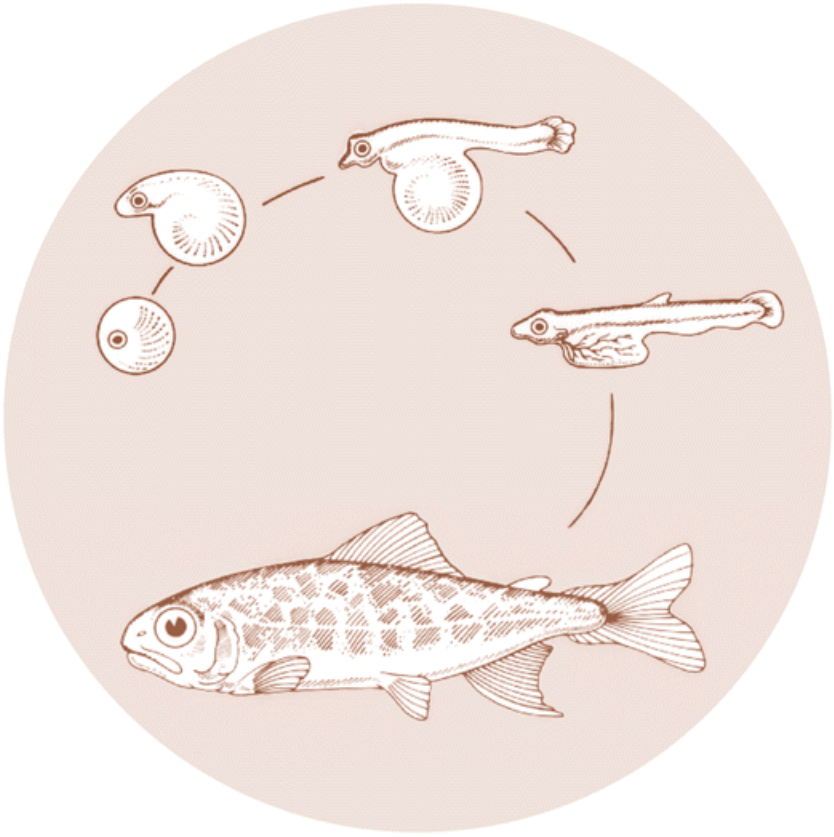
May 1991

AUGMENTED FISH HEALTH MONITORING

Part One of Two Volume

Completion Report



DOE/BP-63461-5

This report was funded by the Bonneville Power Administration (BPA), U.S. Department of Energy, as part of BPA's program to protect, mitigate, and enhance fish and wildlife affected by the development and operation of hydroelectric facilities on the Columbia River and its tributaries. The views of this report are the author's and do not necessarily represent the views of BPA.

This document should be cited as follows:

Michak, Patty; R. Rogers, K. Amos, Washington Dept. of Fisheries, Augmented Fish Health Monitoring, Completion Report (Part 1 of 2 Volume) to Bonneville Power Administration, Portland, OR, Contract DE-AI79-86BP63461, Project 86-54, 25 electronic pages (BPA Report DOE/BP-63461-5)

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AUGMENTED FISH HEALTH MONITORING

Completion Report
(Part One of Two Volume)

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Project No. 86-54
Contract No. DE-AI79-86BP63461

May 1991

Table of Contents

List of Tables

List of Figures

INTRODUCTION

The Bonneville Power Administration (BPA) initiated the Augmented Fish Health Monitoring project in 1986. This project was a five year interagency project involving fish rearing agencies in the Columbia Basin. Participating agencies included: Washington Department of Fisheries (WDF) Oregon Department of Fish and Wildlife, Idaho Department of Fish and Game, and the U.S. Fish and Wildlife Service (USFWS).

Historically, all agencies involved with fish health in the Columbia Basin were conducting various levels of fish health monitoring, pathogen screening and data collection. The goals of this project were; to identify, develop and implement a standardized level of fish health methodologies, develop a common data collection and reporting format in the area of artificial production, evaluate and monitor water quality, improve communications between agencies and provide annual evaluation of fish health information for production of healthier smolts.

