BONNEVILLE POWER ADMINISTRATION

Pathogen Screening of Naturally Produced Yakima River Spring Chinook Smolts

Yakima/Klickitat Fisheries Project Monitoring and Evaluation

Annual Report 2004 - 2005 May 2005 DOE/BP-00017478-1

This Document should be cited as follows:

Thomas, Joan, "Pathogen Screening of Naturally Produced Yakima River Spring Chinook Smolts; Yakima/Klickitat Fisheries Project Monitoring and Evaluation", 2004-2005 Annual Report, Project No. 199506325, 11 electronic pages, (BPA Report DOE/BP-00017478-1)

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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 in this report are the author's and do not necessarily represent the views of BPA.

This report covers one of many topics under the Yakima/Klickitat Fisheries Project's Monitoring and Evaluation Program (YKFPME). The YKFPME is funded under two BPA contracts, one for the Yakama Nation and the other for the Washington Department of Fish and Wildlife (Contract number 00017478, Project Number 1995-063-25). A comprehensive summary report for all of the monitoring and evaluation topics will be submitted after all of the topical reports are completed. This approach to reporting enhances the ability of people to get the information they want, enhances timely reporting of results, and provides a condensed synthesis of the whole YKFPME. The current report was completed by the Washington Department of Fish and Wildlife.

PATHOGEN SCREENING OF NATURALLY PRODUCED YAKIMA RIVER SPRING CHINOOK SMOLTS

Annual Report 2004

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Project Number 1995-063-25

May 2005

PATHOGEN SCREENING OF NATURALLY PRODUCED YAKIMA RIVER SPRING CHINOOK SMOLTS

Abstract

In the spring of 2004 naturally produced smolts outmigrating from the Yakima River Basin were collected for the sixth year of pathogen screening. This component of the evaluation is to monitor whether introduction of hatchery produced smolts would impact the prevalence of specific pathogens in the naturally produced spring chinook smolts. Increases in prevalence of any of these pathogens could negatively impact the survival of these fish. Since 1999 the Cle Elum Hatchery has been releasing spring chinook salmon smolts into the upper Yakima River to increase natural production. In 1998 and 2000 through 2004 naturally produced smolts were collected for monitoring at the Chandler smolt collection facility on the lower Yakima River. Smolts were collected from mid to late outmigration, with a target of 200 fish each year. The pathogens monitored were infectious hematopoeitic necrosis virus, infectious pancreatic necrosis virus, viral hemorrhagic septicemia virus, *Flavobacterium psychrophilum*, *Flavobacterium columnare*, *Aeromonas salmonicida*, *Yersinia ruckeri*, *Edwardsiella ictaluri*, *Renibacterium salmoninarum* and *Myxobolus cerebralis*. Of these pathogens, only *R. salmoninarum* was detected in very low levels in the naturally produced smolts outmigrating in 2004. To date, only bacterial pathogens have been detected and prevalences have been low. There have been small variations each year and these changes are attributed to normal fluctuations in prevalence. All of the pathogens detected are widely distributed in Washington State.

Introduction

Pathogens present in the Yakima River basin are possible strong interactants that can impact the success of a fish population. Pathogens can debilitate, increase susceptibility to predation, or cause mortality in spring chinook, particularly during times of physiological stress such as during smoltification, poor environmental conditions, or situations of high population density.

The purpose of this task is to determine the prevalence of specific pathogens in this stock of naturally produced smolts at the time of outmigration and to monitor these pathogens after introduction of spring chinook reared at the Cle Elum Hatchery. Other factors such as environmental conditions will also have to be taken into consideration when evaluating the presence of these pathogens. Smolts were collected in the spring of 1998 and 2000 through 2004. Due to an oversight, no fish were collected in 1999 and due to poor water conditions in 2002 they were unable to collect fish in June, decreasing the numbers of samples to 130 from the target of 200 fish.

Methods

Each year spring chinook smolts are collected at the Chandler smolt collection facility during outmigration. A portion of the naturally produced fish are selected from the peak to the end of the migration, sacrificed, placed on ice and shipped to the Washington Department of Fish and Wildlife Fish Health Laboratory. Due to small numbers of fish entering the collection facility near the end of the outmigration, the fish may be collected over a period of several days and held live until shipping to the lab in Olympia. All specimens are received and dissected within 24 hours. Samples are not frozen prior to dissection. The goal is to sample 200 smolts each spring.

Upon arrival in Olympia the fish are dissected and any gross abnormalities noted. Kidney tissue from each fish is inoculated onto agar plates for detection of the target bacteria by culture. Tryptone yeast extract plus salts agar (TYESA) is used for detection of *Flavobacterium psychrophilum*, causative agent of coldwater disease, and *Flavobacterium columnare*, causative agent of columnaris. Brain heart infusion agar (BHIA) is used for detection of *Aeromonas salmonicida*, the causative agent of furunculosis; *Yersinia ruckeri*, the causative agent of enteric redmouth; *Edwardsiella ictaluri*, the causative agent of enteric septicemia of catfish. TYESA is incubated at 15°C and BHIA is incubated at 20°C for 7-10 days with examination for colonies of typical morphology. Identification is done using biochemical assays or, for *Flavobacterium*, typical colony and cellular morphology consistent with isolation techniques. *F. psychrophilum* can also be confirmed with an agglutination test or a polymerase chain reaction assay.

