

# National Wild Fish Health Survey

California-Nevada  
Fish Health Center

Annual Report for fiscal year 2006



**National Wild Fish  
Health Survey  
Annual Progress Report FY 2006**  
Prepared by Kimberly True  
and Jessica Gray

**California-Nevada Fish Health Center**

Center staff conducted the National Wild Fish Health Survey (NWFHS) in the 2005/2006 fiscal year by collecting fish tissue samples and performing laboratory tests for major fish pathogens in accordance with standardized procedures (NWFHS Laboratory Procedures Manual – 2005, ). This data is entered into a national database and is accessible to the public and resource managers, via the web, and can be viewed at:

<http://wildfishsurvey.fws.gov/> or <http://www.esg.montana.edu/nfhdb/>

Kimberly True, Assistant Project Leader  
Lyn Rosten, Biological Science Technician  
Jessica Gray, Biological Science Technician

Ken Nichols, Fishery Biologist  
Scott Foott, Project leader  
Ron Stone, Fishery Biologist  
Also assisted with field collections and lab work.

## Abstract

The National Wild Fish Survey (NWFHS), conducted by the U.S. Fish and Wildlife Service's Fish Health Centers, assesses the prevalence and distribution of major fish pathogens in wild fish populations.

In 2005-2006, the California-Nevada Fish Health Center (Ca-Nv FHC) focused on disease monitoring in the upper Klamath River basin. Pathogens associated with diseased fish in the Klamath River include bacteria (*Flavobacterium columnare* and motile aeromonad bacteria), and myxozoan parasites (*Parvicapsula minibicornis* and *Ceratomyxa shasta*). The incidence of two parasites *Ceratomyxa shasta* and *Parvicapsula minibicornis* in juvenile chinook salmon is of special concern.

Another focus in 2005-2006 was done in collaboration with Nevada Division of Wildlife's regional biologists. The Nevada Division of Wildlife (NDOW) has been commissioned to protect, manage and restore the threatened Lahontan cutthroat trout (*Oncorhynchus clarki henshawi*) and Bonneville cutthroat trout (*Oncorhynchus clarkii utah*) populations in Nevada rivers, streams and lakes. Subsets of LCT and BCT donor populations were collected from various streams throughout Nevada, sacrificed and tested for major fish pathogens. No virus was detected in all locations as well as culturable bacteria and the causative parasite of whirling disease, *Myxobolus cerebralis*. Suspect levels of the *Renibacterium salmoninarum* (Rs) antigen were found in all locations and QPCR confirmed all but one stream positive for the presence of Rs DNA.

*Renibacterium salmoninarum* (Rs) was recently detected in Lahontan cutthroat trout during a routine hatchery inspection at Lahontan NFH. This finding has increased concern for the presence of this pathogen in wild trout populations, specifically in waters within the Tahoe Basin. A very low incidence of Rs was found in Brook Trout (+1/60 confirmed by QPCR) sampled in Sagehen Creek, CA. An additional lot was sampled from Sagehen more recently and only 2/60 were confirmed positive for RBT and 1/60 in BNT. Continued monitoring is projected for this next sampling year.

In the Sacramento-San Joaquin Delta, a project called Delta POD (Pelagic Organisms in Decline) was initiated to monitor the populations of longfin smelt and threadfin shad. The Ca-Nv Fish Health Center's participation with this project began in April of 2006 and continued through October of 2006. In partnership with University of California at Davis, a trawl was used to collect a target of \_\_\_ fish on numerous sampling dates. After being visually examined, the samples were brought back to the Center for further testing. Here, they were processed and tested by tissue culture for any known replicating agent. Thus far, none have been found. The remaining tissues were then cut in sagittal sections and examined by histology for parasites or tissue lesions; none were found.

