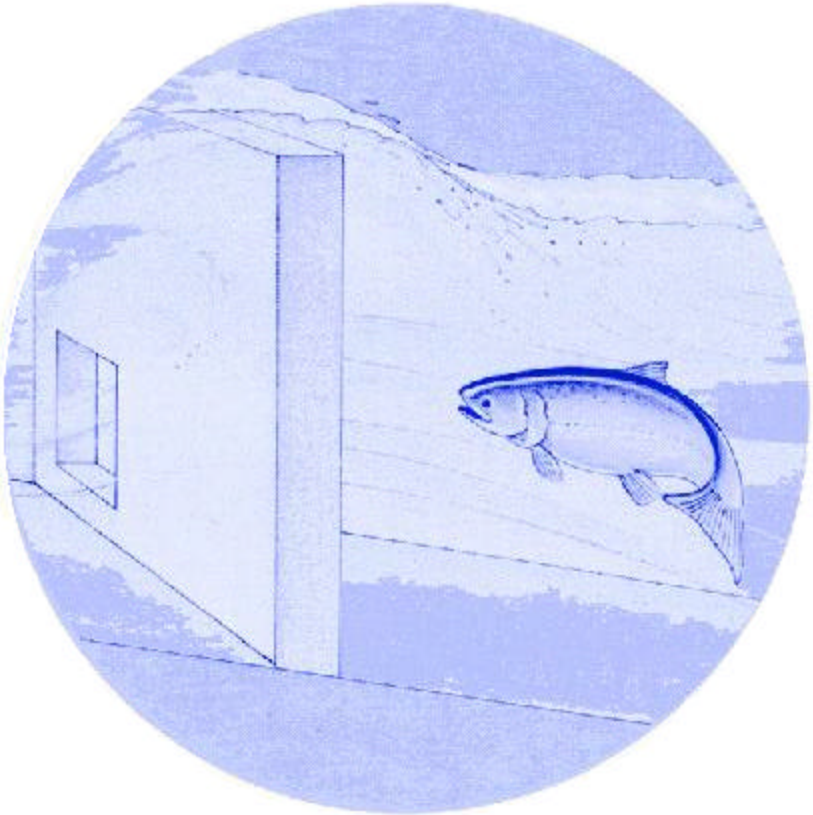
Pathogen Screening of Naturally Produced Yakima River Spring Chinook Smolts

Yakima/Klickitat Fisheries Project Monitoring and Evaluation

Annual Report
2001



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PATHOGEN SCREENING OF NATURALLY PRODUCED YAKIMA RIVER SPRING CHINOOK SMOLTS

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PATHOGEN SCREENING OF NATURALLY PRODUCED YAKIMA RIVER SPRING CHINOOK SMOLTS

Abstract

The change in pathogens prevalence to wild fish is probably the least studied ecological interaction associated with hatchery operations. In 1999, the Cle Elum Hatchery began releasing spring chinook smolts into the upper Yakima River to increase natural production. Part of the evaluation of this program is to evaluate 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. Approximately 200 smolts were collected at the Chandler smolt collection facility on the lower Yakima River during 1998, 2000 and 2001 and monitored for specific pathogens. The pathogens monitored were infectious hematopoeitic necrosis virus, infectious pancreatic necrosis virus, viral hemorrhagic septicemia, *Flavobacterium psychrophilum*, *Flavobacterium columnare*, *Aeromonas salmonicida*, *Yersinia ruckeri*, *Edwardsiella ictaluri*, *Renibacterium salmoninarum* and *Myxobolus cerebralis*. In addition, the fish were tested for *Ceratomyxa shasta* spores in 2001. Not all testing has been completed for every year, but to date, there have only been minimal changes in levels of the bacterial pathogens in the naturally produced smolts. At this point, due to the limited testing so far, these changes are attributed to normal fluctuation of prevalence.

Introduction

Pathogens present in the Yakima River basin are possible strong interactors 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, in poor environmental conditions, or in situations of high population density.

The purpose of this task is to determine the pathogen prevalence in this stock of naturally produced smolts at the time of out migration and to monitor these pathogens after introduction of spring chinook reared and released from 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. Samples were collected in the spring of 1998, 2000, and 2001. Due to an oversight, no samples were collected in 1999.

In addition, hatchery smolts were collected at the Chandler smolt trap facility and examined for select pathogens in the spring of 2001. This year was a drought year with higher than normal water temperatures, particularly in the lower river. The hatchery

smolts were taken to determine if there had been a change in their pathogen load due to the poor environmental conditions.

