2007

PARASITES AND PATHOGENS OF FISH FROM DEVILS LAKE, SHEYENNE RIVER, RED RIVER, AND THE RED RIVER DELTA.

IRRB

THE

INTERNATIONAL

RED RIVER

BOARD

Parasites and Pathogens of Fish from Devils Lake, Sheyenne River, Red River and the Red River Delta

Report for the Fall 2006 Program

INTERNATIONAL JOINT COMMISSION • COMMISSION MIXTE INTERNATIONALE 1909

PARASITES AND PATHOGENS OF FISH FROM DEVILS LAKE, SHEYENNE RIVER, RED RIVER, AND THE RED RIVER DELTA.

REPORT FOR THE FALL 2006 PROGRAM

SUMMARY OF 2006 RESULTS

- Fish from all sites in Canada and USA did not show clinical signs of disease from bacteria

- No evidence of viral infection in USA; 2 of 60 walleye from Canada with a common skin cancer and 1/60 with an iridovirsus.

- Nine parasites detected in Devils Lake; 28 in Red River Delta.

- Two parasite taxa found in Devils Lake, *Gyrodactylus hoffmani* and *Epistylis* sp. have not yet been detected downstream in Canada or USA.

- A foreign and invasive parasite, the Asian tapeworm, has recently colonized the Red River Delta from an unknown source.

- Histopathology of fish from the Red River Delta show evidence of a wide variety of parasite induced lesions on a variety of tissues, as would be expected. However, a relatively high incidence of lesions in the heart of walleye is of concern.

- Whirling disease was not detected in Lake Whitefish collected from Lake Winnipeg.

INTRODUCTION:

The International Joint Commission and International Red River Board contacted the AEC Co-Chairs and requested that a program be developed to monitor parasites and pathogens in Devils Lake. The objective of this program was to be a further analysis of the potential for transfer of species that might exist in Devils Lake but not in the Sheyenne River, Red River or Lake Winnipeg. Previous studies conducted on Devils Lake (Hudson and Peters, 2005, Williamson et al. 2005, Arroyo 2005) focused on fish parasites and pathogens, phytoplankton, zooplankton, aquatic macrophytes, and terrestrial plants.

The IJC requested a proposal that would continue the work on Devils Lake in 2006. The IJC also asked that the proposal include an analysis of multiple species of concern in addition to fish parasites and pathogens and tributaries be included in the program. The AEC Co-Chairs were charged with developing a proposal and budget for the proposed work. The IJC further requested that sampling begin in the spring of 2006.

As a first step in the development of a proposal the AEC Co-Chairs contacted fish health experts in the U.S. and Canada for technical assistance. As a result of early consultations with these experts the AEC Co-Chairs made the following recommendations to the IJC regarding development of the proposal:

1. Work for 2006 should focus on fish parasites and pathogens and be an extension of the previous 2005 work.

2. Samples should also be collected from the Sheyenne River, Red River and Lake Winnipeg as well as Devils Lake.

3. The monitoring program should be conducted twice each year and be carried out in 2006, 2007, and 2008.

4. A risk assessment should be conducted using data collected from previous studies and incorporating the 2006 data.

5. A long term program should be developed that includes tributaries and other species of concern.

6. Funding be provided by each country to the respective agencies conducting the sampling and analysis in each country. The U.S. would fund the work in Devils Lake, Sheyenne River and Red River and Canada would fund the work in Lake Winnipeg.

During the spring and early summer of 2006, the AEC Co-Chairs worked closely with fish health experts to develop a proposal and budget for a three year sampling program. This program would focus on fish parasites and pathogens and include a risk assessment as recommended. The proposal was submitted to the IRRB and IJC at the summer meeting in Winnipeg in July 2006.

The first milestone in implementing the proposal was convening a meeting of the fish health experts from both countries. The objective of this meeting was to discuss specific field and laboratory methods for collection, preservation, identification and analysis of fish parasites and pathogens.

While there were differences identified in specific methods the outcome of the meeting was agreement that both countries methods were comparable and compatible and the work could proceed. There was also agreement that regular coordination between scientists from both counties would be critical to a successful and scientifically defensible monitoring program.

It is emphasized that the 2006 sampling was the first year of an anticipated 3 year program. Future sampling will be designed to capture different conditions in the watershed. The reader is cautioned to not draw any conclusions about the state of the watershed until all of the anticipated future results have been compiled and analyzed. It is the intent of the International Red River Board to develop a final report after the completion of the third year.

PREVIOUS STUDIES

2001 and 2002

In 2001, the COE contracted the U.S. Fish and Wildlife Service, Bozeman Fish Health Center to perform a fish pathogen survey at Devils Lake and the Sheyenne and Red rivers. The survey was performed under the biota transfer section of the COE EIS scope of work. During October 2001 and August 2002, more than 500 fish were collected from the three bodies of water and tested for a list of specific fish pathogens according to procedures of the *National Wild Fish Health Survey* (U.S. Fish and Wildlife Service 2004). At Devils Lake, 180 fish were collected over two consecutive days of sampling. Nets and traps were set in a northeastern area of the lake known as Six Mile Bay. The catch was composed of walleye, northern pike, black crappie, and yellow perch. Antigen of *Renibacterium salmoninarum*, causative agent of bacterial kidney disease (BKD), was detected in very low levels from northern pike, walleye, and yellow perch. Active infection could not be confirmed in these populations using a highly specific DNA-based polymerase chain reaction (PCR) assay. Overall, fish appeared in good general condition. We did not observe any external or internal signs of BKD or any other disease or abnormality. No other listed fish pathogens were detected. A total of 275 fish, representing ten species, were collected among two sampling sites on the Sheyenne River. On the Red river, 83 fish, representing eight species, were collected from two sampling sites. Antigen of *R. salmoninarum* was detected in low or medium levels in all species tested however antigen was not detected in northern pike, smallmouth bass, walleye, and white sucker from the downstream site near Valley City. Nearly 40 kidney samples from fish collected in the Red and Sheyenne rivers were tested with the PCR assay and all were negative for *R. salmoninarum*. Most of the ELISA OD values were just slightly above the negative-positive threshold. No clinical signs of BKD or other diseases or abnormalities were observed, and no other fish pathogens on the survey list were detected. Recommendations for future work included sampling fish at various times of the year, sampling other areas of Devil Lake in coordination with area biologists, and maintaining a sample size of at least 60 fish.