## Overview of the National Wild Fish Health Survey

In 1997, the U.S. Fish & Wildlife Service issued a national directive to all Fish Health Centers to conduct a National Wild Fish Health Survey. The catalyst for this directive was the discovery

of the destructive impact of Whirling Disease on wild trout populations in Montana and the intermountain west states. Fiscal Year 2006 marks the ninth year of involvement in the Survey for the California-Nevada Fish Health Center. To date, the Center has partnered over 100 times at over 200 sites (many duplicate sites) to collect a total of 13,498 samples comprised of a rich diversity of species throughout California and Nevada.

The study and detection of pathogens is increasingly important and vital to the prevention of outbreaks and widespread distribution. The virulence of particular pathogens and potential geographical spread of disease is a significant threat to natural resources. An example of the importance of early detection in preventing the distribution of disease is demonstrated in the LCT/BCT recovery strategy. The goal of this program is to recover the historical population size and range that was negatively influenced by a population boom and natural disasters in Nevada. The state of Nevada has implemented a reintroduction plan that relies on healthy donor populations being reintroduced into historic areas that are now absent of sustained populations of native fish. With fish health knowledge gained through monitoring, pathogens can be detected and prevented from being introduced into other watersheds or basins.

#### **Disease Monitoring in the Klamath River *UPDATE- Need report***

Ceratomyxosis (due to *C. shasta*) has been identified as the most significant disease for juvenile salmon in the Klamath Basin (Foott et al. 1999, Foott et al. 2004). *P. minibicornis* is prevalent at nearly 100% in the main stem resulting in numerous fish found dually infected with these two myxozoans. Monitoring in 2006 detected the onset of *P. minibicornis* infections in emigrating chinook smolts as early as March 16<sup>th</sup>. Prevalence quickly rose to 100% 7 weeks after the first detection (May 4<sup>th</sup>) therefore nearly all fish infected with *C. shasta* were also infected with *P. minibicornis*. The overall prevalence of infection (POI) in 2006 for *C. shasta* is **22%** by histology and **48%** by PCR. For *P. minibicornis*, POI is **83%** by histology and **91%** by PCR. Kidney and intestinal functions are likely to be impaired by these infections, at the time when chinook salmon are already undergoing physiological changes associated with smoltification and requiring increased energy for the demands of down river migration.

#### **Surveys for *Renibacterium salmoninarum* in the Tahoe Basin *UPDATE- Need ELISA results***

Recent isolations of *Renibacterium salmoninarum*, the causative bacterium for Bacterial Kidney Disease (BKD), in the Pilot Peak Lahontan cutthroat population at Lahontan NFH raised concerns about the disease impacts to planned restoration waters for this threatened species. Two surveys on Fallen Leaf Lake and Sagehen Creek were conducted to determine if *R. salmoninarum* was present in resident salmonid species (Brook trout, Rainbow trout, and Brown trout) as well as other species in Fallen Leaf lake. In Sagehen Creek *R. salmoninarum* antigen (P57) was detected in **85% (n=60)** Brook trout, **5% (n=42)** Rainbow trout, and **32% (n=31)** Brown trout tested by ELISA. ELISA-positive samples from all three species were confirmed for the presence of *R. salmoninarum* DNA by PCR. Infection levels, as determined by QPCR Ct are very low, indicating low bacterial loads or asymptomatic infections with this bacterium. Fallen leaf Lake was very similar to Sagehen Creek. **Kokanee salmon and lake trout were tested and the Rs prevalence was 17% (n=6).**

## Laboratory Methods

The methods used in the NWFHS to collect, process, and test fish tissues are standardized throughout the country. The detailed procedures and laboratory protocols can be found in The National Wild Fish Health Survey Procedures Manual (True 2004) at the following websites:

NWFHS

<http://fisheries.fws.gov/FHC/FHCNational.htm>

CANV Fish Health Center

<http://www.fws.gov/canvfhc/nwfhsman.htm>

Some studies conducted in 2003/2004 required additional tests and/or analysis as requested by partners or as specified in contracted fish health services which overlapped with the Survey.

