INTERNATIONAL RED RIVER BOARD

INTERNATIONAL JOINT COMMISSION

Devils Lake - Red River Basin Fish Parasite and Pathogen Project

Qualitative Risk Assessment

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Prepared by the Aquatic Ecosystem Committee

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for

International Red River Board

and

International Joint Commission

October 2011

[1] U. S. Fish and Wildlife Service, Bozeman Fish Health Center, Bozeman, Montana, USA

[2] University of Manitoba, Winnipeg, Manitoba, Canada

[3] University of Guelph, Fish Pathology Laboratory, Department of Pathology, Ontario Veterinary College, Guelph, Ontario, Canada

[4] Department of Fisheries and Oceans, Winnipeg Fish Health Laboratory, Winnipeg, Manitoba, Canada

[5] Environment Canada, Regina, Saskatchewan, Canada

[6] U. S. Fish and Wildlife Service, Bismarck, North Dakota, USA

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Executive Summary

In 2005 the International Joint Commission requested that the International Red River Board investigate the risk that an outlet from Devils Lake in North Dakota would release invasive species and lethal fish parasites and pathogens into the Red River and Lake Winnipeg. Devils Lake supports an outstanding sport fishery and recreational industry valued at $56 million per year. The Red River in Canada and the USA supports a sport fishery mainly for channel catfish and walleye. In Canada, the Red River recreational fishery at $10-15 million is 10% of the total value of the recreational fishery for Manitoba. Lake Winnipeg supports the largest walleye commercial fishery in North America, with total annual revenues in excess of about $15 million (2005 statistics).

Devils Lake was a closed basin within the Hudson Bay watershed that probably has not been hydraulically connected into the Hudson Bay basin for more than 1000 years. However, water levels began to increase in Devils Lake in the 1940s and by 2010 had increased by more than 15.5 m (50.8 ft). By 2010, water levels were within 2 metres (6.6 ft) of the elevation of the natural outlet of Devils Lake, Tolna Coulee. In 2003, North Dakota began construction of an outlet with a discharge capacity of 7.1 m^3/s (250 ft^3/s) from Devils Lake to the Sheyenne River in the Hudson Bay Basin. Construction of the outlet was completed in 2005 and the outlet was first operated in 2006, connecting Devils Lake with the Hudson Bay basin for the first time in more than a millennium. Operation of the outlet was restricted to the open water period (April to November) with discharge depending on sulfate concentration and natural flows in the Sheyenne River. In 2006, less than 2.8 m^3/s (100 ft^3/s) was discharged from the outlet for a few days during summer. Full operation began in 2007 at less than the full capacity of the outlet. By 2010, the outlet was operated at near full capacity (7.1 m^3/s or 250 ft^3/s) throughout most of the open water period. The outlet was fitted with a course mesh screen at the inlet to prevent entry of large fish. This screen was followed by a gravel and rock filter constructed near an intake point at the lake.

In 2006, Canadian and USA fish pathologists initiated a three year study to identify fish parasites and pathogens of Devils Lake that were not found at downstream locations and therefore could pose a risk to downstream fish and fisheries. At total of 7 species and 1616 fish were collected

from Devils Lake and 21 species and 4272 fish from six other locations in the Red River Basin including the Red River Delta and Lake Winnipeg in Canada. The fish were examined externally and internally for parasites, and appropriate tissues were collected for standardized microbiological assays and histology. Microbiological assays were conducted on a targeted group of known fish pathogens including viral and bacterial agents as well as other selected microbes.

A total of 2 viral isolates, 29 bacterial isolates, 76 parasite taxa, and more than 40 different tissue-species specific lesions were detected from fish in the Red River Basin. Eight bacterial agents, no viral infections, and 25 parasites were found in Devils Lake. A similar community of parasites was found both macroscopically and microscopically in fish collected from Devils Lake and elsewhere in the Red River Basin. However, one parasite (a gryporhynchid larval tapeworm), three bacterial agents (*Pseudomonas mendocina, Yokenella regensburgei, Brevundimonas diminuta*) and 17 tissue-species specific lesions were identified only from fish collected from Devils Lake. None of these organisms or lesions was found at downstream locations (Lake Ashtabula, Red River (ND), Lake Traverse, or the Lake Winnipeg – Red River Delta area).

The qualitative risk of disease to fish from the single parasite, three bacterial agents, and the lesions found in Devils Lake but not downstream was evaluated by four Canadian and four USA fish pathologists. These eight experts were asked to address the following five questions and rank risk from the above agents to downstream fish and fish communities where appropriate.

1. Based on the life histories and life cycles of the parasites, pathogens and histopathology anomalies found in Devils Lake and not found downstream in USA and Canada, what are the mechanisms for transport from Devils Lake to other aquatic ecosystems in the Red River basin and Lake Winnipeg.

The probability of transport of parasites and bacteria by discharge through the constructed outlet, and by discharge through the natural outlet (should water levels continue to increase) and by

birds was judged to be high by the fish pathologists. Intentional or unintentional transport by human-related vectors of fish and their associated parasites was judged to be low by the fish pathologists.

2. Using your best professional judgment and best available scientific information what environmental factors are known to trigger a disease outbreak for the identified parasites, pathogens and lesions found in Devils Lake and not downstream?

Environmental factors and variables that could trigger disease outbreak from those parasites and bacterial agents that were present in Devils Lake and elsewhere in the Red River basin included high densities of fish, high intensity of infection, and stress from low dissolved oxygen, high levels of carbon dioxide and ammonia, elevated temperature and toxic substances. Eutrophication of Lake Winnipeg could also promote disease outbreak in fish in that lake. Invasion of exotic fish or parasites could modify a local food web and change the population dynamics which could lead to a disease outbreak.

3. Using your best professional judgment, best available scientific information, and considering environmental triggers, if parasites, pathogens, and histopathological lesions and tumors found in Devils Lake and not found downstream were transferred elsewhere to the basin, what risk to downstream fish and fisheries could these organisms pose and what is their likelihood of causing disease?

The non-targeted bacterial agents found in Devils Lake but not elsewhere in the Red River basin were *Pseudomonas mendocina, Yokenella regensburgei* and *Brevundimonas diminuta.* The fish pathologists agreed that the risk to downstream fish and fisheries from these three bacteria was low, and the likelihood of any of them causing disease was judged to be unlikely.

A single gryporhynchid metacestode parasite was found in Devils Lake but not elsewhere in the Red River basin. The risk to downstream fish and fisheries from this organism was judged to be low because it was rare and because the literature suggests that gryporhynchids do not compromise fish health. Therefore, the potential to cause disease was judged to be unlikely.

Of the 17 tissue specific lesions identified from Devils Lake fish, most were caused by myxosporeans and protozoans. The risk of transfer of these organisms from Devils Lake is high and the potential for infection of fish by these organisms downstream of Devils Lake was also high. Overall, however, the fish pathologists agreed that the risk to downstream fish and fish communities from the myxosporeans and protozoans identified by histology was low, and the probability of causing disease was unlikely.

4. For those fish parasite, pathogens, histopathological lesions and tumors found in Devils Lake but not elsewhere in the basin, what is the North American distribution of these parasites, pathogens, and lesions, and what is their relative abundance elsewhere in North America?

The bacterial agents found in Devils Lake but not elsewhere in the Red River basin included *Pseudomonas mendocina, Yokenella regensburgei* and *Brevundimonas diminuta.* These bacteria are ubiquitous in soil and water throughout North America. The fish pathologists agreed that they are probably present in the aquatic ecosystems downstream from Devils Lake.

The single gryporhynchid metacestode found in a single yellow perch from Devils Lake could not be identified to species and thus the exact geographical distribution of this organism could not be determined. It was hypothesized that this family of parasites is likely to be more widely distributed than the present scientific literature suggests. Furthermore, there is no evidence of this parasite family compromising fish health. Thus, it is unlikely that this parasite would pose any significant risk to fish in the Red River Basin in the future.

The 17 tissue-specific lesions identified from Devils Lake fish and not found downstream in the Red River Delta were caused by myxosporeans, sporozoans, protozoans, nematodes, and a leech. All were widespread in North America and either common or abundant. Therefore, it is unlikely that these parasites would pose any additional risk to fish in the Red River Basin if they were transported from Devils Lake by any one of multiple mechanisms or vectors. Seven myxosporeans could not be identified to genus, and therefore information on distribution and risk could not be determined by the fish pathologists. Identification of all parasites including myxosporeans to genus is difficult or impossible with histology because the technique does not provide the morphological detail required to provide taxonomic resolution.

5. The existing outlet from Devils Lake is equipped with a combination of screens, rock, and gravel filter intended to prevent the downstream transfer of fish. Given the life histories of parasites and pathogens in Devils Lake, does this mitigation measure reduce the impacts for potential of transfer of these organisms to downstream ecosystems?

The design of the existing filter was intended to reduce the risk of adult fish being released to the downstream environment. It was not designed to reduce risks associated with transfer of smaller organisms such as larval fish, bacteria, and free-living life stages of parasites. There are multiple pathways of transport of the bacteria and parasites found in Devils Lake to other locations in the Hudson Bay Basin.

Background

Construction and operation of the outlet from Devils Lake connects a closed basin in North Dakota to the Hudson Bay drainage. The outlet flows into the Sheyenne River and then the Red River which are southern watersheds in the Hudson Bay basin (Fig. 1). The outlet could potentially transfer fish parasites and pathogens from Devils Lake into the Hudson Bay drainage to the detriment of fish populations in that basin. Of special concern are the commercial and sport fish populations in the Red River and Lake Winnipeg that are of economic and recreational value.

Governments agreed in August 2005 that additional, basin-wide scientifically-sound information on aquatic invasive species focusing on fish parasites and pathogens was needed for the Red River Basin. Governments requested this work be undertaken by the International Joint Commission's International Red River Board.

The objectives were developed by the International Red River Board's Aquatic Ecosystem Committee to provide direction to the three year monitoring program. These objectives were as follows:

(1) Determine the presence/absence of fish parasites and pathogens in resident fish from Devils Lake, the Sheyenne River, Red River, and Lake Winnipeg building on previous work (*e.g.* Reinisch 1981, Forstie and Holloway 1984, Peters 2002, Arroyo 2005, Hudson and Peters 2005, Williamson *et al*. 2005).