WDF has actively participated in this project through an interagency technical committee formed to determine minimum levels of monitoring for specific pathogens and pathogen testing methodologies. This completion report will contain a project evaluation, review of the goals of the project, evaluation of the specific fish health analyses, an overview of highlights of the

project and concluding remarks. Data collected in 1990 and 1991 will be published in a supplemental report (Volume 2), approximately December, 1991. Previous years data and evaluation of those data can be found in the following annual reports: State of Washington Augmented Anadromous Fish Health Monitoring Annual Report 1986, Amos et al, 1987; Augmented Fish Health Monitoring Annual Report 1987, Michak and Rogers, 1989; Augmented Fish Health Monitoring Project 86-54 Annual Report 1988, Michak et al, 1989; Augmented Fish Health Monitoring Annual Report 1989, Michak et al, 1990.

DESCRIPTION OF STUDY AREA

The Columbia Basin is historically divided into the lower and upper basins by the Bonneville Dam. At the inception of this project WDF operated 8 hatcheries in the lower Columbia Basin and 6 hatcheries in the upper Columbia Basin. Geographical location of each facility can be found in Figure 1. Species reared at these facilities include spring, summer and fall chinook, early (Type S) and late (Type N) coho. Hatchery watershed and rearing program are listed by basin in Table 1.

DISCUSSION - Project **Review and Evaluation.**

The goals set out to be accomplished during the Augmented Fish Health Monitoring project are listed in Table 2. From these broad goals many specific projects and technological advances occurred,

