

## Viral Isolate Assays for Devil's Lake

### Devil's Lake-Viral Sample Collection and Viral Assay Methodology

A standard sample size of 60 fish was the objective for each of the target species of fish. A 60 fish sample size provides a 95% confidence level that a pathogen in an infected fish will be detected given in a 5% presumed prevalence of infection in that population (>2000). Fish were captured by used of experimental gill nets, and modified Fyke nets. After capture the fish were transported to a temporary field laboratory at Devil's Lake and held alive with Devil's Lake water until time of necropsy, examination, and sample collection.

Fish were humanely euthanized with tricaine methanesulfonate (MS-222/Finquel). Following an external examine for clinical signs of disease, the body cavity of the fish was aseptically opened by a ventral incision to provide a sterile access to the internal organs. Kidney and spleen tissue were the organs of target for fingerling to adult fish and whole viscera samples were collected from fry. Swim bladder was also collected from *Centrarchidae* and *Moronidae* for Large Mouth Bass Virus detection. The viral assay tissue samples were pooled to a maximum of five fish per pool in a saline transport media (Hank's Balance Saline Solution-HBSS with antibiotics). Samples were Fed Ex'd priority overnight. All samples were processed within 48 hours of collection and assays were initiated within 72 hours post sampling as outlined by the standard methods for virology cell culture assays.

Sample processing consisted of decanting the transport media, weighing the tissue to allow appropriate sample dilution with HBSS, and sample homogenation by maceration with a stomacher to liberate any possible viral particles from the collected tissue. After appropriate sample dilutions, the sample homogenate was inoculated in replicate onto confluent monolayers of multiple cell lines in 24 well tissue culture plates. For detection of a wide range of viral agents, the following cell lines and incubation temperatures were used:

Cell lines		Incubation Temps
EPCs	Epithelioma papulosum cyprini	15°C
CHSE-214	Chinook salmon embryo-214	15°C
EPCs	Epithelioma papulosum cyprini	22-25°C
FHM	Fathead minnow	22-25°C
BF-2	Bluegill fry-2	22-25°C
CCO	Channel catfish ovary	25°C
BB	Brown bullhead	25°C
KF-1	Koi fin-1 (Hedrick, et al 2000)	25°C

All wells of the viral assays were monitored for cytopathic effects (CPE) by light microscopy using an inverted light microscope. At 14 days, the replicate wells were blind passed onto corresponding cell lines and also observed for CPE. Each original well was monitored for a minimum of 28 days and each of the blind passed wells were monitored for a minimum of 14 more days.

## Devil's Lake - Viral Assay Results:

The following table lists the species of fish assayed by cell culture for replicating viral agents and the results:

2006		
Species	Pools Assayed	Cytopathic Effects (CPE) from viral agents
Black Crappie	12	not detected
Fathead Minnow	12	not detected
Northern Pike	12	not detected
Walleye	12	not detected
White Bass	12	not detected
White Sucker	6 (27 fish)	not detected
Yellow Perch	12	not detected

2007		
Species	Pools Assayed	Cytopathic Effects (CPE) from viral agents
Black Crappie	12	not detected
Fathead Minnow	12	not detected
Northern Pike	5 (24 fish)	not detected
Walleye	12	not detected
White Bass	12	not detected
White Sucker	2 (8 fish)	not detected
Yellow Perch	4 (17 fish)	not detected

2008		
Species	Pools Assayed	Cytopathic Effects (CPE) from viral agents
Black Crappie	3 (14 fish)	not detected
Fathead Minnow	12	not detected
Northern Pike	7 (34 fish)	not detected
Walleye	12 (59 fish)	not detected
White Bass	12	not detected
White Sucker	1 (5 fish)	not detected
Yellow Perch	12	not detected

Viral assays by cell culture did not isolate any replicating viral agents during 2006, 2007, and 2008. Previous sampling of Devil's Lake fish from October 2001, August 2002 and July 2005 resulted in all viral assays by cell culture being free of cytopathic effects.

Risk due to Life cycle/Life history of viral pathogen from Devil's Lake-----none detected to evaluate.  
 Environmental risk potential of a viral pathogen triggering a disease outbreak Devil's Lake-----none  
 Risk of transfer of a replicating viral agent from Devil's Lake-----none  
 Geographic Distribution of replicating viral agents-----none detected to evaluate.