Pathogen Screening of Naturally Produced Yakima River Spring Chinook Smolts

Yakima/Klickitat Fisheries Project Monitoring and Evaluation Report 6 of 7

Annual Report
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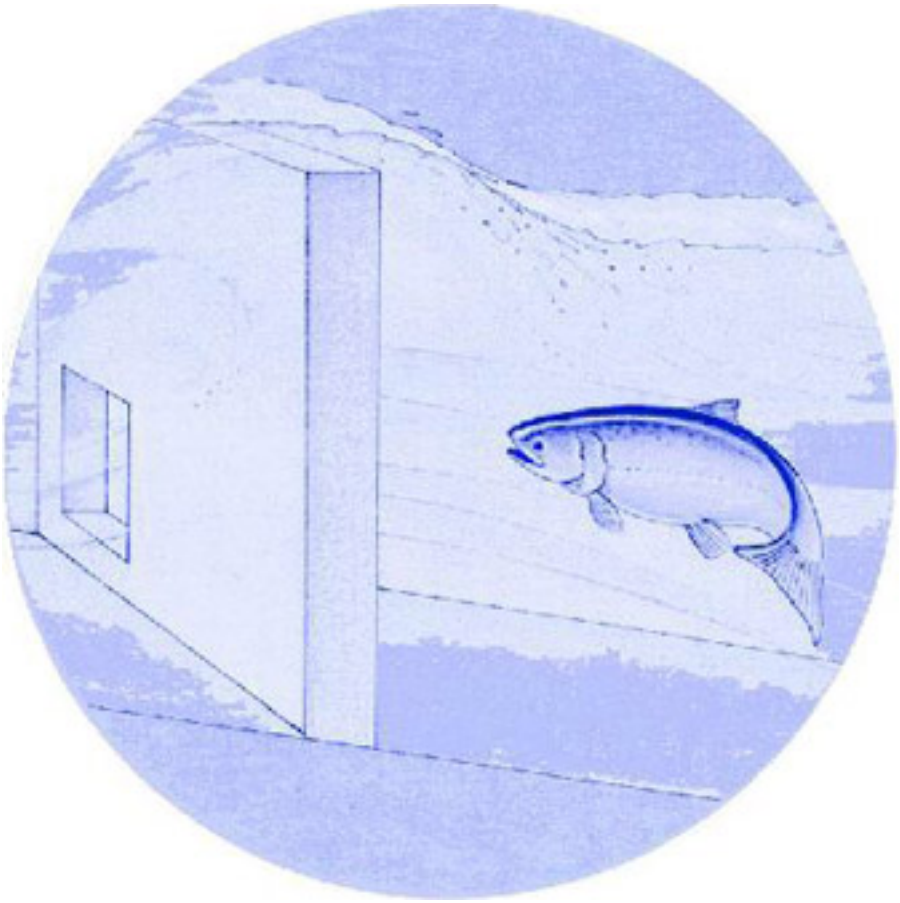

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PATHOGEN SCREENING OF NATURALLY PRODUCED YAKIMA RIVER SPRING CHINOOK SMOLTS

Annual Report 2003

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PATHOGEN SCREENING OF NATURALLY PRODUCED YAKIMA RIVER SPRING CHINOOK SMOLTS

Abstract

In 1999 the Cle Elum Hatchery began releasing spring chinook salmon smolts into the upper Yakima River to increase natural production. Part of the evaluation of this program 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. In 1998 and 2000 through 2003 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*. To date, only the bacterial pathogens have been detected and prevalences have been low. Prevalences have varied each year and these changes are attributed to normal fluctuation of 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 2003. 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.

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

The data from the naturally produced Yakima River spring chinook smolts collected in 2003 was similar to that seen in the previous years. Table 1. indicates the numbers and dates of fish sampled each year. To date only three of the targeted pathogens, *F. psychrophilum, F. columnare* and, *R. salmoninarum* have been detected in the naturally produced smolts. No IHNV, VHSV, IPNV or *M. cerebralis* have been detected in any of the years tested (Tables 4. and 5.). Of the cultured bacterial pathogens only *F. psychrophilum* was detected in 2003 at a very low levels in one fish (Table 2.). The level of *R. salmoninarum* antigen in the smolts remained at relatively low levels with only one fish having a level of antigen in the high range (Table 3.).

Discussion

The results to date have not indicated any significant changes in levels of the target pathogens in the population of naturally produced spring chinook at smoltification. None of the smolts have exhibited gross signs of disease. Some slight external hemorrhaging has been observed, but it was not typical of disease and was most likely due to the handling and euthanization.

F. psychrophilum was detected this year, but again at very low levels. It was previously detected in 2000. This pathogen is widely distributed in the environment and occasional low level detections are probably not indicative of a problem.

There has been little variation in the *R. salmoninarum* profile of the fish to date. The levels of *R. salmoninarum* are most likely to be the strongest indicator of impact by the hatchery produced smolts. Kidney/spleen samples from the 2003 outmigrants were tested using the new batch of conjugated antisera developed by Kirkregaard and Perry. Dilution levels of this new conjugated antisera were evaluated to match OD values of the previous antisera against positive controls, but it was reported to be more sensitive at some ranges than the prior batch. However, the results for the 2003 outmigrants were no higher than seen in the past and had lower overall prevalence than the early years of testing. There will be year to year differences in the results due to normal annual variations of prevalence in a population and specific characteristics of the ELISA test. Three of the fish tested this year had OD levels of *R. salmoninarum* antigen high enough to indicate a potential for disease in the fish. All of these fish were in the normal size range and none of the fish had clinical signs of disease. Although studies have indicated poorer survival in fish with high levels of *R. salmoninarum*, the majority of smolts examined 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.

Of the pathogens this study is monitoring, prerelease testing of the hatchery reared smolts performed by the USFWS Fish Health Lab has only detected *R. salmoninarum*. Due to differences in methodology of the ELISA test, the comparison of OD levels between labs will 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. There will also be variations inherent in the assay between labs that need to be taken into consideration

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, which were not the focus of this investigation, would not be identified. Due to the presence of these pathogens in the natural environment, and the affect of environmental conditions on their prevalence, it will require a significant increase in prevalence of a pathogen in the naturally produced smolts to demonstrate a relation to the hatchery production.

Acknowledgments

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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		
6/15/00	11		
TOTAL 2000	182		
4/25/01	20		
5/02/01	6		
5/09/01	40		
5/16/01	40		
5/23/01	48		
6/06/01	25		
6/13/01	34		
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*

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

Table 4. Prevalence of 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

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