### Organosomatic Indices and Parasitology

Individual fish were weighed (0.1 g) and measured (total length, mm) to determine condition factor ( $KTL = W/L^3$ ). Fish were then examined externally and internally for clinical signs of disease and any abnormalities. Mucus samples (skin scrape), gill tissues and intestine (wetmounts) were examined for presence and morphology of parasites with light microscopy at 40-450x magnification.

### Bacteriology

A sample of kidney tissue from each fish was streaked onto 100 mm petri plates, or 20 x 125 mm test tube slants, of Brain Heart Infusion Agar (BHIA) and incubated at room temperature for 72 hours. If growth appeared on the BHIA media, isolated colonies were subcultured onto fresh BHIA plates to supply pure cultures of bacteria for phenotypic characterization and presumptive identification. Subcultured isolates were screened for bacterial fish pathogens by standard microscopic characteristics (Gram stain, morphology, motility and cytochrome oxidase) and appropriate biochemical tests. Bacterial isolates that are ubiquitous in freshwater and without associated clinical signs were identified to a general group, while those that are potential fish pathogens such as *Aeromonas salmonicida*, *Yersinia ruckeri*, or *Edwardsiella tarda* were examined to a presumptive identity. Corroborative testing for positive results included Fluorescent Antibody Testing (FAT), using specific antibodies against each bacterial pathogen.

### *Renibacterium salmoninarum* by ELISA

Kidney tissue from each fish was removed and diluted 1:8 with Phosphate Buffer Saline (PBS) with Tween 20, homogenized, and separated by centrifugation. The samples were then loaded onto 96-well plates to be assayed by Enzyme Linked Immunosorbent Assay (ELISA) for the presence of *Renibacterium salmoninarum* antigen. The ELISA tested samples in replicate when the quantity of kidney tissue from individual fish was sufficient. The absorbency values (optical density, OD) were averaged and the distribution of ELISA values for separate groups were evaluated. Individual fish with ELISA OD values greater than 2 standard deviations above the negative reference control OD, and up to 0.499, were defined as low level infections, 0.500-.999 moderate level, and values of 1.00 or higher were considered high infection levels. Corroborative testing for positive results was done by Quantitative Polymerase Chain Reaction (QPCR).

## Virology

Samples of kidney and spleen, or visceral tissue in the case of smaller fish, were removed from each fish to assay for the prevalence of Infectious Hematopoietic Necrosis virus (IHNV), Viral Hemorrhagic Septicemia virus (VHSV), and Infectious Pancreatic Necrosis virus (IPNV) using accepted cell culture techniques. Kidney and spleen tissues were tested individual, or from 3 fish pooled into one sample, and occasionally 4-5 fish were pooled when the total number of fish was not a multiple of three. For cell culture assay, tissue samples were weighed and diluted to 1:10 in Hank's Balanced Salt Solution (HBSS) and homogenized with a Stomacher 80 Lab Blender®. Samples were centrifuged at 5000 x g for 15 m and then 1.0 mL of the supernatant was combined with 1 mL of HBSS supplemented with antibiotics and antimycotic (200 IU mL<sup>-1</sup> penicillin G, 200 IU mL<sup>-1</sup> streptomycin, 0.5 µg mL<sup>-1</sup> amphotericin B and 40 µg mL<sup>-1</sup> gentamycin). Final sample dilutions of 1:20 and 1:100 were inoculated onto confluent Chinook Salmon Embryo 214 (CHSE-214), Epithelioma Papillosum Cyprinid (EPC), and Fat Head Minnow (FHM) cell lines in replicate wells of 48-well plates. Samples were incubated on a platform rocker for 30-60 m at 15°C. Wells were overlaid with 0.5 mL Minimum Essential Media with 10% Fetal Bovine Serum (MEM10) or MEM10 with methylcellulose (EPC cell line). Plates were incubated at 15°C for 21 d and were examined bi-weekly for evidence of viral cytopathic effects (CPE). Corroborative testing, if positive, was done by Immunohistochemistry (IHC).

