CHAPTER 1

Overview of the National Wild Fish Health Survey

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Background

The Fish and Wildlife Service requested and received a $1 million annual increase in appropriations for fish disease work. Six hundred thousand dollars was used to initiate a *National Wild Fish Health Survey* (Survey) under the leadership of Service Regional Fish Health Centers (Centers), and in cooperation with stakeholders such as states, Tribes, and the aquaculture industry. This project incorporates standardized diagnostic and data management methods to ensure national comparability, identifies target pathogens, fish species, and habitats for survey, and is developing a systematic and interagency approach to fish health management of important watersheds.

Because initial funds were limited, every effort has been made to collaborate with those collecting fish for other purposes with the aim of maximizing efforts in pathogen and parasite analysis rather than sample collection. In addition, a National Wild Fish Health Survey Database (Database) has been established to receive data from the Survey. The Database is accessible electronically via the Internet.

In November, 1996 a group composed of fish health biologists from each of the Service's nine Regions, state fishery managers from Oregon and Alaska, researchers in fish disease from the University of California-Davis, the Leetown Science Center and Western Fisheries Research Center (USGS), and a representative from the private aquaculture industry met in Denver, CO to develop an implementation plan for the Survey. The initial document (1997 Protocols & Procedures) provided a framework and procedures for implementation of the Survey as developed by this group. Given this was the first endeavor of its kind, this group recognizes that this plan would change as new information arose. In the first year of implementation, as the document was widely distributed, we received many comments and suggestions for revisions. The *NWFHS Laboratory Procedures* Manual (Manual) was further developed in 1998-1999 by contributions from fish health biologists across the country to provide a comprehensive Manual that includes optimum detection methods and standardized protocols for all aspects of the Survey. This Manual is meant to be dynamic and adaptive to best meet the needs and intent of the project. We expect to incorporate comments and suggestions received through yearly revisions of the document.

Acknowledgements

Many individuals have contributed to the Survey throughout its development and implementation. William E. Knapp and Mary Ellen Mueller, of the Division of National Fish Hatcheries, were catalysts for the conceptual development and funding initiatives that brought this Survey to light. Many other researchers made significant contributions to the procedures and protocols developed over the first and second years. We would like to especially thank Dr. Jim Winton of the Western Fisheries Research Center (USGS) in Seattle, and Dr. Ron Hedrick and Dr. Karl Andree of University of California, Davis. These researchers offered immeasurable help in transferring technical capabilities to Fish Health Centers through hands-on training and workshops.

In developing this Manual, the Service would like to recognize and thank Dr. Theodore Meyers who graciously offered the Alaska Fish Pathology Section Laboratory Manual as a template for our protocols, as well as support through technical and editorial review of this Manual.

Many fish health biologists from the nine regional Fish Health Centers developed and contributed individual chapters for the sampling methods and laboratory assays. The following individuals made significant contributions:

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Purpose

The purpose of the National Wild Fish Health Survey is to determine the distribution of specific pathogens in wild fish population.

Justification

Knowledge of the distribution of pathogens in wild fish will contribute to:

- Protect threatened or endangered species;
- Provide more options for better fish management;
- Provide a cohesive national perspective for better fish health management; and
- Develop standardized fish health and fish transport regulations that are scientifically defensible.

Why a National Wild Fish Health Survey?

- The discovery that whirling disease was decimating wild trout in the intermountain west focused the Nation's attention on the fact that very little is known about the diseases among wild fish.

- The most important weapon needed to control or prevent fish diseases is knowledge. Currently, there is very little information about the relationship between presence of the pathogen in wild fish and its likelihood of producing disease in either wild or hatchery reared fish.

- Valuable stocks of fish are at risk because of our lack of knowledge about the distribution of pathogens and parasites in wild fish.

- A standardized approach is necessary to allow for comparisons from state to state or watershed to watershed to help identify why a pathogen or parasite in one area has negative impacts on certain fish stocks while not in others.

- Scientific information is needed to provide a biological basis for management decisions regarding stocking and fish transport activities.

Partnerships

The success of the Survey depends on establishing productive partnerships. Within the Fish and Wildlife Service, fish health biologists involved in the Survey work closely with other fishery biologists in the Fisheries, Ecological Services, and International Affairs programs. This ensures cohesiveness between the Survey and related aquatic activities, such as those involving environmental contaminants, endangered species, refuges and aquaculture. It also adds a valuable fish health dimension to those activities.

Partnerships will continue to be formed with other organizations active in fish health, fishery biology and fishery management. Included are other Federal agencies, State and Tribal agencies, conservation and professional organizations, universities and foreign nations. Care has been taken to identify and include partners early in designing and planning the Survey. Priority has been placed on adopting an overall approach that is broadly inclusive and one that is flexible in attracting and accommodating a variety of different kinds of partners.