2005

At the request of the Council On Environmental Quality, the U.S. Fish and Wildlife Service performed a fish pathogen survey at Devils Lake in North Dakota in July, 2005. The survey was conducted in response to the need for a baseline survey of the distribution of fish pathogens and the concerns for biota transfer.

More than 300 fish were collected from Devils Lake and tested for fish pathogens and parasites using protocols and procedures of the U. S. Fish and Wildlife Service *National Wild Fish Health Survey*. Eight fish health biologists from the Bozeman and LaCrosse Fish Health Centers worked cooperatively with the Missouri River Fish and Wildlife Management Assistance Office, North Dakota Game and Fish Department, and the Spirit Lake Nation to collect samples from seven different species of fish. Fish were sampled with a variety of gear types from two main areas of the lake over a five day period. The catch was composed of black crappie, fathead minnow, northern pike, walleye, white bass, white sucker, and yellow perch. Testing for fish pathogens and parasites involved four main components. First, immediately upon capture, fish were examined externally and internally for gross signs of disease or other abnormalities. Next, representative samples from each species were examined for external and internal parasites. Then, specific tissues samples were collected using aseptic field techniques and were transferred to the laboratories for pathogens screening using standardized assays. Finally, tissue samples were further tested with highly specific corroborative or confirmatory assays whenever suspect pathogens were detected with screening methods. Results of the pathogen survey were completed within 30 d of sampling. No viral fish pathogens were detected in standard cell culture assays from any species of fish. Two ciliated protozoan parasites, *Epistylis sp*. and *Trichodina sp*., were observed in wet mounts of skin scrapings during parasite screening. Additionally, larval forms of the parasitic nematode *Contracaecum sp*. were recovered from walleye. Three parasitic cestodes were found including *Bothriocephalus custpidatus* in walleye, *Proteocephalus pinguis* in northern pike, and *Ligula intestinalis* in fathead minnow and yellow perch. Major microbial findings included the isolation of six species of bacteria representing both Gram-negative and Gram-positive organisms. Motile aeromonids, such as *A. hydrophila*, were the most common Gram-negative bacteria and were recovered from six of the seven species of fish sampled. Other less common species included *Pleisomonas shigelloides* and *Pseudomonas putrifaciens.* The Gram-positive organism *Corynebacterium renale* was also isolated in bacterial culture. In addition, antigen of *Renibacterium salmoninarum* was detected by enzyme-linked immunosorbent assay (ELISA) in very low levels from all species. However, since active infection with *R. salmoninarum* was not confirmed in these populations by the highly specific polymerase chain reaction (PCR) assay, there was reason to believe the low ELISA optical density values represented false-positive readings. Other than *R. salmoninarum*, none of the other fish pathogens listed in the *National Wild Fish Health Survey* were detected in fish from Devils Lake. Likewise, none of the prohibitive fish pathogens found in most state or federal regulations or policies were recovered during the survey.

2006 PATHOGEN AND PARASITE SAMPLING SUPPORTED BY THE IJC

In the fall of 2006, the IRRB proceeded with a sampling program that was planned to be the first year of an anticipated 3 year program.

The objectives of the joint United States and Canadian project were to:

1. Determine the presence and estimate the prevalence of fish parasites and pathogens in resident fish from Devils Lake, the Sheyenne River, Red River, and Red River Delta;
2. Provide a comprehensive and scientifically credible survey of fish parasites and pathogens in fish from the Red River Delta that may be used in a risk analysis of the potential transfer of fish parasites and pathogens from the outlet on Devils Lake to aquatic ecosystems in the Red River basin including Lake Winnipeg; and
3. Use the comprehensive 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 fish species that were targeted are shown in Table 1.

Table 1. Fish species in Devils Lake and the Red River Delta that were captured and assessed for pathogens and parasites.

	Devils Lake	Red River Delta
Fathead Minnow	+	+
Yellow Perch	+	+
Northern Pike	+	+
White Bass	+	+
Walleye	+	+
Brook Stickleback	+	+
Channel Catfish		+
Goldeye		+
Sauger		+
Emerald Shiner		+
Black Bullhead	+	
Black Crappie	+	
White Sucker	+	

The following is a discussion of the approach and results for 2006 presented by country.

UNITED STATES

This report provides results and discussion of the 2006 fish pathogen and parasite survey of Devils Lake, the Sheyenne River, and the Red River of the North. Survey results are available through the U.S. Fish and Wildlife Service National Wild Fish Health Survey database on the worldwide web at 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 1). 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 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.

