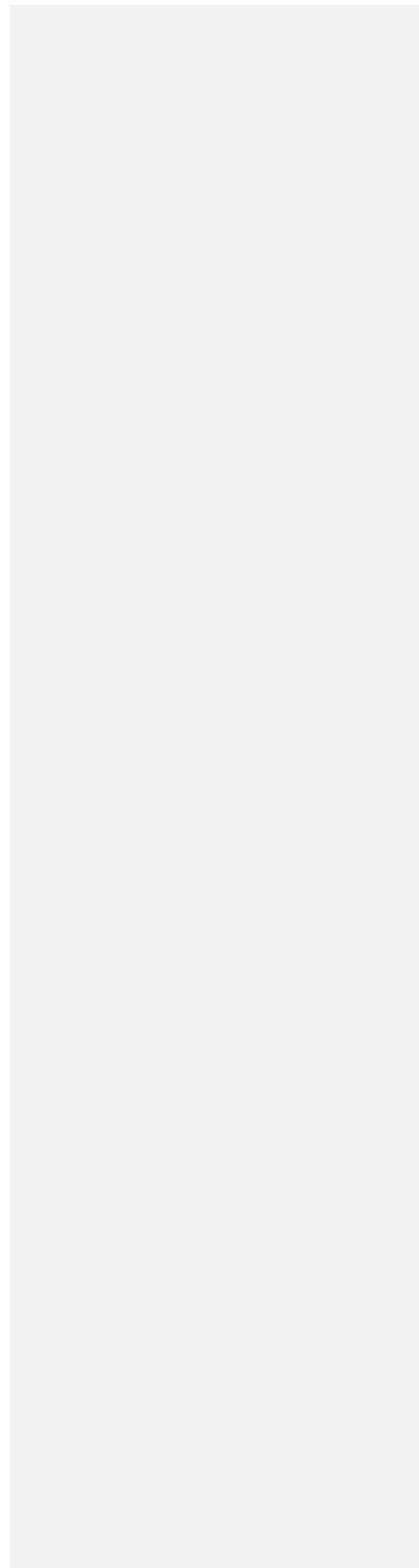


National Wild Fish Health Survey

California-Nevada
Fish Health Center

Annual Report for fiscal year 2006-2007



**National Wild Fish
Health Survey
Annual Progress Report FY 2007**
Prepared by Kimberly True
And Lisa Ratcliff

California-Nevada Fish Health Center

Center staff conducted the National Wild Fish Health Survey (NWFHS) in the 2006/2007 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.esq.montana.edu/nfhdb/>

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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.

One focus in 2006-2007 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. *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 (+7/18 for Rs antigen and 0/18 (0%) confirmed by QPCR) sampled in Sagehen Creek, CA.

In 2006-2007, 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. IGH.....

Another focus in 2006-2007 was done in collaboration with Arizona Game and Fish

In February, 2006, Samples from Rainbow and Brown trout as well as Speckled dace and Desert suckers were collected on February 28, 2006 by Jimmy Fulmer, a Wildlife technician for Arizona Game and Fish Department. Samples were collected by electro-shocking using a backpack shocker, to gain a general fish health assessment of the Black River in Arizona at UTM coordinates 12S 0642598 3728541, 12S 0643360 3730401, and 12S 0643723 3730817.

Comment [MSOffice1]: I'm not sure what to put for this sampling as an abstract.

Another focus in 2007 was done in collaboration with California Department of Fish and Game (CDFG) located in Yreka and Fortuna, California. Fish samples were taken June, 2007 from Iron Gate Reservoir and the Eel River in August, 2007. Rainbow trout (*Oncorhynchus mykiss*) and Yellow perch (*Perca flavescens*) were collected from Iron Gate Reservoir, and Pike minnow (*Ptychocheilus grandis*) and California Roach (*Hesperoleucus symmetricus*) were collected from the Eel River. Culturable bacteria, viruses, and the causative agent of whirling disease, *Myxobolus cerebralis* were not detected. Suspect levels of the *Renibacterium salmoninarum* (Rs) antigen were found in both locations and QPCR confirmed Iron Gate Reservoir positive for the presence of Rs DNA.

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 one sampling season which ended October, 2006. A second sampling season

started in April, 2007 and continued through to October, 2007. In partnership with University of California at Davis, a trawl was used to collect a target of 597 fish in 2007 and 453 fish in 2006 during 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; epithelialistis.....