Partnerships are based on common interests, responsibilities and activities. Some partners, like Federal, Tribal, and State fishery managers have been involved in several ways. Some provide fish taken from areas identified as high priority sampling areas. Others have provided funds to expand the Survey to areas that otherwise might not be targeted. Still others may not have participated in the Survey itself, but may have

benefited from the data generated, or may have voiced their support for the Survey at critical times when national priorities and budgets were being established.

As more and more people and organizations become aware of the Survey and benefit from it, interests in forming partnerships will grow. Initially, the Service has focused on reaching four primary constituencies:

- Other Federal agencies with fishery management responsibilities either on their lands or through cooperative management arrangements;
- States and Tribes;
- Conservation and professional organizations; and
- Universities and other research institutions

During the first year, FY 1997, attention was focused on planning and designing a scientifically sound survey that could provide additional fishery management capabilities in both the public and private sector. Survey design was coordinated carefully with representatives of each of the four primary constituencies to ensure its utility and attractiveness. As the Survey became operational and sampling began toward the later half of FY 1997, the Service broadened its efforts to increase understanding and awareness of the Survey and establish partnerships. Awareness of the Survey will be expanded by presentations made at professional and industry meetings, articles in professional and trade journals, and by communications and interactions among professionals engaged in private and public fishery management.

The Survey will always benefit from new partnerships and, in turn, will be shaped and directed by those partnerships. The Service will be challenged to maintain a flexible outlook in order to be responsive to diverse group of partners and at the same time guide the Survey in the direction intended by Congress.

Fish of Primary Interest

The initial focus of the Survey has been on the following fish: trout, salmon, paddlefish, perch, sturgeon, suckers, sunfish, herring, catfish, bass, carp and minnows.

Target Pathogens

Each fish is evaluated for target pathogens and parasites that are known to infect that particular species. In addition, the standard methods used in the Survey will detect the major salmonid fish pathogens should they exist in other species. Refer to Appendix Z – Glossary of Terms for terms and pathogen abbreviations. The following list includes bacterial, viral, and parasite pathogens of interest, and their abbreviation.

Viruses include:
Infectious Hematopoietic Necrosis Virus (IHNV)
Infectious Pancreatic Necrosis Virus (IPNV)
Viral Hemorrhagic Septicemia Virus (VHSV)
Oncorhynchus Masou Virus (OMV)
Largemouth Bass Virus (LMBV)

Bacterial pathogens include:
Aeromonas salmonicida (AS), Furunculosis
Edwardsiella ictaluri (ESC), Enteric Septicemia
Renibacterium salmoninarum (RS), Bacterial Kidney Disease
Yersinia ruckeri (YR), Enteric Redmouth

Parasites include:
Myxobolus cerebralis (WD), Whirling Disease

Pathogens of Regional Importance (PRI):
In addition to the pathogens and parasites listed above, the Service's Fish Health Centers have identified several Pathogens of Regional Importance (PRI) for which additional diagnostic procedures may be conducted as part of the Survey. These parasites and pathogens are included in laboratory protocols when wither fish health professionals or fishery managers identify them as a potential risk to fish health in watershed or ecosystem. PRI include the following:

Viruses:
Infectious Salmon Anemia Virus (ISAV)
White Sturgeon Iridovirus (WSIV)
White Sturgeon Herpesvirus (WSHV2)

Bacteria:
Flavobacterium columnare
Flavobacterium psychrophilum
Citrobacter freundii
Edwardsiella tarda

Parasites:
Ceratomyxa shasta (salmonid ceratomyxosis)
Bothriocephalus acheilognathi (Asian tapeworm)

The following table lists primary fish pathogens that are targeted by the Survey and may be associated with specific fish families. Pathogens of Regional Importance are denoted with (PRI).