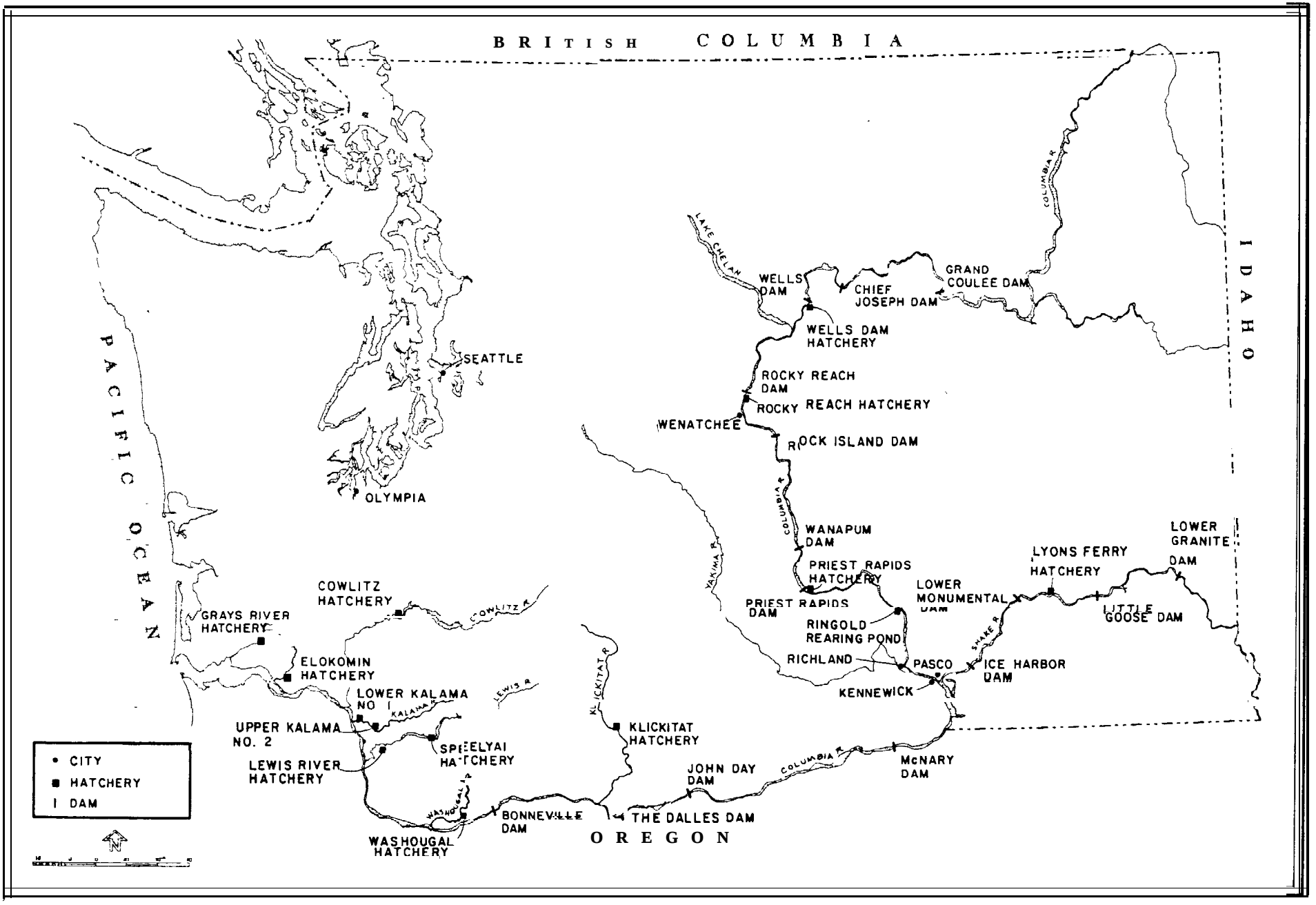

Figure 1. Washington Department of Fisheries Columbia Basin Hatcheries.

TABLE 1. Washington Department of Fisheries Columbia Basin Hatcheries.

Hatchers	Watershed	Rearing Program
LOWER COLUMBIA		
Cowlitz	Cowlitz River	Spring and fall chinook, and late coho.
Toutle	Toutle River	Fall chinook and early coho.
Elokomin	Elokomin River	Fall chinook and late coho.
Grays River	Grays River	Fall chinook and early coho.
Kalama River	Kalama River	Spring and fall chinook and late coho.
Lewis River	Lewis River	Spring chinook, early and late coho.
Lower Kalama	Kalama River	Fall chinook and early coho.
Speelyai	Lewis River	Spring chinook and early coho.
Washougal	Washougal	Fall chinook, early and late coho.
UPPER COLUMBIA		
Klickitat	Klickitat	Spring and fall chinook, and late coho.
Lyon's Ferry	Snake River	Fall chinook.
Tucannon	Tucannon	Spring chinook.
Priest Rapids	Columbia River	Fall chinook.
Ringold	Columbia River	Spring and fall chinook.
Rocky Reach	Columbia River	Fall chinook, early and late coho.
Wells Spawning Channel	Columbia River	Summer chinook.

these will be outlined below.

Table 2. Augmented Fish Health Monitoring Project Goals.
GOAL 1 - Standardization of fish health technologies.
GOAL 2 - Develop common data collection and reporting.
GOAL 3 - Evaluate and Monitor Water Quality.
GOAL 4 - Improve Communications.
GOAL 5 - Annual Evaluation of Fish Health Information.

GOAL 1 - Standardization of fish health technologies.

This goal was accomplished through the steering committee meetings. The committee was composed of administrative and technical representatives from each agency. These agencies participated in developing standardized pathogen detection methods (Table 3) and sampling levels. With all agencies sampling at the same level and using common methodologies, management agencies were provided with comparable fish health information. These establishedmethodologies have also helped WDF in setting standards for non-Columbia Basin hatchery sampling such as adult monitoring, and Erythrocytic Inclusion Body Syndrome (EIBS) detection. As a result of this when dealing with co-managers on issues outside the Columbia Basin most detection methodologies are consistent among co-managers. The BPA project has set the standard within WDF for fish health monitoring on a statewide basis.

