Fish Disease Risk Study Associated With Potential Anadromous Fish Passage at the Pelton Round Butte Project: Summary Report 1997–2002

PELTON ROUND BUTTE HYDROELECTRIC PROJECT

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Prepared by

H. Mark Engelking
Project Leader

Oregon Department of Fish and Wildlife
Fish Pathology Section
Corvallis, Oregon

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ABSTRACT

We have examined more than 8,228 fish since 1997 for fish pathogens in the Deschutes River watershed in central Oregon, from above and below the Pelton Round Butte Hydroelectric Project (Project). In 2002, 1397 fish were examined. We used a fish disease risk matrix that had been developed in 1997 to guide decision making and as the basis for sampling specific groups of fish and performing selected diagnostic tests. Our results show that the genetically different strain of Infectious Hematopoietic Necrosis Virus (IHNV) Type 2 detected below the Project (RB 97) is more virulent to rainbow (redband) trout (*Oncorhynchus mykiss*), summer steelhead trout (*O. mykiss*), spring chinook (*O. tschawytscha*) and kokanee salmon (*O. nerka*) by challenge assays than the IHNV found in the kokanee salmon in Lake Billy Chinook (ME 96). Bull trout (*Salvelinus confluentus*) were found to be relatively resistant to all strains of IHNV tested. Three other fish pathogens — erythrocytic inclusion body syndrome virus (EIBS virus), *Myxobolus cerebralis* (the causative agent of whirling disease) and *Aeromonas salmonicida* (the causative agent of furunculosis) — were detected only in fish from below the Project. Evidence of *Renibacterium salmoninarum* (the causative agent of bacterial kidney disease) was found in fish throughout the study area. Spores of *M. cerebralis* were detected only in anadromous adult fish found below the Project. These results were confirmed by microscopic examination of histology sections that revealed the presence of lesions and spores in cartilage from the head. None of the resident fish examined above or below the Project had detectable spores of *M. cerebralis*. No anadromous fish of Deschutes River origin have been detected with spores resembling *M. cerebralis*. We have created a database to store and organize information gained from the study. The survey work is essentially completed for this project. A risk assessment is being prepared.

INTRODUCTION

The Pelton Round Butte Fish Disease Risk Analysis is a study to assess the attendant risk to native resident fish from introducing anadromous fish and their associated organisms into waters above the Pelton Round Butte Project (Project). This study will also attempt to determine if achieving sustainable natural production above the Project may be constrained by infectious disease. The potential infectious disease impacts to the lower river and hatchery program will also be evaluated. The fish pathogen issues are complex in the Project area. These issues are the result of the Project, the biology of the fish pathogens, and the alterations of the environment that have affected the fish and the pathogens. Our role is to address those issues that have developed as a result of these conditions and make recommendations consistent with our findings.

Fish pathogens may deleteriously impact certain resident and anadromous fish stocks, if anadromous fish transfer such pathogens as they are reintroduced above the Project. Portland General Electric (PGE) and the Confederated Tribes of the Warm Springs Reservation of Oregon (Tribes), as they propose to achieve this reintroduction goal, need to understand any disease constraints that may be important in attaining sustainable natural production of anadromous stocks and preserving the native resident fish stocks. The dynamics of fish and fish pathogen interactions resulting from reintroduction will be more clearly understood and evaluated with a solid base of information.

We have used the fish disease risk matrix, which was developed in 1997 as a cooperative effort of Oregon Department of Fish and Wildlife (ODFW) and PGE biologists, and as a guide for framing our pathogen studies. This fish disease matrix defines areas where key information is lacking and serves as decision-making criteria for the reintroduction of anadromous fish above the Project (see 1998 Study Plan). Study tasks were developed with the matrix as a guide to obtain the information needed to increase our knowledge. Studies reported in this report were funded by PGE and the Tribes and conducted by the Oregon Department of Fish and Wildlife (ODFW), Fish Pathology Section. Other studies funded by PGE and the Tribes on the fish parasites, *Myxobolus cerebralis* and *Ceratomyxa shasta,* are being conducted by the Department of Microbiology, Oregon State University, and are reported separately.

METHODS

Fish Pathogen Surveys

Since 1997 samples have been collected for detection of specifically identified fish pathogens of concern. Summer run steelhead, rainbow, brown (*Salmo trutta*), brook (*Salvelinus fontinalis*) and bull trout, mountain whitefish (*Prosopium williamsoni*), sockeye (*O. nerka*), kokanee and spring chinook salmon were examined from above and below the Project. Blood smears from sampled fish were stained and examined for viral inclusions and other fish pathogens. We also performed Bacterial Kidney Disease (BKD) ELISA tests for *Renibacterium salmoninarum* antigen. The bacteriological media used to culture kidney samples support most common pathogenic fish bacteria, other than *R. salmoninarum*. Tissue and fluid samples were taken for cultivable virus and bacteria. The virus exams are capable of detecting infectious hematopoietic necrosis virus (IHNV), infectious pancreatic necrosis virus (IPNV), viral hemorrhagic septicemia virus (VHSV), erythrocytic inclusion body syndrome virus (EIBS), paramyxovirus, and aquareovirus. Parasite presence was determined microscopically, and head skeletal tissues were

preserved for *Myxobolus cerebralis* histology assays. In our studies, all fish were examined for the presence of *M. cerebralis* and *Ceratomyxa shasta*. We also performed microscopic examinations for other external and internal parasites on subgroups of up to ten fish per stock. Representative isolates of the fish pathogens from these surveys were stored for future analysis. Survey data are still being collected and processed.

All methods for detecting and identifying fish pathogens were based on standard procedures from the AFS Fish Health Blue Book (Thoesen 1994) and/or those of the ODFW Fish Pathology laboratories. The detailed methods for detecting and identifying bacterial, parasitic and viral fish pathogens are described in Appendix II.

Myxobolus cerebralis (Whirling disease) Diagnosis

Presumptive Infection

Using a microscope we observed spores that appeared morphologically (size, shape and other characteristics) similar to those of *M. cerebralis*. Spores from *M. cerebralis* infected fish are circular in shape with a diameter of 7 to 10 µm and possess two polar capsules of equal size that extend less than one half the diameter of spore. The spores also have thin envelopes without ticks. Such spores were found in the material obtained from trypsin and pepsin enzyme digestion of head samples. Two separate microscopic examinations were conducted by different laboratory workers on each enzyme digest sample to verify the presence or absence of spores.

Confirmed Infection

An infection was confirmed when lesions and spores were observed microscopically in the bone and cartilage in the head. Each fish head was cut into thin sections and attached to a microscope slide. The slide was then stained to enhance the appearance of skeletal structures and the polar capsules of the spores. This procedure is called histology. The detection of typical *M. cerebralis* spores within the cartilage or bone by a fish health specialist is required to confirm any findings.

PCR Determination

Graduate students in Jerri Bartholomew's laboratory at Oregon State University performed Polymerase Chain Reaction Assays (PCR) on samples from the enzyme digestion of head material. DNA was extracted from this sample and compared with standards to determine if it was the same as that of *M. cerebralis*. This method was used to corroborate but not confirm

results since the methodology has not been validated. Occasionally, false negative results were obtained because the DNA in the sample was lost or other experimental errors arose.

in situ Hybridization Methodology

In this examination we looked at a thin section of a fish head that was attached to a microscope slide to determine if DNA complimentary to that of *M. cerebralis* would bind to the spore DNA in the section. Staining or radioisotopes made this DNA complex (hybrid) evident. Because of the difficult methodology and experimental variations, false negative results could arise. This method was used to corroborate but not confirm results. All these tests were performed by Dr. R. Hedrick's Laboratory University of California, Davis.

Infectious Hematopoietic Necrosis Virus (IHNV) Genetics and Challenges

Genetic Analysis of IHNV

The genetic differences of the IHNV isolates found above and below the Project were determined by ribonuclease protection assays (RPA). The genetic material of IHNV is ribonucleic acid (RNA); similar to deoxyribonucleic acid (DNA), which is the genetic material in all higher organisms. The difference between these two compounds is that the sugar backbone of RNA contains an additional oxygen atom. The RPA methodology can estimate the heterogeneity (differences) among large numbers of related RNA molecules. This allows the determination of the variation in IHNV populations and evolution of IHNV in the Deschutes system. A second method involving partial sequencing of the IHN virus glycoprotein gene variable region was used to roughly group the virus into Type 1 or Type 2 strains. These groups are designated clades for phylogenetic analysis.

Virulence Testing of IHNV Isolates

Conducting challenge experiments with resident and anadromous fish stocks has identified differences in virulence of IHNV strains found above and below the Project. Relative susceptibility of these stocks to the IHNV strains was also assessed.

Virus (IHNV) Stocks for Challenge Studies

Three IHN virus strains were used to conduct the challenge studies. These strains were isolated from a spawning adult steelhead trout at the Round Butte Hatchery in 1975 (RB1) (Hsu et al.,

1986), a spawning steelhead trout in January 1997 at Round Butte Hatchery (RB 97), and a spawned out kokanee salmon from the Metolius River in 1996 (ME 96).

Fish for IHNV Challenge Experiments

Bull and rainbow (redband) trout and kokanee salmon from the Metolius River and redband trout from the Crooked River were captured and spawned to produce young for disease challenges. The Metolius River rainbow trout, however, did not produce enough fry. We captured naturally produced fry in the Metolius River in May with a seine net. Round Butte Hatchery production steelhead trout and spring chinook salmon were also used in the challenges. Unmarked (wild) steelhead and chinook taken to Round Butte Hatchery were used to produce "wild" fry from these fish stocks. All fish were obtained through the Oregon Department of Fish and Wildlife. Steve Marx, ODFW biologist, and PGE crews assisted in adult rainbow capture. Mike Reihle and his U. S. Forest Service crews assisted with trapping and spawning bull trout and netting Metolius rainbow trout.

Challenge Experiments with IHNV Strains

Virus challenge experiments were performed at the Oregon State University Salmon Disease Laboratory. Triplicate lots of 25 fish were used when possible. If fewer fish were available, experiments were done in duplicate tanks of 35 fish or as many as possible. The fish were challenged with IHNV by immersion in a liter of water for 12 hours containing different concentrations of the virus (Johnson et al., 1982a and 1982b). The virus challenge levels used were from 10^2, 10^3, 10^4 and 10^5 $TCID_{50}$ (Tissue culture infective dose for 50 per cent infection) per milliliter of water. Dead fish were collected daily and examined for visible signs of disease. Every dead fish was processed by standard methods (Thoesen, 1994) to determine if the fish had died from an IHNV infection. Mortality was scored if IHNV was detected in the fish.

Virus (IHNV) Assays from Fish Challenges

Virus assays were performed using confluent CHSE-214 or EPC cell monolayers grown in 24-well tissue culture plates (Falcon). Samples from infected fish were prepared as described (Thoesen, 1994). All virus strains were prepared by growing the virus at a multiplicity of infection of 0.01 to 0.001 $TCID_{50}$ per cell on CHSE-214 cells as previously described (Engelking and Leong, 1981). The virus used for challenge trials was prepared from a stock of

virus, which had undergone no more than three passes in tissue culture after isolation from infected fish.

RESULTS AND DISCUSSION

Fish Pathogen Surveys

Fish were collected and sampled from above and below the Project area for the presence of certain virus, bacteria, and parasites. Most samples have been examined and tests completed. However, some *C. shasta*, *M. cerebralis* and BKD ELISA samples are still in progress. Results are grouped by fish pathogen and summarized below. More detailed information can be found in Appendices I and III.

Sampling 2002

In the first quarter of 2002, the production spawning of summer steelhead occurred at Round Butte Hatchery. More than 300 fish sampled were summer steelhead hatchery fish as well as stray hatchery fish, which were obtained at Pelton Trap. This quarter more than 540 fish were sampled for specific fish pathogens. Smolts from both the hatchery summer steelhead (n=60) and spring chinook (n= 90) programs were intensively sampled. Two bull trout mortalities and 32 kokanee from Lake Billy Chinook were examined. Both bull trout had clinical BKD infections. 28 Kokanee from Lake Simtustus were also examined. Stray steelhead were tested for the presence of *M. cerebralis.*

In the second quarter of 2002, about 155 fish records of sampled fish were entered into the database. Samples were taken from rainbow trout and mountain whitefish from the lower Deschutes River. Steelhead from Trout Creek were sampled. Bull trout mortalities taken in the Merwin trap in the forebay of Round Butte Dam were obtained. Stray chinook and rainbow trout were obtained at Pelton Trap. Bacterial results were for the most part unremarkable. The exception was the clinical BKD infections of the bull trout.

In the third quarter of 2002, spring chinook salmon were spawned at Round Butte Hatchery for production. Pathogen samples were taken at both spawning periods in August and September. This quarter about 524 fish records of sampled fish were entered into the database. Nine of the spring chinook were hatchery strays.

In the final quarter of 2002, 4 adult sockeye salmon were spawned at Round Butte Hatchery. The fry, which had thermally marked otoliths, will be evaluated in outmigration and survival studies. One sockeye mortality was taken for examination. Kokanee were also obtained from Pelton Trap. There were 138 fish sampled this quarter. Among the other fish sampled were groups of live and dead spawned out kokanee salmon from the Metolius River and adult stray hatchery steelhead obtained at Pelton Trap. Fall chinook salmon heads and kidney spleen samples from spawned out carcasses from below Sherars Falls were taken by spawning ground survey groups. Samples of the same stock were obtained from above Sherars Falls also. Previous years (1997, 1998, 1999 and 2000) of sampling have been described in detail in the 1997, 1998, 1999 and 2000 Annual Reports to PGE (Engelking, 1998, 1999, 2000, 2001).

Viral Fish Pathogens

During our studies, Infectious Hematopoietic Necrosis Virus (IHNV) has been detected in the Round Butte summer run steelhead trout and spring and fall run chinook salmon below the Project, but not in the mountain whitefish, rainbow trout, or sockeye salmon we sampled from the lower Deschutes River.

In 2002 the incidence of IHN virus in adult spawning steelhead was 23 percent overall in the first spawn on January 29 and only 5 percent on February 26. In the first spawn 33 of the 80 female fish were IHN virus infected and four of 73 male fish were infected. In the second spawn only six of 74 females and two of 74 males were IHN virus infected (Table 1).

This was somewhat lower than the previous year. In 2001 the incidence of IHN virus in adult spawning steelhead was 39 percent overall in the first spawn on January 31and only 9.4 percent on February 27. In the first spawn 52 of the 89 female fish were IHN virus infected and nine of 76 male fish were infected. In the second spawn only five of 48 females and four of 48 males were IHN virus infected (Table 1). The virus was confirmed by indirect fluorescent antibody assay reactions to both antisera B9/C6 and 2NH105B. RNA protection assay results indicated a similar genetic profile to that seen from these fish in previous years

Previously about eight percent of the summer steelhead trout in 1999 were confirmed positive for IHNV in 1998 (Engelking 2000). In 2000 only the hatchery adult steelhead trout were IHN virus positive at 6.1 percent. No unmarked steelhead trout were IHN virus infected, and stray hatchery fish were not sampled.

For the second year in a row, a high number of adult spring run chinook salmon were infected with IHN virus in 2002. Overall in the first production spawning of 293 fish, 66.6 percent were infected with virus. Of the females 87 percent, and 39.7 percent of the males, had detectable IHN virus infections in the first spawn. The second group spawned was slightly less infected with 58.8 percent IHN virus infected of 221 sampled. Again more females than males were infected; 68.75 percent of the females and 40.3 percent of the males. Two hundred forty four females and 81 males were infected from the 514 fish sampled during spawning.

In 2001, 59.5 percent were infected from the 475 spring chinook sampled during spawning (Table 1). In 2000, only 5.4 percent were infected with IHN virus. Of these infected fish 4.4 percent of the hatchery fish, 15 percent of the stray hatchery fish, 5.9 percent of the unmarked fish and 8.3 percent of the unidentified fish were infected with IHN virus. In 1999, 3 percent of the hatchery, 16 percent of the unmarked and 22 percent of the stray spring chinook were IHN virus positive (Engelking 2000). Previously about 8 percent of the spring chinook were confirmed positive for IHNV in 1998, and more than 58 percent in 1997 (Table 1).

No virus was detected in the kokanee or bull trout obtained during population sampling in Lake Billy Chinook. Kokanee salmon from Lake Simtustus were not infected with virus. This year samples were obtained from spawned out fall chinook salmon carcasses. Of the 63 samples taken 45 (72%) were positive for IHN virus. These appear to be Type 2 strain virus.

The Metolius River and Link Creek kokanee salmon were the only populations previously sampled above the dam from which IHN virus was isolated. This year (2002) was similar in results to 2001; all the fish tested were infected with IHN virus. In 2001 surprisingly only 19 percent of the kokanee spawning adults were IHN virus infected from the first sample. Results showed 93 percent (64 of 69 fish) of the Metolius kokanee were infected with IHN virus in 2000 when sampled on four occasions over two months. However, in 1999 about 63 percent of the 134 spawning kokanee examined (113 females, 21 males) were detected with IHN virus infections. Link Creek was not sampled in 2000, 2001 or 2002.

Table 1. Number of Summer Steelhead Trout and Spring Chinook Salmon by Origin (Hatchery, Stray Hatchery, Unmarked or Unknown) infected with IHN virus.

		IHNV INFECTED			
Fish Species Year	**Total** [a]	**Hatchery**	**Hatchery Strays**	**Unmarked**	**Unknown origin**
Summer Steelhead Trout 1997-1998	57/413	46/187	2/200	9/26	None
Summer Steelhead Trout 1998-1999	20/238	19/174	0/40	1/24	None
Summer Steelhead Trout 1999-2000	14/247	14/228	Not Done	0/19	None
Summer Steelhead Trout 2000-2001	70/269	70/269	Not Done	Not Done	None
Summer Steelhead Trout 2001-2002	45/301	45/301	Not Done	Not Done	None
Spring Chinook Salmon 1997	135/231	106/172	Not Done	29/59	Not Done
Spring Chinook Salmon 1998	26/311	20/255	3/23	3/14	0/19
Spring Chinook Salmon 1999	15/437	10/406	2/9	3/19	0/3
Spring Chinook Salmon 2000	28/516	19/430	5/33	1/17	3/36
Spring Chinook Salmon 2001	282/475	257/440	16/21	None	9/14
Spring Chinook Salmon 2002	325/524	320/515	5/9	None	None

a. Number IHNV positive to the total number of fish per group.