Table 1 – FISH FAMILY AND TARGET PATHOGENS*

Family	Bacterial Pathogens	Viral Pathogens	Parasites
Acipenseridae (Sturgeon)	*A. salmonicida* *E. ictaluri* *Y. ruckeri*	IPNV WSIV (PRI) WSHV2 (PRI)	
Catostomidae (Suckers)	*A. salmonicida* *E. ictaluri* *R. salmoninarum* *Y. ruckeri*	IPNV	
Centrarchidae (Sunfishes)	*A.salmonicida* *E. ictaluri* *R.salmoninarum* *Y.ruckeri*	IPNV LMBV	
Clupeidae (Herring)	*A. salmonicida* *E. ictaluri* *R. salmoninarum* *Y. ruckeri*	IPNV VHSV	
Cyprinidae (Minnows/Carp)	*A.salmonicida* *E. ictaluri* *R. salmoninarum* *Y. ruckeri*	IPNV	*B. acheilognathi (PRI)*
Ictaluridae (Catfish)	*A. salmonicida* *E. ictaluri* *R. salmoninarum* *Y. ruckeri*	IPNV	
Percichthyidae (Temperate Basses)	*A. salmonicida* *E. ictaluri* *R. salmoninarum* *Y. ruckeri*	IPNV	
Percidae (Perch)	*A. salmonicida* *E. ictaluri* *R. salmoninarum* *Y. ruckeri*	IPNV	
Polyodontidae (Paddlefish)	*A. salmonicida* *E. ictaluri* *Y. ruckeri*	IPNV WSHV-2	
Salmonidae (Trout/Salmon)	*A. salmonicida* *E. ictaluri* *R. salmoninarum* *Y. ruckeri*	IHNV ISAV (PRI) IPNV OMV VHSV	*M. cerebralis* *C. shasta (PRI)*

*** Targeted pathogens may not be found in all families.**

Priority Selection Criteria for Determining Areas of Focus

Available resources are not sufficient to accommodate all requests to sample specific waters. Therefore, certain ranking criteria will be applied to determine which areas to sample:

- presence of pure wild (unmanipulated) stock of fish;
- area has never been sampled for fish diseases;
- species of special management concern (high concern e.g., threatened or endangered species);
- study area of special management concern (high concern e.g., recovery project or suspected disease);
- sampling site is Federally managed;
- historical data available from site (population, biodiversity, water quality, etc.);
- area is a broodstock or egg collection site;
- other relevant data is being collected that enhances survey context (examples: contaminants, population estimates, year classes, species abundance/diversity or community structure, environmental parameters such as D.O., temperature, habitat type, pH, hardness, flow rate, etc.)
- partnerships will significantly leverage funds.

Fish Collection Protocols

The Survey uses existing collection activities by cooperators to the fullest extent possible. Methods include either active or passive types of collection as described by Murphy and Willis, 1996. All collection methods described have advantages and disadvantages that must be recognized.

Study objectives, environmental characteristics, animal behavior, and size are additional factors that influence sampling methods. For the purpose of the Survey, collection methods that accurately reflect the relative abundance of animals sampled and allow the investigator to obtain live specimens are preferred.

For these reasons, investigators should focus on active collection methods that include, but are not limited to, electrofishing, seines, trawls, and dredges that generally define a more accurate sampling effort and are more likely to provide live or fresh samples. Passive collection methods include, but are not limited to, gill nets, hoop nets, fyke nets, scoop traps, and rotary screw traps. Care must be used in passive collections to ensure fresh samples suitable for fish health analysis. However, since the study parameters are national in scope and include diverse aquatic habitats and cooperators, any collection method that gives a close approximation of the population for each habitat and provides opportunities for valid tissue collection should be considered.

Fish collection methods must be identified by each investigator and included in the database to enable valid comparison of health data collected.

References:

Murphy, Brian R., and Willis, David W., editors. 1996. Fisheries Techniques, 2nd edition. American Fisheries Society, Bethesda, Maryland.

Specimen Receiving and Custody Procedures

General

Good sample documentation ensures proper identification and storage of samples, and proper tracking of the samples as they move through the diagnostic procedures in the fish health laboratory.

Procedures

All submissions should comply with the following:

1. Each submission will be documented on a NWFHS SUBMISSION FORM and specimens will be collected in accordance with AFS Blue Book. For those tests specified that are currently not found in AFS Blue Book, for example ELISA for *Renibacterium salmoninarum* and Polymerase Chain Reaction (PCR) for *Myxobolus cerebralis*, the collection and processing of samples will follow those outlined in this Manual.

2. Each Submission Form will be reviewed to verify that it contains all appropriate information to process the accession. For most routine submissions this information includes:

- Submitter (fish biologist, fish health specialist, other);
- Date of collection;
- Location of collection (GIS coordinates and common name);
- Capture procedure;
- Site description;
- Remarks;
- Number of samples submitted;
- Sample I.D. Numbers (i.e. 1-15 = samples numbered 1 through 15);
- Genus and species and/or common name of fish samples (age, size and sex if known);
- Specimen type (tissue);
- Media type (if submission is by culture or preserved histology sample);
- Number of samples for this group of fish; and
- Number of fish per sample (pooled samples).

3. The section labeled for *Lab Use Only* will be completed as laboratories receive the samples, therefore initiating the chain of custody tracking for each submission. Information entered will be:

- Date received;
- Coordinating Inspector/Pathologist;
- Case Number (if applicable); and

- Condition of samples
- Remarks

Receiving laboratory personnel will check and verify by signature all samples received.

Further information on sample tracking and details of chain of custody procedures can be found in Chapter 3 – Sample Receipt and Laboratory Tracking.