## *Myxobolus cerebralis* (Whirling Disease)

Screening for *Myxobolus cerebralis*, the causative agent of Whirling Disease, was done by Pepsin-Trypsin Digest (PTD) of cranial elements consisting of bone and cartilage. Sampled salmonids were decapitated and the heads grouped into pools of 5 fish, and then frozen until laboratory analysis could be performed. The heads were heated in a 60°C water bath for 60 m, so that the cranial elements could be removed from the soft flesh. The cranial elements were then ground in a blender and placed in a pepsin solution of 20 mL g<sup>-1</sup> of tissue, and incubated at 37°C for 40-60 m, depending on sample size. The samples were centrifuged, supernatant removed, and the pellet digested in a solution of trypsin at 20 mL g<sup>-1</sup> of tissue. Samples were incubated at room temperature on a rocker plate for 30 m. The larger remaining particles were filtered and the samples were centrifuged a final time to concentrate spores, if present. A small amount of water was added to the pelleted preparation to provide adequate solution volume in which the samples could be examined by phase contrast microscopy at 200-400x. Corroborative testing for positive results was done by PCR.<sup>1</sup>

---

<sup>1</sup> National Wild Fish Health Survey Laboratory Procedures Manual, 2004

## California

### Pathogen Survey

#### Infectious Hematopoietic Necrosis Virus in Juvenile and Adult Fall Chinook

##### Sacramento-San Joaquin Delta, CA

##### *Sagehen Creek, CA – Tahoe Basin*

Sagehen is a small stream that flows from the Castle Peak area in the Sierra Nevada into the Little Truckee River, which eventually adds to the Reno, Nevada water supply. There is evidence that much of the basin was logged in the late 1800's. This spring-fed stream supports a healthy native fishery that includes Lahontan reddsides (*Richardsonius egregius*), speckled dace (*Rhinichthys osculus*), Tahoe and mountain sucker (*Catostomus tahoensis*, *Catostomus platyrhynchus*), and the Paiute sculpin (*Cottus beldingii*). Anglers appreciate its healthy trout fishery that supports brown (*Salmo trutta*), brook (*Salvelinus fontinalis*) and rainbow trout (*Oncorhynchus mykiss*).

##### *Fallen Leaf Lake, CA – Tahoe Basin*

Connected to Lake Tahoe by its outlet Taylor Creek, Fallen Leaf lake is similar in elevation, clarity and depth to its larger counterpart. Located just southwest of Lake Tahoe, Fallen Leaf Lake is found at an elevation of 6377 feet reaching depths of up to 430 feet. At three miles long by three-quarters of a mile wide, it is surrounded by the Tahoe National Forest. The Mackinaw or Lake Trout (*Salvelinus namaycush*) population is strong and is considered the main game fish in the lake but is complimented by browns, rainbows and kokanee salmon (*Oncorhynchus nerka*).



At coordinates W38.9016, N120.0616, forty-five mackinaws and thirteen kokanee salmon were collected by John Stead of UC Davis and his team. These collections occurred on various dates in the months of July-August, after which the fish were frozen and sent to the Ca-Nv Fish Health Center. When they arrived at the Center, the heads and kidneys were removed. The heads were tested for the parasite *Myxobolus cerebralis* (Mc), the causative agent of Whirling Disease. The kidneys were tested for the presence of *Renibacterium salmoninarum* (Rs) by ELISA (Enzyme Linked Immunosorbent Assay). Mc was found in \_\_\_/\_\_\_ (\_\_\_%) of samples and Rs was found in \_\_\_/\_\_\_ (\_\_\_%) of samples.

### Spawning Surveys

The completion of Shasta dam in 1945 had an inevitable impact on Chinook salmon and steelhead. A significant loss of natural spawning areas above the dam was mitigated through the completion of Coleman National Fish Hatchery. Production of FCS, STT and LFS were successful at Coleman. Because of water temperatures, however, aquaculture efforts for the

spring Chinook were unsuccessful and suspended in the mid-late 1950's. The Service concluded that the spring run was more likely to succeed if left undisturbed assuming ecological conditions such as water temperature and flow were satisfactory below the dam in the main stem Sacramento River. Continuing efforts to non-lethally monitor spring run and natural steelhead spawning adults are important to understanding the success of these species in wild populations.