Comment [MSOffice2]: Need Histo results from Scott

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 2007 marks the tenth 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 15,024 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

Ceratomyxosis (causative agent *Ceratomyxa shasta*) has been identified as the most significant disease for juvenile salmon in the Klamath Basin (Foott et al. 1999, Foott et al. 2004). *Parvicapsula minibicornis* is prevalent at nearly 100% in the main stem resulting in numerous fish found dually infected with these two myxozoans. 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. Monitoring in 2007 detected the onset of *P. minibicornis* infections in emigrating chinook smolts as early as April 15th. Prevalence quickly rose to 100% 6 weeks after the first detection on April 15th, and nearly all fish infected with *C. shasta* were also infected with *P. minibicornis*. The overall prevalence of infection (POI) in 2007 for *C. shasta* is 8% by histology and 17% by PCR. For *P. minibicornis*, POI is 28% by histology and 77% 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

Recent isolations of *Renibacterium salmoninarum*, the causative agent 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 Rainbow trout 17% (n=12), and 61% (n=18) Brown trout tested by ELISA. ELISA-positive samples from both species were confirmed for the presence of *R. salmoninarum* DNA by PCR. Infection levels, as determined by QPCR Ct values were very low, indicating low bacterial loads or asymptomatic infections. Fallen Leaf Lake was very similar to Sagehen Creek. Kokanee salmon and lake trout were tested and the *Rs* prevalence was 69% (n=45) for lake trout and all Kokanee were negative.

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 such as 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), which uses 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, 0.500-0.999 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 1mL of HBSS supplemented with antibiotics and antimycotic (200 IU mL⁻¹ penicillin G, 200 IU mL⁻¹ streptomycin, 0.5 µg mL⁻¹ amphotericin B and 40 µg mL⁻¹ 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 minutes at 15°C. Wells were covered with 0.5ml of liquid overlay which contained 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⁻¹ 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⁻¹ 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.¹

¹ National Wild Fish Health Survey Laboratory Procedures Manual, 2004

California

Pathogen Survey

Sacramento-San Joaquin Delta, CA

Add summary/results for 2006/2007

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 reidsides (*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) antigen by ELISA (Enzyme Linked Immunosorbent Assay). Mc was found in 0/58(0%) of samples and Rs antigen was found in 31/45 (69%) of LKT samples. Three samples were confirmed using PCR methodology, 1/3 (33%) were positive for Rs.

Iron Gate Reservoir, Yreka CA

Iron Gate reservoir is a large man made body of water that is fed from the mainstem portion of the Klamath River. The Iron Gate dam was built in 1962 and is the home to Iron Gate Fish hatchery located below the dam. Iron Gate Reservoir is home to an abundance of yellow perch (*Perca flavescens*), Rainbow trout (*Oncorhynchus mykiss*), large mouth bass (*Micropterus salmoides*), small mouth bass (*Micropterus dolomieu*) and blue gill (*Lepomis*

macrochirus).

At coordinates N 41 58.356, W 122 22.088, fourteen Rainbow trout (*Oncorhynchus mykiss*) and forty Yellow perch (*Perca flavescens*) were collected by Jim Whelan in collaboration with the Ca-Nv FHC. These fish were collected June 8, 2007, after which the fish were euthanized and dissected for key tissues. The fish tissue samples were then transported to the CA-Nv Fish Health



Center on ice. The Rainbow trout heads were tested for the parasite *Myxobolus* (Mc), the causative agent of Whirling Disease. The kidneys were processed for Culturable bacteria, virology testing (IHNV, VHSV, IPNV, and OMV), presence of *Renibacterium salmoninarum* (Rs) by ELISA (Enzyme Linked Immunosorbent Assay), and viable *Parvicapsula minibicornis* (Pm) trophozoites by QPCR (quantitative-Polymerase Chain Reaction) as well as intestinal tissue, which were tested for viable *Ceratomyxa shasta* (Cs) trophozoites. Mc was found in 0% of samples, Rs was found in 3/3 (100%) of samples, Pm was found in 2/14 (14.2%) of samples, and Cs was found in 42.8% of samples. There were no culturable bacteria or viruses detected in all samples.

Eel River, Fortuna CA

The Eel River is a major river system located on the northern Pacific coast of California. The Eel River is approximately 200 miles long and runs northwest, parallel to the coast. The Eel River is also home to several species of fish including Winter Steelhead (*Oncorhynchus mykiss*), Chinook salmon (*Oncorhynchus tshawytscha*), Coho salmon (*Oncorhynchus kisutch*), Pike minnow (*Ptychocheilus grandis*), and California roach (*Hesperoleucus symmetricus*).