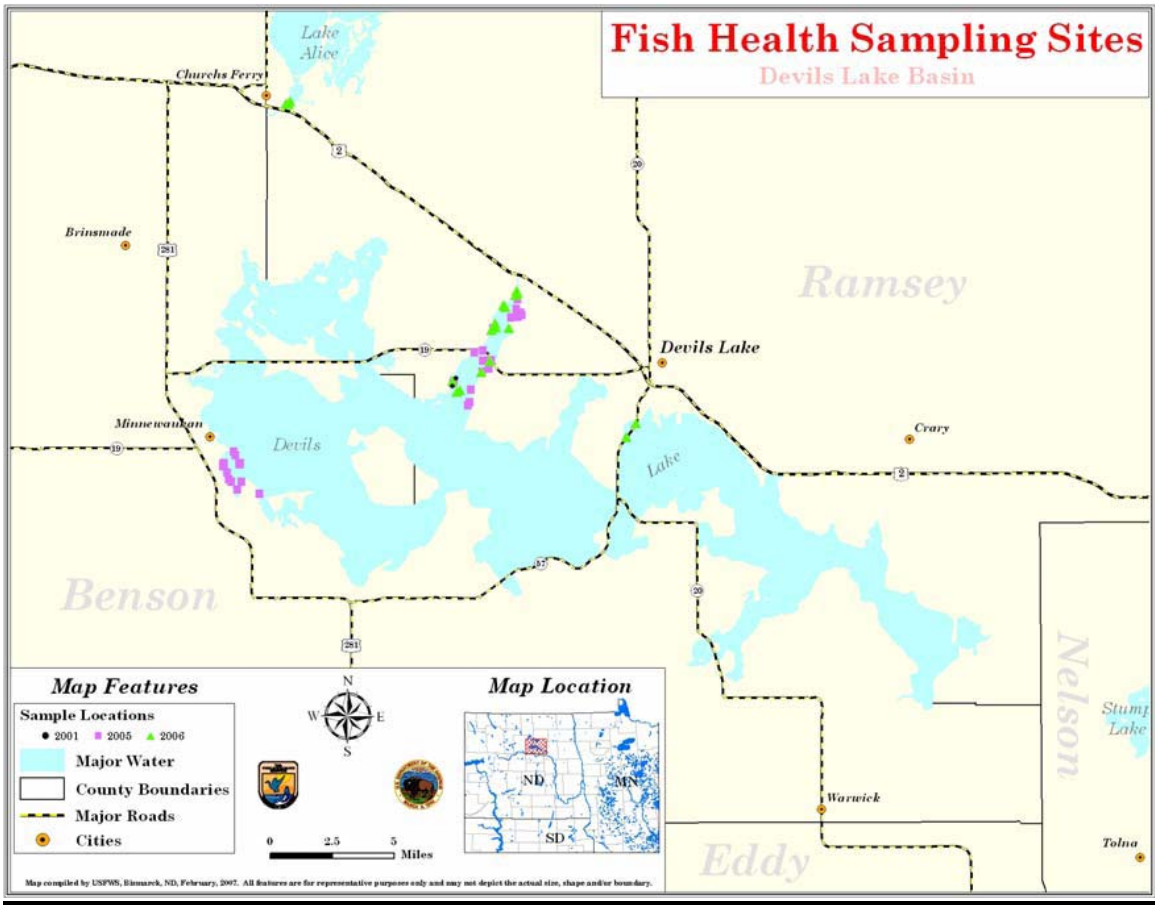

Figure 1. (USFWS 2007) - Locations of fish sampling sites on Devils Lake, North Dakota.

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 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 River 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 and processed during the same day as capture.

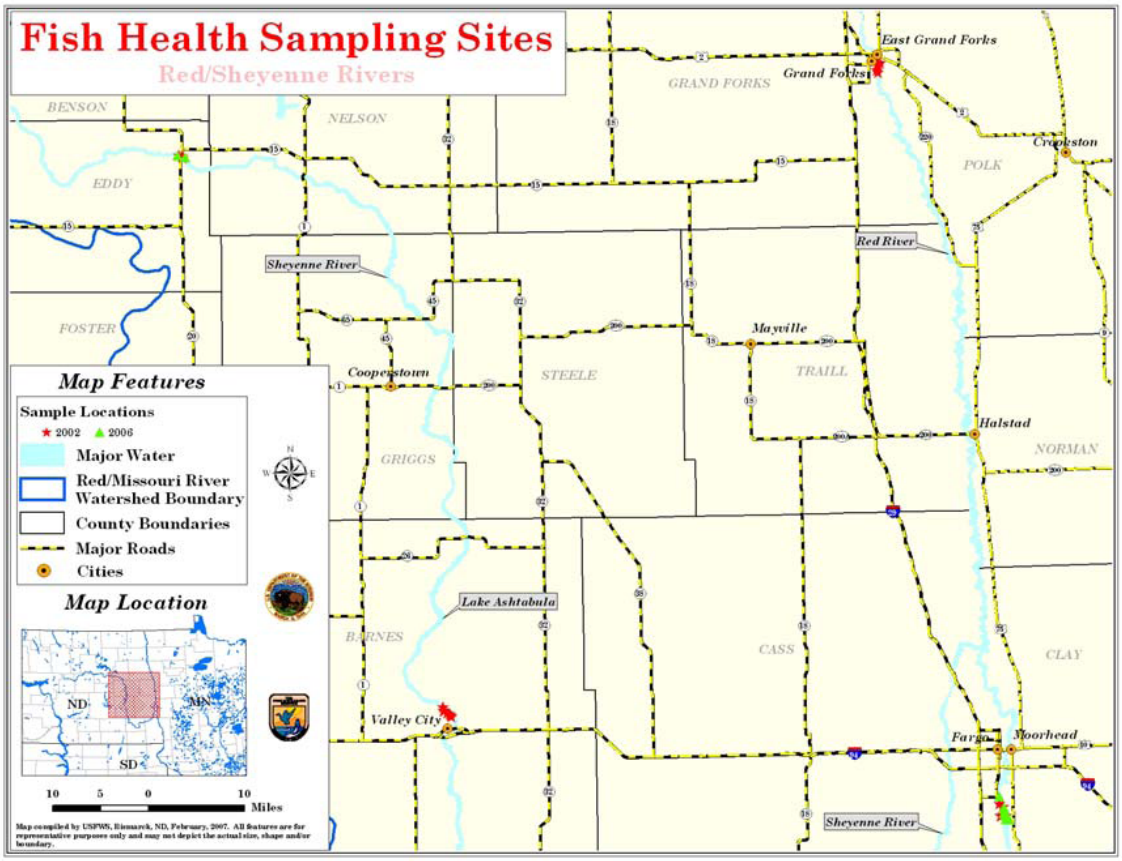

Figure 2. (USFWS 2007) - Geographic locations of sampling sites on the Sheyenne River and Red River.

