

National Wild Fish Health Survey

California-Nevada
Fish Health Center

Annual Report for fiscal year 2005



**National Wild Fish
Health Survey
Annual Progress Report FY 2005**
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California-Nevada Fish Health Center

Center staff conducted the National Wild Fish Health Survey (NWFHS) in the 2004/2005 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/>

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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.

In 2004-2005, 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), digenetic trematode (presumptive *Nanophyetus salmincola*) 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 2004-2005 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.

Fish health examinations conducted in Upper Klamath lake for the second year in a row, on non-salmonid species including Tui chub (*Gila bicolor*) and Fathead minnow (*Pimephles promelus*), tested negative for all fish NWFHS pathogens.

Hat creek's wild trout section is one of the most sought after fly-fishing locations in Northern California. Partnering with California Department of Fish and Game the Center had the opportunity to sample wild rainbow and brown trout as well as sacramento suckers. All samples were negative for virus and culturable bacteria. The parasite *M. cerebralis* was not seen upon microscopic examination and is therefore negative. Many ELISA suspect and one ELISA positive samples were sent to confirm for the presence of *Renibacterium salmoninarum* by QPCR where 8/9 RBT and 1/4 BNT confirmed positive.

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.

Additional monitoring for 2004-2005 included continued survey work in the Yuba watershed to assess the strain and temporal movement, or “viral traffic”, of IHNV in returning Fall Chinook adults. Carcass surveys conducted in November 2004 detected IHN virus incidence of 28% (n=44), compared to previously testing; 61% in 2003.

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 2005 marks the eighth 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 12,180 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 (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 2005 detected the onset of *P. minibicornis* infections in emigrating chinook smolts as early as March 16th. Prevalence quickly rose to 100% 6 weeks after the first detection (Apr 28th) therefore nearly all fish infected with *C. shasta* were also infected with *P. minibicornis*. The overall prevalence of infection (POI) in 2005 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

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).

Ongoing Monitoring and Applied Research

The health of the Upper Klamath Lake in Klamath Falls, Oregon has long been a controversial issue. This sampling year marked the second consecutive year the Center has partnered with USGS Klamath Falls to assess the health status of common fish species. While Upper Klamath Lake is crucial habitat to the endangered Klamath Lake suckers, lethal sampling is avoided due to low population numbers. However, we can achieve an accurate window into the health of the environment and these sensitive species by sampling co-inhabiting species with similar susceptibility to disease such as Tui chubs and fathead minnows.

In the fall of 2003, carcass surveys were performed in an effort to identify the presence and specific strains of Infectious Hematopoietic Necrosis Virus (IHNV) in the Feather and Yuba River watersheds. Dr. Ron Hedrick of UC Davis positively identified the viral strains from this sampling effort as unique to the Yuba River drainage and follow-up efforts were continued in November of 2004. Assessing IHNV in natural populations provides a broader content to assess disease interactions between hatchery and wild stocks, and helps us understand “viral traffic”; the movement of specific strains from one watershed to another.

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⁻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 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⁻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

Infectious Hematopoietic Necrosis Virus in Juvenile and Adult Fall Chinook

Yuba River, CA

A vital tributary to the Sacramento River system, the Yuba River provides essential habitat for native runs of chinook salmon and steelhead. Spring-run chinook (SCS) and steelhead are federally-listed as threatened. Large scale feasibility studies are underway to determine the effects of removing Daguerre Dam on naturally reproducing fish populations in this watershed. Baseline data on the distribution and disease impacts on natural populations is needed to fully assess management alterations for this watershed.

In a continued effort in 2004, fall chinook (FCS) adults were tested to determine the incidence and specific strain of IHNV in naturally reproducing fish populations. Kidney tissues were collected from fresh mortalities collected in annual carcass surveys performed by Jones and Stokes.

Ca-Nv FHC collected fish tissues during peak returns, on November 16, 2004. Fish were sampled from the mid Rose Bar reach to upper Park's Bar reach. Kidneys were accessed through a lateral cut on the side of relatively un-



decomposed fish. Samples were taken by stabbing the aseptic kidney with a cotton swab, which was submerged in a tube of antibiotic solution and kept cold until laboratory processing. The fish were examined for viral fish pathogens and IHNV was detected in 61% of the 44 fish tested. To compare to hatchery incidence of IHNV, historical data from the past 10 years was analyzed for FCS adults returning to CNFH on Battle Creek in Northern California. This data shows a mean incidence of IHNV at 55.5% for the period 1993 – 2003, in mixed samples of male and female tissues tested during routine fish health exams of returning FCS adults collected at CNFH.

Viral isolates from the Yuba River samples were sent to Ron Hedrick of UC Davis to perform viral strain typing in an effort to monitor the presence and movement of IHNV isolates that are unique to this drainage. Results from a two-year study of juvenile and adult disease monitoring can be found in the draft report, Fish Health Monitoring of Fall Chinook and Steelhead in the Yuba and Feather Rivers (2002-2003) (True, 2004, USFWS).