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, the fish may be collected over a period of several days. All specimens are received and dissected within 24 hours. Samples were 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 the *Flavobacterium*s, typical colony and cellular morphology consistent with isolation techniques. *F. psychrophilum* can also be confirmed with an agglutination test.

Kidney and spleen tissues are harvested from individual 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 viruses for a minimum of 14 days at 15°C. Both the supernatant from the diluted homogenate and a 10^{-1} dilution of the supernatant are plated on cells. Confirmation of any positives 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 1:9 with PBS and assayed with the ELISA plate method using antisera produced by Kirkegaard and Perry Laboratories. Results are obtained by recording optical density (OD) of a color reaction indicating levels of antigen to *R. salmoninarum* in the sample.

Each year heads are removed for testing of the cartilage for *Myxobolus cerebralis*, the causative agent of whirling disease. The head is removed behind the opercules operculum?, split in half longitudinally and each half head frozen in pools of five. One set of pools is tested using the pepsin trypsin digest assay for spores. The other set of pools is held in the freezer for confirmation by histopathology if spores are detected by pepsin trypsin digest. Histopathology will determine if spores of the typical size and morphology of *M. cerebralis* are located within lesions in the cartilage of the cranium.

In 2001 a portion of the hindgut was also removed, pooled in groups of five, and frozen for later examination by microscopy for the spores of *Ceratomyxo shasta*. This parasite was added to the testing that year because it has been seen in the adults and can cause significant losses in some watersheds with the correct environmental conditions. It was decided to only test for this pathogen periodically.

The hatchery smolts collected in 2001 were only tested for the bacterial and viral pathogens, since the temperature stressors would have most likely affected their prevalence since release. All testing was done as above except the virology and *R. salmoninarum* was done using pools of three fish, which is how the hatchery smolts are tested prior to release from the hatchery.

Results

To date only minimal pathogens have been detected in the outmigrating naturally produced smolts. The numbers and dates of fish sampled each year are presented in Table 1. No IHNV, VHSV or IPNV have been detected in any of the years tested. Of the cultured bacterial pathogens only *F. psychrophilum* and *F. columnare* have been detected at very low levels (Table 2.). *R. salmoninarum* is a common pathogen in Washington and is often present in smolting chinook. The level of *R. salmoninarum* antigen has been relatively low in the naturally produced Yakima River spring chinook smolts all years tested, although the prevalence was slightly higher in 1998 than 2000 in 2001 (Table 3.). To date no *C. shasta* or *M. cerebralis* has been detected, but due to staffing shortages, not all of the testing has been completed (Table 4.)

Discussion

The results have not indicated any significant health problems in this population due to pathogens, including 2001, when higher than normal water temperatures could have precipitated bacterial infections. None of the smolts have ever had gross signs of disease other than some slight hemorrhaging, which was probably due to the handling and euthanization. The levels of *R. salmoninarum* will be the most significant indicator of an impact of the hatchery smolts but there is often natural variation of *R. salmoninarum* levels in the population which will need to be considered. Another factor in measuring *R.*

salmoninarum by ELISA is that results can vary year to year due to different lots of antisera and the best comparisons are done within a season. Calculating run corrections may compensate for some of this difference. However, we will soon be changing to antisera manufactured by a new company which could also affect the results. At this time we are only guessing how these levels of antigen in the fish will affect the ultimate survival of the fish.

In this study we are examining year to year variations in pathogen prevalence. These results should also be compared to those from the hatchery smolts at time of release to look for possible relations. That testing is done by the USFWS Fish Health Lab.

None of the pathogen isolations seen so far in this study indicate a health problem due to interactions with the hatchery fish. Although affects on earlier or later life-stages, which were not the focus of this investigation, would not be identified. Due to the pathogens' presence in the natural environment and their prevalence is affected by environmental conditions it will require a significant increase in prevalence in the naturally produced smolts to demonstrate a relation to the hatchery production.

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

Date Sampled	**No. of fish tested**
5/18/98	30
5/19/98	55
5/20/98	20
5/26/98	30
5/27/98	30
5/31/98	14
6/03/98	12
TOTAL 1998	191
Spring 1999	No samples taken
5/10/00	20
5/17/00	20
5/22/00	40
5/23/00	21
5/25/00	20
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
Hatchery fish	
6/06/01	30
6/12/01	32
Total 2001	62

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*
2001 Hatchery fish	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
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

2001 – Hatchery Fish			
	OD	**number**	**percent**
below low	<0.099	38	61.3
low	0.100-0.199	18	29.0
mod	0.200-0.499	0	0.0
high	>0.500	6	9.7

Table 4. Prevalence of parasites between 1998 and 2001.

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