For necropsy, fish were anesthetized with tricaine methanesulfonate (Finquel®), weighed (g) and measured (Lt, 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 prohibited 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. Culture tubes were discarded if no growth appeared after 10 d. 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. 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 disconnected 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. 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 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.

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. *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. 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.

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. 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 percent 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.

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. 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. 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.

Viruses

A total of 80 pooled tissue samples were collected among the seven species of fish captured at Devils Lake. On the Sheyenne River, we collected a total of 17 pooled tissue samples representing 5 species of fish. A total of 18 pooled samples representing 6 species of fish were collected from the Red River. 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.

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. 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. The motile Peritrich *Trichodina* sp. was observed in wet mounts of skin scrapings from walleye and yellow perch from Devils Lake. 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. 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*. *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.

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. *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 and fathead minnow. Goldeye collected from the Red River where host to the digenean trematode *Paurorhynchus hiodontis*. Gravid adult forms of *P. hiodontis* were observed in the abdominal cavity of two fish. During the survey we also recovered three parasites from the family Cestoidea. Mature forms of *Bothriocephalus cuspidatus* were found in walleye from Devils Lake. Additionally, metacestodes of *Bothriocephalus* sp. lacking mature proglottids were recovered from black crappie, fathead minnow and walleye. Other cestodes recovered during the survey at Devils Lake were *Proteocephalus pinguis* in northern pike 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. Another nematode, presumptively identified as *Raphidascaris acus*, was found in yellow perch from Devils Lake. 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.

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 were 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.

Gram-negative bacteria *Aeromonas hydrophila and Aeromonas* sp. - *A. hydrophila* and other motile *Aeromonas* are among the most common bacteria in freshwater habitats worldwide. *Aeromonas* are routinely isolated from fish in warm, cool, and coldwater fish populations. However, *A. hydrophila* flourishes in warm water environments between 25 - 30°C. These ubiquitous agents are generally opportunistic pathogens or secondary invaders of fish predisposed by other infectious or non-infectious diseases. As with many courses of disease, acute and chronic stressors are important factors affecting *Aeromonas* infections among free-ranging and hatchery reared fish. Disease expression may be influenced by the physiological condition of fish as a result of adverse environmental conditions such as rapid increases in water temperature, low dissolved oxygen, high

levels of un-ionized ammonia, and high organic loadings (Esch and Hazen 1980; Walters and Plum 1980). While motile *Aeromonas* are capable of causing septicemic conditions, these bacteria also compose part of the normal intestinal microflora of healthy fish (Trust et al. 1974). *A. hydrophila* normal flora may become pathogenic if fish are sufficiently compromised.

Enterobacter cloacae.- *E. cloacae* was isolated from Red River sauger. The bacterium is widely distributed and commonly found in freshwater sediments. *E. cloacae* is considered an opportunistic pathogen of fish. *Enterobacter sp.* has been recovered from market fish as a part of bacteriological quality monitoring programs. Members of the family are found on plants, in water, and in the human intestinal tract as a part of normal flora.

Hafnia alvei.- The genus *Hafnia* is a member of the family *Enterobacteriaceae.* Other genera of the family are import causes of disease in animals worldwide. *H. alvei* has been reported to cause hemorrhagic septicemia in both brown trout and rainbow trout and is routinely isolated from freshwater fish. H. *alvei* was isolated from fathead minnow, northern pike, and white bass from Devils Lake, although no clinical signs of disease were noted.

Pantoea sp.- Pantoea was isolated from fathead minnow and white bass from Devils Lake. *Pantoea sp.* is frequently found on surface of plants and seeds, soil, and water, as well as from animals and human clinical specimens. They are generally regarded as opportunistic pathogens.

Pseudomonas sp.- Pseudomonas, like the *Aeromonas,* are ubiquitous bacteria commonly found in soil and aquatic environments. Growth of *Pseudomonas* covers a broad temperature range from 4 - 43°C allowing them to flourish in warm, cool, and coldwater environments. *Pseudomonas* is so widespread and numerous that secondary infections are common among fish compromised by other infectious and non-infectious agents. *Pseudomonas* is frequently found on eggs (Bell et a1 1971; Sugita et al. 1988), the skin and gills (Colwell 1962; Horsley 1973) and the intestines (Trust and Sparrow 1974; Austin and A1 Zahrani 1988) of a variety of fish species. Free-ranging fish may have increase susceptibility to *Pseudomonas* infections during extremes of temperature, pH, pollution and other environmental factors.

Brevundimonas diminuta.- B. diminuta was isolated from northern pike from Devils Lake. *Brevundimonas* is closely related to *Pseudomonas.* They grow slowly on nutrient agar and require 48 hours incubation at 37°C. *Brevundimonas* may be found in water, soil, and on plants, including fruits and vegetables. They are rare in clinical specimens and of doubtful clinical significance for northern pike.