(2) Provide a scientifically credible survey of fish parasites and pathogens in fish from Devils Lake, Sheyenne River, Red River and the Red River Delta. The data can be used to perform a risk analysis associated with the transfer of fish parasites and pathogens through the outlets on Devils Lake to aquatic ecosystems in the Red River Basin including Lake Winnipeg.

(3) Use the survey of fish collected during this proposed survey to meet the overall framework for biological monitoring in the Red River Basin that is included in the "Work Plan" of the International Red River Board.

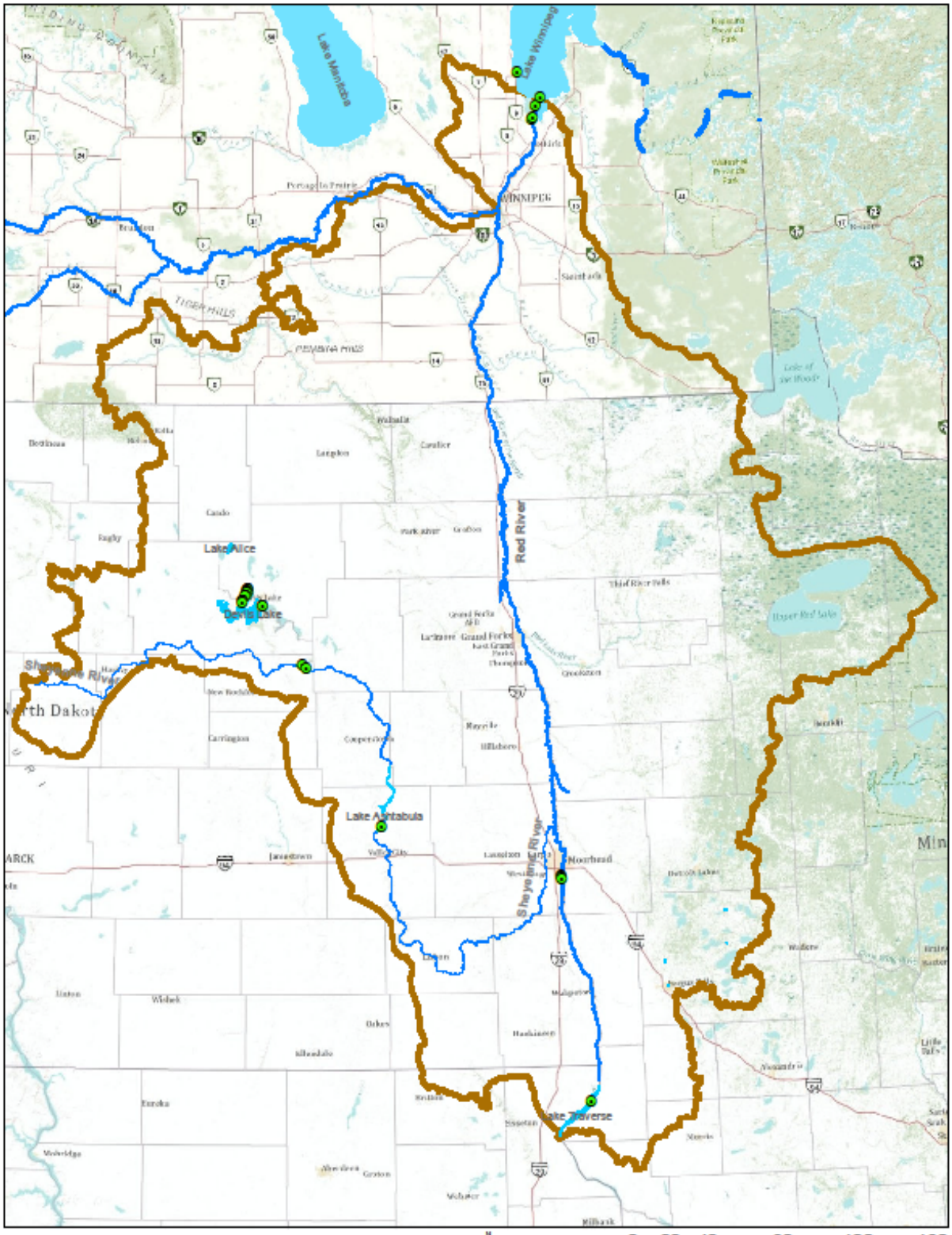
The co-chairs of the Aquatic Ecosystem Committee of the International Red River Board assembled an *Ad Hoc* Group of Experts to scope out a valid and scientifically defensible parasite and pathogen sampling program for Devils Lake and the Red River basin including Lake Winnipeg.

The *Ad Hoc* Group of Experts identified the following principles to guide the work:

(1) Fish in the United States would be collected and analyzed by United States Fish and Wildlife Service. Fish in Canada will be collected and analyzed by Fisheries and Oceans Canada and Canadian universities.

(2) Laboratories in Canada and the United States would use equivalent methods and analytical approaches to determine the diversity of pathogens and parasites in fish. As soon as the project is approved and funded in Canada and the United States, participating laboratories will meet to ensure methods are consistent for fish samples collected on both sides of the international boundary.

In August 2006, the *Ad Hoc* Group of Experts met in Winnipeg, Manitoba to discuss specific field and laboratory methods for collection, preservation, identification and analysis of fish parasites and pathogens. Although it was recognized at the outset that each country would use slightly different methods, the methods were considered equivalent and the results would be comparable and compatible. Both countries agreed to start sampling in the fall of 2006. In 2007 and 2008, fish were collected during the months of June and July to address the question of seasonality. Specifically, the researchers wanted to determine whether warmer water temperatures and spawning stress could significantly alter the bacterial and viral findings reported in the fall 2006 survey. A sample size of 60 individuals from each species was targeted. The assessment focused on five areas: parasitology, histopathology, bacteriology, and virology,



Location of Sampling Sites
Red River Basin (2006 - 2008)

as well as the risk of transfer of these organisms between water bodies. The five pathogens (and related disease) that were known to compromise fish health in other lakes and rivers were included in the study. These were:

Aeromonas salmonicida (furunculosis)

Yersinia ruckeri (enteric redmouth disease)

Edwardsiella tarda (edwardsiellosis)

Edwardsiella ictaluri (enteric septicemia)

Renibacterium salmoninarum (bacterial kidney disease).

The first year of sampling began in the fall of 2006. Samples were collected from the Devils Lake, the Sheyenne River, Red River, and Lake Winnipeg (Fig 1). In following years, samples were also collected from Lake Ashtabula and Lake Traverse because these lakes were known to have an abundant and diverse fish community. Six fish species were targeted for microbiology, histology, and parasite assessment in both USA and Canada. These six species were walleye, white bass, yellow perch, northern pike, fathead minnow, and brook stickleback. Except for brook stickleback, these species are relatively abundant in Devils Lake.

Methods

Methods - United States

In the USA, fish were collected from Devils Lake, Sheyenne River, Red River, Lake Ashtabula, and Lake Traverse (Fig. 1). A standard target sample size of 60 fish for each species was used to determine the presence or absence of bacterial and viral fish pathogens (Table 1). This widely accepted sample size provides a 95% confidence level that an infected fish will be detected given a 5% presumed prevalence of infection within a population of ≥ 2000 or more individuals (Ossiander and Wedemeyer 1973).

At Devils Lake, fish were sampled from two areas between September 25 and 29, 2006 (Peters and Hudson 2007). The primary sampling 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 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 (38.1 m X 1.8 m with 5 panels

Table 1. Number of fish (and number of fish species) assessed for bacteria, virus, parasites, and histology in USA and Canada. ND = no data.

2006			
Virus	Bacteria	Parasites	Histology
United States			
Devils Lake			
387 (7)	387 (7)	237 (7)	ND
Red River			
72 (6)	72 (6)	72 (6)	ND
Sheyenne River			
78 (5)	75 (5)	78 (5)	ND
Lake Traverse			
ND	ND	ND	ND
Lake Ashtabula			
ND	ND	ND	ND
Canada			
Red River Delta and Lake Winnipeg			
547 (10)	547 (10)	302 (9)	547 (10)
2007			
Virus	Bacteria	Parasites	Histology
United States			
Devils Lake			
289 (7)	139 (7)	407 (7)	289 (7)
Red River			
392 (22)	236 (22)	392 (22)	ND
Sheyenne River			
100 (13)	54 (13)	100 (13)	ND
Lake Traverse			
550 (18)	263 (16)	674 (19)	ND
Lake Ashtabula			
ND	ND	ND	ND
Canada			
Red River Delta and Lake Winnipeg			
511 (9)	511 (9)	299 (10)	511 (9)
2008-09			
Virus	Bacteria	Parasites	Histology
United States			
Devils Lake			
292 (7)	292 (7)	347 (6)	294 (7)
Red River			
ND	ND	ND	ND
Sheyenne River			
ND	ND	ND	ND
Lake Traverse			
522 (15)	522 (15)	596 (17)	ND
Lake Ashtabula			
390 (10)	390 (10)	435 (14)	ND
Canada			
Red River Delta and Lake Winnipeg			
583 (10)	583 (10)	666 (9)	ND

incorporating 1.9, 2.5, 3.8, 4.4, 5.1 cm mesh sizes); 2) 300 ft X 6 ft with 3 panels of 3, 4, and 5 inch mesh (91.4 m X 1.8 m with 3 panels of 7.6, 10.2, and 12.7 cm mesh). Gill nets were checked in 1 to 3 hour 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 (6.4 and 12.7 mm mesh). Nets with ¼ inch (6.4 mm) 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 alive in large totes or in boxes with lake water until examined.

On October 11, 2006, fish were collected from the Sheyenne River along a 0.5 km (0.3 mile) reach up and downstream from the bridge on State Highway 20. The reach extended along the south-eastern border of the Spirit Lake Nation. Fish were collected from the Red River on 12 – 13 October 2006 in a 2.0 km (1.2 miles) reach upstream of the bridge at 52nd Avenue south in Fargo, North Dakota. Sampling gear was composed of 38.1 m (125 ft) of multi-mesh gill nets similar to those used on Devils Lake. In addition, modified fyke nets were deployed with hoop nets with 3.8 cm (1.5 inch) mesh. Nets and traps were set for 18 – 24 hour intervals. 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 and processed during the same day as capture.

