low catch rates in select reaches of both rivers resulted in fewer fish than the target sample size. Poor catch rates were attributed to reduced seasonal movement of fish resulting from falling water temperatures and winter-like weather conditions.

Table 1.— Composition of fish collected from Devils Lake and the Red and Sheyenne rivers.

Fish sampled			Number sampled		
Scientific name	Common name	Abbr	Devils L.	Red R.	Sheyenne R.
Ameiurus melas	Black bullhead	BLB	0	0	27
Promoxis nigromaculatus	Black crappie	BLC	60	0	0
Ictalurus punctatus	Channel catfish	CCF	0	21	0
Pimephales promelas	Fathead minnow	FHM	60	0	0
Aplodinotus grunniens	Freshwater drum	FRD	0	15	0
Hiodon alosoides	Goldeye	GDE	0	28	0
Esox lucius	Northern pike	NOP	60	0	3
Stizostedion canadense	Sauger	SAR	0	3	0
Noturus flavus	Stonecat	SNC	0	2	0
Noturus gyrinus	Tadpole madtom	TPM	0	0	20
Sander vitreus	Walleye	WAE	60	3	24
Morone chrysops	White bass	WHB	60	0	0
Catostomus commersoni	White sucker	WHS	27	0	4
Perca flavescens	Yellow perch	YEP	60	0	0

Bacteria.— For samples from Devils Lake, primary bacterial culture tests were negative for reportable bacterial fish pathogens listed in federal inspection policy in the United States. Additionally, none of the bacterial pathogens listed in the *National Wild Fish Health Survey* program were isolated. Important bacteria not detected included *Aeromonas salmonicida*, *Yersinia ruckeri*, *Edwardsiella ictaluri*, *E. tarda*, *Flavobacterium columnare*, *F. psychrophilum*, and *Citrobacter freundii*. There was however considerable growth of other bacteria on the primary isolation medium. We sub-cultured for purity from 135 primary cultures with presumed mixed isolates which resulted in 260 pure cultures. A large proportion of these isolates were from fathead minnow. Upon screening with preliminary biochemical and motility tests, we arrived at about 50 pure cultures that required further differentiation and identification with commercial test systems listed in the preceding methods section. The majority of the isolates were either aerobic or facultative anaerobic, Gram-negative motile rods from the Families Aeromonadaceae, Enterobacteriaceae, and Pseudomonadaceae (Table 2). *Aeromonas hydrophila, Hafnia alvei, Pseudomonas fluorescens* and *Pseudomonas sp.* were the mostly commonly isolated species from these groups. Isolates of *Hafnia alvei* from fathead minnow, northern pike and white bass had API-20E bio-chemical profiles similar to *Y. ruckeri*. *H. alvei* isolates were tested against *Y. ruckeri* by IFAT and were negative. Other less frequently isolated species included *Brevundimonas diminuta* from northern pike, and *Pseudomonas mendocina* and *Yokenella regensburgei* from fathead minnow. *Acinetobacter lwoffii,* a Gram-negative aerobic bacterium in the family Neisseriaceae was isolated from walleye. We did not isolate any Gram-positive bacteria from fish samples at Devils Lake.

Similar to Devils Lake, we did not isolate any of the reportable or regulated bacterial fish pathogens in fish samples from the Sheyenne and Red rivers. For samples from the Sheyenne River, we sub-cultured for purity from 19 primary cultures resulting in the isolation of 30 pure cultures. From these we identified two motile, Gram-negative bacteria, one each from Aeromonadaceae and Pseudomonadaceae families. *A. hydrophila* was found in black bullhead and *P. fluorescens* in tadpole madtom (Table 2). There was no bacterial growth on BHIA cultures of kidney tissue from northern pike (n = 3). For the Red River, we sub-cultured from 14 primary cultures resulting in the isolation of 28 pure cultures. From the pure cultures, we identified three species of bacteria representing Enterbacteriaceae and Pseudomonadaceae families. *Pantoea sp.* (previously *Erwinia*) was the most common bacterium from fish in the Red River and was found in freshwater drum, goldeye, and walleye. *Enterobacter cloacae* were cultured from sauger and *Pseudomonas fluorescens* from channel catfish. There was no growth on primary cultures of kidney tissue of stonecats (n = 2).

None of the fish we examined had any external or internal clinical signs of bacterial disease regardless of sample site or body of water surveyed. Fish infected with one or more of these environmental and opportunistic bacteria could best be described as asymptomatic carriers.

Table 2.— Identification of Gram-negative bacteria and species of fish from which isolates were cultured for samples collected at Devils Lake and the Red and Sheyenne rivers. Abbreviations of fish common names are explained in Table 1.