Yokenella regensburgei.- *Yokenella sp.* are gram-negative, oxidase-negative, fermentative, motile rods possessing the characteristics of the family Enterobacteriaceae. *Yokenella regensburgei* is an opportunistic pathogen that phenotypically resembles *Hafnia alvei.*

Gram-positive bacteria *Renibacterium salmoninarum.*- Several kidney samples tested for R. *salmoninarum* by ELISA had OD readings above the negative threshold. However, the majority of these values were considered very low. Because active infection by R. *salmoninarum* could not be confirmed with the PCR assay, there is reason to believe the ELISA data may represent false-positive readings. Testing methods used in this survey may contribute to observed variations in prevalence because they examine the pathogen differently. Active infection is necessary for detection with PCR because bacterium genomic DNA is required to prime the amplification procedure. The ELISA measures a major extracellular protein of *R. salmoninarum,* known as p57 antigen, which is released in large amounts during infection and which accumulates in the kidney and other tissues (Barton et al. 1997). The antigen is known to persist in kidney tissue (Pascho et al. 1997) and may be present in measurable levels with ELISA for an unknown period of time subsequent to our ability to detect the bacterium. In this respect, the ELISA may detect active infection and/or prior exposure to R. *salmoninarum.* Additionally, false-positive reactions have been reported for ELISA (Dixon 1985; Turaga et al. 1987) while polyclonal ,antisera against R. *salmoninarum* used in the assay has been show to cross-react with other bacteria (Brown et al. 1995; Wood et al. 1995). In protocols for the *National Wild Fish Health Survey,* samples examined in PCR are not necessarily selected at random. In general, all samples taken from a population are first screened with ELISA and then a minimum of three samples with the highest ELISA OD values are selected for corroborative testing with PCR. In this way, the investigator assumes that as ELISA OD values increase the likelihood of PCR confirming active infections also increases. Should the first set of select samples be negative with PCR, the investigator may choose to examine other ELISA-positive samples although this is not routinely done mainly because of additional expense. For the *National Wild Fish Health Survey,* sample sites are considered *suspect* for R. *salmoninarum* and results are viewed as inconclusive when samples test positive with ELISA and negative with PCR. There is mounting evidence from surveys in other areas of the United States that positive ELISA OD values do not necessarily predict whether or not samples will also be positive when examined with PCR. Another explanation centers on the nature of the negative reference tissue used to establish the negative-positive threshold for antigen detection. In this and previous surveys of the study area, negative threshold OD values for ELISA were determined using standardized reference tissue obtained from fall chinook salmon for use in the *National Wild Fish Health Survey.* In the present survey, we calculated negative threshold values between 0.079 - 0.089. These values may be considered conservative. It is possible certain proteinaceous elements or other constituents of non-salmonid kidney may interfere with the ELISA and result in higher background readings thus producing false-positive results.

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. 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.

Viruses

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; Dogie1 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 a1.1987). 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.

CANADA

Table 1. Fish species in Devils Lake and the Red River Delta that were captured and assessed for pathogens and parasites.

	Devils Lake	Red River Delta
Fathead Minnow	+	+
Yellow Perch	+	+
Northern Pike	+	+
White Bass	+	+
Walleye	+	+
Brook Stickleback	+	+
Channel Catfish		+
Goldeye		+
Sauger		+
Emerald Shiner		+
Black Bullhead	+	
Black Crappie	+	
White Sucker	+	

PARASITOLOGY

The objective of this study was to determine the parasite community in, pike, walleye, sauger, yellow perch, goldeye, white bass, emerald shiners, fathead minnows and brook stickleback.

Considering the lack of efforts to collect historical and contemporary baseline data, and no sustained monitoring of biota across the Canadian-United States border, it was not surprising that two recent alien parasite species have been discovered by this preliminary study. The implications of the Asian tapeworm, *Bothriocephalus acheilognathi* and the blood dwelling trematode, *Sanguincola occidentalis*, on the commercial fishery of Lake Winnipeg are unknown at this time. *B. acheilognathi* could impact the food web by causing mortality in young of the year emerald shiner (*Notropis atherinoides)* which is one of the most important prey species of pike, walleye and sauger. The impacts of *B. acheilognathi* on other young-of- the- year cyprinids and the young-of-the-year commercially fished walleye, sauger, pike and goldeye is currently unknown since most of these species are new host records for this parasite.

Materials and Methods

Sample Sites

The sample locations are outlined in Figure 3. Most of the specific locations are in the vicinity of the mouth of the Red River.

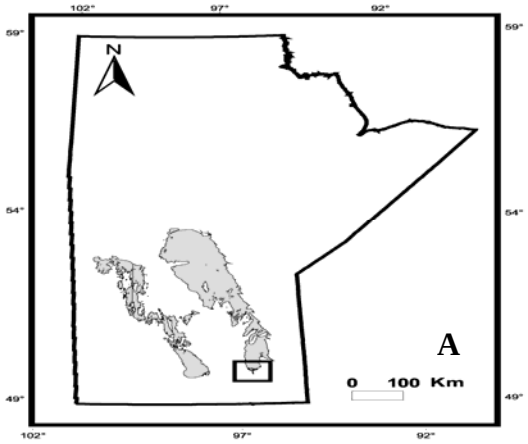

Figure 3. Sampling locations in the Red River Delta, Manitoba.

Fish collections and data acquisition The species of fish sampled are shown in Table 1. Fish samples were collected by seining and with gillnets. 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 and otoliths, opercles, and cleithrum collected and used for aging. Spines were collected for aging goldeye but at the writing of this report sections were not available for counting growth rings.

Food collected from the stomach was identified and counted. Any identifiable parts of food items in the intestine were also recorded and identified as fish otoliths or vertebrae and insect parts (Diptera eyes, wings or chironomid head capsules), etc.