Kidney and spleen tissues are individually harvested from the fish for detection of viral pathogens and *Renibacterium salmoninarum*, causative agent of bacterial kidney disease. These tissues are processed immediately by emulsifying in a 1:10 dilution of phosphate buffered saline (PBS), centrifuged, and the supernatant decontaminated in a solution of gentamicin, penicillin, streptomycin and fungizone. This solution is then inoculated onto CHSE 214 and EPC cell lines to monitor for the cytopathic effect from viruses for a minimum of 14 days at 15°C. The decontaminated supernatant from the homogenate placed on cells both undiluted and at a 10^{-1} dilution. Confirmation of any viral isolates is done using specific antibody tests. Methodology is used that will detect infectious hematopoietic necrosis virus (IHNV), infectious pancreatic necrosis virus (IPNV) or viral hemorrhagic septicemia virus (VHSV).

The pellets from the kidney and spleen tissues are frozen at –75°C for later assay by enzyme-linked immunosorbant assay (ELISA) for detection of the antigen to *R. salmoninarum*. The pellet is thawed and diluted 1:10 with PBS and assayed by the ELISA plate method using antisera produced by Kirkegaard and Perry Laboratories. Results are obtained by recording optical density (OD) of a color reaction which indicates relative levels of antigen to *R. salmoninarum* in the sample.

Each year heads are removed for testing of the cartilage for spores of *Myxobolus cerebralis*, the causative agent of whirling disease. The heads are removed behind the

operculums, split in half longitudinally and each half head frozen at –75° in pools of five. One set of half head pools is tested using the pepsin trypsin digest assay for spores. The other set of half head pools is held in the freezer for confirmation by histopathology if spores are detected by the pepsin trypsin digest assay. Histopathology will determine if spores of the typical size and morphology of *M. cerebralis* are located within lesions in the cartilage of the cranium.

Results

In 2004 the smolts appeared healthy with no clinical signs of disease. The only pathogen detection in these fish were low levels of the antigen to *R. salmoninarum (*Table 3). The only other targeted pathogens that have been detected in the naturally produced smolts, *F. psychrophilum* and *F. columnare*, were not detected in 2004 (Table 2). No IHNV, VHSV, IPNV *Ceratomyxa shasta* or *M. cerebralis* have been detected in any of the years tested (Tables 4 and 5). Table 1 indicates the numbers and dates of fish sampled each year.

In 2004 genetic samples were also collected from the smolts to determine if there were differences in pathogen levels in the various stocks in the basin. Due to lack of funding these samples have not yet been analyzed.

Discussion

In 2004 less pathogens were detected from the naturally produced Yakima River spring chinook smolts than any season to date. The only pathogen detected was *R. salmoninarum* and all positive fish had OD levels in the very low end of the range of detection. This was the first year that no fish had OD levels in the moderate or high range. The levels of *R. salmoninarum* are most likely to be the strongest indicator of either direct impact by the hatchery produced smolts or an indirect impact caused by increased production in the system. Any difference in prevalences between years seen to date are within normal variations of prevalence in a population and specific characteristics of the ELISA test. Although studies have indicated poorer survival in fish with high levels of *R. salmoninarum*, all of the smolts examined in 2004 had low levels of *R. salmoninarum* antigen. This test also measures the amount of antigen present rather than actual pathogen so it is not always indicative of disease. *R. salmoninarum* is a common pathogen in Washington and is generally present at some level in smolting chinook, both naturally and hatchery produced.

Prerelease testing of the hatchery reared smolts performed by the USFWS Fish Health Lab has only detected *R. salmoninarum* to date. This will also decrease the likelihood that the hatchery smolts would shed the other target pathogens, thereby increasing prevalence in the naturally produced smolts. In 2004 the hatchery smolts released from Cle Elum Hatchery also had the lowest OD values for *R. salmoninarum* of any years of rearing (Ray Brunson, USFWS, personal communication). This was also one of their

highest production years, 819,815 smolts released. A direct comparison of OD levels between labs is not possible due to differences in methodology of the ELISA test. The test results can be used to indicate trends in the population rather than be a direct comparison of values. The USFWS data is also only for kidney material instead of kidney and spleen tissue, but this should not cause substantial differences. None of the other targeted pathogens have been detected in the hatchery smolts so it is unlikely that they would increase the prevalence of the other pathogens in the naturally produced fish.

None of the pathogen isolations seen so far in this study indicate a health problem due to interaction with the hatchery reared fish. Although affects on earlier life-stages would not be identified since they were not the focus of this investigation. Due to the presence of the pathogens detected in the natural environment, and the affect of environmental conditions on their prevalence, it will require a significant increase in prevalence of these pathogens in the naturally produced smolts to demonstrate a relation to the hatchery production. The lack of detection of IHNV, VHSV, IPNV or *M. cerbralis* in the smolts is not surprising since the only previous detection in this system, including adult isolations, was an isolation of IPNV from adult summer steelhead held at WDFW's Yakima Hatchery in 1991. However, considering IHNV is very prevalent in the anadromous zone of the Columbia River Basin, it would not be unusual if this pathogen were eventually detected, at least in returning adults.