	Name of bacteria		
Body of water	Genus	Species	Species of fish infected
Devils Lake	*Acinetobacter*	*lwoffii*	WAE
	Aeromonas	*hydrophila*	BLC, FHM, NOP
	Brevundimonas	*diminuta*	NOP
	Hafnia	*alvei*	FHM, NOP, WHB
	Pseudomonas	*fluorescens*	FHM
	Pseudomonas	*mendocina*	FHM
	Yokenella	*regensburgii*	FHM, NOP
Sheyenne River	*Aeromonas*	*hydrophila*	BLB
	Pseudomonas	*fluorescens*	TPM
Red River	*Enterobacter*	*cloacae*	SAR
	Pantoea	*sp.*	FRD, GDE, WAE
	Pseudomonas	*fluorescens*	CCF

At Devils Lake, antigen of *R. salmoninarum* was detected by ELISA in kidney tissues of northern pike, white sucker, white bass, fathead minnow, and yellow perch (Table 3). Antigen was not detected in black crappie or walleye. Because of their small size we had to pool 20 fathead minnow to obtain sufficient kidney tissue for testing with ELISA. The ELISA negative threshold OD value (cut-off) determined from standardized reference tissue ranged between 0.079 – 0.089. The overall mean ELISA OD value for samples from Devils Lake was 0.083. Of the 216 samples tested antigen was detected in 20.4%. Most samples (98%) with OD values above the negative threshold were in the low antigen level category. Only one sample, collected from northern pike, had an OD value in the medium antigen level category. Positive ELISA samples assayed with the nested-PCR for *R. salmoninarum* were negative for all species tested (Table 3).

Similar to Devils Lake, we detected low levels of antigen of *R. salmoninarum* in kidney samples from four species of fish from the Sheyenne River (Table 4). A total of 40 samples were tested with ELISA of which 57.5% were above the negative threshold (0.081). Tadpole madtom were not tested because of their small size and insufficient kidney tissue remaining after sampling for other assays. One black bullhead sample had a medium ELISA OD value (0.239) while all other positive samples were in the low antigen level category. The overall mean ELISA OD value for the Sheyenne River was 0.090. All positive ELISA samples tested with PCR for *R. salmoninarum* were negative.

For the Red River, three of six species tested with ELISA had positive OD values (Table 5). The negative threshold value for ELISA was identical to the value for the Sheyenne River samples (0.081). A total of 57 samples were tested with ELISA of which 24.6% had OD readings above the negative threshold. The overall mean OD value was 0.088. Similar to Devils Lake and the Sheyenne River, most samples from the Red River with positive ELISA values were categorized as low. Only one sample from freshwater drum had a medium ELISA OD value (0.215). All ELISA-positive samples from the Red River tested with PCR for *R. salmoninarum* were negative (Table 5).

Table 3.— 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 seven species of fish collected 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	10	0	BDL	0	
Fathead minnow	3	100	Low	3	0
Northern pike	60	40	Low	4	0
Walleye	44	0	BDL	0	
White bass	59	7	Low	3	0
White sucker	25	12	Low	3	0
Yellow perch	15	20	Low	3	0

Table 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 five species of fish collected from the Sheyenne River.

	ELISA			PCR Assay	
Fish species	Number tested	Percent positive	Mean antigen level	Number tested	Percent positive
Black bullhead	18	50	Low	3	0
Northern pike	3	67	Low	2	0
Walleye	15	60	Low	3	0
White sucker	4	75	Low	3	0

Table 5.— Percent of samples with detectable antigen of *R. salmoninarum* and mean antigen level category as measured by the ELISA, and corroborative testing with a nested PCR assay for seven species of fish collected from the Red River. BDL = below detection limits.

	ELISA			PCR Assay	
Fish species	Number tested	Percent positive	Mean antigen level	Number tested	Percent positive
Channel catfish	21	5	BDL	1	0
Freshwater drum	7	71	Low	3	0
Goldeye	22	36	BDL	3	0
Sauger	3	0	BDL	0	
Stonecat	1	0	BDL	0	
Walleye	3	0	BDL	0	

Virus.— A total of 80 pooled tissue samples were collected among the seven species of fish captured at Devils Lake (Table 6). On the Sheyenne River, we collected a total of 17 pooled tissue samples representing 5 species of fish (Table 7). A total of 18 pooled samples representing 6 species of fish were collected from the Red River (Table 8). All samples were screened for viral fish pathogens using multiple cells lines at two different incubation temperatures and monitored for 28 d. Cytopathic effect or evidence of viral fish pathogens was not detected in any sample from any body of water.

Table 6.— Number of pooled tissue samples assayed using multiple cell lines at two incubation temperatures for seven species of fish from Devils Lake. CPE = cytopathic effect, ND = not detected.

		Cell lines and incubation temperature		
Species sampled	Pools tested	15°C	25°C	CPE
Black crappie	12	CHSE-214	BF-2	ND
Fathead minnow	12	CHSE-214	FHM	ND
Northern pike	12	EPC, CHSE-214	FHM, BF-2, KF-1	ND
Walleye	12	EPC, CHSE-214	FHM, BF-2, KF-1	ND
White bass	12	EPC, CHSE-214	FHM, BF-2, KF-1	ND
White sucker	6	EPC, CHSE-214	FHM, BF-2, KF-1	ND
Yellow perch	12	EPC, CHSE-214	FHM, BF-2, KF-1	ND

Table 7.— Number of pooled tissue samples assayed using multiple cell lines at two incubation temperatures for five species of fish from Sheyenne River. CPE = cytopathic effect, ND = not detected.