Necropsies. — 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.

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

The cestode *Bothriocephalus acheilognathi* — The discovery of the asian tapeworm *B. acheilognathi* in several fish hosts from the Red River delta samples led to additional sampling of emerald shiners.

Results

Fish Data

Individual fish data were recorded including length, weight, weight of soma, gonads, liver and visceral fat for each fish.

Fish Food

The brook stickleback consumed chironomids, amphipods, and a few copepods. Emerald shiner food was mostly cladocerans, copepods and insects. The fathead minnow stomachs were mostly empty but cladocerans and chironomids were occasionally noted. Goldeye food items included cladocerans, chironomids, copepods, water boatman, coleopterans and fish including small white bass. The most common food item in the goldeye samples was water boatman. The food of northern pike was mostly fish but cladocerans, chironomids and gammarids were note occasionally. The food of sauger was mostly fish but occasionally Odonata and other insect parts were noted. The most common food item of walleye was fish with an occasional gammarid noted. The main food items found in white bass were cladocerans, gammarids, copepods and water boatman. The main food recorded from yellow perch were cladocerans, chironomids, copepods, gammarids and water boatman.

Parasites

The brook stickleback had very few parasites and most were larval forms. The most common parasite of emerald shiner was the tapeworm, *B. acheilognathi*, in the foregut and occasionally a Myxozoan on the gills. Two larval trematodes, *Ornithodiplostomum ptylochleidus* and *Bolbophorus confuses*, were common in fathead minnows. A total of five parasite species were recovered from goldeye, with the trematode *Crepidostomum illinoiensis* and the tapeworm *Bothriocephalus cuspidatus* the most common. The most common parasite of pike was the tapeworm, *Proteocephalus pinguis,* but *B. acheilognathi*, *Pomphorhychus bulbicollis* and *Camallanus oxycephalus* were also noted. A total of seven parasite species were recorded from sauger. The two most common parasites of sauger were the crustacean *Ergasilus luciopercarum* and the tapeworm *B. cuspidatus*. Walleye harbored eight parasite species with the three most common species being the crustaceasn, *E. luciopercarum*, and the tapeworms *B. cuspidatus* and *P. pearsei*. White bass had 11 species of parasite recorded with the monogenean, *Onchcleidus chrysops,* and the two tapeworms *B. claviceps* and *B. cuspidatus* mostly frequently recorded. Eight parasite species were recovered from yellow perch and of these eight the tapeworm, *P. pearsei,* was the most common.

Bothriocephalus acheilognathi was found in six species of fish with the most common fish host being the emerald shiner. Prevalences of *B. acheilognathi* of ~ 14 and 32 % for sauger and pike, respectively indicates that these two fish hosts likely play an important role in egg dissemination.

The distribution of *B. acheilognathi* in emerald shiner indicates that all stages of development are present in the September sample from the Red River delta. This suggests that not only are emerald shiners an important host for the dissemination of the eggs of the gravid worms but emerald shiners continue to acquire the parasite in the fall and may

be an important source of new infections the following spring. The levels of *B. acheilognathi* in emerald shiner from different sample locations around the lake indicate it is now widespread in Lake Winnipeg. Although the sample of older emerald shiner examined for *B. acheilognathi* was small, no infections were found in emerald shiners greater then 1 year old.

Since samples of emerald shiner from the Winnipeg River were negative for *B. acheilognathi*, the route of entry into Canada is still unresolved. There is an urgent need for a comprehensive study to determine 1) the route of entry into Canada of this parasite, 2) its pathogenicity in all ages of Lake Winnipeg fishes, especially the commercially fished species and 3) its transmission dynamics in a north temperate region of the world since this parasite has been largely restricted, in the past, to south temperate and tropical areas.

Parasite Summary Nine parasite taxa were identified from Devils Lake, and 28 taxa were identified from the Red River Delta (Table 2). Two external parasite taxa, *Gyrodactylus hoffmani* and *Epistylis* have not yet been identified from fish collected in Canada. Future assessments of presence/absence of these two parasites species should be a focus of future work in Canada. Two other parasite species found in the eyes of fish, *Diplostomum spatheceum* and *Postodiplostomum* sp., were not detected in fish from Red River Delta in fall 2006. However, both are known to occur in the Lake Winnipeg basin in Canada.

Presence and relatively high levels of infestation of the Asian tape worm, *B. acheilognathi*, in several fish species from the Red River Delta is of concern. This species is a foreign and invasive species that in the future may cause mortality in some species present in the Red River Delta and in Lake Winnipeg.

Table 2. Parasite taxa identified in Devils Lake and the Red River Delta.

Taxa	Devils Lake	Lake Winnipeg
Protozoa	1	0
Myxozoa	0	1
Monogenea	1	1
Digenea	2	9
Cestoda	3	10
Nematoda	2	5
Insecta	0	1
Crustacea	0	1

HISTOPATHOLOGY

The purpose of this particular portion of the project (and the subject of this interim report) was to perform a fish health survey of 10 species of fish from Lake Winnipeg using light microscopy. Sixty fish from each species (with the exception that only 7

channel catfish were caught) were examined. This is an interim report, and only the results of the survey are included. The final report will follow a similar format but will include additional fish ('spring 07' collection), the methods used, and interpretation based on the literature.

For this interim report, summaries for each species are included at the beginning of each segment of the report. The summary includes the tissues examined, common lesions present that were not systematically evaluated and any issues peculiar to a species. Next, the notable lesions that were systematically evaluated are described as well as the number of fish affected. For each of these a morphologic diagnosis (summary phrase) is also included. The majority of these lesions/organisms are illustrated with a photograph (powerpoint file for each species). These photographs are low resolution images to allow easy transmission, and will be reproduced at higher resolution for the final report. The remainder of the document is a list of the morphological diagnoses for each individual fish. Occasionally, a short description is given for an additional lesion affecting a fish that was not common or more noteworthy. Rarely, these have an accompanying photo.