### ***Winter Chinook Adult Spawning – Livingston Stone National Fish Hatchery***

The winter run Chinook salmon found itself in dire straights in 1988 and was listed as endangered by Cal Fish and Game in 1989 and the National Marine Fisheries Service in 1994. All captive broodstock attempts to imprint the juveniles to the main-stem Sacramento River failed and in 1997, the Bureau of Reclamation developed a main-stem rearing facility to ensure winter run imprinting and adult returns to the main-stem Sacramento River below Shasta Dam. Livingston Stone NFH is the substation of Coleman NFH that serves this purpose of supplementing declining natural populations. The hatchery's ultimate purpose is to eventually reach such a healthy population size that the facility will no longer be needed because the run will be self-sustaining.



Wild Fish are captured at the base of Keswick Dam and spawned into the genetic pool at the hatchery each year to maintain genetic health and diversity. A total of 92 samples were taken in 2006 from spawned wild fish (39 males, 53 females). Eighty-four fish were sampled for viral testing with 64% kidney samples and 73% ovarian fluid samples positive for IHNV. Out of 92 individual samples tested for various bacteria, there were no positives. Individual samples of kidney and ovarian fluid (n=84) were tested by QPCR for the presence of *Renibacterium salmoninarum* DNA. Intestinal tissues were processed and tested by QPCR for the parasites *Parvicapsula minibicornis* (80/88 positive, 91%) and *Ceratomyxa shasta* (88/92 positive, 96%).

### ***Steelhead Adult Spawning – Coleman National Fish Hatchery***

The Steelhead propagation program began in 1952 following Central Valley Project when steelhead spawning habitat was also reduced. Health information on these populations collected at the hatchery is important as the steelhead run has become greatly dependent upon hatchery operations for maintaining the populations. Fish health data in natural populations is also important for possible management decisions within these watersheds.

Natural spawning fish are collected and spawned into the genetic pool at Coleman National Fish Hatchery and then released. A total of **21** natural female ovarian fluids were collected, pooled and tested for virus and *Renibacterium salmoninarum*. **Thirty percent (30%)** of the natural population tested (**10 pooled samples**) were positive for IHNV and out of **9** pools tested for Rs, **7 (78%)** were positive by Direct Fluorescent Antibody Technique (DFAT).

## Klamath River Health Monitoring Project

The Klamath River has been in the midst of much controversy which was heightened during the 2002 fish kill. Many regional, state, local and tribal biologists have made research efforts to try and better understand what biological factors influence this river. The incidence of the two parasites, *C. Shasta* and *P. minibicornis*, especially acting as a potential dual infection, is

Sample Week	Date	n	Detected	%
1	9-Mar	60	0	0
2	16-Mar	73	0	0
3	23-Mar	37	0	0
4	30-Mar	88	0	0
5	6-Apr	87	9	10
6	13-Apr	63	8	13
7	20-Apr	87	23	26
8	28-Apr	87	60	69
9	4-May	67	47	75
10	11-May	69	69	100
11	18-May	85	65	76
12	25-May	86	62	72
13	2-Jun	83	46	55
14	8-Jun	41	23	56
15	15-Jun	82	26	32
16	22-Jun	88	45	51
17	29-Jun	89	32	36
18	6-Jul	132	44	33
19	13-Jul	40	0	0

of concern specifically for the outmigrating juvenile Chinook salmon in the mainstem river.