		Cell lines and incubation temperature		
Species sampled	Pools tested	15°C	25°C	CPE
Black bullhead	6	EPC, CHSE-214	BB, CCO, FHM, BF-2	ND
Northern pike	1	EPC, CHSE-214	FHM, BF-2	ND
Tadpole madtom	4	EPC, CHSE-214	BB, CCO, FHM, BF-2	ND
Walleye	5	EPC, CHSE-214	FHM, BF-2	ND
White sucker	1	EPC, CHSE-214	FHM, BF-2	ND

Table 8.— Number of pooled tissue samples assayed using multiple cell lines at two incubation temperatures for six species of fish from the Red River. CPE = cytopathic effect, ND = not detected.

		Cell lines and incubation temperature		
Species sampled	Pools tested	15°C	25°C	CPE
Channel catfish	4	EPC, CHSE-214	BB, CCO, FHM, BF-2	ND
Freshwater drum	3	EPC, CHSE-214	FHM, BF-2	ND
Goldeye	7	EPC, CHSE-214	FHM, BF-2	ND
Sauger	2	EPC, CHSE-214	FHM, BF-2	ND
Stonecat	1	EPC, CHSE-214	BB, CCO, FHM, BF-2	ND
Walleye	1	EPC, CHSE-214	FHM, BF-2	ND

Parasite survey.— We examined a minimum of 30 fish from each species collected from Devils Lake except for white sucker because only 27 were caught during the week of sampling (Table 9). We exceeded our target goal of examining at least 5 fish of each species fresh at the temporary field station at Six Mile Bay. In most cases 10 or more fish of each species was examined fresh. A total of 78 fish were frozen and examined later at the Bozeman Fish Health Center. All fish collected from the Sheyenne and Red rivers were examined fresh during necropsy and tissue collection procedures and none were frozen. Parasites recovered from river fish were those observed grossly as we did not perform comprehensive microscopic tissue surveys with those samples.

External Parasites.— We recovered two external parasites from fish collected at Devils Lake (Table 10). The motile Peritrich *Trichodina sp.* was observed in wet mounts of skin scrapings from walleye and yellow perch from Devils Lake (Figure 4). We also observed Trichodina sp on gill filaments of yellow perch but not walleye. The incidence of infestation with *Trichodina sp.* in fish examined fresh at the field station was 73.3% (22/30) for yellow perch and 100% (15/15) for walleye. The intensity of infestation appeared quite broad with only a few parasites observed in some samples to relatively large numbers in others. We did not observe any *Trichodina sp.* on

the gills or in skin scraping from walleye that had been frozen-thawed and examined at the Health Center. The Monogenea trematode *Gyrodactylus hoffmani* was observed on the fins of 36.7% of fathead minnow examined fresh at the field station (Figures 5 and 6). On affected fish, we observed *G. hoffmani* with greater frequency on the dorsal, caudal, and anal fins compared to pectoral and pelvic fins. We did not observe *G. hoffmani* or any other species of Gyrodactylidae on six other species of fish from Devils Lake. The single external parasite recovered from fish from the rivers was the leech *Piscicola punctata* (Figure 7). *P. punctata* was observed primarily on the dorsal and caudal fins of channel catfish from the Red River. We did not observe *P. punctata* on black bullhead or tadpole madtom from the Sheyenne River or stonecat from the Red River.

Table 9. — 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 examined		
Fish host	Fresh	Frozen-thawed	Total
Black crappie	20	10	30
Fathead minnow	60	0	60
Northern pike	6	24	30
Walleye	15	15	30
White bass	10	20	30
White sucker	18	9	27
Yellow perch	30	0	30

Table 10.— Parasites recovered from fish collected at Devils Lake.

Parasite		Host	Location
Protozoa	*Trichodina sp.*	WAE, YEP	skin, gill
Monogenea	*Gyrodactylus hoffmani*	FHM	fins
Trematoda	*Diplostomum spathaceum*	FHM	eye lens
	Neascus of *Posthodiplostomum sp.*	BLC, FHM	mesentery, viscera
Cestoidea	*Bothriocephalus cuspidatus*	WAE	intestine
	Bothriocephalus sp.	BLC, FHM	intestine
	Ligula intestinalis	FHM	visceral cavity
	Proteocephalus pinguis	NOP	intestine
Nematoda	*Contracaecum sp.* (larvae)	BLC, WAE, WHB	mesentery, viscera
	Raphidascaris acus	YEP	mesentery, viscera

Internal Parasites.— At Devils Lake, we recovered several internal parasites representing the Classes Trematoda, Cestoidea, and Nematoda. The Digenea trematode *Diplostomum spathaceum* was recovered from the lens of the eye of fathead minnow (Figure 8). *D. spathaceum* was detected in 5.0% of fathead minnow examined. We found cysts containing *Posthodiplostomum sp.* from mesenteric and visceral tissues of black crappie (Figure 9) and fathead minnow (Figure 10). Goldeye collected from the Red River where host to the digenean trematode *Paurorhynchus hiodontis*. Gravid adult forms of *P. hiodontis* where observed in the abdominal cavity of two fish (Figure 11). During the survey we also recovered three parasites from the family Cestoidea. Mature forms of *Bothriocephalus cuspidatus* were found in walleye from Devils Lake (Figure 12). Additionally, metacestodes of *Bothriocephalus sp.* lacking mature proglottids were recovered from black crappie, fathead minnow and walleye (Figure 13). Other cestodes recovered during the survey at Devils Lake where *Proteocephalus pinguis* in northern pike (Figure 14) and *Ligula intestinalis* from fathead minnow. Larval forms of the nematode *Contracaecum sp.* was observed encysted in mesenteric tissues of numerous fish including black crappie, walleye, and white bass from Devils Lake, and black bullhead, tadpole madtom, and walleye from the Sheyenne River (Figure 15). Another nematode, presumptively identified as *Raphidascaris acus*, was found in yellow perch from Devils Lake (Figure 16 and 17). During examination of muscle tissues of 30 yellow perch, we did not observe any lesions that would suggest infection by the microsporidian parasite *Heterosporis sp.*

Figure 4.— Wet mount of *Trichodina sp.* observed in skin scrapings from walleye and yellow perch collected at Devils Lake.