The vast majority of agents found were metazoan or protozoan (including myxosporeans and microsporeans) that caused local tissue reactions. In the majority of instances, parasites that are well-adapted to their host cause relatively minimal mortality but may impair growth, reproduction, etc. depending on location and numbers. Parasites that have moved to a new host/location typically cause the greatest impact. A relevant example to this study is the discovery of the Asian tapeworm in fish from Lake Winnipeg. Almost all of the species of fish examined by light microscopy had intestinal cestodes. The majority of the intestinal cestodes produced no, or limited, histological lesions, which is not unusual. The effects of the Asian tapeworm will vary markedly among species, but are not best assessed by tissue lesions and/or overt mortality. Unless fortuitous sections reveal discriminating features, cestodes are typically not easy to identify using light microscopy to a family, let alone species, level. This is also true for trematodes and less so for nematodes, crustaceans, myxosporeans, protzoans, etc. Detailed light microscopic examination with a micrometer, special stains and in some cases electron microscopy can speciate some protozoans, myxosporeans and microsporeans. Nematodes can often be identified to a family based only on visible characteristics. For this interim report organisms were only identified to the level of class.

Lesions associated with bacterial and viral agents were rare. These were limited to epitheliocystis (ricketsia-like bacteria) in white bass, yellow perch and fathead minnow that are visible in light microscopic sections, and lymphocystis in walleye, a piscine iridovirus that produces characteristic histological lesions and is known to exist in Lake Winnipeg.

There were numerous lesions present in these fish, as would be expected for most wild fish. Many of these lesions, however, were not associated with a visible organism. The majority of the lesions not directly associated with an agent are most likely remnants of metazoan migrations, etc. or lesions left after the inflammatory/immune response has removed the agent.

The walleye was the only species in which a neoplastic lesion was noted (dermal sarcoma). This is a well-recognized lesion in walleye and has been recorded from Lake Winnipeg previously. A few hepatic altered foci were noted, and these can be considered to be potential pre-neoplastic lesions. However, these were rare. Judged by the lesions produced and number of fish affected, the lesions that are most likely to have a significant impact on fish health are: branchial myxosporeans of emerald shiners; branchial epitheliocystis of white bass; branchial trematodes in white bass; and the intracardial trematodes of walleye and sauger. A single agent that would likely have the greatest impact on fish health (but this could still be relatively minimal) is listed for each species below.

Of those agents specifically noted, the intracardial trematodes of walleye (and to a lesser extent sauger) are likely to have the most substantial impact on fish health. The majority of walleye had significant lesions present in the ventricle, atrium and bulbous arteriosis (in that order). If anything, the number of fish affected as determined here is an underestimate as the heart was not collected from several walleye and was not always sectioned exactly across the location of the parasite. The heart valves were often involved to an extent that would interfere with function. Many of these fish are likely anemic (due to red cell breakdown – as evidenced by splenic hemosiderin accumulation) and would be less fit. A morphologically similar trematode and lesion was also seen in the sauger (a closely related fish) however the number of fish affected and the severity of the lesions were less. There were trematodes (one had a haptor and they are therefore most likely monogenetic trematodes) found on the gills of the fathead minnow. *Gyrodactylus* sp. are somewhat similar (Gyrodactylus are monogenetic trematodes) however classification can best be performed with skin scrapes.

Those agents judged to have the most significant impact on fish health for each species are:
Emerald Shiner (ES - *Notropis atherinoides*) – branchial myxosporeans;
Brook stickleback – intralenticular trematode;
Fathead minnow – hepatic nematodes (also present in BS);
Yellow perch – intestinal cestodes;
Northern pike – not clearly a single choice;
Walleye and Sauger – intracardial trematode;
Channel catfish – unknown – only 7 examined;
Goldeye – intestinal cestodes;
White bass – branchial monogenetic trematodes.

PATHOLOGY

Methods

For pathology 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 were to be 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 Oct 2006 and ended 26 Oct 2006.

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 mxosprean parasite *Myxobolus cerebralis* (causative agent of whirling disease in salmonids).

Testing for the presence of viral pathogens of concern 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). This method differs slightly from the method described in National Wild Fish Health Survey procedure used by the USFWS, but both methods are considered equivalent. Bacterial isolation and identification methods were also similar. The method used in the Winnipeg Fish Health Laboratory was that described in the 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.

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).

Results

Virology

114 pools of kidney/spleen tissue were prepared from the 10 targeted species. Cytopathic effect (CPE) indicative of a viral infection in any of the species tested was not observed in any of the three cell lines used.

Bacteriology

A total of 100 bacterial isolates were cultured from the 10 targeted species. Only 14 of the 100 isolates were identified with any degree of confidence using the API-20E identification system. *Aeromonas hydrophila* was the most common identifiable isolate (present in yellow perch, fathead minnows, white bass and channel catfish); followed by *Pseudomonas aeruginosa* (present in yellow perch, fathead minnows, and white bass); and one isolate each of *Hafnia alvei* (yellow perch); *Pseudomonas fluorescence* (emerald shiners); *Flavobacterium* sp. (sauger) and *Plesiomonas shigelloides* (white bass). Bacterial pathogens of concern such as *Aeromonas salmonicida* (causative agent of furunculosis) and *Yersinia ruckeri* (causative agent of enteric redmouth disease) were not detected.