Results of RPA analysis of the IHNV isolates in 2002 revealed that the virus found above and below the Project continues to be genetically distinct. Challenge experiments described below

continued to show that these two strains of virus differ in their virulence. No losses from IHNV infection in spring chinook salmon juveniles occurred at Round Butte Hatchery since 1997.

Erythrocytic Inclusion Body Syndrome Virus (EIBS)

Spring chinook salmon were the only fish stock surveyed that were infected with Erythrocytic Inclusion Body Syndrome (EIBS) virus (Table 2). Testing of blood smears from spring chinook adults revealed that four hatchery fish of 100 tested had presumptive infections in 2002. Blood smears were not available for no-tag and lost-tag fish (Table 2). Previously Erythrocytic Inclusion Body Syndrome viral inclusions were detected in 9.5 percent of the hatchery, 25 percent of the unidentified hatchery fish (n=8) and none of the unmarked spring chinook salmon in 2001. There is no evidence that EIBS virus is present above the Project, because no resident fish have been determined to have the EIBS virus inclusions (Engelking 2001).

Table 2. Detection of EIBS inclusion bodies in erythrocytes in blood smears of spring run chinook salmon in the Deschutes watershed in 2002.

Fish Stock	Number Fish Sampled	Number Tested Samples	EIBS Negative	EIBS Positive
Round Butte Hatchery	100	100	96	4
Unmarked	None	None	None	None
No Tag/ Unknown	0	0	0	0
Hatchery Stray	0	0	0	0
Totals	100	100	100	4

Bacterial Fish Pathogens

Renibacterium salmoninarum (BKD) antigen was found in all populations tested in 2002. Spring chinook and sockeye salmon, mountain whitefish, rainbow and summer steelhead trout below the Project carried BKD antigen. Rainbow, bull, and brook trout and kokanee salmon from above the Project also revealed detectable levels of antigen to *R. salmoninarum.* In 2002, 871 fish were tested for the presence of *R. salmoninarum* antigen. Of those tested 548 were negative for the antigen.

In 2002, most of the samples examined were from below the Project (92 percent). These exams revealed that 30 percent of the fish below the Project had detectable levels of BKD antigen, while more than 27 percent of those examined above the Project had detectable antigen levels. However most of these fish exhibited only low levels of antigen (about 16 percent). Less than 10 percent of the fish above or below the Project had high levels of antigen present (Table 3). Spring chinook from the lower Deschutes River had the highest incidence of antigen and kokanee the lowest. Sockeye, rainbow trout and mountain whitefish from the lower Deschutes River also had detectable levels of BKD antigen. Bull and rainbow trout examined this year also show the presence of this antigen with many medium to high levels being detected (Appendix I). It appears that BKD may contribute to premature mortality of fish in the Deschutes River watershed. Bull trout from above the Project were found with clinical cases of BKD. Indeed, four of five fish tested were infected. Based on the incidence and prevalence of antigen found in fish surveyed, mortality likely occurs in fish that become susceptible through stress or other factors. With more than 10 percent of the fish above the Project carrying medium to high levels of antigen to *R. salmoninarum,* and more than 15 percent with low levels, this pathogen is likely a significant cause of mortality in the upper watershed (Table 3).

Table 3. Summary by location to Project of BKD ELISA results for 2002.

Number Fish Sampled Year	Number Samples Tested	BKD Negative	BKD LOW	BKD MEDIUM	BKD HIGH
2002					
Total	871	548	158	59	106
100%	100%	69.2%	13.6%	8.2%	9%
Fish Below PRB	801	497	147	58	99
%	92%	62%	18%	7%	12%
Fish Above PRB	70	51	11	1	7
%	8%	73%	16%	1.4%	10%

Flavobacterium psychrophilum (the causative agent of cold water disease, CWD)*, Aeromonas salmonicida* (the causative agent of furunculosis), and aeromonad/pseudomonad (APS) bacteria were among the bacteria detected in populations sampled. One hundred eighty one fish were sampled for bacteria from the kidney. Furunculosis bacteria were found in hatchery summer

steelhead trout, spring chinook salmon and kokanee from below the Project. There was no evidence of furunculosis in the fish populations sampled above Project (Table 4).

Flavobacterium psychrophilum was found in fish populations throughout the Project area, in the summer steelhead, kokanee and spring chinook populations. Among the kokanee it was found in the spawning fish in the Metolius River. This cold water disease bacterium was found in wild sockeye salmon (Table 4). *Carnobacterium piscicola* was present in low numbers in spring chinook salmon, bull trout, steelhead, and kokanee from below the Project. APS was isolated from spring chinook salmon mountain whitefish and kokanee this year (Appendix I). Much of this year's decrease in detecting these bacteria is from the limited testing of only 181 fish. Of those tested, 120 were negative for bacterial infections. The results are consistent with previous years' results.

Table 4. Fish species infected with specific bacterial fish pathogens.

BACTERIA	SPECIES INFECTED BELOW PROJECT	SPECIES INFECTED ABOVE PROJECT
Renibacterium salmoninarum (BKD)	ChS, StS, KK, RB, MWF, SS	BUT, KK, RB, BR, MWF
Flavobacterium psychrophilum (CWD)	StS,ChS,KK, SS	KK, BUT, RB
Aeromonas salmonicida (Furunculosis)	ChS,StS,SS,KK	None

Table abbreviations:
StS-Summer Run Steelhead Trout, Rb-Rainbow Trout, MW-Mountain Whitefish, SS-Sockeye Salmon, KK-Kokanee Salmon, BUT-Bull Trout, BR-Brown Trout, and ChS-Spring run Chinook Salmon

Genetic Analysis of IHNV - RNase Protection Assays (RPA) and Glycoprotein Gene Sequencing

We conducted a genetic analysis above and below the Project using the RNA protection assay, an accepted method for estimating the amount of heterogeneity among large numbers of related

RNAs. This method can be applied to evaluating the variations in the genetics of virus from fish at different locations or from one location from year to year. It was applied here to determine the genetic differences in the IHNV isolates found above and below the Project and to compare these to previously detected isolates from these areas. It was also used to identify the level of heterogeneity (differences) within the virus populations for a group of fish at a particular time. This was important in deciding how many samples would be needed for challenges and genetic analysis.

Further phylogenetic analysis was performed using sequence analysis of a 303 nucleotide variable region of the glycoprotein gene of IHN virus. This analysis is less stringent than the RPA analysis, but can be performed more quickly. This method described differences among the isolates in the Deschutes River basin. All the isolates fell within the U clade, which is rapidly evolving as described by Kurath and co-workers (Kurath et al. In press). This information also confirmed that the strains above and below the Project have remained different from one another during the study (Table 5). The strain from below is consistently a U-1-9 clade, and the IHN virus from the kokanee has remained in the U-2-8 clade group (Garver et al. In review D.A.O.).

Isolates of IHNV detected in summer run steelhead trout in 2002 were analyzed with one RNA probe for the glycoprotein (G) IHNV gene. The IHNV isolates came from early and late steelhead trout spawning dates. The RPA analysis indicated that all isolates were identical to each other and to the 1997 isolate (RB97) from adult summer steelhead. Of the IHNV isolates from hatchery spring chinook salmon examined, all appeared identical to each other. They were also identical to the IHNV strains isolated from spring chinook salmon in 1996 through 1999 and to the RB97 isolate. In 2000 isolates obtained from spawning adult spring chinook, summer steelhead and kokanee were genetically analyzed as previously described. The results indicated that the IHN virus strains were the same as found in previous years' studies. The IHN virus from kokanee in the Metolius River/Lake Billy Chinook is genetically different than the virus isolated from spring chinook and steelhead adults returning to Pelton Trap below the Project. These strains are genetically different from recent IHNV isolates isolated from fish above the Project, such as the 1991, 1992, and 1993 Lake Billy Chinook kokanee salmon isolates and the 1996 and 1997 Metolius River kokanee salmon isolates. These isolates from above Project are all very similar and represent the Lake Billy Chinook haplotype or Type 1 strain (Engelking 1998). Since the IHNV from above the Project passes through the dam and infects fish in the lower river, both haplotypes or Type 1 and 2 strains are found below the Project (Anderson et al. 2000, Engelking 2001). The IHNV isolated from spawned out kokanee salmon in fall 2001 from the Metolius River by comparison are identical to previous isolates from 1996 and 1997 and are all Type 1 strains of the IHN virus.

Table 5. RNA Haplotypes and clades of IHN virus found in the Deschutes River watershed.

Location	Species Year	G gene haplotype	N gene haplotype	NV Gene haplotype	Clade mid-G gene Sequence [1]
Round Butte Hatchery	Summer Steelhead 1997	A	A	A	U-1-9
	Summer Steelhead 1998	A			U-1-9
	Summer Steelhead 2002				U-1-9
	Summer Steelhead 2000				U-1-9
	Summer Steelhead 2001				U-2-8
	Summer Steelhead Fry Loss 1997	B	B	B	U-2-8
Round Butte Hatchery	Spring Chinook 1996	A	A	A	U-1-9
	Spring Chinook 1997	A	A	A	U-1-9
	Spring Chinook Fry Loss 1997	A	A	A	U-1-9
	Spring Chinook 1998	A			
	Spring Chinook 2000				U-1-9
	Spring Chinook 2001				U-1-20
	Spring Chinook 2002				U-1-9
Unmarked (Wild)	Spring Chinook 1998	A			
Dworshak Hatchery Stray	Spring Chinook 1998	A			
Round Butte Hatchery	Summer Steelhead 1995	B	B	B	
Round Butte Hatchery	Summer Steelhead 1994	B	B	B	
Lake Billy Chinook	Kokanee 1991 to 1995	B^{V} [2]	B^{V}	B	U-2-8
Metolius River	Kokanee 1995	B	B	B	U-2-8
Metolius River	Kokanee 1996	B	B	B	
Metolius River	Kokanee 2000				U-2-8
Metolius River	Kokanee 2001				U-2-8

1. U, M, or L refer to the clade, The first number refers to the nucleotide change group, (All of U-1 have nucleotide 9 the same but different from U-2 which has a change in nucleotide 8) The second number refers to the specific nucleotide that is changed.
2. B^{v} = a minor variant of the B haplotype.

These findings further reinforce the concept that certain strains of IHN virus from below the Project are distinct from strains found above the dam. They also indicate the displacement of strains that occurs in the virus population below the Project. The frequent introduction of IHNV-infected fish, in other words, introduces new genetic strains and may displace the strain that is currently present.

IHNV Challenge Experiments for Virulence and Susceptibility

In order to determine the relative virulence of the IHNV isolates found above and below the Project, certain fish stocks are being challenged with these isolates. These virulence challenges provide information on the relative differences in susceptibilities of these fish stocks to various IHNV strains.

The fish in all experiments were challenged with these IHNV isolates obtained from: spawning steelhead trout in January and February of 1997 at Round Butte Hatchery (RB 97) (A haplotype); spawned out kokanee salmon from the Metolius River in 1996 (ME 96) (B haplotype); and adult spawning summer run steelhead trout from Round Butte Hatchery 1975 (RB 1). RB1 is the prototypic IHNV isolate that has been most extensively studied. The prototypic RB1 strain is also the comparator IHNV isolate in the RPA genetic analysis. Results from 1997 experiments indicated that the RB97 IHNV strain is the most virulent virus tested (Appendix I).

1997 Challenge Experiment Results

Rainbow Trout, Crane Prairie and Crooked River Stocks

The Crane Prairie and Crooked River rainbow trout were most susceptible to the RB 97 isolate and most resistant to the RB1 IHNV isolate. The above-Project strain, ME 96 IHNV isolate, was found to be intermediate in virulence as compared to the two other IHNV strains used in these experiments (Engelking 1999).

Spring Chinook Salmon, Round Butte Hatchery Stock

In 1997 we examined the lethal doses required to obtain 70 percent or 50 percent mortality of the fish exposed to various IHNV strains. In particular we determined the LD_{70} or LD_{50}s for hatchery spring run chinook salmon. In our comparison of the LD_{50} values, we found that the

spring chinook salmon fry were most resistant to the RB1 IHNV strain. No difference in virulence between the other two strains of IHNV could be determined using the LD_{50} values. However our comparison of LD_{70} values revealed that the spring chinook were more susceptible to the RB97 strain than the ME96 strain.

1998 Challenge Experiment Results

Bull Trout, Metolius River Stock

Metolius River bull trout fry, obtained from fish trapped in Jack and Canyon creeks and spawned, were challenged in 1998 with the same three strains of IHNV used in the previous challenges. The challenges revealed that this species is relatively resistant to IHN virus infection. Bull trout appear to be similar to other members of this genus, brook trout (*S. fontinalis*) and Arctic char (*S. alpinus*), in their relative resistance to IHNV infection.

Kokanee Salmon, Metolius River Stock

Challenge experiments with IHNV strains were completed for Metolius River kokanee salmon. Kokanee salmon fry were most susceptible to IHNV RB97, the strain found only below the Project. The IHN virus RB1 strain was the least virulent.

Summer Steelhead Trout, Round Butte Hatchery Stock

Challenge experiments with IHNV strains were completed for Round Butte Hatchery summer run steelhead trout fry. The steelhead were more resistant to the RB1 isolate than the kokanee. The RB1 strain has been considered a sockeye and kokanee adapted strain. Again the IHNV RB97 strain was most virulent to this fish stock.

Rainbow Trout, Metolius River Stock

Challenge experiments with IHNV strains were completed for Metolius River (redband) rainbow trout. Rainbow trout fry obtained from the river by seine netting were used in these experiments. The study results showed that again the RB97 isolate was more virulent at all challenge levels to these fish. The losses correspond to what LaPatra and co workers (1993) found using a type 2 isolate of IHNV from Idaho. The type 2 strain of IHN virus killed about tenfold more rainbow trout at a specific challenge dose than did either type 1 (RB1) or type 3 (southern Oregon, northern Californian chinook isolates). However, comparisons among the results should be

made with caution. Since the fish were more than twice as large and probably much older than in the previous experiments, the experiments were performed with fewer fish, reducing the validity of the results.

Rainbow Trout, Crooked River (Opal Springs) Stock

Challenge experiments with IHNV strains were also completed for Crooked River (redband) rainbow trout obtained from Opal Springs. The results of this experiment were similar to results obtained in 1997 with fry obtained from spawning adult rainbow caught below Bowman Dam on the Crooked River. As has been the case in all virulence tests performed, the RB97 strain of IHNV was the most virulent of those tested.

Challenge Experiments with IHNV Strains, 1999

Rainbow Trout, Metolius River Stock

Challenge experiments with IHNV strains were completed for fry from Metolius River rainbow trout spawned at the Salmon Disease Laboratory in January 1999. The adults had been captured in December of 1997 and 1998. The LD_{50} for the RB97 IHNV strain revealed that it was about 3 times more virulent to Metolius rainbow trout than the other two strains (Engelking 2000).

Summer-Run Steelhead Trout, Unmarked Stock

Challenge experiments with IHNV strains were completed for fry from unmarked summer steelhead spawned at Round Butte Hatchery on February 26, 1999. Similarly, the RB97 strain was observed to be almost nine times more virulent than the above-Project ME 96 strain to summer steelhead fry from unmarked adults. In comparison to the RB1 strain, the RB97 IHN virus strain was 100-fold more virulent in challenges of unmarked summer steelhead progeny. The results of this experiment are similar to results obtained in 1997 with fry obtained from hatchery summer steelhead (Engelking 2000).

Rainbow Trout, Crooked River Stock

A challenge experiment with IHNV strains was completed for fry from Crooked River rainbow trout reared at the Salmon Disease Laboratory. The adults had been captured in June of 1997. On April 15, 1999 Crooked River rainbow trout adults were spawned at the Salmon Disease Laboratory. The challenge began in June and ended July 1999. The results of this challenge

were inconclusive, because a chronic IHN virus infection developed instead of an acute infection necessary to assess virulence. In spite of this, the results suggested the RB97 strain of IHNV was the most virulent in these challenges. It also suggests that there may be some increased mortality among the resident and anadromous fish above the Project if this strain of IHNV is introduced there. The greater virulence of the RB97 strain of IHNV was demonstrated for the Metolius rainbow and the progeny of unmarked adult steelhead trout previously (Engelking 2000).

Challenge Experiments with IHNV Strains 2000

Spring Chinook Salmon, Unmarked (wild) Stock

Challenge experiments with IHNV strains were completed for fry from unmarked (wild) spring chinook parents that were spawned in August 1999. The adults had been captured in Pelton Trap. The LD_{50} for the RB97 IHNV strain revealed that it was about 2 to 30 times more virulent to these spring chinook fry than the other two strains . These results correspond closely to the virulence that was demonstrated with fry from hatchery spring chinook in 1997 (Engelking 2001).

Summer-Run Steelhead Trout, Unmarked (wild) Stock

Challenge experiments with IHNV strains were completed for fry from unmarked (wild) summer steelhead trout parents that were spawned in January 2000. The adults had been captured in Pelton Trap. The LD_{50} for the RB97 IHNV strain revealed that it was about 3 to 25 times more virulent to these summer steelhead fry than the other two strains. These results correspond closely to the virulence that was demonstrated with fry from hatchery summer steelhead trout in 1998 and fry from unmarked (wild) summer steelhead in 1999 (Engelking 2001).

Challenge Experiments with IHNV Strains 2001

Bull trout fry, originating from those adults that entered the jump pool from Lake Simtustus and were spawned at Round Butte Hatchery in the fall of 2000, were exposed to three strains of IHN virus and different concentrations of those viruses. Rainbow trout fry of hatchery origin (Cape Cod stock 72.01) were similarly exposed to IHN virus challenges. In order to determine the relative virulence of the IHNV isolates found above and below the Project, these fish stocks were challenged with three IHN virus isolates. These virulence challenges provide information on the relative differences in susceptibilities of these fish stocks to various IHNV strains.

Bull Trout, Lake Simtustus Stock

Mortality occurred in some groups of bull trout from each virus strain tested, however IHN virus was detected only in the two fish that died in the RB 1 challenge group at the lowest dose (Dilution of virus stock; 10^{-2}). Uninfected fish were exposed to 10 to 50 times the highest dose of virus (1 and 5 X 10^{6} virus), and no mortality occurred. After 30 days the remaining fish from each challenge virus group (about 200 fish) were pooled, and 60 of those fish were sampled for virus examination. No virus was detected in these fish that had been exposed to virus. Surviving fish were tested in 10 fish groups monthly for eight months with no virus detected in any fish.

Rainbow Trout, Hatchery Stock (Cape Cod 72.01)

Rainbow trout fry of hatchery origin (Cape Cod stock 72.01) were similarly exposed to IHN virus challenges. This is the first case in which RB 97 was not the most virulent of the strains based on the LD_{50} values. From those values, the RB 1 strain is the most virulent by about 3.5 times that of RB97 and 19 times that of ME 96. It is likely that this stock of fish is so sensitive to IHN virus infection that clear differences in virulence among strains is not evident.