Juvenile Chinook Salmon were collected by the Center's partners, using a



combination of beach seines, rotary screw traps, frame nets, and electrofishing from a total of **eleven sites** over the course of **nineteen weeks** (only **11** of which were known wild fish with no hatchery fish influence) during the spring

and summer of 2006. Each week the goal was to examine 30 fish from three sampling sites for a total of 90 fish per week. The crews collecting fish for the project held fish using in-river live boxes up to 48 hours prior to sampling, depending on the number of fish captured each day. Fish were euthenized in MS222, measured for fork length and examined for abnormalities. The degree of abnormality was scored according to a set of predetermined criteria. Tissue samples were collected for PCR analysis, histological assays and bacteriology.

*Ceratomyxa shasta* (Cs) incidence ranged from 0% in the first few weeks of the study to **100%** by the **tenth week (May 11, 2005)** (Table 1). The incidence of infection (IOI) in fish above the Shasta River and between the Shasta and Scott River were correlated but the **35.9%** IOI in fish captured between the Scott and Shasta was greater than the **28.1%** of infected fish capture above the Shasta. There was no correlation in weekly incidence of Cs infection in fish caught in the reach between the Scott and Shasta and the reach between the Trinity and Salmon Rivers.

Table 1. Percent Cs infection in Klamath River study; gray section represents wild fish without hatchery influence

## Bibliography

Foott JS, JD Williamson, and KC True. 1999. Health, physiology, and migration characteristics of Iron Gate Hatchery Chinook, 1995 Releases. U.S. Fish & Wildlife Service, CA-NV Fish Health Center, Anderson CA.

Foott JS, R. Harmon, and R. Stone. 2004. FY2003 Investigational report: Abundance of *Ceratomyxa shasta* in Iron Gate and Copco Reservoirs. U.S. Fish & Wildlife Service California – Nevada Fish Health Center, Anderson, CA.

Foott, JS 2004. Health Monitoring of Adult Lost River Sucker (*Deltistes luxatus*) and Shortnose Sucker (*Chasmistes brevirostris*) in Upper Klamath Lake, Oregon, April-September 2003. Joint FWS and USGS project. U.S. Fish & Wildlife Service, CA-NV Fish Health Center, Anderson CA.

**Appendix I - NWFHS SUMMARY TABLE for FY 2006**

<b>Case #</b>	<b>Date Collected</b>	<b>Location</b>	<b>Species</b>	<b>Number of Fish</b>	<b>Total Fish From Site</b>
06-025	02-28-06	Black River, Az	Speckled Dace	36	36
06-026	02-28-06	Black River, Az	Desert Sucker	48	48
06-027	02-28-06	Black River, Az	BNT RBT	16 23	39
06-036	03-29-06	Delta POD	Longfin smelt	70	70
06-040	04-05-06	Little Colorado River	BNT RBT Speckled Dace	21 39 60	120
06-046	05-01-06	Delta POD	Longfin smelt	96	96
06-055	04-26-06	Delta POD	Longfin smelt	3	3
06-062	04-27-06	Delta POD	Longfin smelt	17	17
06-066	05-10-06	Delta POD	Longfin smelt	130	130
06-083	05-24-06	Delta POD	Longfin smelt	70	70
06-100	06-21-06	Delta POD	Longfin smelt	7	7
06-116	07-17-06	Delta POD	Longfin smelt	60	60
06-134	08-29-06	Delta POD	Longfin smelt	23	23
06-136	09-13-06	Sagehen Creek Stampede Reservoir	RBT BNT	12 18	30
Various Cases	Various Dates	Livingston Stone NFH	Winter Chinook Adults	92	92
Various Cases	Various Dates	Klamath River- Juvenile health monitoring	Chinook	446	446
<b>Total To Date (09-18-2006):</b>				<b>1260</b>	

## Appendix 2 – Sample summary report tables

### General Methodology

**VIROLOGY:** Incidence of infection for major fish viruses in 1 to 3-fish pools of kidney and spleen tissue (K/S) and/or viscera (VISC). Viruses include Infectious Hematopoietic Necrosis Virus (IHNV); Infectious Pancreatic Necrosis Virus (IPNV); Viral Hemorrhagic Septicemia Virus (VHSV); Oncorhynchus Masou Virus (OMV); Cutthroat Trout Virus (CTV); Spring Vireamia of Carp Virus (SVCV); Koi Herpes Virus (KHV), and Largemouth Bass Virus (LMBV). Tissue homogenates are inoculated on species-specific cell lines (EPC and CHSE or EPC, CHSE and KF1), incubated at 15°C for 21 days. Cell cultures are observed for viral cytopathic effects (CPE).