Renibacterium salmoninarum was not detected in the IFAT stained kidney smears prepared from the 547 fish tested at the WFHL (2 smears per fish X 547 fish = 1094 smears). Nor was *R. salmoninarum* ,detected by IFAT in any of the kidney smears provided by the Bozeman Laboratory. The Bozeman lab obtained positive ELIZA results from 32 of 48 fish from which we obtained kidney smears. Confirmatory testing conducted at the Bozeman lab on 11 ELIZA positive fish using polymerase chain reaction (PCR) failed to confirm their positive results. The positive ELIZA results may be considered suspect as they were not confirmed by PCR, or if the pathogen was truly present in these samples it was at a level not detectable by IFAT staining; although it is curious that PCR, which is considered to be a highly sensitive test method, failed to detect the presence of *R. salmoninarum* nucleic acid.

Parasitology

(*Myxobolus cerebralis*): This myxosporean parasite infects members of the Salmonidae family. To our knowledge, the pathogen has never been detected in Canada; however, it does exist in the eastern U. S. as well as the U. S. inter-mountain west. Because the pathogen is present in the U. S. and lake whitefish (a member of the Salmonidae family), is a commercially important species inhabiting Lake Winnipeg, it was decided that a 60 fish sample would be tested for the presence of the pathogen. Test results were negative for *M. cerebralis*.

All fish sampled in this initial survey appeared healthy and there was no clinical evidence of infection with bacterial or viral pathogens of concern. Lymphocystis and dermal sarcoma which are neoplasia having a viral etiology were observed in a small number of walleye and sauger. The bacterial isolates that were positively identified are ubiquitous water-borne organisms that can be readily isolated from the gastrointestinal tract and external surface of fish. They are considered opportunistic pathogens because they are capable of producing septicemic disease in fish that become stressed when subjected to less than optimal environmental conditions such as elevated water temperatures, low oxygen concentrations, and poor water quality.

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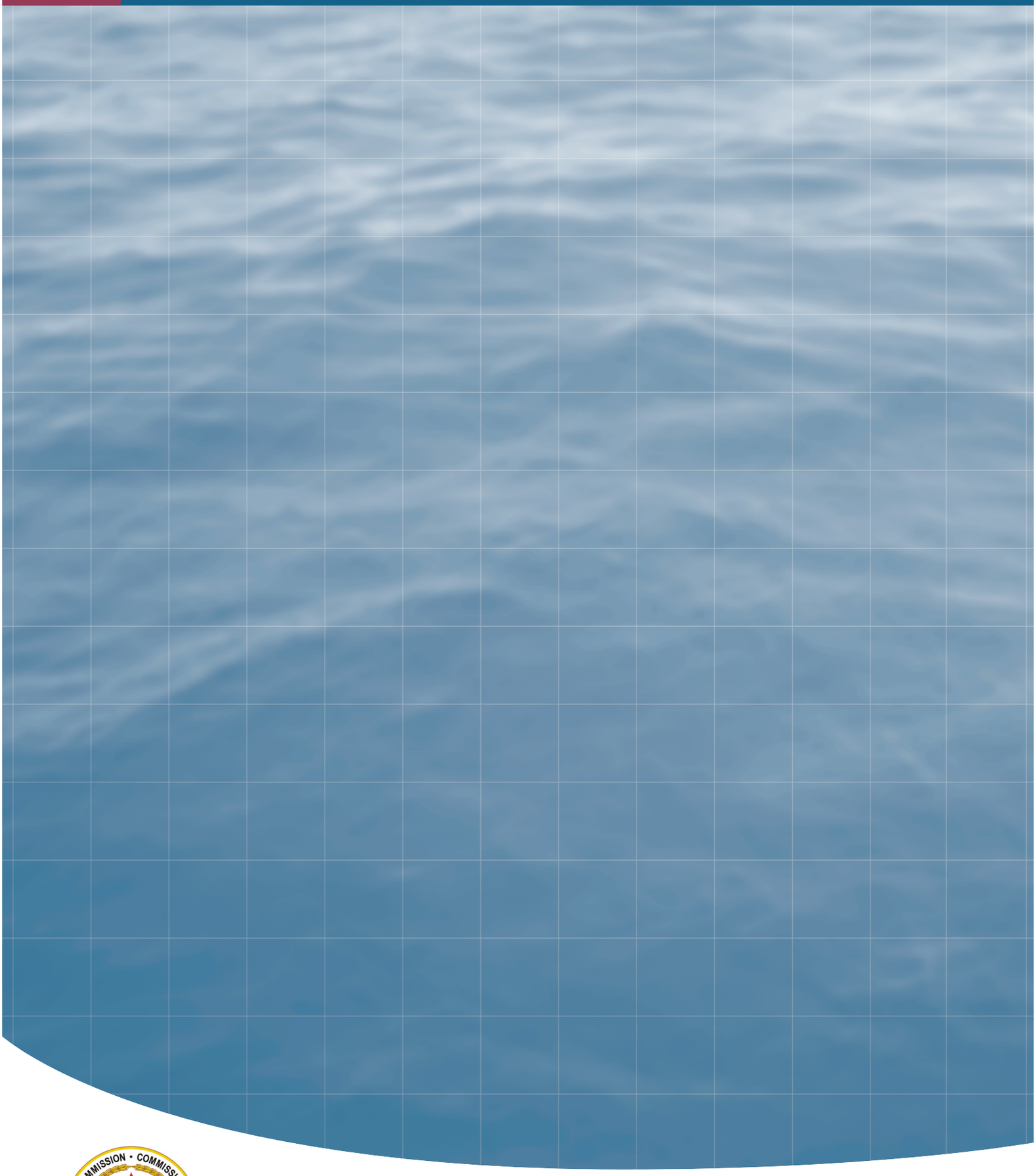


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