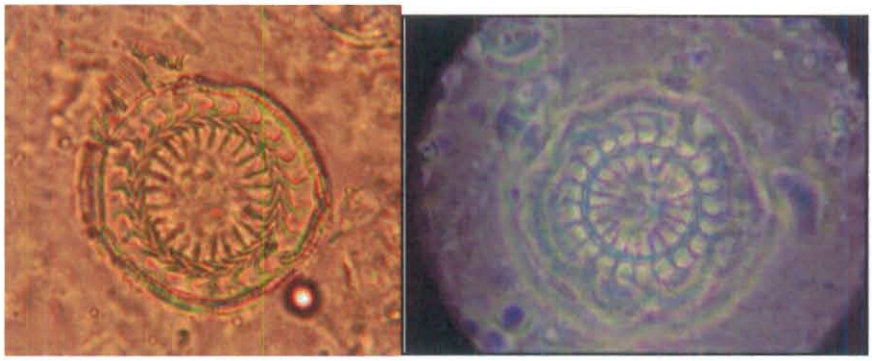


Figure 5.— Fresh wet mounts of *Gyrodactylus hoffmani* observed on the fins of fathead minnows from Devils Lake. Image on left shows parasite attachment near dorsal fin ray.

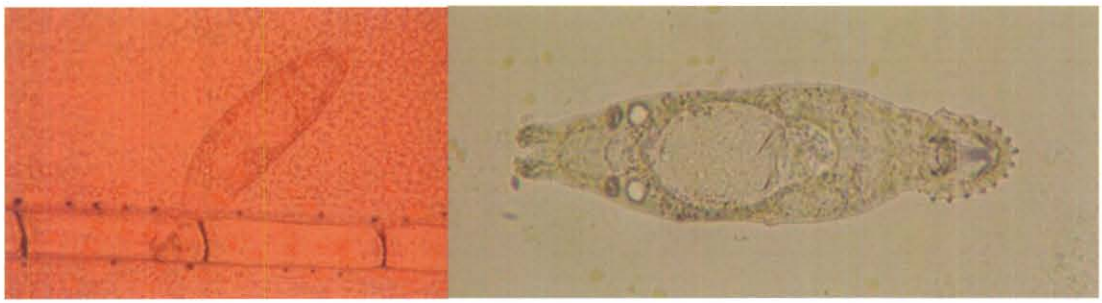


Figure 6.— Acetocarmine-stained images of *Gyrodactylus hoffmani* from fathead minnow.

Figure 7.— The parasitic leech *Piscicola punctata* attached to fins of channel catfish (left) from the Red River. Stained specimen on right.

Figure 8.— *Diplostomum spathaceum* in the lens of the eye of fathead minnow (left) and acetocarmine-stained lens with *D. spathaceum* (right).

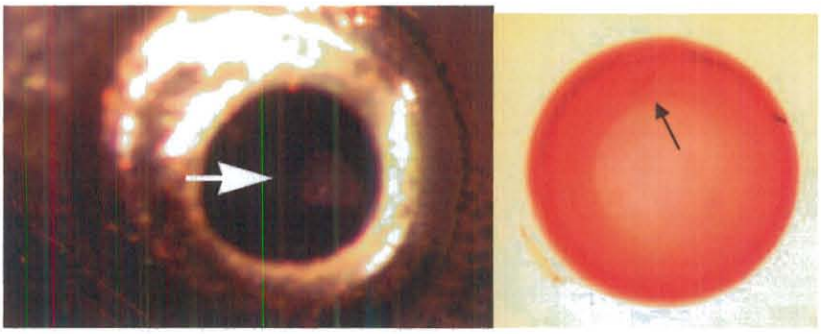


Figure 9.— Encysted metacercariae of *Posthodiplostomum sp.* recovered from black crappie.

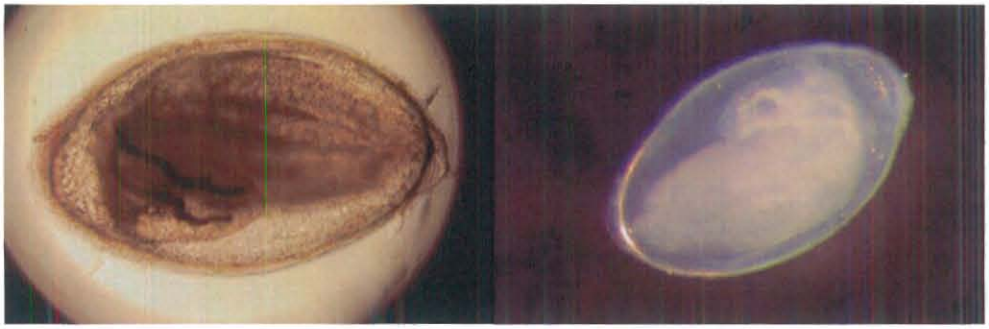


Figure 10.— *Neascus* of *Posthodiplostomum sp.* from fathead minnow.

