9-2005

Survey of Specific Fish Pathogens in Free-Ranging Fish from Devils Lake, North Dakota

Crystal Hudson
U.S. Fish & Wildlife Service

Kenneth Peters
U.S. Fish & Wildlife Service

http://georgewbush-whitehouse.archives.gov/ceq/fish2005.html

Survey of Specific Fish Pathogens in Free-Ranging Fish from Devils Lake, North Dakota

Prepared for Council on Environmental Quality
Washington, DC
Prepared by
Crystal Hudson
and
Kenneth Peters
U. S. Fish & Wildlife Service
Bozeman, Montana 59718
Bozeman Fish Health Center
Technical Report 05-02
September 2005

Executive Summary

Several biological surveys have been performed at Devils Lake, North Dakota to provide timely information to resource managers to assess the potential for biota transfer from the operation of an outlet designed to carry water to the Sheyenne River. In July, 2005 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 where

recovered from six of the seven species of fish sampled. Other less common species included *Pleisomonas shigelloides* and *Pseudomonas putrifaciens.* Two Gram-positive bacteria were also cultured including *Corynebacterium renale* and *Streptococcus sobrinus.* 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. Overall, fish appeared in good general health. Further discussions of major findings from the Devils Lake survey are presented here.

Acknowledgements

This survey could not have been accomplished within the allotted time without the strong spirit of cooperation shared by all those involved. We would like to express our appreciation to the staff of the Bozeman Fish Center including Molly Bensley, Crystal Hudson, Beth MacConnell, Ken Peters, Marlene Rodarte, Linda Vannest, Tammy Weiss, and to the staff of Lacrosse Fish Health Center including Becky LaSee, Eric Leis, Rick Nelson, Terry Ott, Ken Phillips, Cory Puzach, and Ashley Umberger. Their diligent efforts in the laboratories to process and test such a large volume of samples in an expeditious and professional manner was commendable. The survey would not have been possible without the tremendous sampling effort put forth by the staff of the Missouri River Fish and Wildlife Management Assistance Office. The sampling crew, lead by Steven Krentz with assistance and technical support from Everett Nelson, Zac Sandness, and Ryan Wilson, endured long hours as well as hot and windy weather to capture fish for testing. We would like to extend our sincere appreciation to Andrew Moran, Fish and Wildlife Director for the Spirit Lake Nation, for his assistance throughout the week of sampling. Clarence Morin and Vince Greyhorn Jr, also of Spirit Lake Nation, provided additional assistance with the netting efforts. We also thank Myra Pearson, Chairwoman, Spirit Lake Nation, for taking time out of her busy schedule to visit the field sampling site at Six Mile Bay. We are especially grateful to Lynn Schlueter, North Dakota Game and Fish Department, who provided us a trailer, affectionately referred to as the “fish-mobile lab”, which allowed us to collect sample in a more pleasant and aseptic environment. We also thank Lynn for providing assistance with field coordination activities and freely shared his knowledge of the Devils Lake fish community. Terri Thorn, GIS specialist for Ecological Services, Bismarck, ND, created the sampling map of Devils Lake. Finally, we would like to thank Bryan Arroyo, Council for Environmental Quality, Washington, DC, for project coordination activities at the National level and his words of encouragement throughout the long week. Funding for the survey was provided by the U. S. Army Corps of Engineers under Interagency Agreement number W81G6752577225.

Table of Contents

List of Tables

List of Figures

Introduction

Devils Lake is located in northeastern North Dakota in southern Ramsey and northern Benson counties and is approximately 143 km west of Grand Forks and 155 km north of Jamestown. Devils Lake, and neighboring Stump Lake, receives most of the surface drainage in the Devils Lake sub-basin which covers approximately 2.4 million acres. The Devils Lake sub-basin lies within the much larger Red River-Hudson Bay drainage. Presently, the lakes have no perennial outlets and water levels are affected primarily by rainfall, snowmelt runoff, and evapotranspiration. Surface runoff in the basin flows through many small coulees, wetlands, and lakes. Major inflows to Devils Lake include Big Coulee (Mauvais Coulee) and Channel A. Historically, the Devils Lake basin has experienced periods of climatic fluctuation which have caused extreme changes in the lake's water surface elevation (Figure 1). The level of Devils Lake dropped significantly during periods of drought in the 1930s and reached a historic recorded low of about 1402 ft-msl (approximately 2 ft deep) in 1940. Since that time, the lake has been rising in a somewhat erratic fashion, with years of decline and increase. During the period from 1993 to 2001, Devils Lake surface area increased from 50,000 acres to about 125,000 acres. In 1999, the lake reached an elevation of 1446.6 ft-msl and water began to spill from East Devils Lake into Stump Lake for the first time in several hundred years. If the elevation of Devils Lake remains above 1446 ft-msl it will continue to spill into Stump Lake until they reach equilibrium. Should the present wet conditions continue beyond that point, Stump Lake will overflow to the Sheyenne River at approximately 1459 ft-msl. Geologic records indicate Devils Lake has overflowed into the Sheyenne River twice in the last 4,000 years.