Comparing the results of the hatchery rainbow trout to those with steelhead trout and wild Metolius rainbow trout, it is noted that the hatchery trout are the most sensitive to IHN virus RB1 (Engelking 2002). The hatchery rainbow trout are intermediate in resistance to the ME 96 IHN virus strain; similar to that of hatchery summer steelhead trout. Wild rainbow and wild steelhead trout are more resistant to this strain. Again the hatchery rainbow trout are very susceptible to the RB 97 strain of IHN virus, with hatchery steelhead and wild rainbow trout being the most resistant species. These results suggest that wild free-ranging fish are not significantly more resistant to IHN virus than hatchery fish.

Challenge Experiments with IHNV Strains 2002

One challenge experiment was performed this year. Bull trout fry, originating from those adults that entered the jump pool from Lake Simtustus and were spawned at Round Butte Hatchery in the fall of 2001, were exposed to one strain of IHN virus at different concentrations. This virulence challenge provided information on the susceptibility of bull trout to a highly virulent IHNV strain. The fish in this experiment were challenged with the Rangen Idaho strain of IHN

virus. This is a standard challenge virus being used in vaccine development trials. This IHN virus strain killed 50% of the rainbow trout at an exposure of 2×10^2 per milliliter. Prior years' studies suggested that bull trout were essentially resistant to infection from IHN virus. The Rangen IHN virus strain had recently been used to infect and kill rainbow trout and was passed once on cells after being recovered from a dead fish. In this bull trout experiment, 12 fish in duplicate tanks were exposed to each concentration of the virus strain. All mortality was collected daily and tested for the presence of virus. The stock virus was 5×10^7 virus per milliliter. Of 216 fish exposed to the virus, 10 died from which IHN virus was recovered (Table 6).

The results of this experiment revealed that only smaller fish became infected and died with IHN virus infections. There were only ten dead fish. Many of these small fish died without signs of infection. No dose response was indicated by the trials. At a dose of 1,000 times the LD_{50} for rainbow trout, only 8% of the bull trout died. An additional 50 bull trout fry were immersed in the Rangen strain of IHN virus at a concentration of 2.5×10^6 virus per milliliter. None of these fish died. The survivors of the immersion challenges were sampled monthly for six months in groups of twenty live fish. These exams revealed no evidence of virus. All fish that died during this six-month period were also tested and showed no signs of virus infection.

Three 110-gram bull trout were injected with 5×10^7 or 2.5×10^7 virus. After 13 days one fish died and IHN virus was recovered. The remaining fish showed no signs of disease and were sampled two months later. No virus was detected in these fish. It is suggested that bull trout are highly resistant to IHN virus infection unless weak or stressed. This species can become infected experimentally with high doses of IHN virus and carry the virus for periods of time.

Additional challenges experiments for this project will occur if there is a shift in the virus strains present. No more challenges have been planned at this time.

Table 6. Results of the 2002 challenge of bull trout. Percent IHNV specific mortality of Metolius bull trout challenged with one strain of IHNV representing a virulent isolate from Idaho.

Challenge IHN Virus Dose ($TCID_{50}$ per ml)	IHN Virus Specific Mortality (%) [a] RA Rangen Idaho Strain
1 X 10^6	2
7.5 X 10^5	4
5.0 X 10^5	1
2.5 X 10^5	3
5.0 X 10^4	0
2.5 X 10^4	0

a. Two groups of 18 fish each (total 36 fish) were challenged at each virus dose. A total of 252 bull trout fry were used in the experiment. Two groups of control fish were maintained.

Discussion of IHNV Challenges

Results of challenge studies indicated that the IHN virus strain found in returning adult steelhead and chinook (Type 2 IHN) is more virulent against all the fish species tested than the IHN virus found in the kokanee population in Lake Billy Chinook (Type 1 IHN). The results also indicated that there were differences in the susceptibility of different fish species to IHN virus. Wild (unmarked) steelhead are among the most sensitive species to the Type 2 IHN virus strain, followed by rainbow trout and kokanee. Chinook appear to be the most resistant anadromous species. This may account for the single loss event of juvenile chinook in twenty-five years at Round Butte hatchery to IHN virus (Table 7, 8, and 9). The results of the various experiments were condensed into susceptibility rankings of the fish stocks (Tables 7 and 8). The virulence comparison of the three strains of IHN virus is determined from the lethal dose information (Table 9).

Susceptibility to IHN Virus

To determine relative susceptibility to an IHNV strain the LD_{50} values (Table 9) were compared. The groups compared were the anadromous stocks, including the above Project kokanee, and the bull and rainbow trout (Tables 7 and 8). From their susceptibility each stock was given a ranking from 1 to 12, where 1 is the most susceptible to that strain of virus and 12 the most resistant fish stock to the virus (Table 7). Three groups were created based on their relative susceptibility ranking to the IHN virus strains. The groups were high, intermediate and low susceptibility. Bull Trout were put in a separate category of very low, because they are refractory to infection with IHN virus. From these rankings (Table 7) an average of the two rankings for susceptibility to IHN virus RB 97 (Type 2, below Project strain) and to IHN virus ME 96 (Type 1, above Project strain) was determined (Table 8). Using these average rankings the various fish stocks were grouped into the three susceptibility groups with bull trout falling into the fourth very low group.

The highly susceptible fish stocks as determined from the average rankings are: unmarked (wild) steelhead trout, hatchery rainbow trout, and wild rainbow trout from Crane Prairie and the Crooked River. The fish stocks that were intermediate in sensitivity to the two virus strains are: the Metolius kokanee and rainbow trout, lower Crooked River rainbow trout, and hatchery steelhead trout. The fish stocks that were low in susceptibility to the two IHN virus strains are: the unmarked and hatchery spring chinook salmon (Table 8).

Table 7. Relative Susceptibility of test fish to three strains of IHN Virus. (The larger the rank number the more resistant the species is to the IHN Virus.)

Fish Species and Stock	IHN Virus Strain		
	RB 97 Below Project	**ME 96 Above Project**	**RB 1 Comparator**
	SUSCEPTIBILITY RANK	**SUCEPTIBILITY RANK**	**SUSCEPTIBILITY RANK**
HIGH		HIGH	
Steelhead Unmarked (Wild 1999)	1	3	7
Steelhead Unmarked (Wild 2000)	2	2	4
Rainbow Trout Crooked River Bowman Dam	2	7	5
Rainbow Trout Crane Prairie	3	1	3
Rainbow Trout Hatchery 72 Stock	4	6	1
INTERMEDIATE		INTERMEDIATE	
Kokanee Metolius River	5	10	8
Rainbow Trout Crooked River Opal Springs	6	9	6
Rainbow Trout Metolius River	7	8	2
Steelhead RB Hatchery	8	4	9
LOW		LOW	
Chinook RB Hatchery	9	5	4
Chinook Unmarked Wild	10	11	10
		VERY LOW	
Bull Trout Metolius River	11	12	11

Table 8. Relative Susceptibility of test fish to the two strains of IHN Virus found in the Deschutes River basin. (The larger the rank number the more resistant the species is to the IHN Virus. The combined susceptibility rank is the average of the two other rankings.)

Fish Species and Stock	IHN Virus Strain		
	RB 97 Below Project	ME 96 Above Project	COMBINED
	SUSCEPTIBILITY RANK	SUCEPTIBILITY RANK	SUSCEPTIBILITY RANK
HIGH		HIGH	
Steelhead Unmarked (Wild 1999)	1	3	2
Steelhead Unmarked (Wild 2000)	2	2	2
Rainbow Trout Crooked River Bowman Dam	2	7	4.5
Rainbow Trout Crane Prairie	3	1	2
Rainbow Trout Hatchery 72 Stock	4	6	5
INTERMEDIATE		INTERMEDIATE	
Kokanee Metolius River	5	10	7.5
Rainbow Trout Crooked River Opal Springs	6	9	7.5
Rainbow Trout Metolius River	7	8	7.5
Steelhead RB Hatchery	8	4	6
LOW		LOW	
Chinook RB Hatchery [1]	9	5	8.5 (7)
Chinook Unmarked Wild	10	11	10.5
NOT SUSCEPTIBLE		NOT SUSCEPTIBLE	
Bull Trout Metolius	11	12	11.5

1. Chinook RB Hatchery data based on an experiment which did not give LD_{50} information. Therefore three ranking points were given the ME 96 data to account for the difference.

The prototypic RB1 Type 1 IHN virus strain was less virulent to all species other than the other two IHN virus strains tested. Bull trout were observed to be essentially resistant to all strains of IHN Virus. A few weak small bull trout fry did die and virus was isolated from them. In order to kill bull trout, 10,000 times more virus is needed than is required to kill the more susceptible fish species. The virus was isolated from the exposed fish that died. Directly injecting bull trout juveniles with massive amounts of IHN virus does not consistently cause infection. Bull trout may be latent carriers of the virus for short periods of time. However, it is unlikely that the IHN virus amplifies itself greatly in this species.

Comparisons of the LD_{50} values show the relative virulence of an IHNV strain to groups or species of fish (Table 9). Such comparisons can now be made for 15 fish of different species or origins. The virulence comparisons of the anadromous fish and kokanee salmon revealed that the IHN virus strain, RB 97 is the most virulent to all species tested, except to hatchery stock rainbow trout. The IHN virus strain from above the Project, ME 96, shows similar virulence in hatchery stocks as the RB 97 strain. However, the unmarked steelhead required 87-fold more IHNV ME 96 and the kokanee salmon required about nine times more of this strain of virus to attain equivalent mortality to the RB 97 strain. In all cases the prototypic IHN virus strain, RB1, was the weakest in virulence to all fish stocks tested with one exception to hatchery rainbow trout (Table 9). These results reflect the virulence that has been seen at Round Butte Hatchery to summer steelhead from IHNV infections from the Type 1 strain of IHNV found in the kokanee salmon. It is somewhat surprising that the historic RB1 isolate, which apparently was responsible for the steelhead losses in the 1970s, is no longer very virulent compared to more recent isolates. This may reflect loss of virulence with continued replication in the laboratory, although the virus is from laboratory infected fish. Previous studies though, have noted that the type 1 isolates such as RB1 are more virulent for kokanee salmon than rainbow and steelhead trout.

In virulence comparisons of the rainbow trout stocks tested, the RB 97 strain was shown to be the most virulent, except in the test of hatchery stock rainbow trout. When compared to the ME 96 IHN virus strain found above the Project, the RB 97 strain required about three to tenfold less virus to achieve the same level of mortality in all other fish stocks. As with the anadromous fish, the RB 1 prototypic IHN virus strain was the least virulent, except again when tested against hatchery rainbow trout. It required three- to 100-fold more virus to achieve a lethal dose to 50 percent of the fish (Table 9). LaPatra and coworkers (1993) had found that Type 2 IHN virus such as the RB 97 strain is the most virulent for rainbow trout and steelhead species. These results suggest that there may be some increased mortality among the resident and anadromous

fish above the Project, if the RB 97 strain of IHN virus is introduced, displaces the Type 1 strain and becomes established.

Table 9. Relative Susceptibility of test fish to three strains of IHN Virus. (The larger the Lethal Dose 50 required the more resistant the fish species is to the IHN Virus strain.)

Fish Species and Stock	IHN Virus Strain		
	RB 97 Below Project	**ME 96 Above Project**	**RB 1 Comparator**
		Lethal Dose 50 Virus Titers	
Steelhead RB Hatchery	8.4×10^2	8.8×10^2	3.7×10^4
Steelhead Unmarked (Wild 1999)	1×10^2	8.7×10^2	1×10^4
Steelhead Unmarked (Wild 2000)	2.8×10^2	8.6×10^2	7×10^3
Chinook RB Hatchery	$< 1 \times 10^3$	$<1 \times 10^3$	7×10^3
Chinook Unmarked Wild	2.3×10^3	5×10^3	7.2×10^4
Kokanee Metolius River	5×10^2	4.4×10^3	8.7×10^3
Rainbow Trout Hatchery 72 Stock	3.5×10^2	1.9×10^3	1.0×10^2
Rainbow Trout Crane Prairie	3.2×10^2	8.2×10^2	2.8×10^3
Rainbow Trout Crooked River Bowman Dam	2.8×10^2	2.4×10^3	7.2×10^3
Rainbow Trout Crooked River Opal Springs	5.6×10^2	2.7×10^3	8.5×10^3
Rainbow Trout Metolius River	8.3×10^2	2.5×10^3	2.7×10^3
Bull Trout Metolius River	$> 10^6$	$> 10^6$	$> 10^6$

Experiments to Determine Virus (IHN Virus) Loads in the Metolius River

A tangential flow apparatus to concentrate virus from solutions was tested and used since 1999. No sampling was performed this year because we had been unable to isolate virus since April of 2000, in spite of most of the kokanee being infected with IHN virus. All of the kokanee sampled in 2002 were infected with IHN virus. A summary of previous findings follows.

The Metolius River water was sampled in September 15, 1999, at a time of year when kokanee begin their spawning migration and early spawning. This water was filtered in order to detect any virus being released from spawning kokanee into the river. No virus was detected in the Metolius River water or the kokanee from this sample early in the spawning season (Engelking 2000). The Metolius River was water sampled eight times from October through November in 1999 and was processed to concentrate virus being released from spawning and dying kokanee into the river. Virus was detected in the Metolius River water and the kokanee. The variations we observed in the lab tests indicate that the quantization is good to about one order of magnitude (Engelking 2000).

Virus at low levels was apparently present at all Metolius River sites from January 2000 until April 2000 (Engelking 2001). It was not possible to confirm the first isolation at the Spring Creek hatchbox. Isolation was confirmed at the other two sites; however, quantization was not possible because of contamination of the cells. No virus was detected in the May sample.

The tangential flow apparatus to concentrate virus from solutions processed samples of water from Metolius River water in 2001. Samples of water were taken on three occasions during the winter and spring of 2001and immediately following kokanee spawning in the fall 2001 (Table 10). Any virus being released from carcasses of kokanee or other unknown sources into the river may be detected. The first sample of kokanee spawning adults revealed IHN virus infection in 19% of the fish (Table 11).

Virus was not detected in the Metolius River water in 2001. The samples came from near a kokanee spawning side channel of the Metolius River north of Camp Sherman. This result is similar to what was found in 2000. IHN virus was found in water samples 1999, although that year the number of IHN virus infected kokanee was less than in 2000 or 2001. Possible ways to account for these results are that the level of virus is very low in the water or something interferes with detection or inhibits infectivity of the virus. Sediment samples were also taken to

attempt to recover virus that may be absorbed to particles of sediment. A cytopathic effect was noted from some of these samples but virus could not be recovered.

Table 10. Detection of IHN Virus in Water from the Metolius River in 2001.

DATE	Primary [1]	Retentate	Backflush	Filtrate	IHNV in Fish
January 16	NEV	NEV	NEV	NEV	No Fish
March 15	NEV	NEV	NEV	NEV	No Fish
April 24	NEV	NEV	NEV	NEV	No Fish

1. Primary sample = a direct sample of the river water tested for virus without any attempt to concentrate the virus. Fungus or other contaminants filtered to reduce contamination from bacteria/fungus before being put on cells.
Retentate = the sample obtained after about a 100 fold concentration of virus into this sample from the river water. Filtered prior to dilution and inoculation on cells.
Backflush = any virus that might adsorb to the concentration filter is washed off by backflushing the system with a balanced salt solution. Filtered prior to dilution and inoculation on cells.
Filtrate = the water that has passed through the concentration filters and should not contain any virus. Filtered prior to dilution and inoculation on cells.

Table 11. Kokanee adults from the Metolius River found to be infected with IHN Virus during and after spawning in October and November 2001 and October 2002.

Sample Date	Ovarian Fluid	Milt	Tissues PKS	Percent IHNV
October 4	7 of 17	2 of 30	7 of 12	19% (26 Females and 34 Males)
October 24	Not Done	Not Done	3 of 3	100% (2 Females and 1 male)
November 8	Not Done	Not Done	0 of 5	NEV (3 females and 2 Males)
Totals	7 of 17	2 of 30	10 of 17	20%
October 9 2002	10 of 10	40 of 40	12 of 12	100% (46 Males and 14 Females)

Pathogenic Fish Parasites

Ceratomyxa shasta (Ceratomyxosis) Survey

Ceratomyxa shasta was identified in adult and juvenile hatchery and stray adult hatchery spring chinook salmon and adult hatchery summer steelhead trout at Round Butte Hatchery in 2002. In 1998, 20 percent of the rainbow trout fry that were examined from the Metolius River were found to carry *C. shasta* spores. Spores of *C. shasta* were found in two rainbow (redband) trout from the lower Deschutes River but not in sockeye salmon from Pelton Trap (Table 12). In 2002 lower intestinal samples from 391 fish were visually examined for *C. shasta.* The results correspond closely to the previous years' results. Very few infected fish are found above the Project. No infected fish of the 37 examined had detectable spores from above the Project. The adult spring chinook salmon and summer steelhead trout are the most commonly infected fish (Table 12). Nine percent of the fish examined in 2002 from below the Project had spores in the intestine.

Table 12. *Ceratomyxa shasta* a summary of detections above and below the Pelton Round Butte Project in 1997, 1998, 1999, 2000 and 2001.

Number Fish Sampled	Number Samples Tested	*C. shasta* Negative	*C. shasta* Positive
1997			
925Total	678	528	150
%	73%	78%	22%
562 Below PRB	505	357	148
%	90%	71%	29%
363 Above PRB	173	171	2
%	48%	99%	1%
1998			
1,143 Total	992	811	181
100%	87%	82%	18%
818 Below PRB	675	513	162
72%	83%	76%	24%
325 Above PRB	317	298	19
28%	94%	94%	6%

Table 12, Continued.