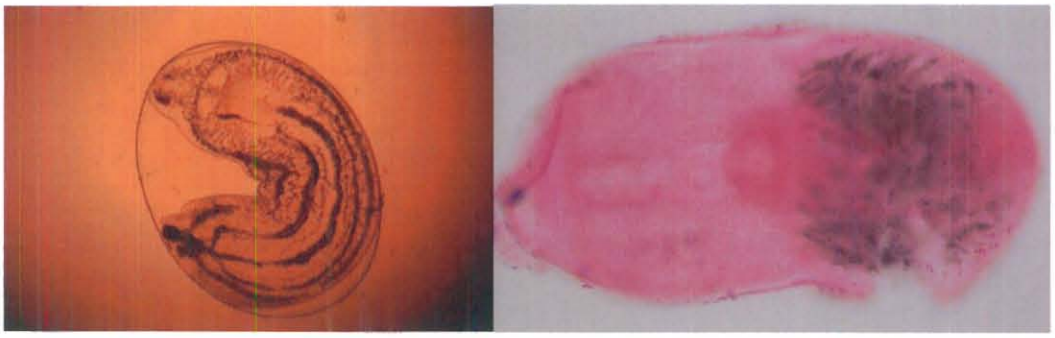


Figure .— 11. Stained and live images of gravid *Paurorhynchus hiodontis* found in the abdominal cavity of goldeye from the Red River.

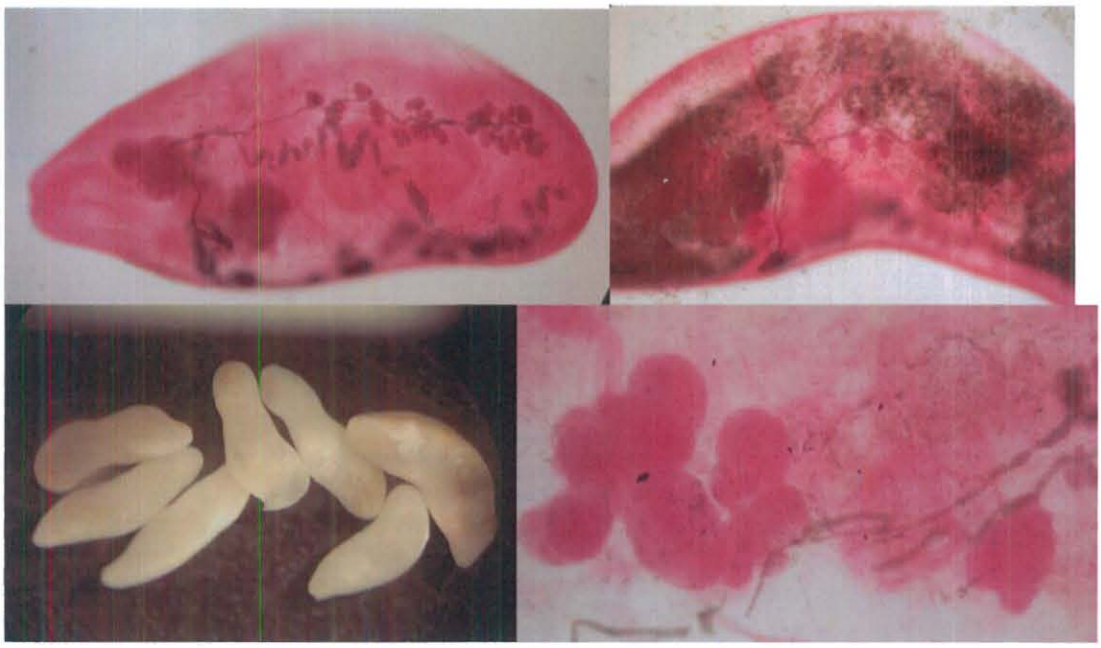


Figure 12.— Whole-mount stained specimens of mature *Bothriocephalus cuspidatus* from walleye (left), scolex stained with acetocarmine (center), and proglottids stained with hematoxylin (right).

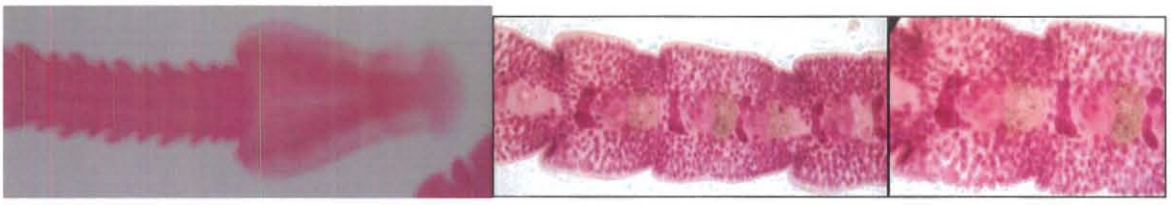


Figure 13.— Mature and immature forms of *Bothriocephalus sp.* observed in black crappie, fathead minnow and walleye from Devils Lake.