GOAL 2 - Develop common data collection and reporting.

Through BPA support WDF developed a fish health database management

system. This system developed methods for data storage, retrieval and numerous reporting formats. Because of existing systems within some agencies, a complete integration of all data into one "super" database was not deemed possible in the scope of the Augmented Fish Health Monitoring project.

GOAL 3 - Evaluate and Monitor Water Quality.

The water quality aspect of the project was not completely developed during the project. Water sources, chemical analysis and sampling levels were identified by each Agency. A contracting lab to conduct the water quality analysis was never selected by BPA due to the Policy Review Group deleting those funds in 1990-1991.

GOAL 4 - Improve Communications.

One of the greatest benefits of this project has been the improved communication between all participating agencies. Participation in steering committee meetings and fish health meetings promoted discussion of fish health matters among project participants and with fish health professionals throughout the western region of the United States and to some extent internationally. Quarterly steering committee meetings provided a forum to discuss fish health status within the Columbia Basin. During the project WDF staff made presentations at the Northwest Fish Culture Conference, Western Fish Disease Workshop, International Fish Health Conference and The European Association of Fish Pathologists meeting.

Fish health information and technology exchange has been enhanced substantially as a direct result of the Augmented Fish Health Monitoring Project. In 1987 Ron Goede from Utah Division of Wildlife Resources conducted a two day training session of his "Autopsy-Base Fish Health/Condition Assessment System". Technical and field personnel from project agencies in Washington attended this well received training session. This system was incorporated into pre-release exams on "index" (coded wire tagged groups) stocks throughout the Basin. In 1988 project technical representatives and lab personnel participated in an EIBS workshop. This workshop allowed all participating agencies to review and develop criteria for identifying this syndrome. This workshop was made possible due to the funding provided by this project.

WDF has also consulted with the USFWS, National Fisheries Research Center-Seattle (NFRC-S) for expert technical assistance in developing new methods in fluorescent antibody technique, and coelomic fluid screening for Bacterial Kidney Disease.

GOAL 5 - Annual Evaluation of Fish Health Information.

WDF has reviewed data collected on an annual basis and provided reports to BPA (reports listed in Introduction) for publication and distribution to interested parties. Review comprised of reporting and evaluating juvenile and adult health data. This review has provided WDF with insight into the magnitude of loss to specific pathogens, prevalence of BKD in adults and potential correlation to

progeny, medicated feed usage and treatments, juvenile health at mid-term and release, and adult health and treatment are the primary areas of expanded information WDF has received from the project.

Evaluation of specific fish health analyses in Table 3.

With completion of the Augmented Fish Health Monitoring project we have found that some of the sampling, as outlined in Table 3, did not provide sufficient information to continue conducting the analysis at the conclusion of the project. Each section will be evaluated below.

Viral Pathogen Detection Evaluation:

Adults: Monitoring of adult broodstock for viral pathogens was found to be extremely valuable. Virology monitoring is required for stock transfers and will be continued after the end of the project. An exception to this is monitoring for EIBS, which proved to be inconclusive. Presence of inclusions was very sporadic, and because so little is known about the etiology of EIBS little benefit can be derived at this time from the labor intensive screening of adults for EIBS.

Juveniles: Conducting viral assays on randomly selected juveniles does not provide health information that could not be obtained through the monthly monitoring visits. We feel that viral detection will occur through clinical cases and

Table 3. Augmented Fish Health Monitoring Project Pathogen Detection Methods and Analyses.