At coordinates N 40' 16 10.08, W 123' 43 40.31, forty Pike minnow (*Ptychocheilus grandis*) and seven California Roach (*Hesperoleucus symmetricus*) were collected by CDFG fisheries biologists in collaboration with the Ca-Nv FHC. These fish were collected August 23, 2007, after which the fish were euthanized and dissected for key tissues used for testing. Kidney tissue were collected for virology testing (IHNV, VHSV, IPNV, and OMV), presence of *Renibacterium salmoninarum* (Rs) antigen by ELISA (Enzyme Linked Immunosorbent Assay), and *Parvicapsula minibicornis* (Pm) trophozoites by QPCR (quantitative-Polymerase Chain Reaction) as well as intestinal tissue, which were tested for viable *Ceratomyxa shasta* (Cs) trophozoites. Rs was found in 9/16 (56%) of samples, Pm was detected in 15/16 (94%) of samples and Cs was detected in 7/16 (44%) of samples. There were no culturable bacteria or viruses detected in all 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. In 2006, 92 samples were collected 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%). A total of 39 samples were collected in 2007 from spawned wild fish (12 males, 27 females). Thirty-nine fish were sampled for viral testing with 94% kidney samples and 62% ovarian fluid samples positive for IHNV. Out of 39 individual samples tested for various bacteria, there were no positives. Individual samples of kidney and ovarian fluid (n=39) were tested by QPCR for the presence of *Renibacterium salmoninarum* DNA. Intestinal tissues were processed and tested by QPCR for the parasites *Parvicapsula minibicornis* (0/39 positive, 0%) and *Ceratomyxa shasta* (0/39 positive, 0%).

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. In 2006, 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). In 2007, **35** natural female ovarian fluids were collected, pooled and tested for virus and *Renibacterium salmoninarum*. **Seventeen percent (17%)** of the natural population tested were positive for IHNV, and 0.33% out of **2** pools tested was positive for Rs.

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 of concern specifically for the out-migrating 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 electro-fishing from a total of **eleven sites** over the course of **eighteen weeks** (only **11** of which were known wild fish with no hatchery fish influence) during the spring and summer of 2006 and 2007. 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 euthanized 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 and histological assays.



Ceratomyxa shasta (Cs) incidence ranged from 0% in the first few weeks of the study to **47%** by the **tenth week (June 17, 2007)** (Table 1). **The incidence of infection (IOI) in fish above the Shasta River and between the Shasta and Scott River was 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.

Comment [MSOffice3]: Not sure what to put as significant IOI.

2006

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

2007

Sample Week	Date	n	Detected	%
1	15-Apr	32	0	0
2	22-Apr	33	0	0
3	29-Apr	38	7	18
4	6-May	50	0	0
5	13-May	78	18	23
6	20-May	67	17	25
7	27-May	155	20	13
8	3-Jun	149	22	15
9	10-Jun	88	20	23
10	17-Jun	95	46	48
11	24-Jun	116	21	18
12	1-Jul	58	3	5
13	8-Jul	93	12	13
14	15-Jul	30	4	13
15	22-Jul	30	6	20
16	29-Jul	21	1	5
17	5-Aug	0	0	0
18	12-Aug	30	2	7

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

Arizona

Black River, Arizona

The Black River is approximately 90 miles long and runs from the Arizona-New Mexico state line and ends near Blue, White, Big Bonita and Little Colorado Rivers. The Black River is known mostly for its excellent small mouth bass (), catfish (), rainbow trout (), native brown trout () and brook trout fishing ().

On February 28, 2006 at UTM coordinates 12S 0642598 3728541, 12S 0643360 3730401, and 12S 0643723 3730817, thirty-six Speckled dace (*Rhinichthys osculus*), forty-eight desert suckers (*Catostomus clarki*), and thirty-nine brown trout (*Salmo trutta*) and rainbow trout (*Onchorhynchus mykiss*) were collected. All fish were collected by Jimmy Fulmer, a wildlife technician for Arizona Game and Fish. The fish samples were then euthanized and tissue samples were collected and sent to Ca-Nv FHC for testing. Kidney tissue was collected for virology testing (IHNV, VHSV, IPNV, and OMV), presence of *Renibacterium salmoninarum* (Rs) antigen by ELISA (Enzyme Linked Immunosorbent Assay), and presence of culturable bacteria. Need to enter results.....

On April 5, 2006.....