In 2007, collections occurred at Devils Lake, Lake Ashtabula (near Valley City, North Dakota), Lake Traverse (near Sisseton, South Dakota) and the Red River (near Fargo, North Dakota) using standard procedures as described in the U.S. Fish and Wildlife Service's Wild Fish Health Survey (USFWS 2009). Additional samples from fish were collected from Devils Lake, Lake Ashtabula, and Lake Traverse in 2008 (Peters 2011). One sampling site in 2008 differed from that sampled in 2007: Lake Ashtabula replaced a sampling site on the Sheyenne River. Also, Lake Traverse was added as a sampling site in 2007 and 2008, and the Red River was not sampled in 2008. The list of fish species collected from North Dakota, Red River Delta and Lake Winnipeg is shown in Table 2.

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For necropsy, fish were anesthetized with tricaine methanesulfonate (Finquel®), weighed (g) and measured (total length, mm), and then examined externally and internally for clinical signs of disease or other abnormalities. Tissue 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 (U.S.FWS, Bozeman, Montana) or the La Crosse Fish Health Center (U.S.FWS, 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 regulated or managed organisms in most state and federal fish health inspection programs. Details of laboratory procedures are available at http://wildfishsurvey.fws.gov.

Thirty fish of each species were randomly selected at Devils Lake to perform a comprehensive parasite evaluation. 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 gastrointestinal 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. Tissue smears were prepared 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. Wet mounts, tissue smears, and gut contents were examined 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.

Methods - Canada

Ten fish species were captured from the lower Red River and south basin of Lake Winnipeg (Table 1). Fish were captured using several methods including seines, gill nets and electro-shocking. Smaller fish were placed in sealed plastic bags, placed on crushed ice in coolers then transported to the Winnipeg Fish Health Laboratory (Fisheries and Oceans Canada). Larger fish were placed on ice in coolers and transported to the lab. The majority of the fish were processed for virology, bacteriology and histopathology within 10 hours of the time of capture. Several smaller species such as fathead minnow, yellow perch, white bass and brook sticklebacks were kept alive and necropsied the morning after their capture.

For parasitology, each fish was placed in a separate plastic bag identified by fish species, site and date of collection. The samples were frozen for full necropsies at a later date. Each fish was weighed and length (fork, total and standard) was recorded. Sex and state of maturity was also recorded. Each fish was necropsied and the following organs checked: external body surface, gills, nares, buccal area, eyes, muscle, body cavity, esophagus, stomach, caecae, intestine, reproductive structures, heart, swim bladder, spleen and liver. Fresh tissues smears were not possible as all samples were frozen immediately after capture but smears were taken from frozen material whenever possible.

Additional fathead minnows were examined to determine if the mongenean *Gyrodactylus hoffmani* was present in the Red River Basin in Canada as it was initially identified as a species of interest. Furthermore, after the unanticipated discovery of *Bothriocephalus acheilognathi* in the year 1 survey, additional samples of emerald shiners were collected to determine the geographical extent of this invasive and pathogenic cestode. Complete necropsies were done on a total of 1279 fish collected in Canadian waters.

All parasites were identified to at least genus and enumerated. Those parasite species not identified to species were either immature or required freshly fixed material rather than specimens from frozen samples.

For microbiology and histology, 10 species were targeted (walleye, sauger, northern pike, channel catfish, white bass, emerald shiners, fathead minnows, brook sticklebacks, goldeye and yellow perch), with 60 fish per species collected. Fish were collected from several sites on the main stem of the Red River north of Selkirk, the south basin of Lake Winnipeg, two tributaries of the Red River (Netley Creek and Wavey Creek), and Willow Creek which drains into the south basin of Lake Winnipeg in the vicinity of Sandy Hook. Sampling commenced on 10 October 2006 and ended 26 October 2006. Fish were collected at these same general locations in 2007.

Sixty fish samples were collected for 9 of 10 species. Only 7 channel catfish were captured. A total of 547 fish were screened for bacterial and viral pathogens of concern. Sixty lake whitefish, obtained from a commercial fisher, were screened for the myxosporean parasite *Myxobolus cerebralis* (causative agent of whirling disease in salmonids).

Testing for the presence viral pathogens involved the use of three cell lines, chinook salmon embryo (CHSE), epithelioma papulosum cyprini (EPC) and Channel Catfish ovary (CCO). The viral testing method used in the Winnipeg Fish Health Lab was that described in the Fish Health Protection Regulations: Manual of Compliance (FHPR: m of c) with the exception that Brain Heart Infusion Agar (BHIA) was substituted for Tryptic Soy Agar (TSA) for initial isolation and culture purification. A commercially produced bacterial identification system, API-20E was used to identify selected representative bacterial isolates. These methods differed slightly from the method described in National Wild Fish Health Survey procedure used by the U.S.FWS, but both methods are considered equivalent. Bacterial isolation and identification methods were also similar.

The method used for detection of *Myxobolus cerebralis* was the cranial digest method using pepsin-hydrochloric acid as described in the FHPR: m of c with the exception that cranial digests

were prepared from five fish pools (12 pools total) and not a single 60 fish pool. Kidney smears prepared from all 547 fish were stained using the Indirect Fluorescent Antibody Technique (IFAT) and examined microscopically for the presence of *Renibacterium salmoninarum* (causative agent of Bacterial Kidney Disease).

The methods used for *R. salmoninarum* detection in the respective labs were different. Therefore, to compare the results obtained in each lab, material was exchanged and processed using the *R. salmoninarum* detection method used in each lab. Kidney/spleen tissue harvested from 59 fish representing 7 species was sent to Bozeman, and the WFHL received kidney smears from 46 fish representing 7 species. Four of the seven species were the same (northern pike, walleye, white bass and yellow perch).

Table 2. Alphabetical listing of common and scientific names of fish species captured in the study from 2006 to 2008 from both the United States and Canada.

Common name	ACRONYM	Genus and Species
Black Bullhead	BLBL	*Ameiurus melas*
Black Crappie	BLCR	*Pomoxis nigromaculatus*
Brook Stickleback	BRST	*Culaea inconstans*
Common Carp	CMCP	*Cyprinus carpio*
Channel Catfish	CHCT	*Ictalurus punctatus*
Emerald Shiner	EMSH	*Notropis atherinoides*
Fathead Minnow	FTMN	*Pimephlales promelas*
Freshwater Drum	FRDR	*Aplodinotus grunniens*
Goldeye	GOLD	*Hiodon alosoides*
Green Sunfish	GRSN	*Lepomis cyanellus*
Mooneye	MOON	*Hiodon tergisus*
Northern Pike	NRPK	*Esox lucius*
Sauger	SAUG	*Sander canadensis*
Smallmouth Bass	SMBS	*Micropterus dolomieu*
Shorthead Redhorse	SLRD	*Moxostoma macrolepidotum*
Stonecat	STON	*Noturus flavus*
Tadpole Madtom	TDMD	*Noturus gyrinus*
Walleye	WALL	*Sander vitreum*
White Sucker	WHSC	*Catostomus commersonii*
White Bass	WHBS	*Morone chrysops*
Yellow Perch	YLPR	*Perca flavescens*

Results and Discussion

Fish Species of Devils Lake

A total of nine fish species occur in Devils Lake including walleye, northern pike, white bass, yellow perch, black crappie, white sucker, fathead minnow, black bullhead and brook stickleback. All these species are known to occur elsewhere in the Hudson Bay basin. During the present study, 1,616 fish were collected over three years. These fish were collected at multiple locations by two methods using a range of mesh of small and large mesh size in the sampling gear. These collecting methods and the large number of fish collected suggest that probably all of the fish species present in Devils Lake have been captured and identified either during previous fish surveys of Devils Lake or during the present parasite and pathogen project. No invasive or foreign fish species have been found in Devils Lake either prior to or during the parasite and pathogen project. However, white bass were not native to the Hudson Bay Basin, but are now widely distributed especially in the Red River subbasin. During the present study, white bass were collected from Devils Lake, Lake Traverse, Lake Ashtabula, and the Red River Delta.

Virology

Over the course of the survey, 1641 fish from Manitoba waters and 3072 fish from North Dakota waters including 968 fish from Devils Lake were screened for the targeted viral pathogens (Table 3). All fish tested were found to be negative for the targeted viral pathogens using the cell culture methods employed. There were no viral agents or viral lesions detected in fish from Devils Lake or elsewhere in the Red River basin by either direct assessment (CHSE, EPC, and CCO cell lines used by both USFWS and DFO). The targeted viral agents were infectious pancreatic necrosis virus, infectious hematopoetic necrosis virus, viral hemorrhagic septicaemia virus, and channel catfish virus. However, two viral lesions, lymphocystis and dermal sarcoma, were detected in walleye from the Red River Delta using histology.

Table 3. Summary of the Virology Test Results for 2006 to 2008 Compiled by the Winnipeg Fish Health Laboratory and the Bozeman Fish Health Center

	Winnipeg Fish Health Laboratory			Bozeman Fish Health Center		
Year	**# of Fish**	**# of Pools**	**Virus Detected (+/-)**	**# of Fish**	**# of Pools**	**Virus Detected (+/-)**
2006	**547**	**116**	**0+**	**537**	**115**	**0+**
2007	**511**	**117**	**0+**	**1331**	**287**	**0+**
2008	**583**	**127**	**0+**	**1204**	**246**	**0+**
Totals	**1641**	**360**	**0+**	**3072**	**648**	**0+**

Bacteria

From the surveys conducted in both Manitoba and North Dakota waters, a total of 29 species of bacteria were identified (Table 4). Of the 29, eight species were identified in fish collected in both surveys. Another eight species were identified in fish from Manitoba waters, but not in fish collected from North Dakota waters. The remaining 13 bacteria were detected in fish from North Dakota waters but not in fish downstream in Manitoba waters. Of these13 bacteria, three were cultured from fish from Devils Lake, and they were not detected in fish from any downstream waters. The three isolates included *Psuedomonas mendocina* from fathead minnow, *Yokenella regensburgii* from fathead minnow and northern pike, and *Brevundimonas diminuta* from northern pike. These three environmental bacteria were isolated in 2006 but not in other years.