Disease/Pathogen	Life Stage	Tissue Sampled	Detection Method
VIRAL			
IHNV	Juvenile	kidney/spleen	Tissue culture EPC w/PEG and CHSE-214.
	Adult	ovarian fluid	Tissue culture EPC w/PEG and CHSE-214.
IPNV	Juvenile & Adult	kidney/spleen	Tissue culture EPC and CHSE-214.
EIBS	Juvenile & Adult	blood film	Pinacynol chloride stain, two (2) minutes at 1000X.
BACTERIAL			
R. salmoninarum	Juvenile	kidney smear	FAT, 30 fields at 600X.
	Adult	ovarian fluid	FAT, 30 fields at 600X.
F. psvchrophilus	Juvenile	kidney or spleen	Gram stain.
A. salmonicida	Juvenile & Adult	kidney or spleen	Culture TSA media.
Y. ruckeri	Juvenile & Adult	kidney of spleen	Culture TSA media.
PARASITE			
M. cerebralis	Juvenile	head cartilage	Digest Method, confirmwithhistopathology.
C. Shasta	Juvenile & Adult	hindgut	Light microscopy.
PKX	Juvenile	posterior kidney	Light microscopy, confirm by histopathology.
MONTHLY MONITORING	Juvenile.		
ORGANOSOMATIC INDEX	Juvenile.		

CHSE - CHinook Salmon Embryo 214 EPC - Epithelioma Papillosum Cyprini PEG - PolyEthylene Glycol
IHNV - Infectious Hematopoietic Necrosis Virus IPNV - Infectious Pancreatic Necrosis Virus
EIBS - Erythrocytic Inclusion Body Syndrome FAT - Fluorescent Antibody Test TSA - Tryptic Soy Agar
PKX - Proliferative Kidney X

that sampling sixty asymptomatic fish is not an effective detecting scheme, and it will be discontinued at the end of the project. In addition pre-release screening for EIBS is also not the best means for management of this disease. Juvenile stocks should be monitored on a monthly basis to detect EIBS as early as possible so that further protection or stress reduction measures can be started to aid the fish in their recovery. Screening for EIBS will be continued in monthly monitoring visits.

Bacterial Pathogen Detection Evaluation:

Adults: Evaluation of adult stocks for the presence of BKD provided very valuable prevalence information. With BKD being difficult to control and transmission occurring both vertically and horizontally any knowledge on prevalence within a population provides insight into possible rearing problems. We will continue, and even expand our adult monitoring to include the use of enzyme-linked immunosorbent assay (ELISA) technology, and to evaluate non-Columbia Basin hatcheries. This effort will be funded by BPA for the implementation work, and State funding for additional Columbia Basin and non-Basin hatcheries.

Regular monitoring (culture and identification) of adult salmon mortalities for Aeromonas salmonicida and Yersinia ruckeri is recommended, particularly at facilities with protracted adult holding periods. Early identification of this

pathogen as a cause of loss in adults, followed by appropriate antibiotic therapy, if necessary, can prevent significant losses.

Juveniles: Determining the prevalence of BKD in juveniles proved to be beneficial only at certain life stages. Sampling of "zereo" age (90 day rearing) releases provided no benefit. Conversely, sampling of yearling groups proved to be beneficial. Yearling groups were sampled at mid-term (6+ months into their rearing cycle) and prior to release. The mid-term sampling gave the current status of the disease and indications to future treatments or problems. This sampling could be completed during the monthly monitoring visit by the station pathologist. The pre-release exam provided valuable information about potential survivability of release groups. As shown by Banner et al, 1986, juveniles with BKD may continue to die after release particularly after entry into the marine environment and therefore survive at lower rates than non-infected cohorts.

Detection of other bacterial pathogens (Aeromonas salmonicida, Yersinia ruckeri, and Flexibacter psychrophilus [Cytophaqa psychrophila]) is most efficiently conducted during monthly monitoring and in clinical cases. This level of monitoring and detection will continue after the Augmented Fish Health Monitoring project is completed.

Parasitic Pathogen Detection Evaluation.

Adults: -Screening for ceratomyxa shasta (Cs) was demonstrated not to be beneficial. Since the identification of spores alone does not imply that the infective stage is present (Hoffmaster et, al,. ,1988), -and.based on historical data that severe losses have not been incurred, continued monitoring of returning adults at all facilities is not warranted. Spot checks at surface water facilities with escapement above the facility water intake would be advisable.