Figure 1.— Water surface elevation (feet above mean sea level) for Devils Lake from June, 1867 through January, 2005. (Source: U. S. Geological Survey, Water Resources of North Dakota).

Stabilization of Devils Lake sub-basin lakes and wetlands has been an issue in the region for many years both in times of low and high water periods. In response to the steep rise in water during the last decade, an outlet has been constructed to carry water from Devils Lake to the Sheyenne River for the purposes of reducing flooding problems. The Sheyenne River flows southeasterly to the Red River which flows north to Lake Winnipeg and Hudson Bay. At the request of the Council On Environmental Quality (CEQ), the U.S. Fish and Wildlife Service performed a fish pathogen survey at Devils Lake during July 2005. The survey was conducted in response to the need for information on the prevalence, if any, of fish pathogens and parasites. Results of the pathogen survey were included with other biological survey information to assess

the potential for biota transfer from the operation of the lake outlet. The survey was performed under the auspices of the U.S. Fish and Wildlife Service *National Wild Fish Health Survey.*

Few studies have been conducted in the either the Devils Lake sub-basin or in the Red River drainage that address the distribution and prevalence of specific bacterial and viral fish pathogens. There have been a number of surveys for fish parasites in regional prairie impoundments and lakes and in select streams in North Dakota (Mizelle and Kritsky 1967; Sutherland et al. 1979; Reinisch 1981; Forstie and Holloway 1984; Holloway 1986). During 2001-2002, the U. S. Fish and Wildlife Service, Bozeman Fish Health Center performed a similar pathogen survey under contract with the U.S. Army Corps of Engineers (COE) (Peters 2002). The survey was performed under the biota transfer section of the COE environmental impact statement scope of work for construction of a lake outlet. Fish were collected from Devils Lake and the Sheyenne and Red rivers and tested for a list of specific bacterial and viral fish pathogens although general parasite screening was not included. Results of the study suggested that antigen of the Gram-positive bacterium Renibacterium salmoninarum, as measured by the enzyme-linked immunosorbent assay (ELISA), was present in several species of fish from all three bodies of water. However, active infection by R. salmoninarum was not confirmed or corroborated by the highly specific nested-polymerase chain reaction assay. The investigators speculated that the relatively low positive ELISA optical density values may have been false-positive readings or that non-salmonid species had a higher background reading compared to the salmonid kidney tissue used to establish the negative-positive threshold for the ELISA. Investigators also comment that future work on Devils Lake should include larger numbers of fish and that sampling should occur at more than one time during the year. As management of Devils Lake water levels continue to evolve, information updates will be important to adequately assess current operations and various future alternatives. Accordingly, this report provides results and discussion of the latest fish pathogen and parasite survey from Devils Lake completed during the summer of 2005.

2005 Survey Objectives

1.— Determine the presenceof specific fish pathogens and parasites in fish from Devils Lake.

2.— Provide fish health specialists, fisheries managers, and other decision makers with a comprehensive pathogen survey report that may be used in performing risk analysis.

3.— Provide survey results for viewing on the world wide web via the U.S. Fish and Wildlife Service National Wild Fish Health Survey database and graphical interface.

Methods

Fish collection and tissue sampling— Based on information provided by North Dakota Game and Fish Department, nine species of fish were known to occur in Devils Lake and were proposed for collection (Table 1). Fish were sampled from two main areas of Devils Lake between 26 July and 30 July 2005 to estimate the prevalence, if any, of specific bacterial and viral fish pathogens and parasites (Figure 2). The first sample area was described as West Bay which is located in the southwestern portion of Devils Lake near Minnewaukan. The second

sample area was in Six Mile Bay located in the north-central section of the lake. Sampling in the Six Mile Bay area extended north into the mouth of Channel A. 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. Finally, we also deployed an experimental deep water fyke net by attaching two nets to one lead end-to-end. We established a target sample size of 60 fish for each species. 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).

Table 1.— Species of fish proposed for sampling at Devils Lake.

Species	Common name and abbreviation	Family
Ameiurus melas	Black bullhead - BLB	Ictaluridae
Esox lucius	Northern pike - NOP	Esocidae
Pomoxis nigromaculatus	Black crappie - BLC	Centrarchidae
Pimephales promelas	Fathead minnow - FHM	Cypriniformes
Catostomus commersoni	White sucker - WHS	Catostomidae
Morone chrysops	White bass - WHB	Percichthyidae
Stizostedion vitreum	Walleye - WAE	Percidae
Perca flavescens	Yellow perch - YEP	Percidae
Culaea inconstans	Brook stickleback - BST	Gasterosteidae

Figure 2.— Map showing geographic locations of sampling sites (red dots) on Devils Lake where fish were collected during the pathogen survey.

Analysis of pathogen prevalence.— Upon collection, fish were transported alive to temporary field laboratories set-up near public access sites on the lake(Figure 3). Fish were anesthetized with tricaine methanesulfonate (Finquel®), sorted by species, measured, and then examined externally and internally for clinical signs of disease or other abnormalities (Figure 4). 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.

Figure 3.— Field laboratory at Devils Lake used for fish necropsy and tissue collections.