In both Canada and the USA five bacterial pathogens were specifically targeted for their presence in fish (targeted pathogens). These important bacterial pathogens were not detected in Devils Lake during the study although three of the five were detected elsewhere in the Red River Basin. The five targeted pathogens (and related desease) included:

Aeromonas salmonicida (furunculosis)

Yersinia ruckeri (enteric redmouth disease)

Table 4. Summary of bacteriology results for Manitoba, Canada and the North Dakota, USA. Manitoba waters were Red River (RR), Netley Creek (NC), Wavey Creek (WC), Willow Creek (WK), and Lake Winnipeg (LW). North Dakota waters were Devils Lake (DL), Sheyenne River (SR), Red River (RR), Lake Traverse (LT), and Lake Ashtabula (LA).

	Bacteria detected (Yes / No)	
Identified bacteria	Manitoba waters	North Dakota waters
Aeromonas hydrophila	Yes	Yes (DL, SR, LT, RR)
Hafnia alvei	Yes	Yes (DL, SR, RR, LT)
Pseudomonas fluorescens	Yes	Yes (DL, SR, RR)
Plesiomonas shigelloides	Yes	Yes (SR, RR)
Acintobacter lwoffi	Yes	Yes (DL)
Edwardsiella tarda	Yes	Yes (LT)
Pseudomonas maltophilia	Yes	Yes (RR, LT)
Pseudomonas sp.	Yes	Yes (LT)
Pasteurella - Acintobacter	Yes	Yes (RR)
Yersinia ruckeri	No	Yes (LT)
Pantoea sp.	No	Yes (RR, LT)
Pseudomonas mendocina	**No**	**Yes (DL)**
Yokenella regensburgei	**No**	**Yes (DL)**
Brevundimonas diminuta	**No**	**Yes (DL)**
Shewanella putrefaciens	No	Yes (DL, LA)
Enterobacter sp.	No	Yes (LT)
Enterobacter cloacea	No	Yes (RR, SR)
Erwinia sp.	No	Yes (LT)
Citrobacter sp.	No	Yes (RR, SR)
Citrobacter freundii	No	Yes (SR)
Myroides sp.	No	Yes (LT, LA)
Salmonella cholerasuis-arizonae	No	Yes (SR)
Pseudomonas cepacia	Yes	No
Flavobacterium sp.	Yes	No
Enterobacter agglomerans	Yes	No
Alcalignes sp.	Yes	No
Klebsiella oxytoca	Yes	No
Pseudomonas paucimobilia	Yes	No
Pseudomonas aeruginosa	Yes	No

Edwardsiella tarda (edwardsiellosis)
Edwardsiella ictaluri (enteric septicemia)
Renibacterium salmoninarum (bacterial kidney disease).

Edwardsiella tarda was detected in the liver of one northern pike and one channel catfish collected from the Red River Delta in 2007. Both cases represent asymptomatic infections as no clinical evidence of infection attributable to this pathogen was evident in either fish. *Edwardsiella tarda* was also detected in black crappie and channel catfish from Lake Traverse in 2007, and *Yersinia ruckeri* was also found in black crappie from Lake Traverse.

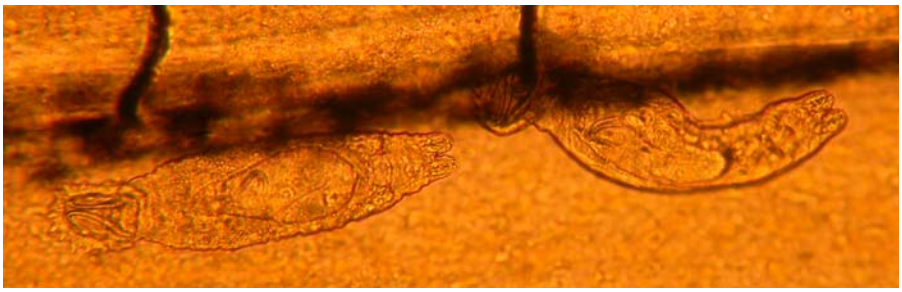
Parasites

A total of 76 parasite taxa were identified from the Red River Basin (Table 5). Twenty-five of the 76 taxa were found in Devils Lake, but only one parasite (a gryporhynchid metacestode) was found in yellow perch from Devils Lake and no other location in the Red River Basin (Table 5 and 6). Devils Lake had fewer parasite species than other locations assessed during the present study probably because the lake has few fish species (7 total) and has only supported fish since 1958. Sports fish have been stocked into Devil Lake, and some species may have been transferred into the Lake as bait or perhaps by overland flooding from the Pembina River under high water and high flow conditions. Lake Traverse, by comparison has more than 20 fish species of fish, and the 20 that were examined for parasites supported 64 different parasite taxa. In Lake Traverse, fathead minnow had the most diverse parasite fauna, with nineteen different parasite species in total.

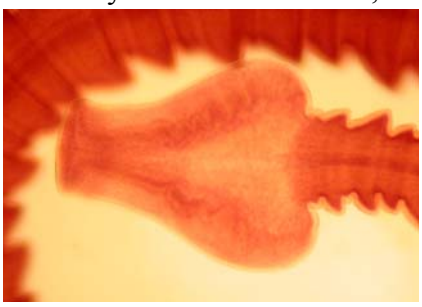
A parasitic pathogen, *Myxobolus cerebralis* (whirling disease) has devastated salmonid populations in western rivers in the inter-mountain region of the United States. Whirling disease was not detected in Devils Lake nor in lake whitefish from Lake Winnipeg. The absence of this pathogen in Devils Lake was expected because whirling disease is only associated with fish species from the family Salmonidae which includes lake whitefish. Salmonid species were not present in Devils Lake.

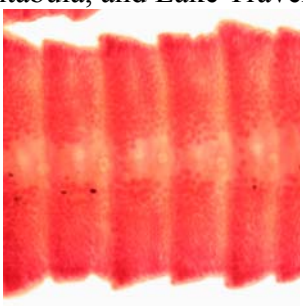
Parasite Photomicrographs

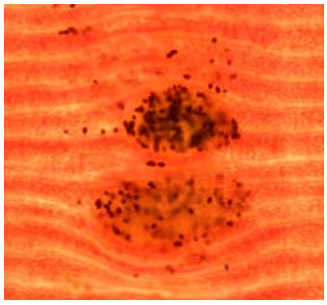
Photomicrograph of wet mount preparation with *Gyrodactylus hoffmani* anchored near dorsal fin ray of fathead minnow from Devils Lake. The parasite was also observed on fathead minnow from Lake Traverse.



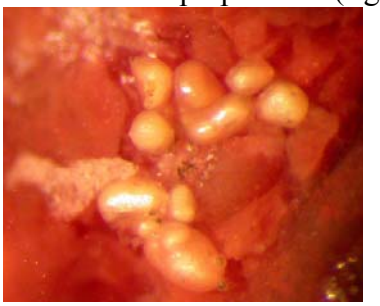
Photomicrographs of *Bothriocephalus cuspidatus* with scolex (left), proglottids (center), and gravid posterior proglottid (right; acetocarmine). Specimens commonly found in the intestines of walleye from Devils Lake, Lake Ashtabula, and Lake Traverse.



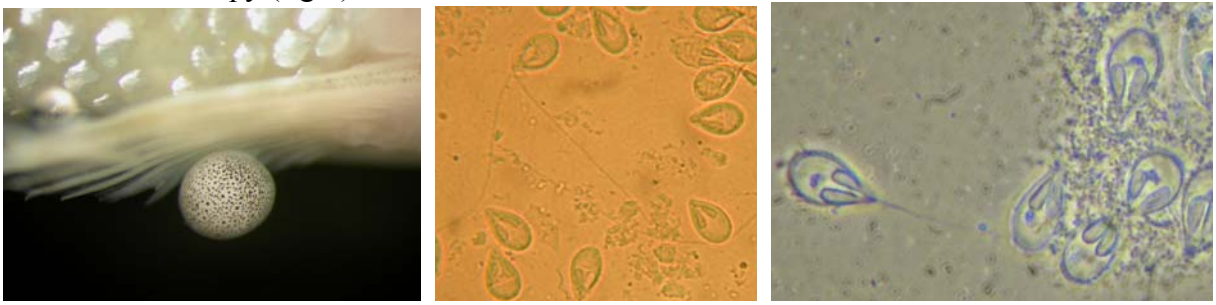




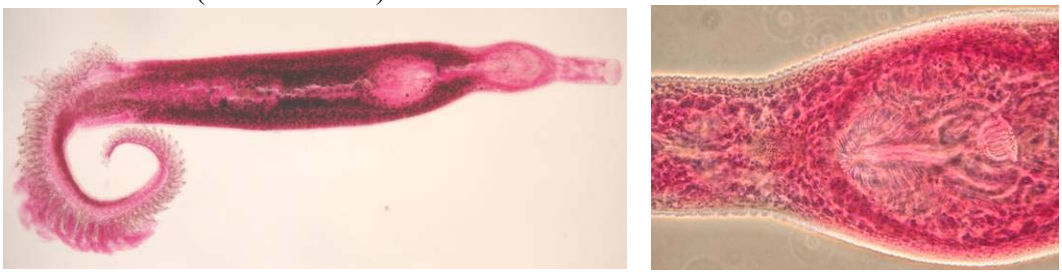
Photomicrographs of *Henneguya sp*. cysts in the kidney of freshwater drum (left) and spore in wet mount preparation (right) from Lake Traverse.



Photomicrographs of *Myxobolus sp*. cyst on the pectoral fin of a fathead minnow from Lake Traverse (left) and wet mount of spores observed with bright field microscopy (center) and phase contrast microscopy (right).