Juveniles: The project identified Mvxobolus cerebralis (Mc), Cs, and Proliferative Kidney X (PKX) as parasitic pathogens of concern and developed sampling protocols and detection methods for each. Evaluation of these strategies recommend that post BPA project we- continue with the established sampling and detection protocol. Screening for Mc should continue annually at mid-term for chinook stocks on surface water (unless trout are sampled in the watershed). Screening for Cs and PKX should continue during the monthly monitoring visit where appropriate.

Monthly Monitoring Evaluation.

The monthly. monitoring exams conducted by staff fish pathologists have been very instrumental in accurately determining causes of loss and attributing monthly mortality to the appropriate cause or pathogen. Monthly health

inspections provided a means for early detection of potential health problems and when coupled with preventative actions, can reduce or eliminate an epizootic. These exams will be continued after the BPA project.

Organosomatic Index (Goede, 1987) Evaluation.

During the project the Organosomatic Index (OSI) for structured necropsy was conducted on four stocks of fish prior to release. Results from these necropsies have provided little information that was not attainable through the monthly monitoring exams. The groups we examined generally fell within normal ranges. Because the sixty fish examined (at a given hatchery) were of the same lot with nearly identical rearing conditions the only apparent result was that hatchery rearing can induce stress that is demonstrated in physiological responses. The hatcheries used in our project presented extreme rearing parameters to the fish and comparisons between hatcheries by release groups would not be appropriate. Comparisons between broods within the same hatchery, assuming rearing conditions were similar between years, have produced some insights, but nothing that was not expected from that facility (based on previous history and monthly monitoring exams).

We feel the best use of the OSI is for experimental lots. For example, feed trials or density rearing studies where repeated monitoring may detect changes between treatments over time.

Though the treatments would have to cause substantial changes to see any physiological response detectable by the OSI.

overview of highlights and new technologies developed through WDF's Augmented Fish Health Monitoring Project.

* We improved our fluorescent antibody technique by arranging a one day training session with the USFWS, NFRC-S. Sample collection, materials, conjugate filtration and standardization of positive criteria were a few of the benefits obtained during the training. In addition WDF implemented the use of p-Phenylenediamine to reduce fading of immunofluorescence (Chapman, 1989).

* With the implementation of sampling of spawning adults for the presence of Renibacterium salmoninarum (Rs), new techniques were desired to provide the best information on Rs levels in spawners that could be related to their progeny. As a result we developed a simple technique to screen coelomic fluid. We felt this would provide the best data because of the close association of coelomic fluid with the eggs. With the assistance of Diane Elliott, USFWS, NFRC-S we developed a simple coelomic fluid centrifugation method for screening adults (Michak and Rogers, 1989). Field sampling was efficient since we were already collecting sixty virus (five fish pooled) samples, obtaining an additional sixty individual

coelomic fluid samples fit easily into our sampling protocol.

* This project placed emphasis on screening juveniles and adults for the presence of EIBS inclusions. Because of the relative newness of this type of screening some confusion was identified by participating agencies as to exactly what is an inclusion, and what is the best staining procedure. Because of these problems a workshop was conducted with technical representatives and lab personnel of the participating agencies (Michak, et al, 1989). As a result of this meeting, criteria for positive status was developed and staining techniques were standardized. All agencies made any modifications necessary to either lab techniques or positive criteria as a result of the workshop.

* With the screening of numerous juvenile samples for Mc, we decided that the plankton centrifuge method was not compatible with our work place. Because of the noise and fumes we discontinued this method and began using the Digest Method (as per "Blue Book", Amos, 1985). We found this method to be more compatible with our available lab facilities.