Number Fish Sampled	Number Samples Tested	*C. shasta* Negative	*C. shasta* Positive
1999			
704 Total	589	505	84
100%	84%	86%	14%
568 Below PRB	495	409	84
81%	87%	83%	17%
136 Above PRB	96	96	0
19%	71%	100%	0%
2000			
1968 Total	1010	946	64
100%	51 %	94 %	6 %
1247 Below PRB	428	365	63
63 %	34%	85 %	15 %
721 Above PRB	582	581	1
37 %	81 %	99.8 %	0.2%
2001			
1622 Total	273	217	56
100%	17%	80%	20%
1446 Below PRB	185	129	56
89 %	13%	70%	30%
176 Above PRB	88	88	0
11%	50%	100%	0%
2002			
1397 Total	391	360	31
100%	28 %	92%	8%
1294 Below PRB	354	323	31
93%	90%	91%	9%
103 Above PRB	37	37	0
7%	10%	100%	0%

Myxobolus cerebralis (Whirling Disease) Survey Results

We conducted examinations for *Myxobolus cerebralis* with more than 6,100 individual fish samples. After the samples were digested with enzymes, they were microscopically examined twice for the presence of spores that resemble those of *M. cerebralis*. Of these samples, more than 4,000 were reexamined. A second examination of all enzyme digest samples was performed to verify the presence or absence of spores. No evidence of the parasite was found in more than 5900 samples. We detected spores of *M. cerebralis* (the causative agent of whirling disease) only in anadromous fish found below the Project (Tables 13 and 14). These results were confirmed by microscopic examination of histology sections, which revealed the presence of lesions and spores in cartilage from the head. None of the resident fish examined above or below the Project had detectable spores of *M. cerebralis* (Appendix I). Through microscopic examination of enzyme digestion samples and the observation and measurement of spores (Table 13) we identified 142 presumptive *M. cerebralis* infections. Of 103 presumptive infections tested, 27 have been confirmed by histology exams in which lesions in cartilage and spores were observed. Polymerase chain reaction assays have corroborated 82 of 125 presumptive infections tested. This method has recently been validated as a diagnostic method by The AFS Fish Health Section.

Diagnostic tests for *M. cerebralis* by the enzyme digest procedure were completed on 790 samples in 2002. Evidence of spores of this parasite were found in out-of-basin hatchery summer steelhead trout. The samples have not been examined by histology to confirm these results (Table 13). A total of seven presumptively infected fish were found this year. None of the seven presumptive infections tested have yet been confirmed by histology exams in which lesions in cartilage and spores were observed. Five were confirmed by PCR assays. Tissues from more than 1,000 other fish processed in 2002 by the enzyme digestion procedure for *M. cerebralis* spores revealed no evidence of presumptive spores (Appendix I).

No unmarked summer steelhead were examined in the last three years (2000-2002), because of the ESA listing of this group of fish. Analysis of hatchery stray steelhead trout in 2002 indicated seven presumptive infections. Examination by histology has not been completed. Five of the fish were confirmed to be infected by PCR analysis.

One of the 57, unmarked spring chinook salmon sampled in 1997 was presumptively infected with *M. cerebralis*. This infection was also confirmed by histology. In 1998, four unmarked spring chinook salmon of 14 examined had presumptive *M. cerebralis* infections. Histology did not confirm the presence of *M. cerebralis*. Interestingly, none of the chinook salmon sampled in

1999 were detected with *M. cerebralis* spores. A number of other *Myxobolus* species spores and *Henneguya* spores were observed. In 2000 one unmarked fish was presumptively infected and also positive by PCR analysis for *M. cerebralis.* Three hatchery stray chinook were presumptively infected, but these were not so identified by histology or PCR analysis.

Nine adult hatchery spring chinook salmon from Round Butte Hatchery in 2001 had spores resembling those of *M. cerebralis.* None of the presumptively positive adult fish showed external signs of disease. These fish were negative by both PCR analysis and histological tests. In 2002 no adult hatchery chinook were presumptively infected with spores. There is still no indication that fish of Deschutes River origin become infected with the parasite during migration.

Most groups of fish we examined showed no signs of spores (Appendix I). Unmarked sockeye salmon, kokanee, rainbow and bull trout and mountain whitefish tested from both above and below the Project tested negative for the presence of *M. cerebralis* spores. Other myxosporean spores were noted in some stocks. The detection of other spores gives assurance that the procedures are being performed correctly and produce results that will allow the detection of *M. cerebralis* spores if present.

The identity of the unmarked *M. cerebralis* positive fish is uncertain because of the high rate of anadromous fish straying in the Deschutes watershed. It is possible that the positive unmarked fish are not wild Deschutes River fish, but stray wild or stray hatchery fish from the upper Columbia or Snake rivers. This area has been endemic for *M. cerebralis* since at least 1986. To date, no Deschutes River resident salmonids have been found to be infected.

Table 13. Summary of the detection of *Myxobolus cerebralis* by four procedures.

Methodology for Detection of *M. cerebralis*	Number of Detections Per Number Tested
Enzyme Digest Spores resembling *M. cerebralis* in size and morphology from enzyme digested head samples	142 Presumptive Infections of 6055 examinations
Histology Spores detected in enzyme digest material and lesions with spores resembling *M. cerebralis* seen in cartilage of head samples by histology	27 Confirmed Infections of 120 Presumptive infections
PCR Spores detected by enzyme digest and *M. cerebralis* DNA detected by PCR	82 PCR Detections of 120 Presumptive Infections
Detections of *M. cerebralis* by all three above methodologies of the same sample	17 by all methods of 68 Presumptive Infections
in situ Hybridization Methodology - A DNA complex (hybrid) is visualized by staining or radioisotopes.	1 Detection of 1 Presumptive Infection

a. Only those samples that were presumptively infected were tested by other methods. A number of samples with no evidence of spores were tested by PCR as negative controls for the assay.

In summary, the presence of *M. cerebralis* spores in three of the four species of fish intended for movement about the Project and the detection of the alternate host, *Tubifex tubifex*, in this area are cause for concern. The worm host has been detected in all three rivers above the Project (Bartholomew 1999). The presence of *T. tubifex* has been confirmed in the Metolius River at four locations, in the Crooked River at three sites, and from one location in the Deschutes River above the Project. Current Oregon Department of Fish and Wildlife rules do not permit the transfer of *M. cerebralis* infected fish to an area that is not known to have the parasite. The benefits of reintroduction must be weighed against the potential jeopardy to native resident stocks, which also include ESA listed bull trout populations. As more knowledge is gained about this pathogen, a better assessment of the degree of risk will be possible. Continued survey work, especially in the watershed below the Project, is necessary when reintroduction begins, as is the monitoring of unmarked and straying summer steelhead and spring chinook returning to the Pelton Trap. There are important questions to be resolved about the potential for establishment of the parasite in the Deschutes River basin.

Table 14. Adult anadromous fish found to be infected with Myxobolus cerebralis.

Fish Species Stock	Years	Number Tested	Number Presumptive *M. cerebralis* Infected	Number Confirmed *M. cerebralis* Infected
Summer Steelhead Trout				
Unmarked [1]	1998-2001	50	5	1
Hatchery Stray	1997-2002	582	109	27
Hatchery [2]	1997-2002	1130	5	2
Spring Chinook Salmon				
Unmarked	1997-2001	114	6	1
Hatchery Stray	1998 - 2001	77	6	0
Hatchery	1997 - 2002	980	15	0
Sockeye Salmon				
Unmarked	1997 - 2002	99	0	0
Hatchery Stray	1997 - 2002	4	1	1
Totals		3036	147	32

1. Unmarked fish are of unknown origin; samples were taken for genetic analysis. The fish could be wild Deschutes River fish, wild stray fish, or unmarked hatchery or hatchery stray fish.
2. The hatchery steelhead that were confirmed positive for the *M. cerebralis* parasite may have been out of basin fish misidentified as Round Butte Hatchery fish.

Discussion of Whirling Disease

There is no evidence that fish become infected with *M. cerebralis* in the lower Deschutes River, although there have been more than 14 years of exposure. No Deschutes basin hatchery or native fish have been confirmed to be infected with the parasite. Differences in habitat above and below the Project are apparent. The lower Deschutes basin tributaries have seasonally varying flows, low gradients and widely varying temperatures. Most of the spawning fish are rapidly removed from spawning areas. In the upper Deschutes basin, the Metolius River is characterized as pristine with tributaries of low water temperatures, constant flows, spring or

snow melt origins, and higher gradients. The upper Deschutes River has constant temperatures in cool canyons and is fed mainly by springs. The gradients are moderate. The Crooked River is degraded and impacted by grazing, blocked by diversions, widely varying temperatures and very warm in the summer. Much of the gradient is very low. These habitat differences may affect the ability of *M. cerebralis* to establish itself in the areas where reintroduction occurs. These variations may lead to local areas in which the fish populations are negatively impacted from the parasite. Few signs of infection are noted on resident fish that have been infected in northeastern Oregon. Fish with some structural deformities have been noted in the upper Columbia and lower Snake rivers (Sandell et al. 2001). The various habitats allow differences in the intensity of infection in the fish and at which life stage the fish are infected.

When and where fish spawn and where early rearing occurs determines in part the intensity and duration of exposure to the parasite. Although salmonids may become infected at any life stage, the greatest susceptibility and damage from infection occurs early in their life history (Downing et al. 2002). Clinical disease and often death occurs in juvenile fish because the parasite destroys the cartilage, which is less present in older fish (Hedrick et al. 1998). Extensive losses in juvenile fish may severely impact the population. Rainbow trout in the Madison River, Montana, have experienced a population collapse from infection with *M. cerebralis.* The severity of infection and loss in this system is attributable to time and space of emergence of young fish. Many of the fish emerge at a time and place where high levels of infectious triactinomyxons are also being released into the water. However some fish rear in areas of low exposure within this system and show correspondingly lower signs of disease and infection levels. Life history difference is postulated to account for some of the differences in effects from the parasite seen in other locations such as northeastern Oregon. No dramatic losses of salmonid populations have occurred in Oregon, although the parasite is present and infects fish (Holt 1996).

This scenario suggests that maintaining or managing for a diversity of life histories among salmonid populations in a watershed and providing areas of low infection risk for spawning and early rearing could reduce or prevent significant population losses from exposure to *M. cerebralis.* However establishment of the parasite in an area may cause localized losses in those vulnerable areas and to those highly susceptible life stages.

SUMMARY

The fish pathogen issues are complex with the Pelton Round Butte Hydroelectric Project. These issues are the result of the Project, the blockage of fish passage to historic spawning and rearing areas, the biology of the fish pathogens, and other alterations of the environment that have affected the fish and the pathogens. The Fish Pathology Section's role is to address those issues that have developed as a result of these conditions. PGE and the Tribes have supported and funded experimental and survey studies to develop the information necessary to make informed decisions in regards to fish health and development of sustainable naturally reproducing anadromous fish populations.

Bacterial kidney disease (BKD), furunculosis, and EIBS represent diseases that may have serious impacts on certain groups of resident and anadromous fish stocks. Although BKD is found throughout the Project area an increase in fish populations may exacerbate the disease's negative impacts on populations. Furunculosis may also become a problem as reintroduction proceeds. Finally EIBS virus, which is specific for chinook and coho salmon, may negatively impact chinook reproduction in the cool waters of the Metolius River. Cooler waters enhance this pathogens' ability to produce disease.

The fish pathogens that have emerged as the most consequential and have the most significant potential to negatively impact fish stocks are the Type 2 strain of IHN virus and *M. cerebralis*. A series of experiments, designed to determine the virulence of the Type 2 strain of IHN virus found below the Project, indicates this virus strain is more virulent than the Type 1 strain found above the Project. It is most probable that reintroduction will transfer this pathogen above the Project. Information also suggests that this strain may then displace the current strain of IHN virus. Evaluations are underway to determine how this more virulent strain of IHN virus may impact both resident and anadromous stocks. Options to reduce or eliminate the virus transferred above the Project by testing and selecting uninfected fish or vaccination procedures may be possible in the future. Removal of some spawned-out carcasses may reduce exposure to emerging fry and reduce potential juvenile losses. However, in the short term it is unlikely that any methods to prevent the reintroduction of fish infected with IHN virus will be available. A continued monitoring of the level of IHN virus infection of stocks in the Project area will be a necessary part of evaluation of the outcomes and impacts of reintroduction.

Whirling disease is the most significant fish pathogen threat to wild trout populations. The transfer of this parasite above the Project may have serious deleterious effects on native resident

stocks. Current information, however, notes a range of effects from the presence of the parasite in a watershed, from benign to catastrophic (Nickum 1999). There are no certain explanations for these vastly different scenarios to have occurred from the introduction of this pathogen into other watersheds. In Northeastern Oregon, *M. cerebralis* has been found since the mid-1980s. Resident trout, anadromous steelhead and spring chinook salmon have been found infected with *M. cerebralis* in this area. Impacts to resident trout and anadromous fish populations have been reported to be minimal, although this is based on limited surveys. Fish with some bone loss and distortion and deformities do exist in these populations (Holt 1996). Similar observations have been reported from New York (Schachte and Hulbert 1998). Although these fish exhibit only minor signs of disease, they remain inapparent carriers of the parasite. Subtle differences in behavior or performance of these fish, that are not easily observed or documented from the infection, may reduce survival or reproductive potential. In California severe losses have occurred in both hatchery and natural populations of trout. Elimination of the sources of infection have reduced the level of infection to below detectable levels in certain areas of California (Modin 1998). Other states have suffered continued extreme losses to natural populations of trout. Both Montana and Colorado lost prized sport fisheries in the 1990s from whirling disease, without any signs of recovery (Vincent and Byroth 1999, Schisler et al. 1997).

The potential options for whirling disease control are: prevention (exclusion of the pathogen from the watershed), control (reducing the disease effects to acceptable levels), and eradication (elimination of the pathogen, or reducing its reproductive success to below levels of detection). Eradication of *M. cerebralis* has not been attained elsewhere. The ability to detect the pathogen in all hosts and to interrupt transmission of *M. cerebralis* does not exist (Stephens 1999). Thus, prevention and control methods must currently be relied on to manage the disease. Effective methods must be put in place that will reduce or prevent the disease without unacceptable ecological or economic consequences. Studies to identify ecological, management or population factors that influence the impacts of *M. cerebralis* are underway in the Deschutes watershed and elsewhere. Risk factors may be identified by these studies and used to manage the pathogen.

Exclusion of the parasite to the best of our ability appears to be a prudent course of action, until the potential for successful emigration and reproduction of anadromous stocks is demonstrated. The management of this pathogen will then require a resolution of the benefits of fish passage to the potential risks and losses from whirling disease. Continued management of whirling disease requires a union of many approaches and jurisdictions in a common goal to maintain and protect a watershed and its salmonid fish inhabitants. Exposure and establishment of this pathogen in this basin would require extreme management measures to attempt to control and minimize its impacts.

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APPENDIX I

Summary Of Fish Pathogen Surveys 2002

(Values indicate numbers positive for the pathogen/ numbers tested)

Fish Source & Type	Sample # & Sex	EIBS Virus	IHN Virus	*M. cerebralis*	ELISA BKD	Bacteria	Parasites
Round Butte Hatchery Summer Run Steelhead Trout HATCHERY	301 Total 147 Males 154 Females	Not Done	46 IHNV positive	0 of 150	Low 2 Medium 1 High 2 Negative 52	CWD 10 A. sal 2 APS 6 Negative 24	*C. shasta* 25 *M. insidiosus* 15 *M. spp* 25
Pelton Trap Hatchery STRAYS Summer run Steelhead	30 Total (Heads only)	Not Done	Not Done	7 infected ND Histol. 5 PCR +	Not Done	Not Done	Not Done
Pelton Trap Rainbow trout Lower Deschutes	16Total 8 Male 8 Female	Not Done	NEV	1 of 16 PCR Negative	Low 3 Med 8 Negative 1	None Detected	*C. shasta* 1 *M. insidiosus* 1 *M. spp* 3 *Henneguya 1*
Pelton Trap Kokanee	5 Total 5 Male	Not Done	NEV	NSS	Negative 4 High 1	A. sal 1 APS 1	No *C. shasta*
Round Butte Hatchery spring chinook Smolts	95 Total Smolts	Not Done	NEV	NSS	Low 19 Medium 3 High 16 Negative 18	Not done	Not Done
Round Butte Hatchery StS smolts	95 Total Smolts	Not Done	NEV	NSS	Not Done	Not Done	Not Done
Lower Deschutes Maupin Rainbow Trout	20 Total 8 Female 12 Male	Not Done	NEV	0 of 20	Low 9 Med 2 High 0 Negative 9	Not Done	*C. shasta* 1 of 19 *M. spp*. 5 *M..insidiosus* 1
Lower Deschutes Maupin Mtn. Whitefish	40 Total 38 Female 12 Male	Not Done	NEV	0 of 40	Low 21 Med 16 High 2 Negative 1	Not Done	*C. shasta* 0/40 *Henneguys* 7
Lower Deschutes Fall Chinook	60 Total (Heads)	Not Done	45 IHNV + 18 NEV	Not Complete	Not Done	Not Done	Not Done
Lower Deschutes Fall Chinook	30Total (Heads) from 2001	Not Done	Not Done	Not Complete	NSS	Not Done	Not Done
Lake Billy Chinook BUT	5 Total 4 Female 1 Male	Not Done	NEV	NSS	Low 1 High 3 Negative 1	3 APS 2 Negative	No *C. shasta*
Lake Billy Chinook KK	32 Total 16Female 16 Male	Not Done	NEV	NSS	Not completed	Not Done	No *C. shasta*

Fish Source & Type	Sample # & Sex	EIBS Virus	IHN Virus	*M. cerebralis*	ELISA BKD	Bacteria	Parasites
Sockeye Salmon SS Pelton Trap UNMARKED	5 Total 2 Females 3 Males	Not Done	NEV	0 of 5	Low 1 High 2 Negative 2	CWD 1	No*C. shasta*
Trout Creek StS	78 Total smolts 22 Males 16 Females 40 unknown	Not Done	NEV	2 of 78 PCR negative	Low 37 Med 11 High 2 Negative 22	Not Done	*M. spp.*2 No *C. shasta*

Summary Of Fish Pathogen Surveys From 2002 (Continued).