Figure 14.— Stained whole-mount of *Proteocephalus pinguis* recovered from northern pike.

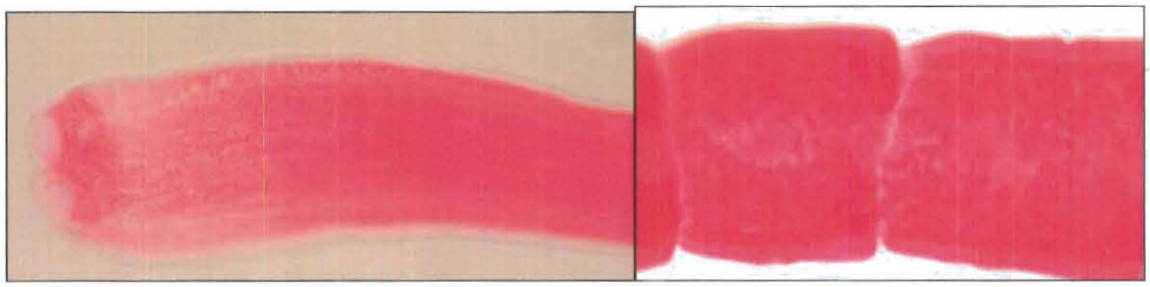


Figure 15.— Larval forms of *Contracaecum sp.* encysted in mesenteric tissue of black crappie (left) and after clearing in glycerin-alcohol and mounted in glycerin-jelly (right).

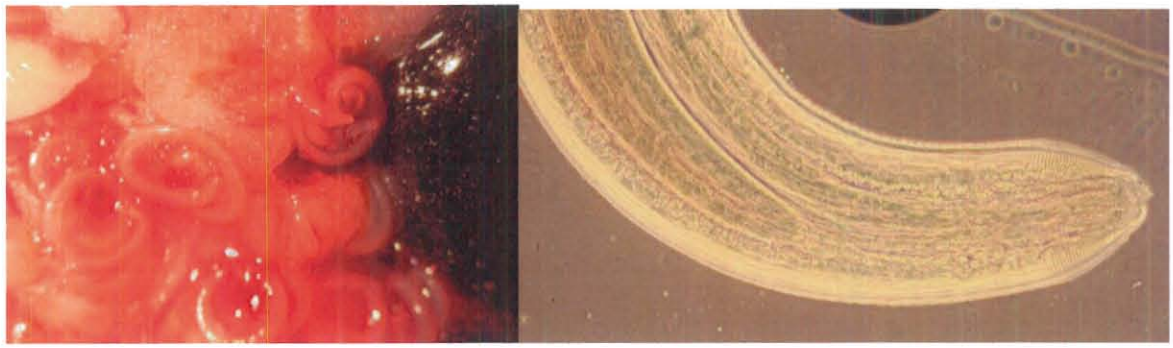
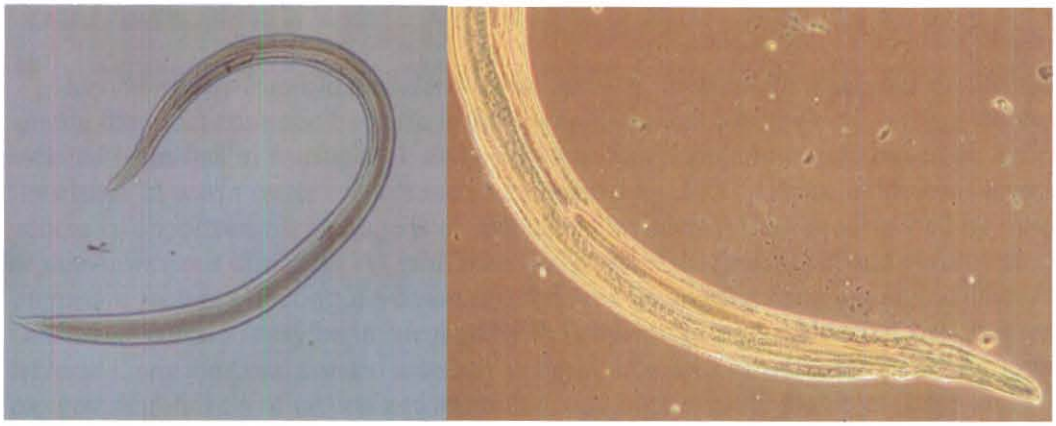


Figure 16.— Encysted larvae of *Raphidascaris acus* (presumptive) found in mesenteric tissues of yellow perch from Devils Lake.

Figure 17.— *Raphidascaris acus* (presumptive) removed from cyst.



Discussion

Sampling

During the fall of 2006, we examined 387 fish collected from Devils Lake, 78 fish from the Sheyenne River, and 72 fish from the Red River. The target sample size for most species was attained at Devils Lake although catch rates on the rivers was poor. Movement of fish in the rivers, an important factor affecting catch rates with stationary nets and traps, was likely affected by falling water temperatures and winter-like weather. Sampling rivers in the late-spring and early-summer when water temperatures are warmer and fish are more active would likely result in greater catch per unit effort.