**BACTERIOLOGY:** Cultured systemic bacteria from individual kidney samples inoculated onto Brain Heart Infusion Agar (BHIA) and presumptive bacteria are further tested by appropriate biochemical tests. Incidence of bacterial infection, *Renibacterium salmoninarum* (Rs-ELISA) by Enzyme Linked Immunosorbent Assay in individual kidney (KD) samples; confirmation by Quantitative Polymerase Chain Reaction (Rs-QPCR).

**PARASITOLOGY:** Incidence of external parasites (Para-External) by microscopic examination of gill and skin tissues or histological examination. Internal reportable parasite *Myxobolus cerebralis* (Para-Mc-TPD) by Pepsin-Trypsin Digest of cranial elements and microscopic examination for characteristic myxosporean spores.

### Case – various (AD WCS – LSNFH)

	# Samples (pool size)	Total Fish	Results	# Positive (% Positive)	Notes
<b>Virus</b>					
Tissue culture (Kd)	33(1)	33	IHNV	21(64%)	
Tissue culture (OF)	51(1)	51	IHNV	38 (73%)	
<b>Bacteria</b>					
BHIA culture (Kd)	92 (1)	92	<i>Aeromonas/Pseudomonas</i>	0 (0%)	
			<i>Aeromonas salmonicida</i>	0 (0%)	
			<i>Yersinia ruckeri</i>	0 (0%)*	
<i>Rs</i> -QPCR (Kd-male)	33 (1)	33	<i>Renibacterium salmoninarum</i>	3 (9%)	
<i>Rs</i> -QPCR (Kd-female)	51 (1)	51	<i>Renibacterium salmoninarum</i>	2(4%)	
<b>Parasites</b>					
<i>Cs</i> -QPCR	92 (1)	92	<i>Ceratomyxa shasta</i>	88 (96%)	
<i>Pm</i> -QPCR	88(1)	88	<i>Parvicapsula minibicornis</i>	80 (91%)	

Case # - various (AD Steelhead – CNFH)

Sample Dates	16 DEC	30 DEC	5-JAN	11-JAN	18-JAN	24-JAN	31-JAN	16-FEB	23-FEB	Incidence
Case no.	04-170	04-174	05-004	05-006	05-008	05-012	05-016	05-023	05-025	
No. natural STT	1	2	2	1	7	2	2	2	2	21
**IHNV Female OvFl	-	-	-	-	1/15	-	-	-	-	2/33 (6%)
-Natural-	1/1	0/1	0/1	0/1	0/2	0/1	1/1	1/1	0/1	3/10 (30%)
**IHNV Male KID	NT	NT	NT	NT	1/1	NT	NT	NT	NT	3/19 (16%)
<b>ELISA RsaI Positive</b> OD > 0.3 OD = NC0.1-0.3  # QPCR confirm	NT	NT	NT	NT	0/4 0/4	NT	NT	NT	NT	5/60 (8%) 14/60 (23%)#  6/8 (75%) conf. POS
OFP-RSAL DFAT	-	-	-	NT	1/1	-	-	0/4	-	1/5 (20%)
-Natural-	1/1	1/1	1/1		2/2	1/1	1/1	0/1	0/1	7/9 (78%)
<b>BACTE</b> A. salmonicida Yersinia ruckeri Pseudomonas/ Aeromonas	NT	NT	NT	NT	0/5 0/5  0/5	NT	NT	NT	NT	0/60 (0%) 0/60 (0%)  4/60 (7%)