(Values indicate numbers positive for the pathogen/ numbers tested)

Fish Source & Type	Sample # & Sex	EIBS Virus	IHN Virus	*M. cerebralis*	ELISA BKD	Bacteria	Parasites
Round Butte Hatchery Spring Chinook HATCHERY	492Total 194 Males 298 Females	4 positive 90 negative	312 IHNV positive	0 of 145	Low 43 Med 21 High 66 Negative 367	*A.sal.*5 *CWD* 12 *APS* 3 *Carno* 3 Negative 17	*C. shasta* 3 *M. spp.* 3 *Henneguya* 19
Round Butte Hatchery Spring Chinook STRAYS	9Total 7 Female 2 Male	2 Negative	5 IHNV positive	0 of 9	High2 Med 2 Negative 5	Not Done	*C. shasta* 1 *M. insidiosus* 1
Round Butte Hatchery Spring Chinook UNKNOWN	13 Total 7 Male 6 Female	2 Negative	7 IHNV positive	0 of 13	High2 Med 1 Low 1 Negative 5	APS 1	*Henneguya* 1
Metolius River Kokanee	60 Total 14 Female 46 Male	Not Done	60 IHNV positive	0 of 60	Low 5 Med 1 High 4 Negative 50	Not Done	*Henneguya* 20 *M.spp* 5
Paulina Lake Kokanee	60 Total	Not Done	NEV	NC	Not Done	Not Done	Not Done
Crescent Lake MWF	5 Total	Not Done	NEV	NC	Not Done	Not Done	Not Done
Hosmer Lake Atlantic Salmon	60 Total	Not Done	NEV	NC	Not Done	Not Done	Not Done
Odell Lake Kokanee	120 Total	Not Done	NEV	NC	Not Done	Not Done	Not Done
Lake Simtustus Kokanee	28 Total 28 Males 0 Females	Not Done	NEV	NSS	Not Completed	Not Done	No Parasites detected

Table abbreviations: Fish: StS-Summer Run Steelhead Trout, Rb-Rainbow Trout, BT - Brook Trout MW-Mountain Whitefish, SS-Sockeye Salmon, KK-Kokanee Salmon, BUT-Bull Trout, BR-Brown Trout, and ChS-Spring run Chinook Salmon

Fish Source RB-Round Butte Hatchery Virus: NEV-No Evidence of Virus Bacteria:CWD-Cold Water Disease, *A. sal-Aeromonas salmonicida,* Carno-*Carnobacterium piscicola,* and APS-Aeromonas/Pseudomonas

General: NC-Not Completed, IP - In Progress and ND-Not Done

Summary Of Fish Pathogen Surveys 2001

(Values indicate numbers positive for the pathogen/ numbers tested)

Fish Source & Type	Sample # & Sex	EIBS Virus	IHN Virus	*M. cerebralis*	ELISA BKD	Bacteria	Parasites
Round Butte Hatchery Summer Run Steelhead Trout HATCHERY	268 Total 129 Males 139 Females	Not Done	69 IHNV positive	0 of 150	Low 1 Negative 59	CWD 3 *A. sal* 3 Negative 24	*C. shasta* 21 *M. insidiosus* 5 *M. spp* 16
Round Butte Hatchery STRAYS Summer Run Steelhead Trout	5Total 4 Males 1 Females	Not Done	NEV	0 of 5	Not Done	Not Done	Not Done
Pelton Trap Hatchery STRAYS Summer run Steelhead	64 Total (Heads only)	Not Done	Not Done	7 infected 2 Histol. 11 PCR	Not Done	Not Done	Not Done
Round Butte Hatchery StS fry	225 Total Fry	Not Done	18 of 27 pools of 5 fish IHNV positive	NSS	Not Done	Not Done	Not Done
Pelton Trap Hatchery Rainbow trout	22 Total 11 Male 11 Female	Not Done	NEV	NSS	Low 3 Med 8 Negative 1	Not Done	No *C. shasta*
Pelton Trap Kokanee	19 Total 16 Male 3 Female	Not Done	NEV	NSS	Negative 19	Not Done	No *C. shasta*
Round Butte Hatchery spring chinook Smolts	150 Total Smolts	Not Done	NEV	NSS		Not done	No Done
Round Butte Hatchery StS smolts	180 Total Smolts	Not Done	NEV	NSS	Not Done	Not Done	Not Done
Lower Deschutes Maupin Rainbow Trout	25 Total 12 Female 13 Male	Not Done	NEV	0 of 25	Low 14 Med 9 High 1 Negative 1	None Detected	*C. shasta* 0/25 *M. spp.* 5 *Henneguya* 1 *M..insidiosus* 1
Lower Deschutes Maupin Mtn. Whitefish	55 Total 38 Female 17 Male	Not Done	NEV	0 of55	Low 5 Med 28 High 21 Negative 1	1 APS	*C. shasta* 0/60 *Henneguys* 25
Lower Deschutes Fall Chinook	77 Total (Heads)	Not Done	Not Done	0 of 77	Not Done	Not Done	Not Done
Mill Creek RB (Tributary of Crooked River)	7 Total Juvenile	Not Done	NEV	NSS	Low 6 Med 1	Not Done	No *C.shasta*
Lake Billy Chinook Kokanee	69 Total 28 Female 32 Male 2juvenile 7 unknown	Not Done	14 IHNV positive	NSS	Low 18 Med 3 High 8 Negative 43	13 CWD 4 APS 3 Negative	No *C. shasta*

Summary Of Fish Pathogen Surveys From 2001 (Continued).

(Values indicate numbers positive for the pathogen/ numbers tested)

Fish Source & Type	Sample # & Sex	EIBS Virus	IHN Virus	*M. cerebralis*	ELISA BKD	Bacteria	Parasites
Sockeye Salmon SS Pelton Trap UNMARKED	27 Total 16 Females 11 Males	Not Done	NEV	0 of 27	Med 3 High 5 Negative 17	None	No*C. shasta* *M. spp.* 1 *M. insisdiosus* 1
Round Butte Hatchery Spring Chinook HATCHERY	440 Total 194 Males 246 Females	8 positive 92 negative	257 IHNV positive	9 digest positive 136 neg PCR neg	Low 38 Med 13 High 28 Negative 359	CWD 31 APS 12 Carno 1 Negative 2	*C. shasta* 35 *M. spp.* 8 *Henneguya* 35
Round Butte Hatchery Spring Chinook STRAYS	22 Total 18 Female 4 Male	None Detected	16 IHNV positive	0 of 18	Low 3 High 4 Negative 15	1 CWD	*C. shasta* 1 *M. spp.* 1 *Henneguya* 5
Round Butte Hatchery Spring Chinook UNKNOWN	7 Total 3 Male 4 Female	None Detected	4 IHNV positive	0 of 7	High1 Med 1 Negative 5	Not Done	*C. shasta* 1
Round Butte Hatchery Spring Chinook LOST TAG	6 Total 3 Females 3 Males	None Detected	4 IHNV positive	0 of 6	High 1 Low 1 Negative 4	Not Done	*Henneguya* 1
Lake Billy Chinook ChS	1 juvenile Total	Not Done	Not Done	Not Done	Low 1	Not Done	*C. shasta* None detected
Lake Billy Chinook Bull Trout	10 Total 2 Males 8 Females	Not Done	NEV	NSS	Low 2 Med 4 High 2 Negative 2	Not Done	*C. shasta* None detected
Metolius River Kokanee	68 Total 31 Female 37 Male	Not Done	40 IHNV positive	NSS	Low 4 Med 2 High 6 Negative 47	Not Done	No *C. shasta*
Lake Simtustus Round Butte Hatchery Bull Trout	4 Total 1 Female 3 Male	Not Done	NEV	NSS	Negative 4	Negative 4	No *C. shasta*
Lake Simtustus Bull Trout	7 Total 5 Males 2 Females	Not Done	NEV	NSS	Low 1 Negative 6	Not Done	*C. shasta* Not Detected
Lake Simtustus Kokanee	30 Total 17 Males 13 Females	Not Done	NEV	NSS	Low 3 High 2 Negative 23	None Detected	*C. shasta* Not Detected
Crane Prairie Kokanee	5 Total 3 Male 2 Female	Not Done	NEV	NSS	Low 3 Negative 2	None Detected	No *C. shasta*
Crane Prairie Rainbow Trout	7 Total 4 Female 3 Male	Not done	NEV	NSS	Low 4 Negative 2	None Detected	No *C. shasta* *Henneguya* 3
Crane Prairie Brook Trout	9 Total 4 Female 3 Male 2 unknown	Not Done	NEV	NSS	Low 6 Negative 3	None Detected	No *C. shasta* *Henneguya* 3
Crane Prairie Chub	1 Female	Not Done	Not Done	Not Done	Not Done	Not Done	Not Done

Table abbreviations: Fish: StS-Summer Run Steelhead Trout, Rb-Rainbow Trout, BT - Brook Trout
MW-Mountain Whitefish, SS-Sockeye Salmon, KK-Kokanee Salmon, BUT-Bull Trout,

BR-Brown Trout, and ChS-Spring run Chinook Salmon

Fish Source RB-Round Butte Hatchery Virus: NEV-No Evidence of Virus Bacteria:CWD-Cold Water Disease, *A. sal-Aeromonas salmonicida,* Carno-*Carnobacterium piscicola,* and APS-Aeromonas/Pseudomonas

General: NC-Not Completed, IP - In Progress and ND-Not Done

Summary Of Fish Pathogen Surveys 2000

(Values indicate numbers positive for the pathogen/ numbers tested)

Fish Source & Type	Sample # & Sex	EIBS Virus	IHN Virus	*M. cerebralis*	ELISA BKD	Bacteria	Parasites
Round Butte Hatchery Summer Run Steelhead Trout 1/00 - 3/00	225 Total 108 Males 117 Females	Not Done	14 of 321	0 of 151	Low 3 Med 0 High 1 Negtive 57	CWD 17 *A. sal* 1 Carno 2 APS 2 Negative 27	*C. shasta* 14 Negative 53
Pelton Trap Hatchery Stray-Summer run Steelhead	Total 77	Not Done	Not Done	23 of 77	Not Done	Not Done	Not Done
Round Butte Hatchery spring ChS Smolts	60 smolts	0 of 12	NEVof 60	0 of 60	Low 17 Negative 43	Not Done	No Done
Round butte Hatchery StS Smolts	96 smolts	Not Done	NEV of 96	0 of 93	Not done	Not Done	Not Done
Rb Deschutes Maupin	60 Total 36 Females 24 Males	Not Done	NEV of 60	0 of 60	Low 26 Med 24 High 9 Negative 1	Not Done	*C. shasta* 0/60 *M. spp.* 24 *Henneguya 3* and *M..insidiosus* 10
MW Deschutes Maupin	60 Total 38 Females 22 Male	Not Done	NEV	0 of 60	Low 14 Med 33 High 13	Not Done	*C. shasta* 0/60 *Henneguys* 25
Trout Creek juvenile sucker	1 juvenile	Not Done	NEV	Not done	Not Done	Not Done	Black spot from *Neascus spp.*
Trout Creek Hatchery Stray StS	10 Total 7 Male 3 Female	Not Done	NEV of 10	0 of 10	Low 6 Med 1 High 2 Negative 1	Not Done	*C. shasta* 9 of 10.
Trout Creek Wild StS juveniles (unmarked)	4Total	Not Done	NEV	0 of 4	Low 2 Med 0 High 1	Not Done	No *C.shasta*
Trout Creek Wild StS (unmarked)	39 Total 4 Males 20 Females	Not Done	10 IHNV positive	0 of 39	Low 5 Med 3 High 7 Negative 24	34 APS 9 CWD 4 Negative	No *C. shasta*
Lake Billy Chinook Kokanee	39 Total 16 Males 20 Females 3 juveniles	Not Done	10 IHNV positive	0 of 39	Low 5 Med 3 High 7 Negative 24	34 APS 9 CWD 4 Negative	No *C. shasta*
Lake Billy Chinook Skimmer Kokanee	10 Total Juveniles	Not Done	5 IHNV positive	0 of 10	Med 1 High 1 Negative 8	2 CWD 6 APS 2 Negative	No *C. shasta*

Summary Of Fish Pathogen Surveys From 2000 (Continued).

(Values indicate numbers positive for the pathogen/ numbers tested)

Fish Source & Type	Sample # & Sex	EIBS Virus	IHN Virus	*M. cerebralis*	ELISA BKD	Bacteria	Parasites
Pelton Trap Sockeye Salmon Hatchery strays 10/2000	4 Total 2 Female 2 Male	Not Done	NEV of 4	1 Confirm by Histol	Low 2 Negative 2	Negative	No *C. shasta*
Pelton Trap Sockeye Salmon Wild (unmarked) 10/2000	12Total 7 Females 5 Males	Nott Done	NEV of 12	NSS	Low 2 Med 0 High 1 Negative 9	Carno 1 APS 4	*C. shasta* 0/12
Round Butte Hatchery Spring Chinook (ChS RB) 8/00-9/2000	491 Total 288 Females 203 Males	4/87	38/491	0 of 221	Low 46 Med 9 High 10 Negative 387	CWD 7 *A. sal* 5 Carno 1 APS 1	*C. shasta* 17/59 *M. sp*p. 7 *H.spp.* 14
Round Butte Hatchery ChS Hatchery Strays	35 Total 20 Female 15 Male	0 of 7	6 IHNV positive	3 infected	Low 2 Med 0 High 0 Negative 29	CWD 1 *A. sal* 1 APS 2 Negative 4	*C. shasta* 3 *M. sp*p. 4 *H.spp.* 1
Round Butte Hatchery ChS unknown	55 Total 31 Female 24Male	2	3IHNV positive	0 of 12	Low 3 Med 20 High 1 Negative 30	*A. sal* 1 APS 1 Carno 1	*C. shasta* 1
Round Butte Hatchery ChS Lost Tags	6 Total 3Female 3 Male	Not Done	NEV	NSS of 6	Low 0 Med 0 High 1 Negative 3	Not Done4	*C. shasta* None Detected
Lake Billy Chinook Bull Trout	5 Total 2 Female 3 Male	Not Done	NEV	NSS	Low 0 Med 0 High 1 Negative 3	Not Done	No *C. shasta*
Metolius River Kokanee	69 Total 26 Females 43 Males	Not Done	62 IHNV positive	0 of 60	Low 7 Med 0 High 1 Negative 19	CWD 19 8 APS	*C. shasta* None detected
Metolius River Kokanee fry	40 Fry	Not Done	NEV	Not Done	Not Done	Not Done	Not Done
Rainbow Trout Lake Simtustus 9/2000	3 Total 2 Female 1 Male	Not Done	NEV	NSS	Low 1 Negative 2	Not Done	*C. shasta* none
Bull Trout Lake Simtustus 7/00-10/2000	21Total 14 Females 7 Males	Not Done	NEV/21	NSS 0/21	Low 7 Med 0 High 0 Negative 14		*C. shasta* 0/21
Kokanee Lake Simtustus 9/00-10/2000	60 Total 33 Females 27 Males	Not Done	NEV/60	0 of 60	Low 4 Med 0 High 3 Negative 53	None Detected in16	No *C. shasta* of 55 sampled
Crane Prairie Kokanee	2 Total 1 Female 1 Male	Not Done	NEV	NSS	Low 1 Negative 1	Not Done	*C.shasta* None detected
Crane Prairie Rainbow Trout	22 Total 11 Female 11 Male	Not Done	NEV	0 of 22	Low 2 High 1 Negative 8	Not Done	No *C. shasta* 18*Henneguya*

Fish Source & Type	Sample # & Sex	EIBS Virus	IHN Virus	*M. cerebralis*	ELISA BKD	Bacteria	Parasites
Crane Prairie Brook Trout	6 Total 3 Female 3 Male	Not Done	NEV	0 of 6	Low 1 Negative 2	Not Done	No *C. shasta* 6 *Henneguya*
Fish Source & Type	**Sample # & Sex**	**EIBS Virus**	**IHN Virus**	***M. cerebralis***	**ELISA BKD**	**Bacteria**	**Parasites**
Metolius River ChS fry	158 Total Fry	Not Done	NEV	Not Done	Not Done	Not Done	Not Done
Jack Creek ChS Fry	60 Fry	Not Done	NEV	Not Done	Not Done	Not Done	Not Done
Crooked River Rainbow Trout	39 Total 26 Female 13 Male	Not Done	NEV	NSS	Low 9 Med 2 Negative 20	Not Done	No *C. shasta* 31*Henneguya* 11 *M. spp.*
Crooked River Mt. Whitefish	60 Total 26 Female 34 Male	Not Done	NEV	0 of 60	Low 40 Med 12 High 5 Negative 2	12 Negative	No *C. shasta* 35 *Henneguya*
Deshutes River (upper) RB Mile Camp	35 Total	Not Done	NEV	0 of 35	Low 4 Negative 1	Not Done	No *C. shasta* 20*Henneguya*
Deshutes River (upper) BT Mile Camp	35 Total	Not Done	NEV	0 of 35	Low 2 Negative 1	Not Done	No *C. shasta* 30*Henneguya*
Paulina Lake Kokanee	60 Total	Not Done	NEV	NSS	Low 2 Negative 57	Not Done	Not Done

Table abbreviations:

Fish:	StS-Summer Run Steelhead Trout, Rb-Rainbow Trout, BT - Brook Trout MW-Mountain Whitefish, SS-Sockeye Salmon, KK-Kokanee Salmon, BUT-Bull Trout, BR-Brown Trout, and ChS-Spring run Chinook Salmon
Fish Source	RB-Round Butte Hatchery
Virus:	NEV-No Evidence of Virus
Bacteria:	CWD-Cold Water Disease, *A. sal-Aeromonas salmonicida,* Carno-*Carnobacterium piscicola,* and APS-Aeromonas/Pseudomonas
General:	NC-Not Completed, IP - In Progress and ND-Not Done

Summary Of Fish Pathogen Surveys 1999

(Values indicate numbers positive for the pathogen/ numbers tested)

Fish Source & Type	Sample # & Sex	EIBS Virus	IHN Virus	M. cerebralis	ELISA BKD	Bacteria	Parasites
Round Butte Hatchery Summer Run Steelhead Trout 1/99 - 3/99 StS RB	174 Total 75 Males 99 Females	Not Done	19/155	0/170	Low 13 Med 1 High 0 Not Detected 145/148	CWD 14 *A. sal* 5 Carno 1 APS 10	*C. shasta* 43/111
Round Butte Unmarked Summer Run Steelhead Trout 2/99 to 3/99 Wild-StS	21Total 10 Females 11 Male	Not Done	1 male of 21	4 of 21	Low 2 Med 0 High 0 Negative 10	CWD 1 APS 2 *A. sal.* 1 *Y. ruckerii* 1	*Myxobolus spp*. 8/21 *C. shasta* 13/21
Pelton Trap Hatchery Stray-Summer run Steelhead StS RB 11/98-5/99	116 Total Females Males	Not Done	NEV	22 of 116	Low 2 Med 0 High 0 Negative 83	Not Done	*C. shasta* 7/46
Round Butte Hatchery spring chinook smolts 4/99	150 smolts	NEV	NEV	0 of 150	Negative 60 of 60	Not Done	No Done
Round butte Hatchery StS smolts 4/99	150 smolts	NEV	NEV	0 of 93	Not done	Not Done	Not Done
Rb Deschutes Maupin 3/99	60Total	Not Done	NEV	0 of 60	Low 26 Med 26 High 2 Negative 5	Not Done	*C. shasta* 5/60 *M. insidiosus* 1/5 *M. spp.* and *M..insidiosus* 1/5
MW Deschutes Maupin 3/99	60 Total	Not Done	NEV	0 of 60	Low 11 Med 35 High 13	Not Done	*C. shasta* 0/60
Trout Creek StS hatchery strays 4/99	5 adults	Not Done	Not Done	2 of 5	Low 0 Med 1 High 0 Negative 3	Not Done	*C. shasta* Negative 3 of 3
Trout Creek StS wild smolts 4/99	83 smolts	NEV of 30 fish	Not Done	0 of 83	Low 15 Med 21 High 6 Negative 9	Not Done	*C. shasta* Negative 83 of 83
Squaw Creek Rb 5/99	7 adults	Not Done	Not Done	0 of 7	Low 1 Med 5 High 1 Negative 0	Not Done	*C. shasta* Negative 7 of 7

Summary Of Fish Pathogen Surveys From 1999 (Continued).