Bacteria

During the surveys we isolated several environmental and opportunistic species of bacteria although we did not observe any clinical signs of bacterial disease. Larger numbers of bacteria were isolated from fish at Devils Lake compared to the rivers. Differences in water temperatures and sample size may have been factors in fewer species of bacteria being cultured from fish collected in the rivers. The majority of bacteria identified in fish samples from Devils Lake and the Red and Sheyenne rivers were species from families Aeromonadaceae, Enterobacteriaceae, and Pseudomonadaceae. These families are characterized as Gram-negative, aerobic or facultative anaerobic, rod-shaped bacteria, which are usually motile. Many are saprophytes and plant and animal parasites with worldwide distribution. 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 agricultural, poultry, cattle, and swine industries. 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 sufficiently stressed.

To the best of our knowledge, *R. salmoninarum* has not been isolated previously from fish in North Dakota. Antigen of *R. salmoninarum* was detected in previous surveys of the study although presence of the bacterium was not confirmed with PCR (Peters 2002, Hudson and Peters 2005). At Lake Sakakawea in western North Dakota, feral fall chinook salmon *Oncorhynchus tshawytscha* have been tested annually for *R. salmoninarum* by the direct fluorescent antibody technique (FAT) and no positive fish have been detected. A query of the *National Wild Fish Health Survey* database for *R. salmoninarum* and all fish species (1997 – 2005) shows numerous sample sites with inconclusive results (Figure 9). In our laboratory, we have examined several samples that were negative with PCR despite a wide range of positive ELISA OD values with antigen levels ranging from low to high. Most regions of the United States with fish populations positive for *R. salmoninarum* occur in areas with high densities of salmonids. These regions include the Pacific Northwest, Rocky Mountains, Great Lakes, and the Appalachian Mountains. According to North Dakota Game and Fish there are no salmonid fish in Devils Lake.

Virus

No viral fish pathogens were detected during the present survey. Likewise, no virus was detected in fish from these study areas during previous surveys conducted in 2001 - 2002 (Peters 2002) and in 2005 (Hudson and Peters 2005)

Parasites

Parasites identified in fish samples from the study areas are not unusual findings. Parasites found during this survey have been described previously from studies in North Dakota and or other areas in North America.

Protozoa

Trichodina sp.— *Trichodina sp.* was observed in skin scrapings from walleye and in skin scrapings and gill filaments from yellow perch. We observed *Trichodina sp.* previously from walleye, white bass, and yellow perch from Devils Lake (Hudson and Peters 2005). Trichodinids are mobile ciliates often found on gills, fins, and skin of many fish species. Trichodinids have low host specificity and are therefore widely distributed. Most families of freshwater fish harbor *Trichodina sp.* (Lom 1995, Hoffman 1999). They have also been reported from amphibians, as well as crustaceans, mollusks and coelenterates inhabiting both fresh and seawater (Schaperclaus 1991). In North America, they are frequently reported from perch, pike, sunfishes, and striped bass (Hoffman 1967 and 1978). According to Hoffman (1999), some *Trichodina* species are pathogenic. Transmission is direct when ciliates swim from one host to another (Lom 1995). Trichodinids do not occur in large numbers on healthy fish and hence irritation caused by attachment of their adhesive disc is negligible. Heavily infected fish may show denuded areas of the gill filaments and epithelial hyperplasia. *Trichodina* feed on newly produced cells and cell debris (Lom 1995).

Trematoda

Gyrodactylus hoffmani.— The mongenean trematode *G. hoffmani* was observed on fins of fathead minnow from Devils Lake. In a previous survey (Hudson and Peters 2005), the parasite was not present on fathead minnow even though fish were collected from the same area of the lake. We are uncertain whether time of year or water temperature influenced prevalence and therefore detection of *G. hoffmani* at Devils Lake. In 2005, fish were collected in July while in the present survey fish were collected in October. In goldfish, investigators reported higher numbers of Gyrodactylus and increased mortality when water temperatures were relatively cooler (Anthony 1969; Byhovskaya-Pavlovskaya 1962; Dogiel et al. 1958). According to Hoffman (1998), *Gyrodactylus* are very host specific in nature with the possible exception of *G. elegans*. Earlier reports of *G. hoffmani* from fathead minnow in the Midwest region include Mizelle and Kritsky (1967) and Holloway (1986) in North Dakota and Molnar et al. (1974) in Ontario, Canada.

Diplostomum spathaceum.— *Diplostomulum* (larval genus) of *Diplostomum spathaceum* are digenean flukes in which fish serve as the second intermediate host. The final host is usually a picsivorous bird. *D. spathaceum* were observed in the lens of eyes from fathead minnow collected at Devils Lake. This parasite is very common with worldwide distribution and does not show host specificity in fish. Fish may survive infection unaffected although there are several reports of *D. spathaceum* causing cataracts and blindness.

Posthodiplostomum sp.— Similar to Diplostomum, *Posthodiplostomum* are digenetic flukes that utilize fish as second intermediate hosts. Snails are first intermediate hosts and piscivorous birds serve as final hosts. *Posthodiplostomum* are also widely distributed in North American and around the world. At Devils Lake, *Posthodiplostomum* were observed encysted in the mesenteric tissues of black crappie and fathead minnow.