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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 2007

Case #	Date Collected	Location	Species	Number of Fish	Total Fish From Site
07-002	10/18/2006	IGH	FCS	N=30	30
07-004	10/26/2006	Suisun Bay Delta Pod	LFS TFS	N=22 N=11	33
07-006	11/9/2006	IGH	FCS	N=30	30
07-007	10/27/2006	Delta Pod	LFS	N=12	12
07-043	3/28/2007	Stanislaus River	FCS	N=5	5
07-053	4/19/2007	Klamath River	FCS COHO	N=1284 N=85	1369
07-055	4/24/2007	Doudy Pond, UT	CUT	N=19	19
07-067	5/29/2007	LSNFH	WCS	N=1	1
07-069	5/31/2007	LSNFH	WCS	N=1	1
07-074	6/4/2007	LSNFH	WCS	N=1	1
07-075	6/4/2007	LSNFH	WCS	N=1	1
07-076	6/5/2007	LSNFH	WCS	N=1	1
07-077	6/5/2007	LSNFH	WCS	N=1	1
07-078	6/7/2007	LSNFH	WCS	N=1	1
07-079	6/7/2007	LSNFH	WCS	N=1	1
07-080	6/8/2007	Iron Gate Reservoir	RBT	N=14	14
07-081	6/8/2007	Iron Gate Reservoir	Yellow Perch	N=40	40
07-083	6/14/2007	LSNFH	WCS	N=1	1
07-084	6/14/2007	LSNFH	WCS	N=1	1
07-085	6/14/2007	LSNFH	WCS	N=1	1
07-088	6/18/2007	LSNFH	WCS	N=1	1
07-089	6/18/2007	LSNFH	WCS	N=1	1
07-090	6/21/2007	LSNFH	WCS	N=4	4
07-091	6/21/2007	LSNFH	WCS	N=4	4
07-092	6/25/2007	LSNFH	WCS	N=1	1
07-093	6/26/2007	LSNFH	WCS	N=1	1
07-095	6/28/2007	LSNFH	WCS	N=1	1
07-095	6/28/2007	LSNFH	WCS	N=1	1
07-096	7/2/2007	LSNFH	WCS	N=4	4
07-099	7/9/2007	LSNFH	WCS	N=2	2
07-103	7/17/2007	LSNFH	WCS	N=1	1
07-104	7/19/2007	LSNFH	WCS	N=3	2
07-105	7/26/2007	LSNFH	WCS	N=2	1
07-106	7/17/2007	Delta Pod	Not	Entered	In DB
07-108	8/1/2007	Delta Pod	Not	Entered	In DB
07-110	6/18/2007	Delta Pod	Not	Entered	In DB
07-117	8/23/2007	Eel River	Pike Minnow California Roach	N=41 N=7	48

Total Fish= 1635

NWFHS SUMMARY TABLE for FY 2006

Case #	Date Collect	Location	Species	Number of Fish	Total Fish from Site	Significant Findings
06-025	2-28-06	Black River, AZ	Speckled dace	36	36	Viral sample sent to UC Davis for further confirmation
06-026	2-28-06	Black River, AZ	Desert sucker	48	48	No pathogens detected
06-027	2-28-06	Black River, AZ	BNT RBT	16 23	39	No pathogens detected No pathogens detected
06-036	3-29-06	Delta POD	Longfin smelt	70	70	
06-040	4-05-06	Little Colorado River	BNT RBT Speckled dace	21 39 60	120	No pathogens detected No pathogens detected Yersinia ruckeri (2% Speckled dace)
06-046	5-1-06	Delta POD	Longfin smelt	96	96	
06-055	4-26-06	Delta POD	Longfin smelt	3	3	
06-062	4-27-06	Delta POD	Longfin smelt	17	17	
06-066	5-10-06	Delta POD	Longfin smelt	130	130	
06-083	5-24-06	Delta POD	Longfin smelt	70	70	
06-116	7-17-06	Delta POD	Longfin smelt	60	60	
06-134	8-29-06	Delta POD	Longfin smelt	23	23	
06-136	9-13-06	Sagehen Creek Stampede Reservoir	RBT Brown Trout	12 18	30	
Various Cases	Various Dates	Livingston Stone NFH	Winter Chinook-AD	92	92	Infectious Hematopoietic Necrosis Virus, Ceratomyxa shasta, Parvicapsula minibicornis, Renibacterium salmoninarum
06-139	Various Dates	Fallen Leaf Lake	Lake Trout Kokanee Salmon	45 13	58	LKT – Rs+ Heads pending
06-100	6-21-06	Delta POD	Longfin smelt	7	7	