* Evaluation of cytoplasmic inclusions, as associated with EIBS, by electron microscopy (EM). In 1988 BPA provided WDF with additional funding to collect blood samples from fish experiencing EIBS. A blood preservation technique was

developed by Andrew Blixt (who conducted the EM work), Montana State University Veterinary Research Lab and Elizabeth MacConnell, USFWS, Bozeman, Montana. This technique was then transferred to WDF. Blood samples were collected and analyzed by EM for the presence of viral particles associated with cytoplasmic inclusion bodies. Photomicrographs were provided to Charlie Smith, USFWS, Bozeman, Montana and WDF for evaluation. Results of this study are to be published in "Diseases of Aquatic Organisms", in 1991/1992.

* Hematocrit baseline data collection. WDF collected hematocrit data on all juvenile release groups. These data were in addition to the required sampling. We felt that valuable baseline data could be easily collected during the pre-release exam. Hematocrit summary information will be presented in the supplemental report.

* Use of acridine orange staining technique (Piacentini, 1989). In 1989 we began using the acridine orange staining technique described by Piacentini for additional confirmation of EIBS inclusions. After working through a few technical problems the procedure seems to work fairly well, though we do get variable staining results on occasion (cell nucleus staining incorrectly). We plan to continue to refine this technique for future application.

CONCLUSIONS

The Augmented Fish Health Monitoring project has been very beneficial to WDF and to Columbia Basin fish health management. This project has brought all fish health labs in the Columbia Basin to the same level of fish health screening capabilities. WDF expanded their lab capabilities, obtained additional personnel and provided training to personnel in fish health management.

This project meet many of the goals that it set out to accomplish, and produced unexpected benefits such as refinement of fluorescent antibody technique for BKD, development of the coelomic fluid analysis for BKD, and evaluation of the EIBS cytoplasmic inclusions. We have also had the opportunity to evaluate fish health sampling regimes and select from this project what is most beneficial to WDF to meet the needs of Columbia Basin fish health management.

Through the development of the project database it became possible to store and retrieve large amounts of data efficiently. Through this system, assessment of released juveniles and returning adults is possible for certain pathogens. Data has been provided to management groups (Water Budget, Fish Passage Center, USFWS and BPA) within the Columbia Basin to aid them in their management and assessment of migrating smolts.

Outlined in Table 4 are the specific analyses conducted during the project. After each is an indication of whether the analyses will

Table 4. Augmented Fish Health Monitoring Project Fish Health Analyses Evaluation.

Disease/Pathogen	Life Stage	Tissue Sample	Continue at Project conclusion ?
VIRAL			
IHNV	Juvenile	kidney/spleen	No
	Adult	ovarian fluid	Yes
IPNV	Juvenile & Adult	kidney/spleen	Juvenile - No Adult - Yes
EIBS	Juvenile & Adult	blood film	Juvenile - Yes Adult - No
BACTERIAL			
R. salmoninarum	Juvenile	kidney smear	Yes - during monthly monitoring.
	Adult	ovarian fluid	Yes - expand statewide.
F. psychrophilus	Juvenile	kidney or spleen	Yes - during monthly monitoring.
A. salmonicida	Juvenile & Adult	kidney or spleen	Yes - during monthly monitoring.
Y. ruckeri	Juvenile & Adult	kidney of spleen	Yes - during monthly monitoring.
PARASITE			
M. cerebral&	Juvenile	head cartilage	Yes - annually in surface water.
C. Shasta	Juvenile & Adult	hindgut	Juvenile - Yes Adult - No
PKX	Juvenile	posterior kidney	Yes - during monthly monitoring.
MONTHLY MONITORING	Juvenile.		**Yes**
ORGANOSOMATIC INDEX	Juvenile.		**No**

IHNV - Infectious Hematopoietic Necrosis Virus IPNV - Infectious Pancreatic Necrosis Virus
EIBS - Erythrocytic Inclusion Body Syndrome PKX - Proliferative Kidney X

be continued at the conclusion of the project.

Overall the Augmented Fish Health Monitoring project has been invaluable to WDF. The level of support for fish health provided through this project greatly enhanced our ability to monitor and evaluate fish health in the Columbia Basin.

ACKNOWLEDGEMENTS

Augmented Fish Health Monitoring Project Staff 1986 - 1991.

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