(Values indicate numbers positive for the pathogen/ numbers tested)

Fish Source & Type	Sample # & Sex	EIBS Virus	IHN Virus	M. cerebralis	ELISA BKD	Bacteria	Parasites
Sockeye Salmon SS Pelton Trap 10/99	7Total 2 Females 5 Males	0/5	NEV/5	NSS	Low 1 Med 0 High 1 Negative 3	Carno 1 APS 1	*C. shasta* 0/7
Round Butte Hatchery Spring Chinook (ChS RB) 8/99-9/99	400 Total 266 Females 128 Males 6 Jacks	3/83	30/401	0 of 161	Low 5 Med 0 High 1 Negative 325	CWD 9 *A. sal* 0 Carno 0 APS 1	*C. shasta* 12/56
Unmarked Spring Chinook (ChS Wild) 8/99-9/99	20 Total 9 Females 11 Males	0/19	4/20	0 of 20 by digest	Negative 20	CWD 3 APS 1	*C. shasta* 2/20 *M. spp*. 1/20 *H.spp.* 1/20
Lake Billy Chinook Bull Trout 2/99-12/99	6 Total 2 Male 2 Females 2 Unknown	Not Done	NEV of 3	0 of 6	Low 2 Med 1 High 0 Negative 3	NOt Done	No *C. shasta*
KK Metolius River 9/99 - 12/99	134 Total 21 Females 113 Males	Not Done	80 of 134	0 of 60	Low 4 Med 3 High 2 Negative 53	CWD 1/1	*C. shasta* 0/60
Metolius River Bull Trout 3/99-12/99	23 total 1 Female 22 Unknown	Not Done	1 of 1	0 of 23	Low 0 Med 0 High 4 Negative 8	Not Done	No *C. shasta*
Rainbow Trout Lake Simtustus 9/99	1 Males	Not Done	NEV	NSS	Negative 1	None Detected	No *C. shasta*
Pelton Trap Summer Steelhead StS 10/99-12/99	70 Total	Not Done	Not Done	13/70	Not Done	Not Done	Not Done
Bull Trout Lake Simtustus 7/99-10/99	21Total 11 Females 8 Males 2 Juvenile	Not Done	NEV/18	0/21	Low 4 Med 1 High 0 Negative 16		*C. shasta* 0/21
Kokanee Lake Simtustus 9/99-10/99	88 Total 26 Females 62 Males	Not Done	NEV/88	0 of 88	Low 1 Med 0 High 3 Negative 84	None Detected	No *C. shasta*
Metolius River Rainbow Trout Various dates 1998	60 adults 59 unknown 1 female	Not Done	Not Done	0 of 60	Low 30 Med 11 High 1 Negative 10		*M. spp*. 4/60 *H.spp.* 1/60
Deschutes River Rainbow Trout Various dates 1998	45 Total 3 Females 42 unknown	Not Done	Not Done	0 of 44	Low 26 Med 4 High 0 Negative 11	Not Done	*M. spp*. 20/44 *H.spp.* 5/44 both 5/44

Table abbreviations:

Fish: StS-Summer Run Steelhead Trout, Rb-Rainbow Trout, MW-Mountain Whitefish, SS-Sockeye Salmon, KK-Kokanee Salmon, BUT-Bull Trout, BR-Brown Trout, and ChS-Spring run Chinook Salmon

Fish Source RB-Round Butte Hatchery

Virus:	NEV-No Evidence of Virus
Bacteria:	CWD-Cold Water Disease, *A. sal-Aeromonas salmonicida,* Carno-*Carnobacterium piscicola,* and APS-Aeromonas/Pseudomonas
General:	NC-Not Completed, IP - In Progress and ND-Not Done

Summary Of Fish Pathogen Surveys From 1998.

(Values indicate numbers positive for the pathogen/ numbers tested)

Fish Source & Type	Sample # & Sex	EIBS Virus	IHN Virus	M. cerebralis	ELISA BKD	Bacteria	Parasites
Round Butte Hatchery Summer Run Steelhead Trout 1/98 -2/98 StS RB	187 Total 78 Males 109 Females	0/156	46/186	0/32	Low 11 Med 0 High 2 Not Detected 143/156	CWD 12 *A. sal* 0 Carno 16 APS 7	*C. shasta* 59/156 *Myxobolus spp.* 8/32 *M. insidiosus* like 4/32
Round Butte Unmarked Summer Run Steelhead Trout 2/98 to 3/98 Wild-StS	26 Total 9 Females 17 Male	0/10	9/26	1 of 13 by digest and histology	Low 2 Med 0 High 0 Negative 10	CWD >? APS	*Myxobolus spp*. 5/13 *M. insidiosus* 1/13 *C. shasta* 2/7
Pelton Trap Hatchery Stray-Summer run Steelhead StS RB 11-10-97 to 4-2-98	151 Total 81 Females 70 Males	0/143 61 ND	2/140 IHNV 11 ND	32/150 by digest	Low 9 Med 0 High 0 Negative 161	CWD 5 APS 7 Carno 1	*Myxobolus spp.* 29/150 *M. insidiosus* 2/150 *C. shasta* 29/149 *Henneguya* 7/150
Kokanee Salmon KK 4/98 Lake Billy Chinook	50 Total 13 juveniles 19 males 18 females	ND	NEV/50	0 of 50	Low 14 Med 0 High 0 Negative 35	APS 9/46	*C. shasta* 0/50
Bull Trout BUT Lake Billy Chinook	1 Total	ND	NEV	0 of 1	Low 1 Med 0 High 1	*R. salmoninarum* clinical infection in one fish	*C. shasta* 0/2
Rb Deschutes Maupin 3-24-98	59 Total 32 Females 24 Male 5 Juvenile	0/49	NEV/59	0/59 *M. spp* 19/59 *H. spp*. 2/59 *M. insid*. 7/59	Low 29 Med 23 High 6	APS 2 Carno 1	*C. shasta* 0/51 Hexamita 3/10 Myxidium 1/10 Trematodes 2/10 Crepidostomum 3/10 Gyrodactylus 2/10 copepods 2/10 Nematodes 1/10
MW Deschutes Maupin 3-24-98	60 Total 50 Female 6 Male 4 Juvenile	0/50	NEV/60	0 of 59	Low 6 Med 20 High 22	APS 4 Carno 3	*C. shasta* 0/45
Rb Crooked River 6/98 Bowman Dam	60 Total 29 Females 21 Male 8 Juvenile 2 unknown	0/60	NEV/60	0 of 53	Low 30 Med 1 High 1 Negative 10	APS 4 CWD Carno	*C. shasta* 0/52
MW Crooked River 6/98 Bowman Dam	51 Total 33 Females 15 Males 3 Juveniles	0/51	NEV/51	0 of 58	Low 19 Med 0 High 0 Negative 30	APS 2	*C. shasta* 0 of 51

Summary Of Fish pathogen Surveys From 1998 (Continued).

(Values indicate number positive for pathogen/ numbers tested)

Fish Source & Type	Sample # & Sex	EIBS Virus	IHN Virus	M. cerebralis	ELISA BKD	Bacteria	Parasites
Sockeye Salmon SS Pelton Trap 9/98	5 Total 2 Females 3 Males	0/3	NEV/5	*H. spp.* 1/5	Low 1 Med 0 High 1 Negative 3	None Detected	2/3 nematodes *C. shasta* 0/5
Round Butte Hatchery Spring Chinook (ChS RB) 8/98-9/98	255 Total 132 Females 91 Males 32 Jacks	15/255	20/254	0 of 161	Low 9 Med 1 High 1 Not Detected 232	CWD 92 *A. sal* 3 Carno 10 APS 6	*C. shasta* 64/255
Unmarked Spring Chinook (ChS Wild) 8/98-9/98	14 Total 8 Females 6 Males	1/14	3/14	4 /14 by digest	Negative 14	CWD 2	*C. shasta* 3/14
Round Butte Stray Hatchery Spring Chinook (ChS Stray unknown) 8/98-9/98	42 Total 29 Females 11 Males 2 Jacks	4/40	3/24	3/24	Low 1 Med 0 High 3 Negative 38	CWD 4 *A. sal* 2 Carno 1	*C. shasta* 9/41 *M. sp*p. 1/24 *H.spp.* 1/24
KK Metolius River 9/98	60 Total 19 Females 40 Males	0/30	8/60	0 of 60	Low 4 Med 3 High 1 Negative 52	CWD 38/60 APS 6/60	*C. shasta* 0/60
KK Link Cr. 9/98	20 Total Females Males	Not Done	3/20	0 of 20	Low 0 Med 0 High 0 Negative 20	CWD 8/20	*C. shasta* 0/20
KK Paulina Lake 9/98	60 Total 60 Females	Not Done	NEV/60	Not Done	Negative by DFAT	Not Done	Not Done
Pelton Trap Summer Steelhead StS 10/98	73 Total 43 Females 30 Males	Not Done	NEV/49	3/25 48 NC	Negative 73/73	Carno 1/49	*C. shasta* 62/62
Opal Springs Rainbow Trout Crooked River 7/98	60 Total 24 Females 35 Males 1 Juvenile	0/59	NEV/60	NC	Low 31 Med 3 High 1 Negative 23	APS 2 CWD 1 Carno 1 Unknown 1	*Chloromyxum* 1/5 *Hexamita* 1/5 *Cestodes* 1/5 Copepods 3/5 *C. shasta* 18/60
Little Summit Creek Loss investigation	34 rainbow trout 10 speckled dace	Not Done	NEV/34	NC	Not Done	*Icthyophthirius* Ich loss High Temps.	*C. shasta* 1/22
Metolius River Rainbow Trout	10 adults 2 male 8 female	Not Done	NEV/8	0 of 8	Not Completed	APS	Not Completed

Table abbreviations:

Fish: StS-Summer Run Steelhead Trout, Rb-Rainbow Trout, MW-Mountain Whitefish, SS-Sockeye Salmon, KK-Kokanee Salmon, BUT-Bull Trout, BR-Brown Trout, and ChS-Spring run Chinook Salmon

Fish Source RB-Round Butte Hatchery

Virus: NEV-No Evidence of Virus

Bacteria: CWD-Cold Water Disease, *A. sal-Aeromonas salmonicida,* Carno-*Carnobacterium piscicola,* and APS-Aeromonas/Pseudomonas

General: NC-Not Completed, IP - In Progress and ND-Not Done
Numbers indicated are the numbers positive over numbers tested.

Summary Of Fish Pathogen Surveys From 1997.

(Values indicate numbers positive for the pathogen/ numbers tested)

Fish Source & Type	Sample # & Sex	EIBS Virus	IHN Virus	M. cerebralis	ELISA BKD	Bacteria	Parasites
Round Butte Hatchery-Summer run Steelhead StS RB 1/97	152 Total 74 Males 78 Females	Not Done	59/183	0/152 47/152 *M. spp.* 9/152 *M. insid.*	Low 43/152	CWD 51/152 *A. sal* 4/152 Carno 4/152 APS 18/152	Copepods 6/10 Nematodes 3/10 Fungus 7/10 Trypanorhyncid 2/10 *C. shasta 5/10* Myxidium 1/10 Syphidia 1/10
Round Butte Unmarked Summer run Steelhead Wild-StS RB 1/97	21 Total 15 Females 6 Male	Not Done	NEV/21	0/17 3/17 *M.spp.*	Low 7/16 Med 1/16	CWD 4/7 APS 1/7	Copepods 3/5 Fungus 3/5 Nematodes 3/5 *C. shasta* 2/5 Myxidium 1/5 Trematode 1/5
Pelton Trap Hatchery Stray-Summer run Steelhead StS RB 1/97	63 Total 26 Females 37 Males	0/30	3/63	0/63 5/63 *M. spp.*	Low 34/62	CWD 3/26 APS 2/26 Carno 1/26	Copepods 7/9 Fungus 4/9 Nematodes 4/9 Myxidium 2/9 *C. shasta* 2/9
Pelton Trap Stray- StS RB 4/97	4 Total	ND	NEV/4	0/4	Low 1/4	ND Too old to culture	*C. shasta* 1/4
KK LBC 1/97	23 Total Not Determined	ND	NEV/23	ND	Low 4/23 Negative 19	ND	ND
KK LBC 4/97	54 Total Not Determined	ND	NEV/54	0/54	Low 39/54 Med 1/54	ND	NC
BUT LBC 4/97	6 Total Not Determined	ND	NEV/6	0/6	Low 2/6 Med 2/6 High 2/6	ND	ND
Rb Abbot Cr. 4/97	8 Total Not Determined	ND	NEV/8	0/8 *M. spp.* 3/8; *H.* 1/8	ND	APS 1/8	*Ambiphyra* 8/8 *Gyrodactylus* 8/8 *C. shasta* 0/8 *Crepidostomum* 2/8
BUT Abbot Cr. 4/97	1 Total Not Determined	ND	NEV/1	0/1	ND	APS 1/1	*Ambiphrya* 1/1 *Gyrodactylus* 1/1 *C. shasta* 0/1
Rb Deschutes River 5/97	4 Total 3 Females 1 Male	ND	NEV/4	0/2	Low 3/4 Med 1/4	APS 1/4	*C. shasta* 0/4
MW Deschutes 5/97	1 Total 1 Female	ND	NEV/1	0/1	Med 1/1	APS 1/1	ND
BR Metolius 5/97	13 Total 8 Females 5 Males	ND	NEV/13	0/13	Low 5/13 Med 7/13 High 1/13	No Bacteria Isolated	Chilodonella 4/13 *Gyrodactylus* 2/13 Myxozoan 1/13 Plerocercoid 1/13
Rb Crooked River 6/97	52 Total 24 Females 28 Male	0/52	NEV/52	0/52 *H.* 38/52 *M. spp.* 6/52	Low 31/52 Med 21/52	APS 6/52 CWD 2/52 Carno 1/52	Copepods 18/52 *Unicauda* 3/52 *C. shasta* 0/52
MW Crooked River 6/97	10 Total 7 Females 3 Males	ND	NEV/10	0/10 *M. spp.* 2/10	Low 9/10 Med 1/10	APS 2/10	*C. shasta* 1/10 unconfirmed 4/10 *Henneguya*

Fish Source & Type	Sample # & Sex	EIBS Virus	IHN Virus	M. cerebralis	ELISA BKD	Bacteria	Parasites
Rb Squaw Cr. 6/97	6 Total Not Determined	ND	NEV/6	0/6	Low 3/6 Med 2/6 High 1/6	APS 1/6	*C. shasta* 0/6

Summary Of Fish pathogen Surveys From 1997 (Continued)

(Values indicate number positive for pathogen/ numbers tested)

Fish Source & Type	Sample # & Sex	EIBS Virus	IHN Virus	M. cerebralis	ELISA BKD	Bacteria	Parasites
SS Pelton Trap 8/97	37 Total 15 Females 22 Males	0/37	NEV/37	0/37	Low 11/37 Med 2/37 High 4/37	APS 9/37 CWD 9/37 *A. sal* 2/37	Copepods 2/10 Trematode 3/10 Nematode 7/10 Cestode 7/10 Fungus 2/10
Round Butte Hatchery-Spring Chinook ChS RB 8/97	150 Total 75 Females 75 Males	3/150	89/150	0/161 *M.spp.* 3/158	Low 97/381 Med 26/381 High 39/381	APS 32/150 CWD 45/150 Carno 1/150 *A. sal* 11/150	*C. shasta* 64/150 Copepods 9/10 Myxidium 1/10 Henneguya 1/161
Unmarked - ChS RB 8/97	55 Total 29 Females 26 Males	5/55	18/55	1/55 *M. spp* 2/58	Low 11/54 Med 5/54 High 11/54	APS 8/45 CWD 13/45 Carno 6/45 *A. sal* 6/45	*C. shasta* 24/55 Copepods 9/10 Myxidium 1/10 Trematodes 2/10
Round Butte Hatchery Prespawn Mortality-ChS RB 8/97	8 Total	ND	ND	ND	ND	ND	*C. shasta* 5/8
BUT Metolius River 9/97	20 Total 8 Females 12 Males	0/20	NEV/20	0/20	Low 7/20 Med 7/20 High 5/20	APS 3/20 CWD 2/20 Carno 1/20	Chloromyxum 7/10 Trematode 2/10 Myxidium 2/10 *C. shasta* 0/20
KK Metolius River 9/97	60 Total 33 Females 27 Males	0/60	24/60	0/59 *H. spp.* 4/12 five fish pools	Low 7/60	CWD 53/60 APS 11/60 Carno 2/60	Chloromyxum 8/10 Fungus 2/10 Costia 1/10 Unknown Protozoan 1/10 Myxobolus 1/10 Copepods 7/10 *C. shasta* 2/60
KK Link Cr. 9/97	23 Total 10 Females 13 Males	ND	1/23	0/23	Low 1/23	CWD 7/23 APS 5/23 Carno 1/23	*C. shasta* 0/23
KK Paulina Lake 9/97	60 Total 60 Females	ND	NEV/60	0/60	Low 41/60 Med 1/60	ND	ND
Pelton Trap Summer Steelhead StS 11 and 12/97	54 34 Females 20 Males	0/54	NEV/54	12/54	Low 11/54	CWD 3/53 APS 4/53	Copepods 4/5 Nematodes 5/5 Fungus 2/5 *C. shasta* 4/54
Rb Metolius River 12/97	14 6 Females 5 Male 3 Unknown	ND	NEV/11	0/8 *M. spp.* 4/8 *H.* 1/8	Low 7/11 Negative 4/11	APS 2/7	Copepods 1/7 Nematodes 1/7 Fungus 1/7 *Crepidostoma* 4/7 *C. shasta* 0/7

Table abbreviations:

Fish: StS-Summer Run Steelhead Trout, Rb-Rainbow Trout, MW-Mountain Whitefish, SS-Sockeye Salmon, KK-Kokanee Salmon, BUT-Bull Trout, BR-Brown Trout, and ChS-Spring run Chinook Salmon

Fish Source: RB-Round Butte Hatchery

Virus: NEV-No Evidence of Virus

Bacteria: CWD-Cold Water Disease, *A. sal-Aeromonas salmonicida,* Carno-*Carnobacterium piscicola,* and APS-Aeromonas/Pseudomonas

General: NC-Not Completed, IP - In Progress and ND-Not Done
Numbers indicated are the numbers positive over numbers tested.