Total Fish = 1318

DELTA POD SUMMARY FY2006

Case #	Date	Species	Virology Results	Histology	Total Fish/Site	Coversheet Notes / Site Information
06-036	3-29-06	LFS	0/12 (5-p) n=60		70	20mm net target size n=70 (viral, RNA/DNA, histo)
06-046	4-12-06	LFS	0/8 (10-p) n=76		96	N=96 (viral, histo) Collected by Gouhoa (UCD)
06-055	4-26-06	LFS	0/1 (2-p) n=2		3	Scott and Ron collected: n=3 and then net broke Site 340 Napa River
06-062	4-27-06	LFS	0/11 (5-6-p) n=11		17	Guohus collected and sent at 9d old (arrived lab 5/5/06): Site 336 (n=11: 1,5-p viral) Site 328 (n=6: 1,6-p viral)
06-066	5-10-06	LFS	0/16 (5-p) n=80		130	Collected by Gouhua
06-083	5-24-06	LFS	0/12 (5-p) n=60		70	Collected by Gouhua n=70 (10 histo, 60 viral)
06-100	6-21-06	LFS	0/7 (1-4-p) n=7		7	Changed to summer tow-net gear, therefore fewer numbers overall. No histo collected. Site 335 (n=1: viral 1-p) Site 341 (n=4: viral 4-p) Site 341 (n=2: viral 2-p)
06-116	7-18-06	LFS	0/12 (5-p) n=60		60	SF collected: Viral 60; Histo 26,1-p (No site info or histo tube id on WFS coversheet)

Appendix 2 – Sample summary report tables

General Methodology

Kidney, spleen, and intestine were dissected from live fish and tested for Bacterial Kidney Disease (*Renibacterium salmoninarum*), *Parvicapsula minibicornis*, and *Ceratomyxa shasta*, respectively. Kidney was also dissected and tested for IHNV, IPNV, OMV, and CTV. A kidney swab was dissected and cultured for *Yersinia ruckeri*, *Aeromonas salmonicida*, and *Aeromonas hydrophila*. Cranial elements were collected and tested for presence of Whirling disease (*Myxobolus cerebralis*).

BACTERIOLOGY: Incidence of bacteria including *Yersinia ruckeri*, *Aeromonas salmonicida*, *Aeromonas hydrophila*, by culture on Brain Heart infusion agar (BHIA) of individual kidney (KD) samples. Pure colonies isolated and biochemical tests performed; confirmation by IFAT.

Rs-ELISA:

Kidney tissues were tested for *Renibacterium salmoninarum* by Enzyme Linked Immunosorbent Assay (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 loaded onto 96-well plates and assayed for the presence of *Renibacterium salmoninarum* antigen using specific Rs antibodies from Kirkegaard and Perry Laboratories (KPL). Rs specific antibodies were used at concentrations of 1:1000 (unlabelled coating antibody) and 1:1500 dilutions (secondary Horse-radish Peroxidase labeled antibody). Test samples with ELISA Optical Density (OD) values greater than 2 standard deviations above the negative reference control, and up to 0.499, were defined as low level antigen levels; 0.500-.999 moderate; and OD values of 1.00 or higher were considered high antigen levels. Corroborative testing of the three highest positive test samples was done by Polymerase Chain Reaction (PCR) to confirm the presence of Rs (viable bacterial) cells*.

Rs PCR:

For PCR, oligonucleotide primers and Taqman fluorogenic probe specific for the 57 kDa protein of Rs (Chase 1998) were utilized in a quantitative PCR assay (Applied Biosystems Sequence Detection System 7300). Total DNA was extracted from kidney tissue with Qiagen DNAeasy kit. 5ul of extracted DNA was added to 25ul of Rs Mastermix containing PCR buffer, primers, probe, nucleotides and molecular grade water. DNA was amplified for 40 rounds and samples above background assay thresholds were analyzed with Sequence Detection software to determine the test status of each sample.

VIROLOGY: Incidence of infection for major fish viruses in 1 to 5-fish pools of kidney (KID) and/or spleen tissue (K/S) tissue. Viruses include Infectious Hematopoietic Necrosis Virus (IHNV); Infectious Pancreatic Necrosis Virus (IPNV); Viral Hemorrhagic Septicemia Virus (VHSV); Oncorhynchus Masou Virus (OMV) and Cutthroat Trout Virus (CTV). Tissue homogenates are inoculated on species-specific cell lines (EPC and CHSE), incubated at 15°C for 21 days. Cell cultures were observed for viral cytopathic effects (CPE), blind passed and incubated for an additional 14 days.

Mc Pepsin-Trypsin Digest (PTD):

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 (Thoesen 1994). Sampled salmonids were grouped into pools of 5 fish or less, depending on the total sample size. The heads were heated in a 60°C water bath for 60 minutes, to remove soft flesh from bone and cartilage tissues. These cranial elements were then ground in a blender and placed in a pepsin solution of 20 mL g⁻¹ of tissue, and incubated at 37°C for 40-60 minutes, depending on sample size. The samples were centrifuged, supernatant removed, and the pellet digested in a solution of trypsin at 20 mL g⁻¹ of tissue. Samples were incubated at room temperature on a rocker plate for 30 m. The larger bone fragments were filtered and the samples were centrifuged a final time to concentrate spores, if present. Samples were examined by phase contrast microscopy at 200-400x.