**Case # 05-111, 05-112 (Andorno Crk. A and North Fork Battle Creek B, NV – LCT)**

	No. SAMPLES (POOL SIZE)	No. POS/ TOTAL	(Percent Positive)	Total FISH Sampled	Comments
--	----------------------------	-------------------	-----------------------	-----------------------	----------

**VIROLOGY:**

Tissue Culture on EPC, CHSE, and FHM cell lines (IHNV, IPNV, VHSV, OMV, CTV)

A – K/S	2 (3-4p)	0/2	(0)	7	
B – K/S	7 (2-3p)	0/7	(0)	20	

**BACTERIOLOGY:**

Rs-ELISA\* (*Renibacterium salmoninarum*) Assay detects antigen only (specific P57 protein).

A – KD	7 (1p)	1/7	(14)	7	Antigen pos. only
B – KD	20 (1p)	1/20	(5)	20	Antigen pos. only

\*Highest Optical Density (OD) value at 405nm = 0.229 for Andorno Creek and 0.205 for NF Battle Creek indicating positive levels of the Rs antigen. Threshold for the assay is 2 STD above the Negative Control Tissue (NCT=0.074) or 0.079 OD. Highest three OD values confirmation tested by QPCR.

Rs-QPCR\*\* (*Renibacterium salmoninarum*) Assay detects specific bacterial DNA.

A – EP	3 (1p)	0/3	(0)	3	
B – EP	3 (1p)	3/3	(100)	3	

\*\* Corroborative testing by Quantitative Polymerase Chain Reaction confirmed one sample positive for Rs (EP – ELISA pellet)

Culturable Bacteria

A – KD	7 (1p)	0/7	(0)	7	
B – KD	20 (1p)	0/20	(0)	20	

No obligate fish pathogens (*Yersinia ruckeri* or *Aeromonas salmonicida*) were detected.

**PARASITOLOGY:**

Para-Mc-TPD – *Myxobolus cerebralis* – Assay allows microscopic identification of parasite spores.

A – Heads	1 (7p)	0/1	(0)	7	
B – Heads	4 (5p)	0/4	(0)	20	

Spores of the parasite *Myxobolus cerebralis* were not seen through microscopic examination of pepsin/trypsin digest.

Case # 05-109, 05-148 (Sagehen, Creek, NV – BKT, RBT, BNT)

	No. SAMPLES (POOL SIZE)	No. POS (p) or SUS (s)/TOTAL	(Percent Positive)	Total FISH Sampled
--	----------------------------	---------------------------------	-----------------------	-----------------------

**BACTERIOLOGY:**

Rs-ELISA (*Renibacterium salmoninarum*). Assay detects antigen only (specific P57 protein)\*.

BKT – KD	60 (1p)	s51/60	(85% sus)	60
RBT – KD	42 (1p)	p2/42	(5%)	42
BNT – KD	31 (1p)	p10/31	(32%)	31

\*Highest Optical Density (OD) value at 405nm = 0.172 indicating suspect levels of the *Rs* antigen. Threshold for the assay is 2 STD above the Negative Control Tissue OD (NCT = 0.077). Highest three OD values confirmation tested by QPCR.

Rs-QPCR (*Renibacterium salmoninarum*). Assay detects specific bacterial DNA\*\*.

BKT – EP	3 (1p)	p1/3	(33%)	3
RBT – EP	3 (1p)	p2/3	(66%)	3
BNT – EP	3 (1p)	p1/3	(33%)	3

\*\* Corroborative testing by Quantitative Polymerase Chain Reaction confirmed one sample positive for *Rs*. (EP – ELISA pellet)

## Appendix 3 –

### Partnerships

List of partners corresponds to sample sites on map

Map Site	Partners
1. Coleman NFH, CA	USFWS – CNFH
2. Sagehen Creek, CA	UC Davis
3. Fallen Leaf Lake, CA	USFWS – Reno FRO
4. Klamath River, CA	Karuk Tribe, Yurok Tribe, USFWS – Arcata FRO, USGS
5. Livingston Stone NFH, CA	USFWS – LSNFH