Samples were assayed for fish pathogens according to protocols and procedures for the *National Wild Fish Health Survey* (U.S. Fish and Wildlife Service 2005) and the *Blue Book: Suggested*

Procedures for the Detection and Identification of Certain Finfish and Shellfish Pathogens (AFS-FHS 2004). Principle fish pathogens of the *National Wild Fish Health Survey* included specific organisms that are known to cause disease in cultured or wild fish and are considered prohibitive organisms in most state and federal fish health inspection programs. A summary of procedures used in this survey is provided below. Details of these procedures may be examined on the worldwide web following the Protocols and Procedures link on the National Wild Fish Health Survey website at http://wildfishsurvey.fws.gov, the American Fisheries Society Fish Health Section at http://www.fisheries.org/fhs/bb_inspection.htm, and the *Standard Procedures for Aquatic Animal Health Inspections* at http://www.fws.gov/fisheries/FHC/Volume1.htm.

Figure 4.—Fish Health Center staff members perform fish necropsy, tissue sampling and examinations for parasites.

Virology.— Standard cell culture techniques were used to test fish for viruses. 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. Tissues 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 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 fathead minnow (FHM) cell lines and incubated at 25°C. Viral assays were monitored for cytopathic effect (CPE) using inverted light microscopy for 28 d.

Bacteriology— Isolation of aerobic bacterial pathogens was performed by inserting a disposable sterile loop (1.0 or 10.0 µL) into the kidney and streaked across the surface of tubes containing brain-heart infusion agar. Tubes were incubated at 22°C and monitored for bacterial growth at 24, 48, and 72 h. If no growth appeared after 10 d culture tubes were discarded. Suspect bacterial growth was sub-cultured for purity and then differentiated using a flow chart with standard biochemical profiling techniques and tests for motility by the hanging drop method. Several commercial systems were used to identify bacteria including the API 20E (bioMérieux Vitek, Inc., Hazelwood, Mo.), Crystal Enteric/Nonfermenter (Becton Dickinson, Inc., Cockeysville, Md.), and Biolog Microbial ID/Characterization (Hayward, Ca.) for Gram positive isolates. Where appropriate, further confirmation of suspect bacterial isolates was performed with either direct or indirect fluorescent antibody tests, serum agglutination tests, or with polymerase chain reaction (PCR) assays. Kidney tissue was also collected to quantify soluble antigen of *Renibacterium salmoninarum* by the enzyme-linked immunosorbent assay (ELISA; Pascho and Mulcahy 1987). When small fish had insufficient kidney for testing of individuals, we pooled tissue from two to five fish until a suitable 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). The prevalence of *R. salmoninarum* was estimated as the percentage of samples with positive ELISA OD values. 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). Pelleted kidney tissue remaining from the ELISA sample was used in the PCR. 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. DNA template was extracted from suspect kidney tissue 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. Kidney samples that had detectable antigen of R. salmoninarum in the ELISA but tested negative with the PCR were considered inconclusive and viewed as suspect.

Parasitology— All fish were examined externally and internally for gross signs of parasitism. In addition, sub-samples of each species of fish were examined for microscopic parasites. Wet mounts were prepared from skin scrapings, fin and gill clips, and intestinal scrapings. Wet mounts were examined with compound light microscopy at 100 – 400X magnification. We also examined the body surface and fins of 60 fathead minnow specifically for *Gyrodactylus hoffmani* with a dissecting microscope at 6 – 40X magnification. Parasites recovered from the peritoneal cavity and the intestines were preserved in either AFA (cestodes) or glycerin-alcohol (nematodes) solutions. Identification of parasites collected in the field was performed by a parasite specialist at the LaCrosse Fish Health Center. Finally, muscle tissue from yellow perch was collected to test for the presence of the microsporidean parasite Heterosporis sp. Muscle tissue was first examined grossly for signs of degeneration or necrosis and then a 50 – 100 mg sample from each fish was macerated for extraction of DNA with the Qiagen DNeasy® extraction kit. Tissue extracts where tested with a nested PCR using methods adopted from D. Sutherland and P. Stelzig (University of Wisconsin – LaCrosse). Amplified 7
DNA was subjected to electrophoresis in a 1.5% agarose gel, and then stained with ethidium bromide, visualized with UV light, and photographed.

Results

Sampling.— Excluding young-of-year fish and fathead minnow, over 500 fish were caught as a result of about 480 net hours of effort. Of these, a total of 313 fish representing seven of the nine target species were collected and processed for pathogen testing (Table 2). Tissue samples were taken from black crappie, fathead minnow, northern pike, walleye, white bass, white sucker, and yellow perch (Figure 5). The target sample size of 60 fish was obtained for fathead minnow, walleye, and white bass however fewer numbers of other target species were found. We did not catch any black bullhead regardless of sample location or gear type used. Only two brook stickleback were caught and they were not tested because of insufficient sample size for tissue collection and pooling. Low catch rates for some species were most likely attributed to either relative low abundance or because seasonal distribution and occurrence in selected sample areas was low. During the last two days of sampling, nets were relocated further north of Six Mile Bay

into Channel A to target additional northern pike, black crappie, white sucker, and yellow perch while avoiding excess by-catch of walleye and whitebass. Use of the modified double fyke net in this area resulted in a substantial increase in the number of adult black crappie captured.