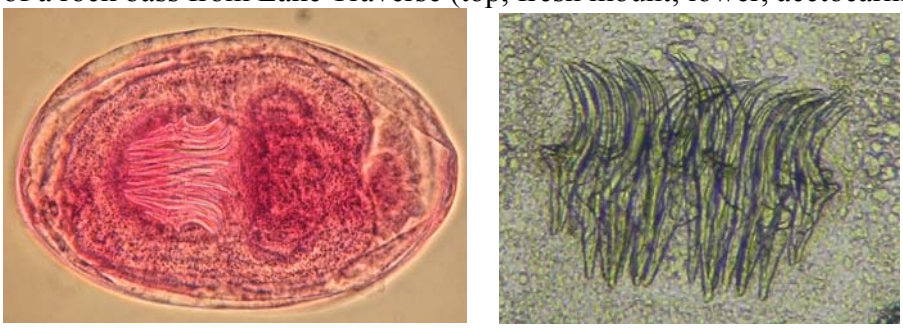


Photomicrographs of *Microcotyle spinicirrus* found on gill lamellae of freshwater drum from Lake Traverse (acetocarmine).



Photomicrographs of *Proteocephalus pinguis* fresh mount (left), scolex with five suckers (right), and acetocarmine-stained view of scolex (center) recovered from the intestines of northern pike from Devils Lake and lake Traverse

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



Photomicrographs of acetocarmine-stained specimen of a gryporhynchid metacestode found in yellow perch from Devils Lake in June 2008.

Table 5. List of parasite taxa found in the Red River Basin. * - parasites identified to species and only reported from Canadian waters in the study.

Actheres pimelodi	*Leptorhynchoides thecatus* *
Alloglossidium corti	*Ligula intestinalis*
Allacanthocasmus sp.	*Megalogonia ictaluri*
Ambiphyra sp.	*Microcotyle spinicirrus*
Apiosoma sp.	*Microsporidea*
Biacetabulum sp.	*Myobolus* sp.
Bothriocephalus claviceps *	*Myxobolus* sp
Bothriocephalus cuspidatus	*Myxidium* sp.
Bothriocephalus acheilognathi *	*Myxosporea*
Bucephalus elegans *	*Myzobdella cyprinaceus*
Bunoderina sacculata *	*Myxobdella lugubris*
Camallanus oxycephalus	*Neascus* sp.
Caprinia sp.	*Neoechinorhynchus cylindratus* *
Capriniana piscium	*Onchocleidus chrysops*
Centrovarium sp.	*Ornithodiplostomum ptychocleilus* *
Chloromyxum sp.	*Paradilepis* sp.
Clinostomum marginatus	*Paurorhynchus hiodontis*
Coccidia	*Polymorphidae cystacanth*
Corallobothrium fimbriatum	*Pomphorhynchus bulbocolli*
Contracaecum sp.	*Posthodiplostomum minimum*
Crepidostomum cornutum	*Prohemistomulum* sp.
Crepidostomum illinoiense *	*Proteocephalus pinguis*
Cryptogonimus sp. ?	*Proteocephalus pearsi* *
Diplostomulum sp. *metacercaria*	*Rhabdochona* sp.
Diplostomum spathaceum	*Rhabdochona bulbicolla*
Diplostomum of Bolbophorus confusus *	*Raphidascaris acus*
Epistylis sp.	*Sanguinicola occidentalis* *
Ergasilis cyprinaceus	*Spinitectus carolini*
Ergasilis luciopercarum *	*Spinitectus gracilis*
Glugea sp.	*Spiroxys* sp.
Gyrodactylus hoffmani	*Spiruridae (immature mematode)*
Gryporhynchidae	*Tretraonchus monenteron* *
Ichthyophthirius sp.	*Trianenophorus nodulosus* *
Heterosporis sp.	*Trichodina* sp.
Henneguya sp.	*Tylodelphys scheuringi*
Hunterella nodulosa	*Unicauda* sp.
Hysteromorpha triloba	*Urocleidis* sp.
Hysterothylacium brachyurum	*Valipora* sp.
Khawia iowensis	

Table 6. Piscine hosts and anatomical location of parasites recovered from fish collected at Devils Lake and at three sites in the Red River Basin. Anatomical abbrevations: e (eye), f (fin), (g) gills, (i) intestine, (k) kidney, (l) liver, (m) musculature, (mt) mesenteries, (pc) peritoneal cavity, (s) skin. Sample site abbreviations: LA = Lake Ashtabula, LT = Lake Traverse, RRD = Red River Delta.

Parasite taxonomic classification			
Class	Family or Genus and species	Devils Lake host and anatomical location	Other Red River Basin sites of detection
Protozoans	*Apiosoma* sp.	BLCR(g), FTMN(g), WALL (g,s)	LA LT
	Epistylis sp.	WALL(g)	LA LT
	Trichodina sp.	BLCR(g,s), FTMN(g), NRPK(s), WALL(g), WHBS(g,s), YLPR(g,s)	LA LT RRD
Myxosporea	*Myxobolus* sp.	FTMN(k)	LA LT RRD
	Unicauda sp.	FTMN(k)	LT
Monogenea	*Dactylogyrus* sp.	FTMN(g)	LT
	Gyrodactylus hoffmani	FTMN(f)	LT RRD
	Gyrodactylus sp.	YLPR(s)	LT
	Onchocleidus chrysops	WHBS(g),	LA RRD
Trematoda	*Diplostomum spathaceum*	FTMN(e)	LA
	Diplostomulum sp.	FTMN(m)	RRD
	Neascus of Posthodiplostomum sp.	BLCR(mt), FTMN(mt)	LA LT
	Neascus sp.	FTMN(m)	LA LT
Cestoida	*Bothriocephalus cuspidatus*	WALL(i),	LA LT RRD
	Bothriocephalus sp. (metacestodes)	BLCR(i), FTMN(i), NRPK(i), WHBS(i), YLPR(i)	LA LT RRD
	Gryporhynchidae	YLPR(i)	
	Ligula intestinalis	FTMN(pc)	RRD
	Proteocephalus sp. (metacestodes)	FTMN(i), NRPK(i), WHBS(i)	LA LT
Nematoda	*Contracaecum* sp. (larvae)	BLCR(mt), WALL(mt), WHBS(mt)	LA LT RRD
	Raphidascaris acus	YLPR(mt)	RRD
	Raphidascaris sp.	FTMN(i), YLPR(i)	LA LT
	Rhabdochona sp.	YLPR(mt)	LA LT RRD
	Spiroxys sp. (larvae)	WHBS(mt)	LA LT
Hirudinea	*Myzobdella lugubris*	YLPR(f)	LA LT

Histology

Histology was conducted only on fish from Devils Lake and the Red River Delta (examples in Figures 3-1 to 3-11). Histology provided another perspective on the observation of several parasites found during more traditional parasite surveys. Both the traditional approach and the histology approach identified similar protozoan, trematode, cestode, and nematode parasites. However, histology identified additional myxosporidian parasites in a variety of fish tissues from Devils Lake that were not identified in the Red River Delta (Table 7). These included myxosporeans from kidney tubules, peripheral nerve tissue, cartilage/bone tissue, and ovary. Seventeen tissue-specific lesions were identified from fish in Devils Lake that were not found in the Red River Delta.

Table 7. Summary of histology for fish collected in 2007 and 2008 from Devils Lake and the Red River Delta.

Location of lesion	Taxon responsible for lesion	Seen in Lake Winnipeg fish	North American distribution	Abundance (rare, common, abundant)	Risk of disease (unlikely, low, high)
Fathead minnow					
Kidney - tubule	Myxosporean (large)	Not seen**			
Kidney - tubule	Myxosporean (small)	Not seen**			
Kidney - interstitium	Myxosporean	Similar			
Skeletal muscle	Myxosporean	Similar			
Skeletal muscle	Trematode - Digenetic (encysted)	Similar			
Nerve - peripheral	Myxosporean	Not seen**			
Brain - meninges	Trematode - Digenetic	Similar			
GI- intestine	Cestode	Similar			
Gill	Myxosporean	Not seen?**			
Gill	Myxosporean – *Henneguya*-like.	Similar			
Gill	Protozoan – *Trichodina* sp.	Similar			
Gill	Protozoan – *Apiosoma* sp.	Not seen	widespread	abundant	unlikely
Gill	Monogenean -*Gyrodactylus* sp.-like	Similar			
Gill	Annelid (leech-like)	Not seen	widespread	abundant	unlikely
GI - intestine	Sporozoan	Not seen	widespread	common	unlikely
Cartilage/Bone	Myxosporean	Not seen**			
Thymus	Myxosporean	Similar			
Walleye					
GI- intestine	Cestode – (possibly Proteocephalus sp.)	Similar			
Gill	Protozoan – *Trichodina* sp.	Similar			
Gill	Protozoan – *Apisoma* sp.	Not seen	widespread	abundant	unlikely
Gill	Protozoan – *Ichthyophthirius*	Not seen	widespread	abundant	low
Gill	Protozoan - flagellate	Not seen	widespread	abundant	unlikely
Heart	Myxosporean	No photo			
Ovary	Microsporea- Suspect *Ovipleistophora* ovariae*	Not seen			unlikely
Skeletal Muscle	Myxosporean	Not seen			
Intestine	Nematode -Contracaecum	Not seen	widespread	common	low
Liver	larval nematode	Similar			
White bass					
GI - intestine	Cestode	Similar			
Gill	Protozoan– *Trichodina* sp.	Similar			
Gill	Monogenean Trematode	Similar			

GI-muscle/liver	Nematode*	Not seen			
Yellow perch					
GI - intestine	Cestode – *Ligula* sp.	Similar			
Gill	Protozoan – *Ichthyophthirius multifiliis*	Not seen	widespread	abundant	low
Gill	Protozoa-Trichodina sp.	Similar			
Gill	Monogenean- *Gyrodactylus**	Not seen	widespread	common	unlikely
Gill	Protozoa-*Costia*- like	Not seen	widespread	abundant	low
Muscle- skeletal	Trematode-Digenetic	Similar			
GI - intestine	Cestode	Similar			
GI - muscle/vis.	Nematode	Similar			
GI-mucosal epithelium	Sporozoan-coccidian	Not seen	widespread	common	unlikely
Skin – oral cavity	Protozoan - *Apiosoma* sp.	Not seen	widespread	abundant	unlikely
Nerve-sp cord	Myxosporean – *Henneguya*?	Similar			
Connective tissue-lower jaw	Myxosporean – *Henneguya*?	Similar			
Northern pike					
GI - intestine	Cestode	Similar - some			
GI - intestine	Nematode	Similar			
Kidney	Myxosporean – *Thelohanellus* sp.	Similar			
Gill	Monogenean – *Gyrodactylus*	Similar			
Gill	Protozoan - *Ichthybodo* sp.	Not seen	widespread		low
Gill	Protozoan - *Trichodina* sp	Similar			
Gill	Protozoan - *Apiosoma* sp.	Not seen	widespread	abundant	unlikely
Gill	Hirudinea: Leech	Not seen	widespread	abundant	unlikely
Kidney	Myxosporean – *Myxidium* sp. (*lieberkuhni* ?)	Not seen			
Black crappie					
GI-viscera	Cestode – *Ligula* sp.		widespread	abundant	low
Gill	Protozoa – *Ichthyobodo* sp.		widespread	abundant	low
Kidney	Myxosporean – *Myxobolus* sp. (*trijugum*?)	**			
Urinary bladder	Myxosporean – *Henneguya*	**			
Gall bladder	Myxosporean – *Chloromyxum* sp.*	**			
GI-viscera	Nematode				
White sucker					
Gill	Myxosporean – *Myxobolus* sp. (*bibullatus*?)	**			

* possibly ***M. bibullatus*** based on tissue and fish species

**For myxosporeans for which a genus is not given no attempt was made to assign risk. It is most important to note that there is little tissue reaction to any myxosporean photographed in the Devils Lake surveys.