Diagnostic Protocols

Viruses

Procedures used for virology and cell culture techniques in this Manual largely follow those outlined in AFS Blue Book. Additional protocols are included for corroborative testing that includes DNA probe and Polymerase Chain Reaction (PCR). Quality Assurance and Control are also addressed in the Manual for virology in regards to cell cultures used in viral testing. Specifically, cell cultures are standardized among all Centers, annually tested for viral sensitivity, and bi-annual tested for mycoplasma infections.

Cell Culture Lines

The following species sampled and applicable cell lines for preliminary viral testing are:

Salmonids and Herring: EPC and CHSE-214
Sturgeon: WSSK1 and CHSE-214
Ictalurids: BB and CHSE-214
Centrarchids: FHM and CHSE-214

All Fish Health Centers use reference cell lines from American Type Culture Collection (ATCC) that have been tested for ivral sensitivity and mycoplasma infections. The cells lines are tested annually by William Batts, USGS – Western Fisheries Research Center in Seattle, or by the Fish Health Centers. Each Center maintains these reference cell lines in liquid nitrogen, or ultra-low cryopreservation. Each year, the existing cell lines are re-tested for viral sensitivity and mycoplasma infection, and the optimum cell cultures are distributed for virology testing performed for the Survey.

Corroborative Methods

For target pathogens and Pathogens of Regional Interest (PRI), the following corroborative methods will be performed.

Virus

Identification by specific antibody tests: serum neutralization, immunoblot, or FAT. Polymerase Chain Reaction (PCR) will only be used for viruses with appropriately defined specific known and labeled nucleotide sequences, i.e. North American VHS virus, IHNV, IPNV, and LMBV.

Bacteria

The procedures described here largely follow those outlined in AFS Blue Book with the following additional methodologies:

Renibacterium salmoninarum (Bacterial Kidney Disease)
Sample: Kidney
Preliminary methods: ELISA
Corroborative testing: Nested PCR with specific primers

Aeromonas salmonicida (Furunculosis)
Sample: Kidney and/or spleen
Preliminary methods: Isolation on BHIA and biochemical assays suggested for identification of *Aeromonas salmonicida*.
Corroborative testing: Specific antibody tests (DFAT, agglutination)

Yersinia ruckeri: type I and type II (Enteric Redmouth)
Sample: Kidney, spleen
Preliminary methods: Isolation on BHIS and biochemical assays suggested for identification of *Yersinia ruckeri*.
Corroborative testing: Specific antibody tests (DFAT, agglutination)

Edwardsiella ictaluri (Enteric Septicemia)
Preliminary methods: Isolation on BHIA and biochemical assays suggested for identification of *Edwardsiella ictaluri*.
Corroborative testing: Specifi antibody tests (DFAT, agglutination)

Citrobacter freundii
Sample: Kidney, Spleen
Preliminary methods: Isolation on BHIA and identification of colony and cell morphology consistent with *Citrobacter freundii*.
Corroborative diagnosis: Specific antibody tests (agglutination test, DFAT)

Edwardsiella tarda
Sample: Kidney, Spleen
Preliminary methods: Isolation on BHIA and identification of colony and cell morphology consistent with *Edwardsiella tarda*.
Corroborative diagnosis: Specific antibody tests (agglutination test, DFAT)

Flavobacterium columnare (Columnaris Disease)
Sample: Kidney, Spleen
Preliminary methods: Isolation on TYES and identification of colony and cell morphology consistent with *Flavobacterium columnare*.
Corroborative diagnosis: Specific antibody tests (agglutination test, DFAT), or PCR.

Flavobacterium psychrophilum (Coldwater Disease)
Sample: Kidney, Spleen

Preliminary methods: Isolation on TYES and identification of colony and cellular morphology consistent with *Flavobacterium psychrophilum*.
Corroborative testing: Specific antibody tests (DFAT, agglutination test), or PCR.

Parasites

Myxobolus cerebralis (Whirling Disease)
Sample: Half head or cartilage plug for preliminary detection method, and remaining half head for archiving for corroborative testing by PCR or histology.
Preliminary methods: Pepsin-Trypsin Digest (PTD) method with observation of typical spores.
Corroborative diagnosis: PCR with nested primer or histological sections showing spores in cranial cartilage.

Bothriocephalus acheilognathi (Asian Tapeworm)
Sample: Intestine
Preliminary methods: Microscopic examination of morphology, intestinal squash for visualization.
Corroborative diagnosis: Morphological criteria for Asian tapeworm.

Ceratomyxa shasta
Sample: Posterior intestine
Preliminary methods: Wet mount observation of multicellular myxosporean trophozoites.
Corroborative diagnosis: PCR with specific primers or histological sections.