Table 2.— Composition of fish collected from two main sample areas on Devils Lake.

	Number of fish sampled by species						
Devils Lake sample sites	Black crappie>	Fathead minnow	Northern pike	Walleye	White bass	White sucker	Yellow perch
Minnewauken Flats and Six Mile Bay	32	60	57	60	60	12	32

Figure 5.— Members of sampling crew display fish collected from Devil Lake. Species shown (left to right) are walleye, northern pike, black crappie, and yellow perch.

Bacteriology.— Primary bacterial culture tests were negative for reportable bacterial fish pathogens listed in most state and federal regulations for fish health inspections in the United States. Additionally, none of the bacterial pathogens listed in the *National Wild Fish Health Survey* program were isolated. These negative findings 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 more than 80 presumed mixed culture isolates which resulted in about 150 pure cultures. Upon screening with preliminary biochemical and motility tests, we arrived at about 60 pure cultures that required further differentiation and identification with commercial test systems listed in the preceding methods section. The majority of these isolates were Gram-negative motile rods (Table 3). *Aeromonas hydrophila* was the most common bacterium and was isolated from five of the six species tested including black crappie, fathead minnow, northern pike, white sucker, and yellow perch. We isolated another motile aeromonid from fathead minnow and walleye but the test system did not predict species with a sufficient level of confidence. The second most common Gram-negative bacterium was *Pleisomonas shigelloides* which was found in black crappie and fathead minnow. *Pseudomonas putrifaciens* was isolated from fathead minnow and *Pseudomonas sp.* was isolated from black crappie. In addition to the preceding Gram-negative organisms, two Gram-positive organisms were also cultured. These were identified as *Corynebacterium renale* isolated from fathead minnow, walleye, and white bass, and *Streptococcus sobrinus* which was isolated from northern pike. None of the fish we examined had any external or internal clinical signs of bacterial disease. Infected fish could best be described as asymptomatic carriers of these bacteria.

Table 3.— Identification of bacteria within two main staining groups and species of fish from which isolates were cultured for samples collected at Devils Lake. Abbreviations of fish common names are explained in Table 1.

	Name of bacteria		
Staining characteristic	Genera	Species	Species of fish infected
Gram-negative	Aeromonas	hydrophila	BLC, FHM, NOP, WHS, YEP

	Aeromonas	sp.	FHM, WAE
	Pleisomonas	shigelloides	BLC, FHM
	Pseudomonas	putrifaciens	FHM
	Pseudomonas	sp.	BLC
Gram-positive	Corynebacterium	renale	FHM, WAE, WHB
	Streptococcus	sobrinus	NOP

Antigen of *R. salmoninarum* was detected by the ELISA in kidney tissues of all seven species of fish tested during the survey (Table 4). The ELISA negative threshold (cut-off) OD value determined from standardized reference tissue was 0.075. Most samples (97.4%) with OD values above the negative threshold were in the low antigen level category. Only two samples, one each from northern pike and yellow perch, had OD values in the medium antigen level category. Antigen was not detected in only 2.6% of the samples tested with ELISA. Three ELISA samples from representing each species of fish were tested with the nested-PCR for *R. salmoninarum* and all samples were negative.

Table 4.— Percent of samples with detectable levels of *R. salmoninarum* antigen and mean antigen level category as measured by the ELISA, and corroborative testing with a nested PCR assay for seven species of fish collected from Devils Lake. Abbreviated common names for fish are explained in Table 1.

	ELISA			PCR Assay	
Fish species	Number tested	Percent positive	Mean antigen level	Number tested	Percent positive
Northern pike	57	100.0	Low	3	0
Walleye	60	96.2	Low	3	0
Yellow perch	32	100.0	Low	3	0
Black crappie	12	100.0	Low	3	0
White bass	53	89.6	Low	3	0
White sucker	12	91.7	Low	3	0
Fathead minnow	60	100.0	Low	3	0

Virology.— Nearly 70 pooled tissue samples were collected from among the seven species of fish captured at Devils Lake. Samples were tested for viral fish pathogens at two different incubation temperatures and monitored for 28 d. No viral fish pathogens were detected with the variety of standardized cell culture assays used during the survey.

Parasitology.— The microsporidian parasite *Heterosporis sp.* was not detected in muscle tissue from yellow perch (n = 7). Also, *Gyrodactylus hoffmani* was not detected during examination of 60 fathead minnow. Two ciliated protozoan parasites were observed on the body surface of fish during the survey. The motile Peritrich *Trichodina sp.* was observed in wet mounts of skin scrapings from walleye, white bass, and yellow perch (Figure 6). The sessile Peritrich *Epistylis*

sp. was detected in skin scrapings from a single yellow perch (photo not available). Amoeba were observed on the gills of both white sucker and yellow perch (Figure 5).

Figure 6.— *Trichodina sp.* observed from skin scrapings from yellow perch (left), and amoeba observed on the gills of white sucker (right).