Parasitology: Incidence of parasites, *Parvicapsula minibicornis* (Pm) and *Ceratomyxa shasta* (Cs) by Quantitative Polymerase Chain Reaction (QPCR) of kidney tissue and intestine. Oligonucleotide primers and TaqMan fluorogenic probe specific for the 18s ribosomal subunit of Pm and Cs were utilized in 7300 Sequence Detection System (Applied Biosystems). Total DNA was extracted from tissues with Qiagen DNeasy kit. 5ul of extracted DNA in 25ul of Master mix was amplified for 40 rounds and samples above background assay thresholds were analyzed with Sequence Detection software to determine the test status of each sample.

Case – various (AD WCS – LSNFH) 2007

	# Samples (pool size)	Total Fish	Results	# Positive (% Positive)	Notes
Virus					
Tissue culture (Kd)	30(1)	39	IHNV	37(94%)	
Tissue culture (OF)	2(5-4)p 27(1)	27	IHNV	26(96%)	
Bacteria					
BHIA culture (Kd)	39 (1)	39	<i>Aeromonas/Pseudomonas</i> <i>Aeromonas salmonicida</i> <i>Yersinia ruckeri</i>	0 (0%) 0 (0%) 0 (0%)*	
<i>Rs</i> -QPCR (Kd-male)	12 (1)	12	<i>Renibacterium salmoninarum</i>	0 (0%)	
<i>Rs</i> -QPCR (Kd-female)	27 (1)	27	<i>Renibacterium salmoninarum</i>	0(0%)	
Parasites					
<i>Cs</i> -QPCR	39 (1)	39	<i>Ceratomyxa shasta</i>	0 (0%)	
<i>Pm</i> -QPCR	39(1)	39	<i>Parvicapsula minibicornis</i>	0 (0%)	

Case – various (AD WCS – LSNFH) 2006

	# Samples (pool size)	Total Fish	Results	# Positive (% Positive)	Notes
Virus					
Tissue culture (Kd)	33(1)	33	IHNV	21(64%)	
Tissue culture (OF)	51(1)	51	IHNV	38 (73%)	
Bacteria					
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%)	
Parasites					
<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) 2007

Sample Dates	12/21/06	12/28/06	01/04/07	1/11/07	1/17/07	1/25/07	02/16/07	Total # of Fish	Incidence
Case no.	07-013	07-015	07-017	07-021	07-022	07-025	07-030		
**IHNV Female OvFl	4(3)p	6(3)p	6(3)p	13(3)p	3(3)p	8(3)p	0	120	
-Natural-	0	1(1)p	0	0	1(1)p	0	4(1)p	6	
IHNV Male KID	4(3)p 1(2)p	2(3)p	1(3)p	4(3)p	0	1(3)p	0	38	
OvFl Pellet- <i>R.sal</i>	9(3)p	5(3)p	6(3)p	10(3)p	3(3)p	0	4(3)p	111	2/111 (0.2%)
#PCR confirmed					2 poss.				2/6 (0.33%)
Natural- <i>Rsal</i> DFAT	0	1(1)P	0	0	1(1)P	0	0	2	
BACTE									
<i>A. salmonicida</i>	0/13	NT	0/4	1/12	NT	0/5	NT	34	1/34 (3%)
<i>Yersinia ruckeri</i>	0/13		0/4	0/12		0/5		34	0/34 (0%)
<i>Pseudomonas/</i> <i>Aeromonas</i>	0/13		2/4	1/12		0/5		34	3/34 (9%)

Case # - various (AD Steelhead – CNFH) 2006

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%)
ELISA R_{sal} Positive 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%)
BACTE <i>A. salmonicida</i> <i>Yersinia ruckeri</i> <i>Pseudomonas/</i> <i>Aeromonas</i>	NT	NT	NT	NT	0/5 0/5 0/5	NT	NT	NT	NT	0/60 (0%) 0/60 (0%) 4/60 (7%)

Case # 07-080, 07-081 (Iron Gate Reservoir, Yreka CA-RBT and YP)

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

Culturable bacteria on BHIA pure slants:

RBT-KD	14(1p)	0/14	(0)	14
YP-KD	40(1P)	0/40	(0)	40

***Renibacterium salmoninarum*:**

ELISA (*Renibacterium salmoninarum*) - Detects bacterial antigen only (specific P57 protein).

*Confirmation by PCR is required for Rs-positive test status.

RBT-KD	3(4&5P)	3/3	(100)	14
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Highest Optical Density (OD) value at 405nm = 2.697 indicating high levels of the Rs antigen. Threshold for the assay is 2 STD above the Negative Control Tissue OD (NCT =0.071). Samples with the five highest OD values were submitted for confirmation testing by QPCR.

