Pathogen Screening of Naturally Produced Yakima River Spring Chinook Smolts

Yakima/Klickitat Fisheries Project Monitoring and Evaluation

Annual Report
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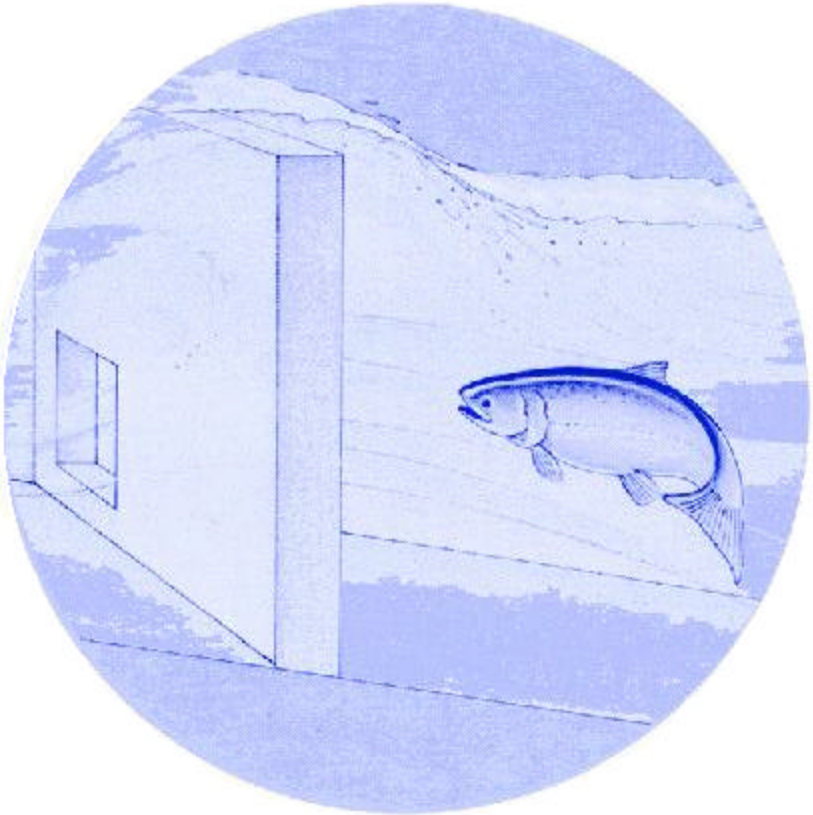

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PATHOGEN SCREENING OF NATURALLY PRODUCED YAKIMA RIVER SPRING CHINOOK SMOLTS

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PATHOGEN SCREENING OF NATURALLY PRODUCED YAKIMA RIVER SPRING CHINOOK SMOLTS

Abstract

In 1999 the Cle Elem Hatchery began releasing spring chinook smolts into the upper Yakima River for restoration and supplementation. This project was designed to evaluate whether introduction of intensively reared 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 130 smolts were collected in 2002 for monitoring 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 2000 and 2001 (a correction from the 2001 report). To date, the only changes have been in the levels the bacterial pathogens in the naturally produced smolts and they have been minimal. 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, poor environmental conditions, or 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 Elem 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, 2001 and 2002. Due to an oversight, no samples were collected in 1999 and due to poor water conditions in 2002 sampling was not completed.

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 since release 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 and held live until shipping to the Olympia lab. 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 the *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 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 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 1:9 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 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.

In 2000 and 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 those two years because it has been seen in adults returning to the Yakima River drainage. *C. shasta* can cause significant losses in some watersheds when the intermediate host and correct environmental conditions exist. Live box studies done by Dr. Lee Harrell, NMFS, in 1992 indicated that the infectious stage of the pathogen was not present, or at least not common, in the Yakima drainage. In addition, ceratomyxosis has not been seen in the spring chinook reared at the Cle Elum Hatchery. Monitoring for this pathogen will now occur only every third year to determine if it does become established.

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

Results

The data from the smolts collected in 2002 was similar to that seen in the previous years. To date only minimal pathogens have been detected in the outmigrating naturally produced smolts. Table 1. indicates the numbers and dates of fish sampled each year. 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 and 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 the other years tested (Table 3.). To date no *C. shasta* or *M. cerebralis* has been detected, but due to staffing shortages, the *M. cerebralis* screening has not been completed for 2000 (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.
There has been little variation in the *R. salmoninarum* profile of the fish to date. Even though levels in returning adults were quite high in the fall of 2001 this did not result in increased levels in the juveniles in the system that outmigrated the following spring.

However, it is possible that *R. salmoninarum* levels of the adults held in the hatchery were not representative of those that spawned naturally. The levels of *R. salmoninarum* are most likely to be the strongest 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 use of different lots of antisera and the best comparisons are done within a season. Calculating run corrections may compensate for some of this difference. At this time there is limited data available to indicate how these levels of antigen in the fish will affect the ultimate survival of the fish. 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. None of these fish had signs of clinical disease.

The results from the naturally produced smolts has yet to 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. Due to differences in methodology that comparison will be of population trends rather than of direct values. The USFWS data is for three fish pools of only kidney material and uses a different dilution of the detecting antisera.

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 the affect on their prevalence 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
5/6/02	20
5/8/03	20
5/14/03	30
5/20/03	30
5/28/03	30
Total 2002	130
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*
2002	No target bacteria detected
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	0.0
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

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.

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