Larval forms of the parasitic nematode *Contracaecum sp.* were recoved from walleye. During the survey we also recovered three different cestode parasites. *Bothriocephalus custpidatus* was found in walleye (Figure 7), *Proteocephalus pinguis* in northern pike(Figure 8), and Ligula intestinalis from fathead minnow and yellow perch.

Figure 7.— Whole-mount stained specimens of *Bothriocephalus cuspidatus* from walleye (left), scolex stained with acetocarmine (center), and proglottids stained with hematoxylin (right).

Figure 8.— Stained whole-mount of *Proteocephalus pinguis* recovered from northern pike.

Fungal pathogens.— No fungal pathogens were observed either externally or internally among the seven species of fish examined during the survey.

Discussion

During July 2005, we examined and tested more than 300 fish collected from two main sampling areas on Devils Lake for fish pathogens and parasites using protocols for the *National Wild Fish Health Survey.* Overall, the health and condition of fish from Devils Lake appeared to be very good. None of the fish examined, regardless of species, size, or sample site had any external or internal clinical signs indicative of disease. Even fish infested with macro- and microscopic parasites appeared to be in good health with no obvious signs of distress or disease.

The survey of Devils Lake resulted in the isolation seven species of bacteria including both Gram-negative and Gram-positive organisms. In addition, antigen of the Gram-positive bacterium *R. salmoninarum* was detected in very low levels from all species of fish sampled, however active infections could not be confirmed with the nested PCR. Major parasite findings during the survey included a single nematode, three cestodes, two ciliated protozoan, and gill amoeba. A discussion of each organism recovered or detected during the survey follows.

Bacteria

Bacteria identified in fish samples from Devils Lake are common bacteria. Stress mediated infections with these bacteria are highly likely in fish populations not only in the Red River Basin but worldwide when water temperatures exceed 25°C.

Gram-negative bacteria

Aeromonas hydrophila and Aeromonas sp.— A. hydrophila and other motile aeromonads are among the most common bacteria in freshwater habitats throughout the world. These ubiquitous agents are opportunistic pathogens that can be associated with disease among a variety of free-

ranging fish species. Although motile aeromonads are capable of causing septicemic conditions in fish, these bacteria also compose part of the normal intestinal microflora of healthy fish (Trust et al. 1974). Aeromonad septicemia is considered to be a stress mediated disease. Disease expression is influenced by many factors including: physiological condition of the fish host, water temperature, dissolved oxygen, carbon dioxide, ammonia, and organic loading (Esch and Hazen 1980; Walters and Plum 1980). Aeromonad species can act as either primary or secondary invaders. Under certain conditions of stress, *Aeromonas hydrophila* occurring as a part of normal gut flora can become pathogenic. *A. hydrophila* flourishes at an optimum temperature range of 25 – 30°C. Aeromonads in general affect a wide variety of warm, cool and coldwater fish species. The identification of these bacterial agents is a routine finding in a warm and cool water fishery population such as Devils Lake.

Pseudomonas sp.— Pseudomonads are ubiquitous in the aquatic environment. They are motile bacteria that are commonly found in soil and water. They are so widespread and numerous, they are often considered to be secondary invaders of fish compromised by other pathogens or factors. Pseudomonads are frequently found on eggs (Bell et al 1971; Sugita et al. 1988), the skin and gills (Colwell 1962; Horsley 1973) and in the intestine (Trust and Sparrow 1974; Austin and Al Zahrani 1988) of a variety of fish species. Most *Pseudomonas sp.* are found in freshwater and are opportunistic pathogens for most fish species. Free-ranging fish become susceptible to Pseudomonad infections during extremes of temperature, pH, pollution and other environmental factors. The temperature range for bacterial growth is 4 - 43°C. They can flourish in a warm and cool water aquatic environment and are capable of becoming pathogenic and producing disease in a variety of fish.

Pleisomonas shigelloides.— These bacterial agents are considered to be normal residents in the gastro-intestinal tract of warm water fish (Vandepitte et al., 1980). *Pleisomonas sp.* grow in an optimum temperature of 30°C and have a range from 39 - 41°C. They have also been isolated from rainbow trout *Oncorhynchus mykiss* (Austin and Austin 1987). These bacteria are not normally associated with fish disease and detection in black crappie and fathead minnow from Devils Lake may suggest they are a normal component of the microbial habitat in this ecosystem.

Gram-positive bacteria

Streptoccocus sobrinus.— *Streptococcus sp.* has been reported in a wide variety of fresh and saltwater fishes. They have been documented to produce disease outbreaks presenting a septicemia that may become systemic (Inglis et al. 1993). Natural streptococcosis has been reported in over 22 fish species comprised of fresh water and marine fishes. The bacterium appears to be associated with disease when water temperatures rise above 20°C and when key environmental stressors are also present. The bacteria can be transmitted horizontally between fish. *Steptococcus sp.* appear to be abundant in water and soils in a variety of habitats worldwide.