***Renibacterium salmoninarum* in LCT recovery waters**

Renibacterium salmoninarum (Rs) is an obligate fish pathogen considered nearly ubiquitous and infecting over 13 species of trout and salmon, both cultured and wild in North America, Europe, Chile and Japan. The bacteria can be transmitted both vertically (through the egg) and horizontally, and infections can occur at any life stage in a salmonid population. Generally clinical signs of disease are less common in fish less than six months old, and chronic forms of the disease are more common than acute and sub-acute disease. Bacterial kidney disease occurs over a wide range of temperatures, can be influenced by fish culture practices and stress, and can exist in a quiescent (carrier) state. Mortality has been reported at water temperatures between 4-20°C, with disease progressing rapidly at temperatures of 15-20°C. Infected salmonid populations are believed to be the principal reservoir of infection.

There has been an increased interest by partners to sample and define the incidence of Rs in threatened wild trout populations, especially those in critical habitat for recovery of the Lahontan cutthroat trout (LCT).

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*).

On July 5, 2005 Virginia Boucher of UC Davis collected Brook trout by electro-fishing at coordinates W39.433, N120.2558. A total of 60 kidney tissues were submitted to the Center for pathogen testing in July, 2005. One of three ELISA-positive samples was confirmed positive by QPCR (+1/60, 1.6%). Additionally, cranial samples from 42 RBT and 31 BNT were collected between July 5 and September 16, 2005 and sent to the center. All samples were negative by microscopic examination for the myxosporidian parasite, *Myxobolus cerebralis*. Further sampling of Sagehen Creek and additional trout waters in the Tahoe basin is expected to continue in the coming year.

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 it's 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, three mackinaws and one kokanee were collected

and frozen on dry ice August 8-9, 2005. Two additional mackinaws were collected and frozen September 29, 2005 and all were sent to the Center where kidneys were processed for detection of *R. salmoninarum*. One of six samples tested positive by ELISA and two had suspect levels of the *Rs* antigen. One of three samples sent to QPCR confirmed positive (+1/6, 17%).

Hat Creek – Wild trout section

Thirty-four years ago the California Department of Fish and Game implemented and has managed a Wild Trout Program for the purposes of providing quality angling experiences and maintaining healthy wild trout populations. In 1998 the California Heritage Trout program was launched as part of the Wild Trout Program with an emphasis on restoration and conservation. Hat Creek has been designated Heritage trout waters because of its location within the historic range of the native rainbow trout. Hat Creek is located in northern California's Shasta County, originating in Lassen Volcanic National Park and flowing north to its end in Lake Britton. The lower 3.5 miles of this creek are reserved for wild trout, more specifically native rainbow and brown trout. This section of Hat Creek has long had a reputation with anglers for being challenging and therefore sees a lot of angling traffic. (Knutson, 2001)

The Center's interest in sampling these fish for major fish pathogens had a specific interest in the occurrence of *Myxobolus cerebralis*, the parasite that causes whirling disease. This watershed is in close proximity to historically positive drainages such as Battle Creek. While Hat Creek itself has not had an historical incidence of whirling disease, regional biologists were interested in performing a fish health evaluation. A total of 30 rainbow trout and 4 brown trout were sampled for all major fish pathogens. Thirty sacramento suckers were also tested for viruses only. All viral and culturable bacteria were negative. Presence of the myxosporidian parasite, *M. cerebralis*, in the salmonids was negative. One kidney in particular was noted upon field examination as abnormally swollen and therefore was tested directly by QPCR. This sample tested positive for *Renibacterium salmoninarum*. Additionally, eight suspect and one positive rainbow trout samples from ELISA were sent to QPCR as well for confirmation and 7 confirmed positive for the presence of *Rs* (+7/30, 23%). One BNT sample confirmed positive by QPCR (+1/4, 25%).

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 mainstem 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 103 samples were taken in 2005 from spawned wild fish (79 males, 24 females). Seventy fish were sampled for viral testing with 33% kidney samples and 43% ovarian fluid samples positive for IHNV. Out of 52 individual samples tested for various bacteria, 7 (13%) were positive for *Aeromonas salmonicida*. Individual samples of kidney (n=79) and ovarian fluid (n=24) were tested by QPCR for the presence of *Renibacterium salmoninarum* DNA. Intestinal tissues were processed for histological examination. *Ceratomyxa shasta* parasites were found in 15 out of 22 samples (68%) observed.

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 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 2005. 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.

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

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

Ceratomyxa shasta 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 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.

Nevada

Restoration

Lahontan Cutthroat Trout

In the Northern Nevada lakes Pyramid, Walker and Tahoe, Lahontan cutthroat trout (LCT) populations were abundant into the mid 1880's until settlement increased. Naturally, this resulted in a significant decrease in habitat and a drastic increase in harvest operations. Water quality decreased from agricultural and industrial pollution and riparian zones were compromised by improper grazing practices. In addition, the introduction of exotic fish that could out-compete the LCT for food and habitat drove their numbers down even more. The LCT was listed as endangered in 1970 and reclassified in 1975 as a threatened species.