Summary of ELISA Bacterial Kidney Disease Results for 2002

Fish Location	Number Sampled	Number Tested	BKD Negative	BKD LOW	BKD MEDIUM	BKD HIGH
Summer Steelhead Round Butte H.						
Hatchery	301	60	55	2	1	2
Smolts	95	0	0	0	0	0
Rainbow Trout Maupin (Lower Deschutes)	20	20	9	9	2	0
Mountain Whitefish Maupin (Lower Deschutes)	40	40	1	21	16	2
Bull Trout Lake Billy Chinook	5	5	1	1	0	3
Rainbow Trout Pelton Trap	16	16	1	13	1	1
Kokanee Pelton trap	5	5	4	0	0	1
Kokanee Metolius River	60	60	50	5	1	4
Kokanee Lake Billy Chinook	34	0	0	0	0	0
Kokanee Lake Simtustus	28	0	0	0	0	0
Spring Chinook Hatchery	497	497	367	43	21	66
Unknown	13	13	9	1	1	2
Stray	9	9	5	0	2	2
Sockeye Salmon Lower Deschutes	5	5	2	1	0	2
Spring Chinook Smolts Hatchery	56	55	17	19	3	16
Totals	1068	785	521	115	48	99

Summary of ELISA Bacterial Kidney Disease Results for 2001

Fish Location	Number Sampled	Number Tested	BKD Negative	BKD LOW	BKD MEDIUM	BKD HIGH
Summer Steelhead Round Butte H.						
Hatchery	268	60	60	0	0	0
Smolts	180	0	0	0	0	0
Rainbow Trout Maupin (Lower Deschutes)	25	25	1	14	9	1
Mountain Whitefish Maupin (Lower Deschutes)	55	55	1	5	28	21
Bull Trout Lake Billy Chinook	10	10	2	2	4	2
Rainbow Trout Pelton Trap	22	12	1	3	8	0
Kokanee Pelton trap	19	19	19	0	0	0
Rainbow Trout Mill Creek	7	7	0	6	1	0
Rainbow Trout Crane Prairie	7	6	2	4	0	0
Brook Trout Crane Prairie	9	9	3	6	0	0
Kokanee Crane Prairie	3	3	0	3	0	0
Kokanee Metolius River	68	59	47	4	4	6
Kokanee Lake Billy Chinook	69	69	43	18	3	8
Spring Chinook Lake Billy Chinook	1 Juvenile	1	0	1	0	0
Bull Trout Lake Simtustus	7	7	6	1	0	0
Kokanee Lake Simtustus	30	30	23	3	0	2

Fish Location	Number Sampled	Number Tested	BKD Negative	BKD LOW	BKD MEDIUM	BKD HIGH
Spring Chinook Hatchery	440	440	359	38	13	28
Unknown	13	13	9	1	1	2
Stray	22	22	15	3	0	4
Sockeye Salmon Lower Deschutes	27	25	17	0	3	5
Spring Chinook Smolts Hatchery	150	119	117	0	0	2
Totals	1432	991	725	112	74	81

Summary of *Ceratomyxa shasta* detections for 2002.

Fish with spores of *Ceratomyxa* detected in the lower intestine in 2002.

Fish Location	Number Sampled	Number Tested	*C. shasta* Negative	*C. shasta* Positive
Deschutes River at Maupin Whitefish	40	40	40	0
Deschutes River at Maupin Rainbow Trout	20	19	18	1
Round Butte Hatchery ChS	497	60	44	16
Hatchery Stock ChS	9	1	0	1
Hatchery Strays ChS Untagged/Lost CWT	13	0	0	0
Round Butte Hatchery ChS smolts	53	26	23	3
Summer Steelhead Trout	302	60	35	25
Round Butte StS Smolts	60	15	15	0
Lake Billy Chinook	32	32	32	0
Kokanee	5	5	5	0
Bull Trout				
Pelton Trap Kokanee	5	5	5	0
Sockeye Salmon	5	5	5	0
Rainbow Trout	15	15	15	0
Lake Simtustus Kokanee	32	32	32	0
Metolius River Kokanee	60	60	0	0
Total	1148	375	225	31

Summary of *Ceratomyxa shasta* detections for 2001.

Fish with spores of *Ceratomyxa* detected in the lower intestine in 2001.

Fish Location	Number Sampled	Number Tested	*C. shasta* Negative	*C. shasta* Positive
Deschutes River at Maupin Whitefish	2	2	2	0
Deschutes River at Maupin Rainbow Trout	5	5	5	0
Round Butte Hatchery ChS Hatchery Stock	59	59	26	33
ChS Hatchery Strays	8	8	7	1
ChS Untagged/Lost CWT	1	1	1	0
Summer Steelhead Trout	60	60	39	21
Lake Billy Chinook ChS	1 juvenile	1	1	0
Kokanee	19	19	19	0
Bull Trout	1	1	1	0
Pelton Trap Kokanee	19	19	19	0
Sockeye Salmon	26	26	26	0
Rainbow Trout	22	22	22	0
Lake Simtustus Kokanee	30	30	30	0
Bull Trout	1	1	1	0
Metolius River Kokanee	60	60	60	0
Mill Creek Rainbow Trout	7	7	7	0
Total	321	321	266	55

Results of enzyme digest procedure and microscopic examination of head tissues from samples processed in 2002.

Sample Site **Fish Species** **Date**	Number Presumptive Positive ***M. cerebralis*** **Detected**	Number PCR Positive for ***M. cerebralis***	**Number of fish with unidentified *Myxobolus* species** **Detected**	**Number of fish with *Henneguya* Species** **Detected**	**Number of fish with *M. insidiosus*** **Detected**
Round Butte Hatchery Spring Chinook 8-27-01	13/156	0 of 12	9/156	34/156	0 of 156
Round Butte Strays Spring chinook 8-27-01 and 9-5-01	1/18	0 of 1	1/18	6/18	0 of 18
Round Butte Lost or No Tag Spring Chinook 8-27-01	0/6	Not Done	0/6	0/6	0 of 6
Pelton Trap Stray Summer Steelhead Oct./Nov 2001	6/45	10 of 45	10/45	3/45	0 of 45
Pelton Trap Sockeye Salmon October 2001	0/26	Not Done	1 of 26	0 of 26	1 of 26
L. Simtustus Jump Pool Bull Trout	0/6	Not Done	0 of 6	0 of 6	0 of 6
Crane Prairie Brook Trout	0 of 10	Not Done	0 of 10	3 of 10	0 of 10
Crane Prairie Rainbow Trout	0 of 7	Not Done	0 of 7	3 of 7	0 of 7
Crane Prairie Kokanee	0 of 2	Not Done	0 of 2	0 of 2	0 of 2
Pelton Trap Stray Kokanee 10-16-01	0 of 2	0 of 2	0 of 2	0 of 2	0 of 2 NSS
Lower Deschutes R. Fall Chinook	0 of 26	Not Done	0 of 26	7 of 26	0 of 26
Lake Simtustus Kokanee 9-5-01	NSS of f28	Not Done	0	0	0
Pelton Trap Kokanee 9-20-01	NSS of 17	Not Done	0	0	0

Sample Site **Fish Species** **Date**	Number Presumptive Positive ***M. cerebralis*** **Detected**	Number PCR Positive for ***M. cerebralis***	**Number of fish with unidentified *Myxobolus* species** **Detected**	**Number of fish with *Henneguya* Species** **Detected**	**Number of fish with *M. insidiosus*** **Detected**
Metolius River Kokanee 10-3-01	0 of 60	Not Done	1 of 60	1 of 60	0 of 60
Round Butte Hatchery Summer Steelhead 1-29-02	0 of 18	0 of 18	3 of 18	0 of 18	3 of 18
Round Butte Hatchery Summer Steelhead 1-29-02	0 of 102	0 of 4	17 of 102	0 of 102	10 of 102
Lake Billy Chinook Kokanee 2/13/02	0 of 7 pools pooled in groups of 3, 4, 5 fish	Not Done	0 of 7 pools	0 of 7 pools	0 of 7 pools
Round Butte Hatchery Summer Steelhead 2/26/02	0 of 30	0 of 2	3 of 30	0 of 30	3 of 30
Round Butte Hatchery/Pelton Ladder Spring Chinook 2/26/02	0 of 10 individual 0 of 17 pools	Not Done	0 of 10 individual 0 of 17 pools	0 of 10 individual 0 of 17 pools	0 of 10 individual 0 of 17 pools
Lake Billy Chinook Bull Trout 3/16/02	0 of 1	Not Done	0 of 1	0 of 1	0 of 1
Lake Billy Chinook Bull Trout 3/30/02	0 of 1	Not Done	0 of 1	0 of 1	0 of 1
Lake Billy Chinook Bull Trout 4/12/02	0 of 1	Not Done	0 of 1	0 of 1	0 of 1

Sample Site **Fish Species** **Date**	Number Presumptive Positive ***M. cerebralis*** **Detected**	Number PCR Positive for ***M. cerebralis***	**Number of fish with unidentified *Myxobolus* species** **Detected**	**Number of fish with *Henneguya* Species** **Detected**	**Number of fish with *M. insidiosus*** **Detected**
Lake Billy Chinook Bull Trout 4/13/02	0 of 1	Not Done	0 of 1	0 of 1	0 of 1
Lake Billy Chinook Bull Trout 5/7/02	0 of 1	Not Done	0 of 1	0 of 1	0 of 1
Pelton Trap Spring Chinook 5/20/02	0 of 2	Not Done	0 of 2	0 of 2	0 of 2
Round Butte Hatchery/Pelton Trap Sockeye 8/28/01	0 of 1	Not Done	0 of 1	0 of 1	0 of 1
Round Butte Hatchery Summer Steelhead Smolts 3/27/02	0 of 10 individual 0 of 10 pools pooled in groups of 5	Not Done	0 of 10 individual 0 of 10 pools	0 of 10 individual 0 of 10 pools	0 of 10 individual 0 of 10 pools
Maupin Rainbow Trout 4/8/02	3 of 20	2 Negative 1 Positive	5 of 20	0 of 20	2 of 20
Maupin Mountain White Fish 4/8/02	0 of 10 individual 0 of 6 pools pooled in groups of 5	Not Done	0 of 10 individual 0 of 6 pools	2 of 10 individual 1 of 6 pools	0 of 10 individual 0 of 6 pools
Pelton Trap Rainbow Trout 5/20/02	2of 16	2 Negative of 2	3 of 16	1 of 16	1 of 16
Trout Creek 4/15/02 StS smolts 13	0 of 13	Not Done	0 of 13	0 of 13	0 of 13

Sample Site **Fish Species** **Date**	Number Presumptive Positive ***M. cerebralis*** **Detected**	Number PCR Positive for ***M. cerebralis***	**Number of fish with unidentified *Myxobolus* species** **Detected**	**Number of fish with *Henneguya* Species** **Detected**	**Number of fish with *M. insidiosus*** **Detected**
Trout Creek 11/19/00 StS stray 2	2 of 2	0 of 2	2 of 2	0 of 2	0 of 2
Trout Creek 5/1/01 StS smolts 3	0 of 3	Not Done	0 of 3	0 of 3	0 of 3
Trout Creek 4/15/02 StS smolts 60	0 of 12 pools	Not Done	0 of 12 pools	0 of 12 pools	0 of 12 pools
Lower Deschutes 11/9/01 ChF 7	0 of 7	Not Done	0 of 7	4 of 7	0 of 7
Pelton Trap 2/01/02 StS stray 15	2 of 15	1 Positive of 2	1 of 15	0 of 15	0 of 15
Pelton Trap 2/15/02 StS stray 15	5 of 15	3 Positive of 5	6 of 15	0 of 15	0 of 15
Lower Deschutes 11/5/01 ChF 10	1 of 10	1 negative	0 of 10	5 of 10	1 of 10
Lower Deschutes 11/7/01 ChF 13	1 of 13	0 of 1	0 of 13	9 of 13	1 of 13
Round Butte Hatchery 8/27/02 ChS 129	8 of 129	8 Negative	10 of 129	15 of 129	0 of 129

Sample Site **Fish Species** **Date**	Number Presumptive Positive ***M. cerebralis*** **Detected**	Number PCR Positive for ***M. cerebralis***	**Number of fish with unidentified *Myxobolus* species** **Detected**	**Number of fish with *Henneguya* Species** **Detected**	**Number of fish with *M. insidiosus*** **Detected**
Round Butte Hatchery 8/27/02 ChS stray 2	0 of 2	Not Done	0 of 2	0 of 2	1 of 2
Round Butte Hatchery 9/4/02 ChS 31	2 of 31	2 Negative	2 of 31	5 of 31	0 of 31
Round Butte Hatchery 9/4/02 ChS stray 3	0 of 3	Not Done	0 of 3	0 of 3	0 of 3
Metolius 10/8/02 KK 60	0 of 10 individual 0 of 10 pools	Not Done	0 of 10 individual 0 of 10 pools	0 of 10 individuals 5 of 10 pools	0 of 10 individuals 0 of 10 pools
Round Butte Hatchery 10/8/02 SS 4	0 of 4	Not Done	0 of 4	0 of 4	0 of 4
Round Butte Hatchery 10/3/02 SS 1	0 of 1	Not Done	0 of 1	0 of 1	0 of 1
Pelton Trap 10/6/02 KK 5	0 of 5	Not Done	0 of 5	0 of 5	0 of 5
Pelton Trap 11/19/ StS Stray 9	0 of 9	Not Done	1 of 9	0 of 9	0 of 9
Lower Deschutes Sherars ChF 12	0 of 12	Not Done	0 of 12	3 of 12	0 of 12
Lower Deschutes Trout Cr ChF 10	0 of 36	Not Done	1 of 36	8 of 36	0 of 36

Results of enzyme digest procedure and microscopic examination of head tissues from samples processed in 2001.

Sample Site **Fish Species** **Date**	**Number Presumptive Positive** ***Myxobolus cerebralis*** **Detected**	**Number Confirmed Positive for *Myxobolus cerebralis* by Histology**	**Number of fish with unidentified *Myxobolus* species** **Detected**	**Number of fish with *Henneguya* Species** **Detected**	**Number of fish with *Myxobolus insidiosus*** **Detected**
Round Butte Sockeye Salmon 10/5/00 and 10/18/00	1/15	1 of 1 hatchery stray (Idaho)	1 of 15	1 of 15	Not Detected
Pelton Trap StS hatchery Stray 11-7-00	12/43	4 of 5 tested	10 of 43	5 of 43	Not Detected
Pelton Trap StS hatchery Stray 1-30-00	2/18	1 of 2 tested	2/18	3/18	Not Detected
Crane Prairie Rainbow 10-19-00	0/22	Not Done	0/22	4 of 5 pools	Not Detected
Crane Prairie Brook Trout 10-19-00	0/6	Not Done	0/6	2 of 2 pools	Not Detected
Crane Prairie Kokanee 10-19-00	NSS/2	Not Done	Not Detected	Not Detected	Not Detected
Metolius River Kokanee	0/60	Not Done	0/60	1 of 12 pools	Not Detected
Crescent Cr. Rainbow 9-29-00	0/40	Not done	1/13 pools	12/13 pools	Not Detected
Crescent Cr. Brown trout 9-29-00	NSS/1	Not Done	Not Detected	Not Detected	Not Detected
Crescent Cr. Mt. Whitefish 9-29-00	NSS/4	Not Done	Not Detected	Not Detected	Not Detected
Paulina Lake Kokanee 10-3-00	NSS/60	Not Done	Not Detected	Not Detected	Not Detected
L. Simtustus Kokanee 10-5-00	NSS/5	Not Done	Not Detected	Not Detected	Not Detected
L. Simtustus Bull Trout 10-5-00, 11-29-00	NSS/21	Not Done	Not Detected	Not Detected	Not Detected
Totals	15 of 297 completed	6 confirmed of 8	14 individuals and groups	28	0

Sample Site **Fish Species** **Date**	**Number Presumptive Positive** ***Myxobolus cerebralis*** **Detected**	**Number Confirmed Positive for *Myxobolus cerebralis* by Histology**	**Number of fish with unidentified *Myxobolus* species** **Detected**	**Number of fish with *Henneguya* Species** **Detected**	**Number of fish with *Myxobolus insidiosus*** **Detected**
L. Billy Chinook Bull Trout 4-30-00, 18-31-00	NSS/2	Not Done	Not Detected	Not Detected	Not Detected
Pelton Trap StS Strays 2-13-01	2 of 7	1 of 1 tested PCR positive	2 of 7	1 of 7	Not detected
Round Butte Unmarked - Wild Spring chinook August 2000	NSS of 4	Not Done	Not detected	Not detected	Not detected
Round Butte Hatchery ChS smolts 3-8-01	NSS of 60	Not Done	Not detected	Not detected	Not detected
Round Butte Hatchery Summer Steelhead Jan/Feb 2001	0 of 150	Not Done	17 of 150	0 of 150	5 of 150
Lake Simtustus Kokanee Sept. 13, 2000	NSS of 32	Not Done	Not detected	Not detected	Not detected
Round Butte Hatchery StS smolts 3-14-01	NSS of 60	Not Done	Not detected	Not detected	Not detected
L. Billy Chinook Bull Trout 8-2-00,3-1-01	NSS of 2	Not Done	Not detected	Not detected	Not detected
Lower Deschutes R ChF 12-00	NSS of 34	Not Done	Not Detected	4/34	Not Detected
Totals	2 of 351	1 of 1	19 of 157	5 of 191	5 of 150

Results of enzyme digest procedure and microscopic examination of head tissues from samples processed in 2001.