Corynebacterium renale.— This bacterium has been documented in aquatic habitats. It is considered to be widely distributed in nature. The optimum temperature for growth is 37°C with a range of 15 - 40°C (Buchanan et al. 1974). It is primarily a disease causing agent in domestic livestock and continues to be of veterinary significance in cattle and swine. It is not documented as a fish pathogen. *C. renale* is often associated with aquatic habitats where agricultural activities

may have an influence on the microbial flora present. The bacterium was found in fathead minnow, white bass, and walleye in Devils Lake. The agricultural influence including cattle grazing occurring in this ecosystem may provide a possible vector for bacterial introduction.

Renibacterium salmoninarum.— Most kidney samples (95.9%) from Devils Lake had OD values above the negative threshold although 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. Therefore, ELISA measures not only active infections but may also indicate 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 is speculating 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 site 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 in the U.S. that positive ELISA OD values are not necessarily an accurate predictor of 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. The negative threshold OD values for ELISA was determined using standardized negative reference tissue from fall chinook salmon for use in the *National Wild Fish Health Survey.* We calculated a negative threshold OD value of 0.075 during this survey, a value considered to be very conservative. It is possible that certain proteinaceous elements or other constituents of non-salmonid kidney 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. Previous screening for the bacterium was performed at Devils Lake and at the Sheyenne and Red rivers (Peters 2002) with results similar to the current survey on Devils Lake. At Lake Sakakawea in western North Dakota, feral fall chinook salmon have been tested annually for *R. salmoninarum* by the direct fluorescent antibody technique and no positive fish have been detected. A query of the *National Wild Fish Health Survey* database for *R. salmoninarum* and all fish species (1997 – 2005) shows numerous sample sites with inconclusive

results (Figure 9). In our laboratory, we have examined several samples that were negative with PCR despite a wide range of positive ELISA OD values with antigen levels ranging from low to high. Most regions of the U.S. 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 Appalachians.

Figure 9.— Map of sample sites (colored dots) where previous testing (1997 – 2005) was performed for *R. salmoninarum* in cooperation with the *National Wild Fish Health Survey.* Green dots indicate sample sites that tested negative, yellow dots are sites that test ELISA positive but are negative by PCR, and red dots are sites that test positive by ELISA and PCR.

Parasites

Parasites identified in fish samples from Devils Lake are not unusual findings. These parasites are commonly found in fish populations throughout the U.S. and other areas of the world. Discussion of the parasites observed in fish from Devils Lake follows.

Protozoa

Trichodina sp.— Trichodina sp. Trichodinids are mobile ciliates often found on gills, fins, and skin of many fish species. They are found in numerous species of fish, amphibians, and even 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 have low host specificity and are therefore, widely distributed. Most families of freshwater fish harbor *Trichodina spp.* (Lom 1995, Hoffman 1999). 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. Trichodinids feed on newly produced cells and cell debris (Lom 1995).

Epistylis sp.— Epistylids are attached sessile, colonial, ciliated ectoparasites which infect the body, fins, and gills of fishes. *Epistylis sp.* has a direct life cycle requiring only the fish host. The epistylids become very numerous in waters containing high levels of organic matter, similar to other protozoans. Fish subjected to chronic and certain acute stressors may be particularly susceptible to infestation (Hazen et al. 1978). *Epistylis sp.* is ubiquitous in fresh water, both in the water column and in sediments (Esch et al. 1976) and has been reported in a variety of fishes in North America (Hoffman 1999). *Epistylis sp.* may affect the bony prominences such as fins, jaws, or gill covers. They may also infect the oral cavity or gills. Epistylids attach or anchor themselves to a hard surface such as fin rays and scales (Noga 1996). High rates of infestation of gills may contribute to morbidity by respiratory distress (Hoffman 1999).

Amoeba sp.— Free living freshwater or soil amoebae are often present on the gills of fishes (Daoust and Ferguson 1985). They are considered to be common symbionts of fish (Post 1983). They are opportunistic pathogens. Amoebae have been documented to be pathogenic to fish under certain conditions (Kent et. al. 1988). They have been associated with nodular gill disease

in salmonids. Amoeba feed on bacteria, detritus, dead host cells, and other organic matter. They are often associated with aquatic habitats with higher organic loading. *Acanthamoeba, Entamoeba, Volkampfia* and *Schizamoeba* have been associated with fish losses. Under certain conditions, amoebae can be an internal parasite of fishes and cause systemic disease (Schlaperclaus 1992). The amoeba observed on yellow perch and white sucker from Devils Lake were found externally.

Nematodes

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

Cestodes

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., primary host birds and mammals. *L. intestinalis* have been reported in many species of sunfishes, suckers, basses, minnows, shiners, chubs, dace, bream and many others, mostly in freshwater fishes. 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). This 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 custpidatus.— 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. custpidatus* 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.

Pathogen Summary

There are several peer reviewed references that suggest the bacteria and parasites found in Devils Lake during the present survey are not unique. There is a reasonable likelihood that the preceding organisms would be detected in fish from other waters from North America under similar sampling conditions.

Recommendations for Future Work

The present survey was based on samples collected during a one week period. Given the occurrence and prevalence of certain fish pathogens may be variably affected by several life history characteristics and elements of environment, especially those causing increased stress, future surveys should consider sample collections at two or more times during the year. It may be particularly important and interesting to examine fish either during or immediately following spawning activities.