Rs-PCR - Detects Rs DNA, confirming presence of viable Rs bacterial cells in kidney tissue.

RBT-KD	3(1p)	1/3	(33)	3
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VIROLOGY:

Cell culture –specific cell lines used; EPC and CHSE-214

RBT-K/S	3 (4&5p)	0-3	(0)	14
YP-K/S	8(5p)	0/8	(0)	40

PARASITOLOGY:

Mc-PTD –Screening test for presence of non-specific Myxoboloid spore. Further testing by PCR is required to confirm the species identify of observed spores as *Myxobolus cerebralis*.

RBT-HD	3 (4&5p)	0/3	(0)	14
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Pm QPCR - Detects Pm 18s DNA, presumably viable *Parvicapsula minibicornis* trophozoites in kidney tissue

RBT-KD	14(1p)	2/14	(4.2)	14
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Cs QPCR - Detects Cs 18 s DNA, presumably viable *Ceratomyxa shasta* trophozoites in intestine

RBT-IT	14(1p)	6/14	(42.8)	14
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HISTOLOGY:

Microscopic examination of kidney and intestine fixed in Davidson's and stained with Hematoxylin and eosin, to test for the presence of *Parvicapsula minibicornis* and *Ceratomyxa shasta*.

RBT-KD	14(1p)	0/14	(0)	14
RBT-IT	14(1p)	4/14	(30.8)	14
YP-KD	5(1P)	0/5	(0)	5
YP-IT	5(1P)	0/5	(0)	5

Case # 07-117-A, 07-117-B (Eel River, Fortuna CA– Pikeminnow and CaR)

	No. SAMPLES (POOL SIZE)	No. POS (p) or SUS (s)/TOTAL	(Percent Total FISH Positive)	Sampled
BACTERIOLOGY: Culturable bacteria on BHIA pure slants:				
Pikeminnow– KD	40 (1p)	0/40	Negative	40
Ca Roach – KD	7(1p)	0/7	Negative	7

***Renibacterium salmoninarum* (Bacterial Kidney Disease):**

Rs-ELISA - Detects bacterial antigen only (P57 protein metabolite). *Confirmation by PCR is required for Rs-positive test status.

Pikeminnow– KD	16 (1p)	9/16	(56%)	16
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Highest Optical Density (OD) value at 405nm = 2.697 indicating high levels of the Rs antigen. Threshold for the assay is 2 STD above the Negative Control Tissue OD (NCT =0.071). Samples with the five highest OD values were submitted for confirmation of viable bacteria by QPCR.

Rs-QPCR - Detects Rs DNA, confirming presence of viable Rs cells in fish kidney tissue.

Pikeminnow– KD	5(1p)	0/5	Negative	5
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VIROLOGY: Cell culture specific cell lines used: EPC and CHSE-214

Pikeminnow– K/S	9 (2, 3 & 5p)	0/9	Negative	40
Ca Roach– K/S	2 (2 & 5p)	0/2	Negative	7

PARASITOLOGY:

Pm QPCR - Detects Pm 18s DNA, presumably viable *Parvicapsula minicorbis* trophozoites in kidney tissue. Only Pikeminnow were tested for this parasite.

Pikeminnow-KD	16(1P)	15/16	94%	16
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Cs QPCR - Detects Cs 18 s DNA, presumably viable *Ceratomyxa shasta* trophozoites in intestinal tissue. Only Pikeminnow were tested for this parasite.

Pikeminnow-IT	16(1P)	7/16	44%	16
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Case #06-139, (Fallen Leaf Lake, CA LKT -KOK)

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

Rs-ELISA (*Renibacterium salmoninarum*) - Detects bacterial antigen only (specific P57 protein).

*Confirmation by PCR is required for Rs-positive test status.

LKT – KD	45 (1 p)	31/45	(69%)	45
KOK-KD	13 (1 p)	0/13	Negative	13

Highest Optical Density (OD) value at 405nm = 0.412 indicating low levels of the Rs antigen. Threshold for the assay is 2 STD above the Negative Control Tissue OD (NCT = 0.069). Samples with the three highest OD values were submitted for confirmation testing by QPCR.

*Rs-PCR - Detects Rs DNA, confirming presence of Rs bacterial cells in kidney tissue.

LKT – KD	3 (1 p)	1/3	(33% of 3 samples tested)
KOK-KD	Negative by ELISA screening method, not tested by PCR		

PARASITOLOGY:

Mc-PTD - Screening test for presence of non-specific Myxobolus spores. Further testing by PCR is required to confirm the species identity of observed spores as *Myxobolus cerebralis*.