Sample Site **Fish Species** **Date**	**Number Presumptive Positive** ***Myxobolus cerebralis*** **Detected**	**Number Confirmed Positive for *Myxobolus cerebralis* by Histology**	**Number of fish with unidentified *Myxobolus* species** **Detected**	**Number of fish with *Henneguya* Species** **Detected**	**Number of fish with *Myxobolus insidiosus*** **Detected**
Oak Springs StS smolts 4-6-01 PGE studies	NSS of 60	NSS of 60	NSS of 60	NSS of 60	NSS of 60
Round Butte Smolts Pond 6 Spring chinook 3-8-01	NSS of 60	NSS of 60	NSS of 60	NSS of 60	NSS of 60
Round Butte Hatchery ChS Ladder #4 smolts 3-8-01	NSS of 90	NSS of 90	NSS of 90	NSS of 90	NSS of 90
Round Butte Hatchery smolts Summer Steelhead 3-14-01	NSS of 60	NSS of 60	NSS of 60	NSS of 60	NSS of 60
Lake Simtustus Kokanee Sept. 13, 2000	NSS of 32	NSS of 32	NSS of 32	NSS of 32	NSS of 32
Mill Creek RbT (Crooked R.) 6-20-00	NSS of 7	NSS of 7	NSS of 7	NSS of 7	NSS of 7
L. Billy Chinook Kokanee 3-2-01,3-16-01	NSS of 8	NSS of 8	NSS of 8	NSS of 8	NSS of 8
Totals	0 of 317	0 of 317	0 of 317	0 of 317	0 of 317
Round Butte Hatchery Spring Chinook 8-27-01	9/150	0 of 12 0 of 12 by Histology	9/150	43/150	NSS of 150
Round Butte Strays Spring chinook 8-27-01 and 9-5-01	0/18	0 of 1 by PCR and Histology (Fish with *M. spp.*)	1/18	5/18	NSS of 18
Round Butte Lost or No Tag Spring Chinook 8-27-01	0/6	Not Tested	0/6	1/6	NSS of 6

Sample Site **Fish Species** **Date**	**Number Presumptive Positive** ***Myxobolus cerebralis*** **Detected**	**Number Confirmed Positive for *Myxobolus cerebralis* by Histology**	**Number of fish with unidentified *Myxobolus* species** **Detected**	**Number of fish with *Henneguya* Species** **Detected**	**Number of fish with *Myxobolus insidiosus*** **Detected**
Pelton Trap Stray Summer Steelhead Oct./Nov 2001	5/45	10 of 45 PCR positive	10/45	3/45	NSS of 45
Crane Prairie Brook Trout	0 of 10	Not Done	0 of 10	3 of 10	0 of 10
Crane Prairie Rainbow Trout	0 of 7	Not Done	0 of 7	3 of 7	0 of 7
Crane Prairie Kokanee	0 of 2	0 of 2	0 of 2	0 of 2	0 of 2
Pelton Trap Stray Kokanee 10-16-01	0 of 2	0 of 2	0 of 2	0 of 2	0 of 2 NSS
Pelton Trap Sockeye Salmon October 2001	0 of 26	Not Done	1 of 26	0 of 26	1 of 26
L. Simtustus Jump Pool Bull Trout	0 of 6	Not Done	0 of 6	0 of 6	NSS of 6
Lower Deschutes R. Fall Chinook	0 of 26	Not Done	0 of 26	7 of 26	0 of 26
Totals	14 of 298	10 of 62	21 of 298	13 of 298	1 of 298
GRAND TOTAL	31 of 1263	17 of 71	54 of 1263	98 of 1263	6 of 1263

APPENDIX II

Detailed Methods

All methods for the detection and identification of fish pathogens were based on standard procedures from the AFS Fish Health Blue Book (Thoesen 1994) and/or those of the ODFW Fish Pathology Section Laboratories.

1. Bacterial Methods

ELISA Assay For BKD Antigen

Enzyme Linked Immunosorbent Assay (ELISA) methods for determining the presence and amount of *Renibacterium salmoninarum* antigen present in fish kidney tissues.

This is a commonly used method for the detection of bacterial kidney disease (BKD, caused by *R. salmoninarum*). It is used because of its sensitivity and the short length of time required obtaining results. The presence of *R. salmoninarum* antigen was determined by the ELISA method as follows: Kidney samples were taken aseptically from each fish and frozen. The kidney tissue was processed by standard methods and run in duplicate (Pascho and Mulcahy 1987). The test was run in the presence of both negative and positive control tissues. An automated plate reader read all samples, and the results were processed by a standard program.

ELISA designations: The presence and quantity of *R. salmoninarum* antigen found in the kidney.

Designation	OPTICAL DENSITY VALUE (O.D. 405nm)
NEGATIVE.	< 0.1
LOW	$\geq 0.1 < 0.2$
MEDIUM	$\geq 0.2 < 0.499$
HIGH	≥ 0.5

Bacterial Culture Methods

A kidney sample was obtained from each fish sampled and streaked on bacteriological media. Growth of bacteria from fish kidney streaks at 17º C on tryptic soy agar (TSA) or trypticase yeast extract with skim milk (TYES) bacterial agar plates was then presumptively identified (below). Bacterial identity of 10% of the ERM, CWD, pseudo kidney disease, and furunculosis positive cultures were confirmed by AFS Blue Book procedures and/or ODFW Fish Pathology laboratory's standard methods. Further analysis performed as necessary. The presumptive bacterial identification was based on the following characteristics:
Aeromonas salmonicida (Furunculosis): White pigmented colonies; gram negative, oxidase positive, non-motile rods; releases a brown water soluble pigment into the agar.
Aeromonas/ Pseudomonas species: (opportunistic secondary bacterial infections) White, tan or pinkish colonies; gram negative, oxidase positive, motile rod shaped bacterium.
Yersinia ruckeri (Enteric Redmouth disease, ERM): Creamy white and often large colonies; gram and oxidase negative large rod shaped bacterium.
Carnobacterium piscicola (formerly Lactobacillus, pseudo kidney disease): Pure white (unpigmented) colonies; gram positive (variable) oxidase negative small rods.

Flavobacterium psychrophilum (Coldwater disease, CWD): Yellow pigmented colonies, proteolysis apparent from clearing of casein on TYES plates; gram negative long spindly rods; no growth above 25º C.

2. Parasite Methods

Parasite exams for *M. cerebralis*

Heads from each juvenile or small adult fish sampled were cut in half. Each large adult fish was sampled using the core method, and the core was divided into two equal parts (Lorz et al. 1989). One half of the head or core was preserved in 10% buffered formalin for histological confirmation of spores. The other half was frozen and analyzed by the enzyme digest method for the presence of myxosporidian spores by standard methods (Thoesen 1994, Markiw and Wolf 1974). All samples were processed individually.

Parasite exams *C. shasta*

The posterior 1 to 1 1/2 inch of the intestine from each fish sampled was taken and the contents were either immediately examined microscopically for the presence of *C. shasta* spores or frozen individually in numbered whirl pac bag for later examination. Frozen samples were thawed in 2 mls of phosphate buffered saline (PBS) and macerated in a Stomacher processor for thirty seconds. One and a half milliliters of the resulting fluid were centrifuged at 6000 rpm for two minutes. The pelleted material was resuspended in 0.1 ml of PBS for producing a wet mount. Wet mounts of intestinal material were scanned in a systematic manner (2 minutes per slide) at 100 X magnification. Confirmatory diagnosis was based on the presence of one or more of the characteristic bean-shaped mature spores of *C. shasta* (Bartholomew et al. 1989)

General fish health examinations for parasites

From each stock of fish, up to ten fish were intensively examined for external and internal parasites, virus, and bacteria. All tests described above were performed. Additional examinations were done on selected organs and tissues.

3. Virus Methods

Cells and virus

The chinook salmon (*Oncorhynchus tshawytscha*) embryo cells, CHSE-214, or *epithelioma papulosum cyprini* (EPC) cells were grown as monolayers in minimal essential medium(MEM) auto pow medium supplemented with fetal bovine serum (10%), penicillin (100 I.U./ml) and streptomycin (100 µg/ml) and glutamine (2mM) as previously described (Engelking and Leong, 1981).

Virus Identification by the Indirect Fluorescent Antibody Test (IFAT)

Cytopathic effect (CPE) on cells was determined to be caused by viral infection by either of two methods. Virus neutralization assays are described below. Routinely IFAT was used to identify

virus cpe. Two mouse monoclonal antibodies were used in the assay. The B9/C6 monoclonal antibody is reactive with all known strains of IHNV. The 2NH105B monoclonal antibody is reactive with the Type 2 strain of IHNV. Infected cells were resuspended in Hanks buffered salt solution (0.5 ml). The suspension was centrifuged for 3 minutes at 8000 rpm . The resulting pelleted material was fixed with cold acetone, triturated and centrifuged as before. The pelleted material was resuspended with 25 µl of monoclonal antibody. The monoclonal antibody was incubated with the pellet for 30 minutes at 37ºC or alternatively the reaction mix was placed in ice water and microwaved on high power for 3 minutes. The reaction mix was centrifuged for 3 minutes at 8000 rpm. The pelleted material was resuspended with goat anti-mouse fluorescence conjugated antibody. The reaction was incubated as above. The resulting pelleted material was resuspended in a tiny drop of Evans' blue stain. The material was placed on a slide and viewed with a fluorescent microscope. A positive reaction was observed by bright apple green fluorescence present on the cells.

Erythrocytic Inclusion Body Syndrome (EIBS) Assay

Blood smears were made on frosted microscope slides of blood samples from freshly killed fish. The smears were allowed to air dry. The slides were fixed within four hours with 100% methanol for at least five minutes. Slides were later stained with pinacyanol chloride for two minutes and air dried to be stored in the dark. The slides were scanned in a systematic manner (2 minutes per slide) at 1000 X magnification. Presumptive diagnosis was based on the presence of the characteristic light purple round inclusion bodies in the erythrocytes. Confirmatory diagnosis requires electron microscopic visualization of the virus particle (Thoesen 1994,Engelking October 1999).

Virus (IHNV) stocks for challenge studies

Virus used in this study was isolated in 1975 from an adult steelhead trout at the Round Butte Hatchery in Oregon (RB1) (Hsu et al. 1986), spawning steelhead trout in January and February of 1997 at Round Butte Hatchery (RB 97); and from spawned out kokanee salmon from the Metolius River in 1996 (ME 96). The virus was propagated in fish cells at a multiplicity of infection of 0.001 at 15ºC for 7 days (Engelking and Leong 1981). The supernatant fluid was then collected and centrifuged at 2,500 x g for 10 min. at 4ºC. The cell-free supernatant fluid contained 0.5-1 x 10^8 TCID50 (50% tissue culture infective doses) per ml. as determined by the method of Reed and Muench (1938). Stocks of virus were frozen immediately after centrifugation at - 80º C in an ultracold freezer. Virus purification when necessary was performed by ultracentrifugation on sucrose gradients as described (Kurath and Leong 1985).

Virus Neutralization assays

Plaque assays were performed as described by Burke and Mulcahy (1980). Serial tenfold dilutions of virus were incubated with equal volumes of various dilutions of antisera at 15ºC for 3 h on a rotating shaker. Duplicate wells in a six well plate (Falcon) containing monolayer cultures of CHSE-214 cells were inoculated with 0.2 ml of each antiserum-virus mixture. After 1 h at 15ºC, the infected cells were overlaid with 0.8% gum tragacanth (Fisher Scientific Co.) in minimal essential medium with 5% fetal bovine serum. After seven to ten days at 15ºC, the cells

were fixed, stained, and counted. The relative virus titers with and without antisera treatment were determined and used to calculate the plaque reduction end point dilutions.

Tangential Flow Filtration for Concentration of Low Amounts of Virus from Large Volumes of Water

Tangential flow filtration passes sample fluids across filter membranes to reduce clogging and allow water and low molecular weight particles to pass through the filters while retaining macromolecules and virus particles. A Pelicon Cassette System (Millipore Corp.) was used for concentrating IHN virus from river water samples. The exclusion, or pore size of the filter was of 100,000 molecular weight with a surface area of 0.46 m^2. Particles with large molecular weights that were excluded returned to the original sample container (retentate). Smaller particles passed through the filter and were collected in a filtrate container (filtrate). Flow circulation reduced the volume of retentate while it increased the filtrate. At the desired retentate volume, the filter was washed with Hank's Buffered Saline Solution (HBSS) to remove an particles (virus) that adhered to it. The water flow was reversed and the filter washed with more HBSS (backflush). The filter was washed of residual virus with a buffered salt solution (backflush) by reversing the flow. To process about 20 liters of water took about 5 hours and reduced the initial volume about 100 fold (retentate). Fetal bovine serum was added (0.1% v/v) to stabilize any virus present. These methods were an adaptation of procedures developed by Watanabe and co-workers (1988). Samples prior to and after filtration were tested for the presence or absence of virus by diluting samples and inoculating EPC cells. The samples tested were: initial unconcentrated water, retentate, backflush and filtrate. The relative efficiency was determined from trial experiments using water "seeded" with IHN virus.

Fish for IHNV Challenge Experiments

Rainbow trout (*O. mykiss*) from Crane Prairie, Metolius and Crooked rivers and summer steelhead trout, kokanee (*O. nerka*), sockeye and spring chinook salmon (*O. tshawytscha*) were obtained through the Oregon Department of Fish and Wildlife. Bull trout (*S. confluentus*) were obtained with the assistance of the U. S. Forest Service.

Challenge experiments with IHNV Strains

The virus challenge experiments were performed at the Oregon Department of Fish and Wildlife, Oregon State University Salmon Disease Laboratory. Triplicate lots of 25 fish were challenged with IHNV by immersion in a liter of water for 12 hours containing different concentrations of the virus (Johnson et al., 1982a and 1982b). Dead fish were collected daily and examined for visible signs of disease. Every dead fish was processed by standard methods (Thoesen, 1994) to determine if the fish had died from an IHNV infection.

Virus assays from fish challenges

Virus assays were performed using confluent CHSE-214 or EPC cell monolayers grown in 24-well tissue culture plates (Falcon). Samples from infected fish were prepared as described (Thoesen, 1994), sterilized by filtration (0.2 um acrodisc, Gelman), and diluted in minimal essential medium (MEM) (without fetal calf serum). Duplicate samples (0.05-0.1 ml) of each

dilution were placed on monolayers in individual wells and allowed to absorb for 60 minutes. Sample inocula were removed from the wells after adsorption and 1.0 ml of MEM growth medium was added to each well. All virus strains were prepared by growing the virus at a multiplicity of infection of 0.01 to 0.001 $TCID_{50}$ per cell on CHSE-214 cells as previously described (Engelking and Leong, 1981). The virus used for challenge trials was prepared from a stock of virus, which had undergone no more than three passes in tissue culture after isolation from infected fish.

4. RNA Protection Assays of IHNV isolates - Genetic Analysis

RNA preparation from infected cells.

RNA was prepared from IHNV infected EPC cells using the commercial RNazol B RNA isolation system (Cinna/Biotecx laboratories, Inc.). EPC cell monolayers in 24 well plates were prepared, and four 2 cm^2 wells (8 cm^2 total) were infected with each IHNV isolate at a multiplicity of infection (moi) of approximately ten. Four other wells were mock infected with media. At 24 hours post infection early CPE was visible in all the IHNV infected wells, and total RNA from each set of four wells was pooled and extracted according to the manufacturer's instructions. This produced 20 µg of RNA preparation from 8 cm^2 of infected cells. The yield of RNA was determined because of the ribosomal RNA in this relatively crude preparation.

Viral genomic RNA was prepared by purifying virions and extracting their RNA as described previously (Kurath and Leong 1985).

Cloning, transcription and probe synthesis of N, NV, and G genes

The NV, N, and G genes of IHNV have been previously cloned into the plasmid vector pT7 (Novagen) and subcloned into pBlueScribe (pBS-, Stratagene Cloning Systems) by Kurath and co-workers (1995 and unpublished results). Template DNA for transcription of plus-sense RNAs was prepared by digesting the specific probe plasmids with Bam .For negative-sense RNAs the probes were digested with Xba 1. Plus sense transcript RNAs were then synthesized in T7 polymerase reactions and minus-sense RNAs were synthesized in T7 polymerase reactions. The transcript products were then purified and quantitated as described by Kurath et al. (1992). For the synthesis of radioactive probes the polymerase reaction conditions were modified to include 20 µCi of ^{32}P -UTP, and the concentration of cold UTP was lowered to 50 µM. Probe synthesis reaction conditions, protocol and quantitation were described by Kurath and co workers (1992).

RNase protection Assays (RPA)

Each RNase protection assay included 1 X 10^5 cpm of probe. Target RNAs were 100ng of RNA transcribed from a plasmid, an unknown quantity of RNazol prepared RNA (2µl of the 20 ul total yield from 8 cm^2 of infected cells). The assay protocol and gel analyses were slight variations on the original protocol (Winter et al. 1985), using an annealing step and a one hour RNase treatment at 37°C.

RT-PCR amplification and sequence analysis

Viral genomic and messenger RNAs were utilized as templates for reverse transcription polymerase chain reactions (RT-PCR). Briefly 5ul of a 1:20 dilution of cell culture supernatant for each viral isolate was combined with 1 ul of 20pmole/ul of each IHN virus glycoprotein (G) gene specific primersk, 5ul of 25 mM $MgCl_2$, 5 ul of 10X PCR buffer (Promega), 5units of AMVreverse transcriptase (Promega), and 2.5 units Taq to produce a 50 ul reaction. The reactions ws then incubated at 50 C for 30 sec, and 72 C for 30 sec, followed by a final extension of 72 C for 7 minutes. Two ul of the RT-PCR reaction were then utilized as template for the second round PCR reaction without the addition of AMV reverse transcriptase and initial 50 ºC incubation. Sequences of the first and second round PVR primers utilized for the G-gene amplification were:

1st 5' AGAGAYCCCTACACCAGAGAC 3'
5' GGTGGTGTTGTTTCCGTGCAA 3'

2ND 5' TCACCCTGCCAGACTCATTGG 3'
5' ATAGATGGAGCCTTTGTCAT 3'

The sequenced product of the second round PCR of the G-gene is a 303 nucleotide (nt) region located in the middle of the gene from nucleotide 686-988 (Genbank accession no. U50401) and is denoted the mid-G region. The mid-G region was sequenced using a fluorescent dye terminator-cycle sequencing kit (Applied Biosystems) using second round PCR primers and following the manufacturer's protocols.

RT-PCR amplification and sequence analysis of the nucleoprotein (N) gene were carried out using identical reaction conditions to those described for the G-gene with the exception of using N-gene specific primers. The sequenced product of the second round PCR of the nucleoprotein gene is a 412 nt region located at the 5' terminus of the gene and is denoted as the 5"N region. This region corresponds to nucleotide 133-544 on the full length WRAC strain IHN virus genome sequenced by Morzunov (Genbank L40883).