While an adequate sample size was obtained for some species at some of the samples sites, too few fish of other species were examined to establish the presence or absence of fish pathogens with an appropriate level of confidence. Obtaining sufficient sample size can be a common problem with large field studies such as this. It can also be problematic when certain species are of low abundance or are not available because of limited seasonal distribution. Often times, prior information regarding species abundance and distribution are limited. Also, upon initiation of such surveys, it is often difficult to know the type(s) of fishing gear to employ that permit collection of multiple species and maximize catch per unit effort. These elements are made particularly difficult during rapidly changing environmental conditions. Future fish pathogen surveys should identify and focus on species of greatest interest or importance. When appropriate, selection of species should also be related to the particular fish pathogens of concern.

In the present survey, fish in Devils Lake were collected from two sample areas. Future work should consider the merits of sampling additional areas of the lake. Sampling efforts should be coordinated with area fisheries biologists to identify key spawning and rearing habitat.

References

AFS-FHS (American Fisheries Society – Fish Health Section). 2004. FHS Blue Book: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, 2004 edition. AFS-FHS, Bethesda, Maryland.

Austin, B. and D. A. Austin. 1987. Bacterial Fish Pathogens: Disease in Farmed and Wild Fish. Ellis Horwood Limited, West Sussex, England.

Austin, B. and A. M. J. Al-Zahrani. 1988. The effect of antimicrobial compounds on the gastrointestinal microflora of rainbow trout *Salmo gairdneri* Richardson. Journal of Fish Biology 33:7-14.

Barton, T. A., L. A. Bannister, S. G. Griffiths, and W. H. Lynch. 1997. Further characterization of *Renibacterium salmoninarum* extracellular products. Applied and Environmental Microbiology 63(10):3770-3775

Bell, G. R., G. E. Hoskins, and W. Hodgkiss. 1971. Aspects of characterization, identification and ecology of the bacterial flora associated with the surface of the stream-incubating Pacific salmon *(Oncorhynchus)* eggs. Journal of the Fisheries Research Board of Canada 28:1511-25.

Brown, L. L., T. P. T. Evelyn, G. K. Iwama, W. S. Nelson, and R. P. Levine. 1995. Bacterial species other than *Renibacterium salmoninarum* cross-react with antisera against *R. salmoninarum* but are negative for the p57 gene of R. salmoninarum as detected by the polymerase chain reaction (PCR). Diseases of Aquatic Organisms 21:227-231.

Buchanan, R.E. and N.E. Gibbons, Co-Editors. 1974. Bergey's Manual of Determinative Bacteriology. Eighth Edition. Williams and Wilkins Company, Baltimore.

Colwell, R.R. 1962. The bacterial flora of Puget Sound fish. Journal of Applied Bacteriology 28:147-158.

Daoust, P. U. and H. W. Ferguson. 1985. Nodular gill disease: A unique form of proliferative gill disease in rainbow trout, *Salmo gairdneri* Richardson. J. Fish Dis. 8:511-522.

Dick, T. A., M. H. Papst, and H. C. Paul. 1987. Rainbow trout *(Salmo gairdneri)* stocking and *Contracaecum spp.* J. wildlife Diseases 23:242-247.

Dixon, J. G. 1985. Rapid detection and identification of the fish pathogens by enzyme-linked immunosorbent assay (ELISA). Pages 11-16 in A. E. Ellis, editor. Fish and Shellfish Pathology. Academic Press, London.

Esch, G. W., T. C. Hazen, R. V. Dimock, Jr., and J. W. Gibbons. 1976. Thermal effluent and the epizootiology of the ciliate *Epistylis* and the bacterium *Aeromonas* in association with centrarchid fish. Trans. Am. Microscop. Soc. 95:687-693.

Esch, G. W., and T. C. Hazen. 1980. Stress and body condition in a population of Largemouth bass: implications for red-sore disease. Transactions of the American Fisheries Society 109(5):532-536.

Forstie, M. and H. L. Holloway Jr. 1984. Parasites of fish from the James and Sheyenne Rivers, Jamestown Reservoir complex, and Lake Ashtabula in North Dakota. Prairie Naturalist 16(1):11-20.

Hazen, T. C., M. L. Raker, G. W. Esch, and C. B. Fliermans. 1978. Ultrastructure of red- sore lesions on largemouth bass

(Micropterus salmoides): Association of the ciliate *Epistylis sp.* and the bacterium *Aeromonas hydrophila.* J. Protozool. 25:351-355.

Hoffman, G. L. 1967. Parasites of North American Freshwater Fishes. University of California Press, Berkley and Los Angeles.

Hoffman, G. L. 1978. Ciliates of freshwater fishes. *In* Parasitic Protozoa, Vol II, J. P. Kreir, editor. Academic Press, New York, pp 583-632.

Hoffman, G. L. 1999. Parasites of North American Freshwater Fishes, 2nd Edition. Comstock Publishing Associates, Ithaca, New York.

Holloway, H. L. 1986. Parasites of fishes in prairie lakes and impoundments. Proceedings of the North Dakota Academy of Science 40:33.

Holloway, H. L. and N. T. Hagstrom. 1981. Comparison of four North Dakota impoundments and factors affecting the development of impoundment parasitofauna. Prairie Naturalist 13:85-93.