Histology Parasite Figures

Figure 3-1. Large myxosporean in fathead minnow kidney tubules.

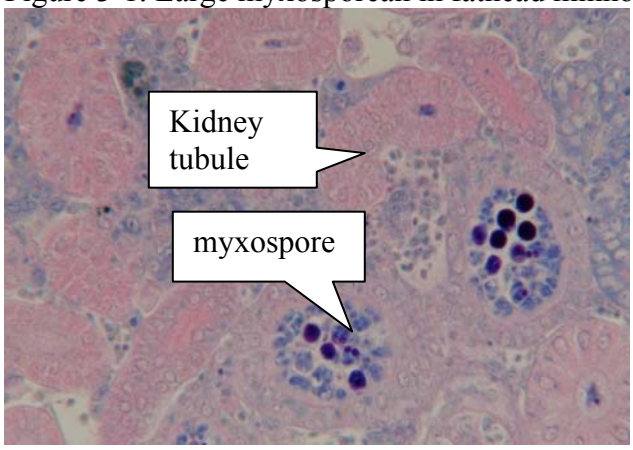


Figure 3-2. Small myxosporean observed in fathead minnow kidney tubules.

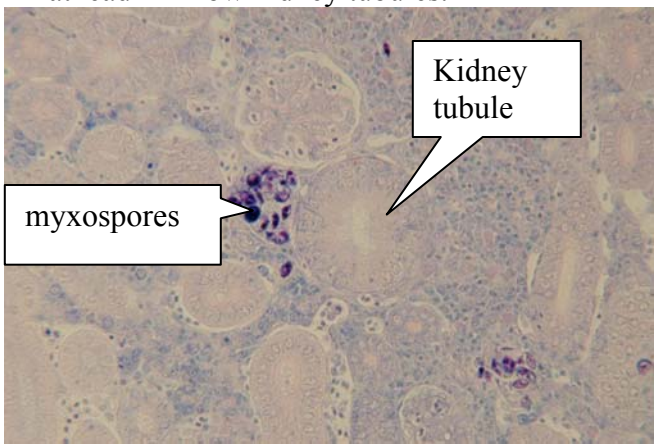


Figure 3-3. Myxosporean from skeletal muscle in fathead minnows.

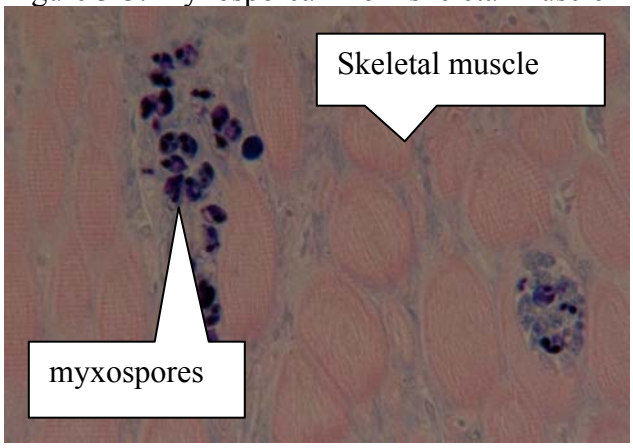


Figure 3-4. Myxosporean observed in fathead minnow peripheral nerve tissue.

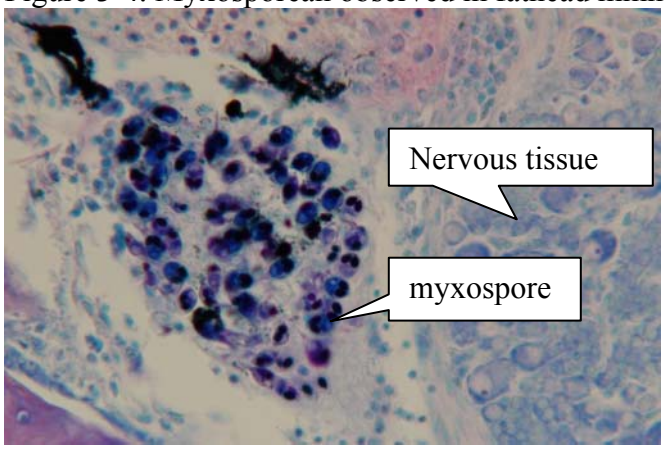


Figure 3-5. Coccidian parasite observed in fathead minnow gastrointestinal tract..

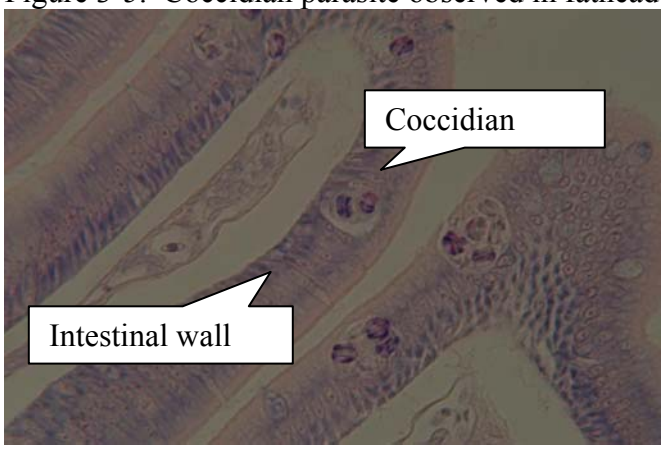


Figure 3-6. Mysospores observed in cartilage of fathead minnow.

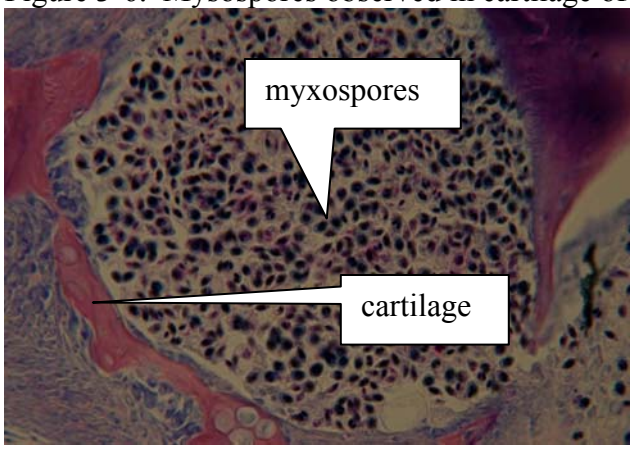


Figure 3-7. Myxosporean in walleye skeletal muscle.

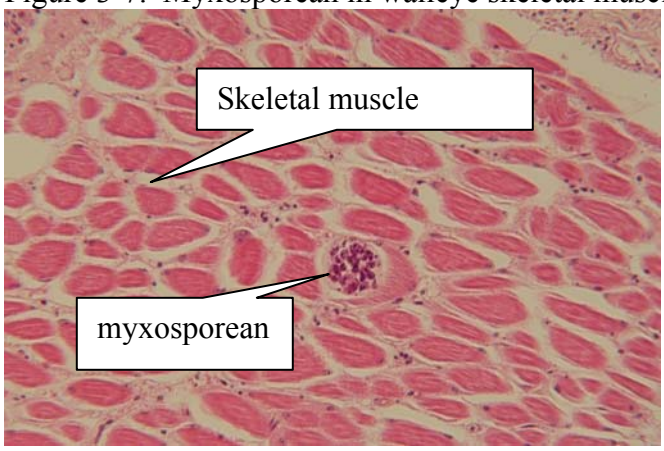


Figure 3-8. Yellow perch. Coccidian sporozoan in GI tract.

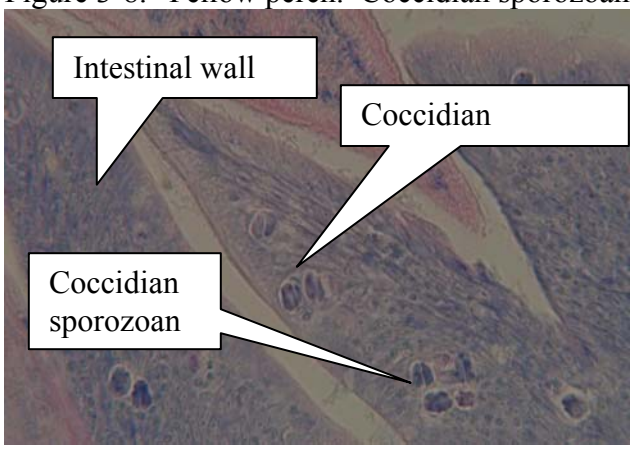


Figure 3-9. Northern pike. Suspect leech.