Since appearance of pathogens is often density dependent we may detect an increase in some pathogens if the number of fish in the system increase. One option for future research would be pathogen monitoring of the smolts over the entire outmigration. Smolts collected later in the outmigration, when water temperatures have increased, may have higher prevalences of some bacterial pathogens. However, other pathogens may be more prevalent in the earlier outmigrating fish and this is also the period of time in which the smolts would be more likely to interact with the hatchery produced smolts. Future research could also include monitoring smolts higher in the system, because an unhealthy fish may not survive to reach the Chandler facility.

Acknowledgments

This study was funded by the Bonneville Power Administration. I would like to acknowledge Leroy Senator of the Yakama Nation and his staff for their work collecting the smolts at the Chandler facility and ensuring their delivery to the WDFW Fish Health Lab. In addition I would like to thank Margo Whipple, Ann West and Keisuke Yamawaki for their work in the Fish Health Lab.

Table 1. Dates and numbers of fish tested each year.

Date Sampled	**No. of fish Tested**	**Date Sampled**	**No. of fish Tested**
5/18/98	30	5/06/02	20
5/19/98	55	5/08/02	20
5/20/98	20	5/14/02	30
5/26/98	30	5/20/02	30
5/27/98	30	5/28/02	30
5/31/98	14	TOTAL 2002	130
6/03/98	12	4/21/03	30
TOTAL 1998	191	4/28/03	30
Spring 1999	No samples taken	5/05/03	20
5/10/00	20	5/12/03	20
5/17/00	20	5/20/03	30
5/22/00	40	5/26/03	30
5/23/00	21	6/09/03	30
5/25/00	20	TOTAL 2003	180
6/08/00	50	4/21/04	20
6/15/00	11	4/27/04	30
TOTAL 2000	182	5/04/04	30
4/25/01	20	5/11/04	30
5/02/01	6	5/18/04	30
5/09/01	40	5/25/04	17
5/16/01	40	6/01/04	23
5/23/01	48	6/09/04	13
6/06/01	25	6/15/04	7
6/13/01	34	TOTAL 2004	200
TOTAL 2001	213		

Table 2. Summary of prevalences of the bacterial pathogens *F. psychrophilum*, *F. columnare*, *A. salmonicida*, *Y. ruckeri*, and *E. ictaluri.*

YEAR	**RESULTS**
1998	No target bacteria detected
1999	No fish tested
2000	2/182 *F. psychrophilum* 1/182 *F. columnare*
2001	1/213 *F. columnare*
2002	No target bacteria detected
2003	1/180 *F. psychrophilum*
2004	No target bacteria detected

Table 3. Summary of *R. salmoninarum* results by ELISA testing.

1998			
	OD	**number**	**percent**
below low	<0.099	127	66.5
low	0.100-0.199	52	27.2
mod	0.200-0.499	8	4.2
high	>0.500	4	2.1

2000			
	OD	**number**	**percent**
below low	<0.099	166	91.2
low	0.100-0.199	15	8.3
mod	0.200-0.499	0	0.0
high	>0.500	1	0.5

2001			
	OD	**number**	**percent**
below low	<0.099	181	85.0
low	0.100-0.199	31	14.6
mod	0.200-0.499	1	0.5
high	>0.500	0	0.0

2002			
	OD	**number**	**percent**
below low	<0.099	123	94.6
low	0.100-0.199	5	3.9
mod	0.200-0.499	0	0.0
high	>0.500	2	1.5

2003			
	OD	**number**	**percent**
below low	<0.099	176	97.7
low	0.100-0.199	1	0.6
mod	0.200-0.499	2	1.1
high	>0.500	1	0.6

2004			
	OD	**number**	**percent**
below low	<0.099	195	97.5
low	0.100-0.199	5	2.5
mod	0.200-0.499	0	0.0
high	>0.500	0	0.0

Table 4. Prevalence of the parasites, *M. cerebralis* and *C. Shasta*.

Year	***M. cerebralis***	***C. shasta***
1998	Not detected	No testing
1999	No testing	No testing
2000	incomplete	Not detected
2001	Not detected	Not detected
2002	Not detected	No testing
2003	Not detected	No testing
2004	Not detected	No testing

Table 5. Prevalence of the viral pathogens IHNV, VHSV, and IPNV.

Year	**IHNV**	**IPNV**	**VHSV**
1998	Not detected	Not detected	Not detected
1999	Not detected	Not detected	Not detected
2000	Not detected	Not detected	Not detected
2001	Not detected	Not detected	Not detected
2001 hatchery release	Not detected	Not detected	Not detected
2002	Not detected	Not detected	Not detected
2003	Not detected	Not detected	Not detected
2004	Not detected	Not detected	Not detected