Horsley, R. W. 1973. The bacterial flora of Atlantic salmon (*Salmo salar* L.) in relation to its environment. Journal of Applied Bacteriology 36:377-386.

Inglis, V. R., J. Roberts, and N. R. Bromage. 1993. Bacterial diseases of fish. Institute of Aquaculture. Halsted Press, New York and Toronto.

Lockard, L. L. and R. R. Parsons. 1975. Some parasites of paddlefish *(Polyodon spathula)* from the Yellowstone River, Montana. Great Basin Nat. 35:425-426.

Lom, J. 1995. Protozoan and metazoan infections, Vol. 1. p 229-262 *In* Fish diseases and disorders. P.T.K. Woo, editor. CAB International, Cambridge.

Mizelle, J. D. and D. C. Kristsky. 1967. Studies on the monogenetic trematodes. XXXIII. New species of *Gyrodactylus* and a key to the North American species. Tranactions of the Microsc. Soc. 86:390-401.

Noga, E.J. 1996. Fish disease. Diagnosis and treatment. Mosby, St Louis. 367 pp.

Ossiander, F. J. and G. Wedemeyer. 1973. Computer program for sample sizes required to determine disease incidence in fish populations. Journal of the Fisheries Research Board of Canada 30:1383-1384.

Pascho, R. J. and D. Mulcahy. 1987. Enzyme-linked immunosorbent assay for a soluble antigen of *Renibacterium salmoninarum,* the causative agent of bacterial kidney disease. Canadian Journal of Fisheries and Aquatic Sciences 44:183-191.

Pascho, R. J., D. G. Elliott, and J. M. Streufert. 1991. Brood stock segregation of spring chinook salmon *Oncorhynchus tshawytscha* by use of the enzyme-linked immunosorbent assay (ELISA) and the fluorescent antibody technique (FAT) affects the prevalence and levels of *Renibacterium salmoninarum* infection in progeny. Diseases of Aquatic Organisms 12:25-40.

Pascho, R. J., D. Chase, and C. L. McKibben. 1998. Comparison of the membrane-filtration fluorescent antibody test, the enzyme-linked immunosorbent assay, and the polymerase chain reaction to detect *Renibacterium salmoninarum* in salmonid ovarian fluid. Journal of Veterinary Diagnostic Investigations 10:60-66.

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

Post, G. 1983. Textbook of Fish Health. T.F.H. publications, Inc., New Jersey.

Reinisch, J. D. 1981. Parasites of fishes from Devils Lake and the Souris River in North Dakota. Master's thesis, University of North Dakota, 99pp.

Schaperclaus, W. 1991. Fish Diseases, Volumes 1 and 2. A. A. Balema, Rotterdam.

Sugita, H., Tsunohara, M., Ohkoshi, T. & Degachi, Y. 1988. The establishment of an Intestinal microflora in developing goldfish *(Carassius auratus)* of culture ponds. Microbial Ecology 15:333-344.

Sutherland, D. R. and H. L. Holloway, Jr. 1979. Parasites of fish from the Missouri, James, Sheyenne, and Wild Rice Rivers in North Dakota. Proceedings of the Helminthological Society of Washington 46(1):128-134.

Trust, T. J., L. M. Bull, B. R. Currie, and J. T. Buckley. 1974a. Obligate anaerobic bacteria in the gastrointestinal microflora of the grass carp *(Ctenopharyngodon idella),* goldfish *(Carassius auratus),* and rainbow trout *(Salmo gairdneri),* J. Fish. Res. Board Can. 36(10):1174-1179.

Trust, T. J. and R. A. H. Sparrow. 1974b. The bacterial flora in the alimentary tract of freshwater salmonid fishes. Canadian Journal of Microbiology 20:1219-28.

Turaga, P. S. D., G. D. Wiens, and S. L. Kaattari. 1987. Analysis of *Renibacterium salmoninarum* antigen production in situ. Fish Pathology 22:209-214.

(USFWS) U. S. Fish and Wildlife Service. 2005. National Wild Fish Health Survey, Laboratory Procedure Manual, 3nd Edition, J. Woodland (Ed). Washington, D.C.

Van As, J. G., and Basson, L. 1987. Host specificity of trichodinid ectoparasites of freshwater fish. Parasitology Today 3:88-90.

Vandepitte, J., VanDamme, L., Fofana, Y. and Desmyter, J. 1980. *Edwardsiella tarda* et *Pleisomonas shigelloides.* Leur role comme agents de diarrhees et leur epidemiologie. Bulletin de Societe Pathologie Exotique 73:139-149.

Walters, G. R., and J. A. Plumb. 1980. Environmental stress and bacterial infection in channel catfish, *Ictalurus punctatus* Rafinesque. J. Fish Biol. 17 (2):177-185.

Wood, P. A., G. D. Wiens, J. S. Rohovec, and D. D. Rockey. 1995. Identification of an immunologically cross-reactive 60-kilodalton *Renibacterium salmoninarum* protein distinct from p57: implication for immunodiagnostics. Journal of Aquatic Animal Health 7:95-103.