LKT – KD	9 (5 p)	0/9	(0%)	45
KOK-KD	3 (5 p)	0/3	(0%)	13

Case #06-136 (Saghen Creek, CA RBT-BNT)

No. SAMPLES	No. POS (POOL SIZE)	(Percent /TOTAL Positive)	Total FISH Sampled
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BACTERIOLOGY:

Rs-ELISA (*Renibacterium salmoninarum*) – Detects bacterial antigen only (P57 protein).

* Confirmation by PCR is required for Rs positive test result.

RBT – KD	12 (1p)	2/12	(17%)	12
BNT – KD	18 (1p)	11/18	(61%)	18

Highest OD value at 405nm = 0.364 indicating low levels of the Rs antigen. Threshold for the assay is 2 STD above the Negative Control Tissue OD (NCT = 0.077).

* Rs-QPCR: Detects Rs DNA, confirming presence of Rs cells in fish kidney tissue.

RBT – KD	3 (1p)	0/2	Negative	
BNT-KD	3 (1p)	0/3	Negative	

PARASITOLOGY:

Para-Mc-TPD – Screening test for presence of Myxobolus species spores. Further testing by QPCR is required to confirm the identity of any spore as *Myxobolus cerebralis*.

RBT – Heads	3 (2-5p)	0/3	Negative	12
BNT – Heads	4 (3-5p)	0/4	Negative	18

Case #06-025,026, 027 (Black River, CA SDC-DSK-BNT/RBT)

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

Culturable bacteria on BHIA pure slants:

SDC – KD	11 (1 p)	0/11	(0)	11
DSK – KD	48 (1 p)	0/48	(0)	48
BNT/RBT – KD	39 (1 p)	0/39	(0)	39

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

BNT/RBT – KD	39 (1 p)	p3/39	(8%)	39
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*Highest Optical Density (OD) value at 405nm = 0.580 indicating moderate levels of the *Rs* antigen. Threshold for the assay is 2 STD above the Negative Control Tissue OD (NCT = 0.0716). Highest three OD values sent to confirmation testing by QPCR. **QPCR results confirmed all samples were negative for *Rs* DNA.**

VIROLOGY:

Cell culture – species specific cell lines used; EPC and CHSE-214

*SDC – K/S	8 (1 & 5p)	1s/8	(SUSPECT)	36
DSK – K/S	9 (3 & 5p)	0/9	(0)	48
BNT/RBT – K/S	8 (4 & 5p)	0/8	(0)	39

***Cytopathic effects (CPE) observed on EPC cell line, indicative of the class of Reovirus. Samples submitted to UC Davis for further confirmation. EM was inconclusive and further tests are being performed.**

Case #06-040 (Black River, CA SDC-BNT-RBT)

No. SAMPLES	No. POS (p) (POOL SIZE)	(Percent TOTAL Positive)	Total FISH Sampled
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BACTERIOLOGY:

Culturable bacteria on BHIA pure slants:

SDC – KD	60 (1 p)	p1/60 <i>Y.ruck</i>	(2)	60
BNT – KD	21 (1 p)	0/21	(0)	21
RBT – KD	39 (1 p)	0/39	(0)	39

Indirect Fluorescent Antibody Technique (IFAT) confirmed one culturable bacteria **positive** for *Yersinia ruckeri*.

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

BNT – KD	21 (1 p)	s20/21	(0)	21
RBT – KD	39 (1 p)	s30/39	(0)	39

*Highest Optical Density (OD) value at 405nm = 0.580 indicating moderate levels of the Rs antigen. Threshold for the assay is 2 STD above the Negative Control Tissue OD (NCT = 0.0716). Highest three OD values sent to confirmation testing by QPCR. **QPCR results confirmed one BNT sample positive for the presence of Rs DNA and all RBT samples negative for the presence of Rs DNA.**

VIROLOGY:

Cell culture – species specific cell lines used; EPC and CHSE-214

SDC – K/S	12 (5p)	0/12	(0)	60
BNT – K/S	5 (4 & 5p)	0/5	(0)	21
RBT – K/S	7 (5p)	0/7	(0)	39

Appendix 3 –

Partnerships

List of partners corresponds to sample sites on map

Map Site	Partners
1. Coleman NFH, CA	USFWS – CNFH
2. Iron Gate Reservoir, CA	CDFG-Yreka CA
3. Eel River, CA	CDFG – Fortuna CA
4. Klamath River, CA	Karuk Tribe, Yurok Tribe, USFWS – Arcata FRO, USGS
5. Livingston Stone NFH, CA	USFWS – LSNFH
6.	