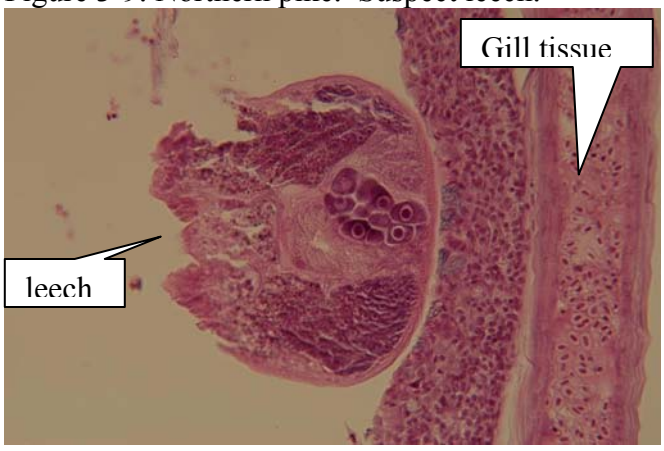


Figure 3-10. Northern pike. *Myxidium sp.* in kidney tubule.

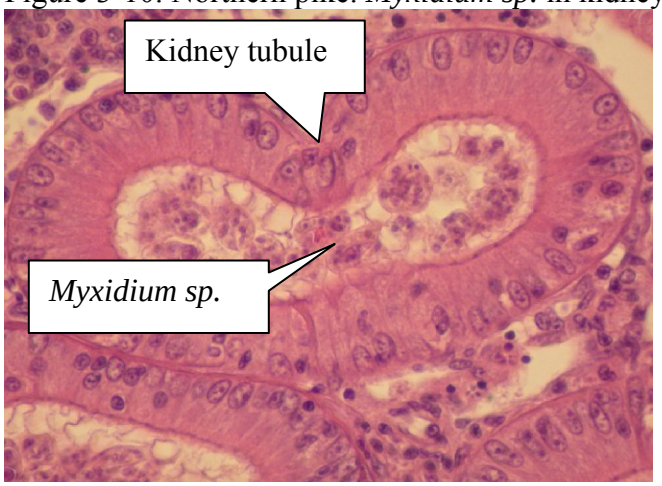
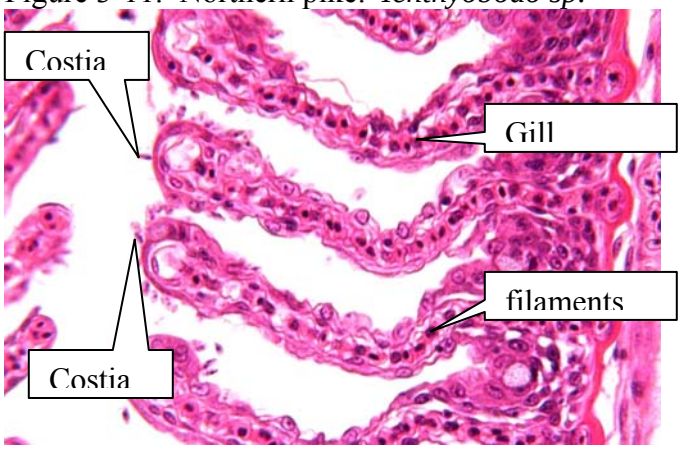


Figure 3-11. Northern pike. *Ichthyobodo sp.*



Qualitiative Risk Assessment

A total of 29 bacterial isolates and 76 parasite taxa and more than 40 different tissue-specific lesions were detected from fish in the Red River Basin. Eight bacterial agents and 25 parasites were found in Devils Lake. However, a similar community of parasites was found both macroscopically and microscopically (histology) in fish collected from Devils Lake and elsewhere in the Red River Basin. One parasite (a gryporhynchid larval tapeworm), three bacterial agents (*Pseudomonas mendocina, Yokenella regensburgei, Brevundimonas diminuta*), and 17 tissue specific lesions were identified only from fish collected from Devils Lake. None of these organisms or lesions was found at downstream locations (Lake Ashtabula, Lake Traverse, or the Lake Winnipeg – Red River Delta area. For the 2695 fish assessed for parasites from Devils Lake, only a single individual of the gryorhynchid parasite was found in a single yellow perch.

The parasite and pathogen species list, from the current study can be compared to a report by Dick et al. (2001) for the Hudson Bay drainage where 53 parasite species from 8 fish species (whitefish, pike, goldeye, brook stickleback, white bass, yellow perch, sauger and walleye) and 6 pathogens were listed. Most of the 25 parasite species identified from Devils Lake have been identified downstream but it is worth noting that in this study 14 fish parasite species found in Canadian waters were not reported from the Red River Basin, south of the US/Canada border (Table 5). Moreover, *Epistylis* sp. and Gyrporhynchidae have not been identified in Canadian waters. However a closely related protozoan genera, *Caprinia* sp., has been identified from fish in the Red River Delta. The leech, *Myzobdella lugubris,* was not identified from the Red River estuary but the genus is common in the Hudson Bay drainage. However, the presence of the Asian tapeworm from a large number of fish hosts and the heartworm (*Sanguiicola occidentalis*) in walleye and sauger, and its absence in the drainage south of the US/Canada border, warrants further assessment of entry routes into the Red River Basin.

The qualitative risk from disease to the structure and function of downstream fish and fish communities from the parasite, three bacterial agents, and the lesions found in Devils Lake but not downstream was evaluated by four Canadian and four USA fish pathologists knowledgeable on the topic of fish health. These eight experts were asked to address the following five questions and rank risk where appropriate.

1. Based on the life histories and life cycles of the parasites, pathogens and histopathology anomalies found in Devils Lake and not found downstream in USA and Canada, what are the mechanisms for transport from Devils Lake to other aquatic ecosystems in the Red River basin and Lake Winnipeg. Using your best professional judgment and relevant scientific information how likely is it that these organisms will be transferred? (Rank: 1 = unlikely, 2 = low, 3 = high).

The Canadian and USA teams identified a variety of mechanisms that would enable the transport of potential pathogens, parasites, and micro-parasites identified by histology to other aquatic ecosystems including those downstream from Devils Lake. These include:

a) transport by fish eating birds. This would be a particularly important transport route for those parasites with fish-eating birds as either an intermediate or primary host for the parasite. For example, present knowledge on gryporhynchid parasites suggests the first intermediate hosts are likely copepods while fresh- and brackish-water fish are secondary intermediate hosts, and fish-eating birds serve as the definitive hosts. **The probability of transport of parasites and bacteria by birds was judged to be high by the fish pathologists.**

b) transport past the screen and through the rock and gravel filter of the current outlet of bacterial agents and certain life-stages of parasites. **The probability of transport of parasites and bacteria through the constructed outlet was judged to be high by the fish pathologists.**

c) transport of fish and their parasites and pathogens through the Tolna Coulee, the natural outlet of Devils Lake. The water level in Devils Lake would have to increase by about 5 feet (1.5 m) before the Tolna Coulee would serve as an outlet to Devils Lake. Once water levels reach the base elevation of Tolna Coulee 1458 ft asl (444.4 m asl), it is expected that significant flow would occur through the Coulee to the Sheyenne River for months if not years. The spill-elevation of the Coulee would be allowed to erode to lower elevations to lower water levels in Devils Lake, allowing the recovery of presently flooded agricultural lands. The probability of transport of parasites and

bacteria through the Coulee was judged to be high by the fish pathologists, but this projection depends on the time of year and the extent of flow due to annual precipitation in the Devils Lake basin. Once the water level in Devils Lake exceeded the base elevation of Tolna Coulee and discharged into the Sheyenne River, more parasites/pathogens and their fish hosts would probably be transported through the Coulee during the summer than in the winter.

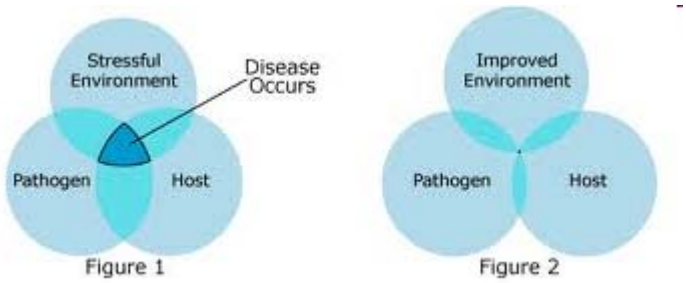
d) intentional or unintentional transport by humans (or their boats) of fish and their associated parasites. **The probability of transport of fish and their parasites and bacteria by humans was judged to be relative low by the fish pathologists compared with transport by birds and through outlets. However, fish and their parasites can be readily transported when live-bait is moved from one major watershed to another, and if bilge water is not drained from boats before they are moved to another waterbody.**

2. Using your best professional judgment and best available scientific information what environmental factors are known to trigger a disease outbreak for the identified parasites, pathogens and lesions found in Devils Lake and not downstream?

There have been no reported historic fish infectious disease outbreaks in Devils Lake. However, Canadian and USA teams identified a number of environmental factors and variables that could trigger disease outbreak from those parasites and bacterial agents that were present in Devils Lake and elsewhere in the Red River basin (Fig. 4). These include high intensity of infection by the pathogen, and fish stressed by environmental variables such as low dissolved oxygen, elevated carbon dioxide levels, elevated levels of ammonia, elevated temperature and toxic substances such as pesticides. Eutrophication of Lake Winnipeg could also promote disease outbreak in fish in that lake from decomposition of algal blooms in summer and the consequent reduction in oxygen concentrations in the water. A nutrient rich environment (eutrophic) that supports population growth of the intermediate invertebrate hosts may increase the transmission of parasites such as *Bothriocephalus*, *Proteocephalus*, *Ligula* and myxosporeans. Eutrophication also promotes higher rates of growth for some bacteria especially the aeromonids and pseudomonids. High densities of

fish may also increase the opportunity for direct transfer of ectoparasites such as protozoans, mongeneans and leeches. Stocking an exotic fish species or a new species invasion by a parasite, invertebrate or fish host can modify a local food web and change the population dynamics which could lead to a disease outbreak.

Figure 4. Schematic showing the relationship between stressful environment and disease in fish (Figure 1) and the similar schematic (Figure 2) for a normal environment with healthy fish.