Paurorhynchus hiodontis.— *P. hiodontis* is an adult digenetic fluke in the family Bucephalidae. The anterior attachment organ (rhynchus) is weakly developed and ovary is opposite the superior testis. According to Hoffman (1999), the life cycle of these fragile trematodes is not presently known. *P. hiodontis* was observed in the body cavity of goldeye collected from the Red River. Previous reports of *P. hiodontis* include mooneye *Hiodon tergisus* from Lake Erie (Dickerman 1954), mooneye from Kentucky (Aliff 1977), and goldeye from Saskatchewan (Margolis 1964).

Cestoidea

Ligula intestinalis.— *L. intestinalis* is geographically ubiquitous, having been reported from all continents. They are not highly host-specific but can develop in a wide variety of copepods, fishes, birds, and mammals. *L. intestinalis* have been reported in numerous freshwater fish including sunfishes, suckers, basses, minnows, shiners, chubs, dace, bream, and many others. Second intermediate host fishes ingest infected copepods and the procercoid stage is released. The procercoid penetrates the intestinal wall and enters the body cavity, where development continues to the plerocercoid stage, which is consumed by piscivorous birds. *L. intestinalis* resides in the intestines of many species of piscivorous birds including gulls, terns, herons,

grebes, loons, and mergansers. *L. intestinalis* was identified in previous studies of fish from Devils Lake. In addition, there have been at least two reports of this cestode from surveys conducted after 1967 in North Dakota (Holloway and Hagstrom 1981; Reinisch 1981).

Proteocephalus pinguis.— *P. pinguis* cestode has been reported in numerous fish species including salmonids and esosids. Sutherland and Holloway (1979) reported *P. pinguis* in northern pike in North Dakota. Forstie and Holloway (1984) also identified this nematode in northern pike in North Dakota surveys of the James and Sheyenne Rivers, Jamestown Reservoir, and Lake Ashtabula. *P. pinguis* has also been found in white suckers from North Dakota by Holloway and Hagstrom (1981). The parasite was detected previously in Devils Lake surveys. This fish appears to be the definitive host in the life cycle with unknown first and second intermediate hosts.

Bothriocephalus cuspidatus.— This cestode is commonly found in the caeca and intestine of many warm water fish species. It has been documented to occur in over 28 fish species. It has been reported in fourteen states and two Canadian provinces (Hoffmann 1999). Sutherland and Holloway (1979) reported *B. cuspidatus* in fish species in North Dakota. The life cycle consists of an adult form in the intestine of fishes and a procercoid stage occurring in copepods. The 2005 survey identified this cestode in the intestine of walleye from Devils Lake.

Nematoda

Contracaecum sp.— Larval stages of these nematodes are often reported in many fish species. The *Contracaecum sp.* identified from Devils Lake walleye were larval therefore could not be identified to species. Sutherland and Holloway (1979) previously reported larval *Contracaecum sp.* in many fish species from North Dakota during a survey of parasites in fishes from the Missouri, James, Sheyenne, and Wild Rice Rivers. It has also been reported in rainbow trout, minnows, and sticklebacks in Manitoba (Dick et al.1987). Lockard and Parsons (1975) reported the nematode in paddlefish in Montana. Forstie and Holloway (1984) reported *Contracaecum sp.* in fish species from selected impoundments and river systems in North Dakota. The life cycle involves a crustacean as the first intermediate host and fish appear to be the second intermediate host (Hoffmann 1999). Some *Contracaecum* species can become pathogenic to fish.

Raphidascaris acus (presumptive).— We recovered the second or third larval stage of a nematode presumptively identified as *R. acus* from the visceral tissues of yellow perch from Devils Lake. Adult forms of *R. acus* are commonly found in piscivorous fish like northern pike. Hoffman (1999) summarizes numerous reports of this nematode from North America and Europe.

Leeches

Piscicola punctata.— *P. punctata* belongs to the family Piscicolidae and are considered the true fish leeches (Hoffman 1999). This leech is widely distributed in North America and Europe

and has been reported to parasitize a very broad range of hosts. *P. punctata* may serve as a mechanical vector for other disease agents including blood parasites, bacteria, and viruses.

Recommendations for Future Work

The present survey and two previous survey of the study areas were based on samples collected during late-summer and early-fall. Given the occurrence and prevalence of certain fish pathogens and parasites may be variably affected by several life history characteristics and elements of environment, especially those causing increased stress, future surveys should consider collecting sample in spring or early summer. It may be particularly important and interesting to examine fish either during or immediately following spawning activities. In the present survey, fish in Devils Lake were primarily collected from one sample areas. Previous surveys at Devils Lake focused sampling in similar areas. Future work should consider the merits of sampling additional areas of the lake. Sampling efforts should be coordinated with area fisheries biologists to identify key spawning and rearing habitat.

While an adequate sample size was obtained for most species at Devils Lake fewer fish were examined from the rivers to establish the presence or absence of fish pathogens with an appropriate level of confidence. Low catch rates in the rivers resulted from the use of stationary sampling gear (gill and fyke nets) during a period of falling water temperatures when movement of fish is slowed. Obtaining sufficient sample size can be a common problem with large field studies such as this. It can also be problematic when certain species are of low abundance or are not available because of limited seasonal distribution. Often times, prior information regarding species abundance and distribution are also limited. These elements are made particularly difficult during rapidly changing environmental conditions. Future fish pathogen surveys should identify and focus on species of greatest interest or importance. When appropriate, selection of species should also be related to the particular fish pathogens of concern.

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