3. Using your best professional judgment, best available scientific information, and considering environmental triggers, if parasites, pathogens, and histopathological lesions and tumors found in Devils Lake and not found downstream were transferred elsewhere to the basin, what risk (Rank: 1 = unlikely, 2 = low, 3 = high) to downstream fish and fisheries could these organisms pose and what is their likelihood of causing disease? (Rank: 1 = unlikely, 2 = low, 3 = high).

The non-targeted bacterial agents found in Devils Lake but not elsewhere in the Red River basin were *Pseudomonas mendocina, Yokenella regensburgei* and *Brevundimonas diminuta.* These bacteria were identified from Devils Lake by the random nature of the assay method used to identify bacterial agents in both Canada and the USA. These assay methods were not a rigorous scientific assessment to determine distributions or bacteria in the Red River Basin. Because the methods to identify bacterial differed, it is not surprising that the Canadian and USA non-targeted

(not reportable) bacterial species lists were different. Since these three species of bacteria are opportunistic pathogens and are ubiquitous in the environment, including soil, it is not surprising that they were isolated from the kidneys of a small number of fish. They are not known to cause disease in healthy fish. However, opportunistic pathogens are capable of causing disease if the health of an organism is otherwise compromised. **The fish pathologists agreed that the risk to downstream fish and fisheries from these three bacteria was low, and the likelihood of any of them causing disease was judged to be unlikely or negligible.**

The gryporhynchid metacestode found in Devils Lake but not elsewhere in the Red River basin was considered a new parasite and host record. To date, there are no known fish kills in Devils Lake from this parasite. Limited laboratory and field data are available on the pathogenicity of this group parasites. However, they are believed to be transmitted by fish-eating birds. The risk to downstream fish and fisheries from this organisms was judged to be low based on the paucity of data on the pathogenicity of this species and the unusual low abundance and consequent low probability of detection,. **The potential to cause disease was judged to be unlikely or low.**

Of the 17 tissue specific lesions identified from Devils Lake fish, most were caused by myxosporeans and protozoans. The risk of transfer of these organisms from Devils Lake is high and the potential for infection of fish by these organisms downstream of Devils Lake was also high. However, there was little pathology caused by any of the myxosporean observed in histology sections photographed from Devils Lake and the lesions in the tissues were minimal. Some of the myxosporeans were very uncommon in the tissues of fish collected from Devils Lake. However, while myxosporeans are unlikely to be a primary source of morbidity/mortality there are documented cases where some species affect fish performance and population numbers. **Overall, the fish pathologists agreed that the risk to downstream fish and fish communities from the myxosporeans and protozoans identified by histology was low, and the probability of causing disease was unlikely or negligible.**

4. For those fish parasite, pathogens, histopathological lesions and tumors found in Devils Lake but not elsewhere in the basin, what is the North American distribution of these parasites, pathogens, and lesions (describe geographical distribution), and what is their relative abundance elsewhere in North America? (Rank: rare, common, abundant).

The non-targeted bacterial agents found in Devils Lake but not elsewhere in the Red River basin included *Pseudomonas mendocina, Yokenella regensburgei* and *Brevundimonas diminuta.* These bacteria are ubiquitous in soil and water throughout North America. The fish pathologists considered these organisms to be common, and very likely present in the aquatic ecosystems downstream from Devils Lake. These bacteria may cause disease in fish from the Red River Basin that are already in poor health for other reasons, but the source of these organisms should not be attributed to Devils Lake.

The gryporhynchid metacestode found in single yellow perch from Devils Lake could not be identified to species and thus the exact geographical distribution of this organism could not be determined. However, this very unusual record for a parasite warranted a literature search and literature review to determine the pathogenicity, distribution, and invasive potential of gryprohynchid cestodes. Previous records were not found in the literature of larval gryprohynchid cestodes in fish from Devils Lake, Lake Ashtabula, Lake Traverse, or from fish in other bodies of water in the Red River basin. The earliest report of gryporhynchids 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 accounts 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). In 2004, Scholtz et al. (2004) published a proposal to replace the Family Dilepididae with Gryporhynchidae

and at the same time presented a review of gryporhynchid metacestode geographical distribution. More recently, Scholz and Harris (2006) reported the first occurrence of *Cyclustera ralli* (Cestoda: Cyclophyllidae) in the livers and mesenteries of mummichog from Virginia. Finally, larval gryporhynchids were found in fish from Lake Traverse that may be metacestodes of *Paradilepis* sp. and *Valipora* sp., and perhaps other unidentified species. The gryporhynchids from Lake Traverse were morphologically different from the specimen collected at Devils Lake. These findings suggest gryporhynchid metacestodes are rare in North America but their distribution is probably wider than indicated by records in the literature. **Given the apparent wide distribution but rare detections of this parasite in North America and no known evidence of this parasite compromising fish health, it is unlikely that this parasite would pose any significant risk to fish in the Red River Basin in the future.**

Of the 17 tissue-specific lesions identified from Devils Lake fish and not found downstream in the Red River Delta, ten were caused by myxosporeans, protozoans, nematodes, and leech which were found to be both widespread in North America and either common or abundant. Given that all of these ten taxa are both abundant and widespread in North America, it is unlikely that these parasites would pose any additional risk to fish in the Red River Basin when they are transported from Devils Lake by any one of multiple mechanisms. **Since seven myxosporeans could not be identified to genus, information on distribution and risk could not be determined by the fish pathologists.**

5. The existing outlet from Devils Lake is equipped with a combination of screens, rock, and gravel filter intended to prevent the downstream transfer of fish. Given the life histories of parasites and pathogens in Devils Lake, does this mitigation measure reduce the impacts for potential of transfer of these organisms to downstream ecosystems?

The design of the existing filter was intended to reduce the risk of adult fish being released to the downstream environment. It was not designed to reduce risks associated with smaller organisms such as larval fish, bacteria, virus particles or free-living life stages of parasites. Because the design of the existing filter was not intended to reduce risks associated with small particle sizes the risk of

these smaller organisms being transferred downstream is high. However, the organisms found in Devils Lake but not downstream do not pose a risk to downstream fish species or communities even if they are transferred through the existing filter. **These organisms (3 parasites and 1 bacteria, no virus particles detected) pose no risk because (1) they are widely distributed (2) they have life cycles and life histories the involve multiple hosts and multiple pathways for transport and (3) there are multiple pathways to transport these organism from Devils Lake.**

Summary

Three bacteria, one parasite, and several lesions were identified from fish in Devils Lake that were not identified elsewhere in the basin. The fish pathologists concluded that the fish parasites and pathogens in Devils Lake could be transferred from the Lake through the gravel and rock filter currently in place, by birds (often the intermediate or final parasite host), and by unintentional and intentional transfer by people (or their boats). The parasites and bacteria found in Devils Lake were generally widely distributed throughout much of North America. All were opportunistic pathogens that could adversely affect fish health only if fish health was compromised for other reasons. None were foreign parasite or pathogen species. For these reasons, all experts concluded that the risk to downstream fish and fisheries was low from the parasites and pathogens found in Devils Lake, and the potential for causing disease was negligible.

Recommendations:

The investigation undertaken in 2006, 2007, and 2008 is a significant effort to isolate and identify pathogens and parasites in a watershed that is shared by North Dakota and Manitoba. Based on the data collected, the risk assessment indicates that, at present, there is limited risk to downstream fish species or communities from the organisms found in Devils Lake. However, the U.S. and Canada fish health experts provided the following recommendations that would help to ensure that risk of certain pathogens and invasive species entering the Red River basin is reduced and would monitor for the presence of invasive species in the basin.

1. Adopt a proactive model and precautionary approach to prevent and monitor transfer of invasive species and certain fish pathogens into the Hudson Bay Basin. To effectively prevent transfer of invasive species and non – endemic fish pathogens into the Hudson Bay Basin, provincial, state, and federal agencies should adopt a general model with the following key components:

A) enact legislation and develop policies to prevent transfer of foreign species and fish pathogens into the Hudson Bay basin,

B) maintain active enforcement of invasive species policies and legislation, and fish disease prevention and control policies and legislation.

C) monitor selected organisms and pathogens in aquatic ecosystems at a few biophysically unique locations to assess the effectiveness of legislation, enforcement, and remedial actions to prevent the introduction and spread of invasive species into the Hudson Bay Basin, and to provide a feedback loop for adaptive fish disease control and invasive species management programs.

2. Use the data generated in the present study to conduct a risk assessment to fish in the Red River Basin from the parasites and pathogens found throughout the Red River Basin, including Lake Winnipeg. Innovative risk analysis methods and techniques such as computer modeling should be used.

3. A fish parasites and pathogens monitoring program should be established based on selected and restrictive criteria. A workshop could be held to develop protocols, methods, and short- and long-term monitoring goals. This focused monitoring program should use multiple approaches and methods, and could include the following key components:

A) one sampling location in Canada and one sampling location in the USA;

B) fish parasites and pathogens should be assessed every three years at these two sites;

C) field and laboratory methods for Canada and the USA should be standardized. Methods should be standardized to ensure that data are comparable and compatible and costs for monitoring are kept at reasonable levels;

D) based on expert input, monitoring should be targeted to specific species of concern and problematic parasites, bacteria or virus;

E) fish species should be selected that are more susceptible than others to disease producing organisms. Ongoing targeted surveillance should be coordinated with the National Aquatic

Animal Health Program (NAAHPs) that is in the process of being implemented in Canada and the USA.

4. State and provincial agencies should continue to maintain and to improve surveillance procedures to prevent transfer of organisms into the Hudson Bay Basin.

5. The science literature and other information should be regularly reviewed by the International Red River Board and member agencies to identify those organisms that are extending their range toward the Hudson Bay Basin possibly because of climate change, biological factors, or anthropogenic activities. The likelihood of these organisms moving into the basin should be modeled, and a risk assessment should be undertaken as part of this process to provide decision makers with information that could be used to prevent invasive species from entering the Basin, or could be used to develop invasive species management strategy should an invasive species become established.

6. Implement a project to determine route of transfer, rate of spread, and distribution of the Asian tapeworm (*Bothriocephalus acheilognathi*) in the Hudson Bay Basin. These population characteristics of the Asian tapeworm could be used as a model to study invasion pathways of foreign species into the watershed.

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