Restoration of the threatened LCT is a high priority for the State of Nevada Division of Fish and Wildlife (NDOW) and the U.S. Fish and Wildlife Service. Remnant populations of this distinct genetic strain are used to implement enhancement programs that utilize the best-suited waters for re-introduction and protection of this native species.

Fish health information provides knowledge about the current health status of these populations to help ensure their success, but it also prevents the spread of disease to new locations if pathogens are detected in the existing brood stock populations. Several watersheds containing Lahontan cutthroat and other trout species were sampled for all major fish pathogens. An emphasis was placed on sampling for *Myxobolus cerebralis*, the parasite that causes Whirling Disease, because of the endemism of this pathogen in Nevada waters. The success of these creeks is important to recovery efforts as it will allow greater genetic diversity among the entire existing population of LCT's and thus more adaptable fish to changing environmental conditions. All samples were collected by Nevada Division of Wildlife biologists by electro-shocking and held on ice until complete necropsy could be performed.



Andorno Creek

Andorno Creek is a 5.7 mile long creek that is fed primarily by springs and snow melt at an elevation of 7,800 feet in the Santa Rosa Range, Humboldt County. The lower section flows are influenced by a pipeline that removes most surface flows and diverts them to a small reservoir. The current resident LCT population was introduced in 1999 from Washburn Creek (Jenne, 2005). A total of 7 samples were collected by NDOW Fisheries biologist Alan Jenne on July 12, 2005 at coordinates 41.4387, 117.739. Some of these LCT's had swollen internal organs but results were negative for all major fish pathogens tested for under the NWFHS.

North Fork Battle Creek

The largest tributary stream to Battle Creek, the North Fork starts at an elevation of 7,520 feet in the Black Rock Range, Humboldt county. A population survey was performed three years after the stream was stocked with 50 LCT's in 2002 and found an average of 79.2 fish per mile throughout 1.9 miles of habitat. Twenty fish were captured on July 12, 2005 by NDOW Fisheries biologist Alan Jenne and held on ice until sampled. There were 9 females and 11 males. Fish were negative for all viruses and parasites sampled for. Three out three samples that were positive or suspect by ELISA and sent to QPCR for confirmation of *Rs*, were confirmed positive for the bacterium DNA (3/20, 15%). The five largest fish were frozen on dry ice and passed back to NDOW for mercury testing.

Santa Fe Creek

A number of surveys have been performed on Sante Fe Creek found in the Toiyabe Range, Lander County. The first population survey performed in 1954 found an average of 482.5 fish per mile while the second survey in 1980 found, in the 2.1 miles of occupied stream, a population of 897.6 fish per mile. The most recent survey in 1997 estimated a population of 1,056 per mile with at least four age classes. Historical records estimate this strain to be genetically pure and recent genetic analysis has confirmed them to be most closely related to the Reese River Strain of LCT. This is useful information as this population has been used for reintroduction in the past and can now confidently be used for recovery efforts in the Reese River Subbasin (Elliot, 1997 and 2005). A total of 29 LCT's were collected at coordinates 39.2509, 117.074 and frozen on dry ice by John Elliot of NDOW on July 13, 2005. Samples were picked up and processed in the laboratory July 14, 2005. All viruses and parasites tested for were negative by the NWFHS procedures. Testing for the bacteria *Renibacterium salmoninarum* confirmed one positive by QPCR (1/29, 3.4%).

Bonneville Cutthroat Trout

Bonneville cutthroat trout face many of the same challenges that LCT's face. They are often subject to water diversions, habitat damage from livestock grazing and competition from non-native trout species that are still being stocked. The BCT has many advocates for it's listing under the endangered species act but has yet to be listed. Despite this, the Partners for Fish and Wildlife under the US Fish and Wildlife Service have in place a program with private landowners to restore BCT habitat in Eastern Nevada. Two creeks supporting BCT populations were sampled for all major fish pathogens under the NWFHS. Chris Crookshanks of NDOW collected BCT's in two Eastern Nevada Streams by method of electroshocking.

Hendry's Creek

Hendry's creek is found in far eastern Nevada in the North Snake Range and is considered the only confirmed remnant population of BCT in Nevada (Crookshanks, 2005). This creek contains approximately 7 miles of habitat, of which all is occupied by BCT at an estimated average density of 1,078 fish per mile (1999). Hendry's Creek BCT's have been used for reintroduction in many creeks. A total of 37 BCT's were collected from Hendry's Creek on July 12, 2005 and kept cold on ice until sampled. The fish appeared very healthy upon external examination and were negative for all viruses and parasites assayed for. QPCR confirmed only 1 sample positive (1/37, 3%) for *Renibacterium salmoninarum*.

Mill Creek

Mill Creek is also found in far eastern Nevada, although in the South Snake Range, and is considered genetically divergent from Hendry's Creek as well as other creeks close in proximity. While this creek is smaller in size than Hendry's with 1.6 miles of habitat, and densities are lower at 300-400 fish per mile, Mill Creek still offers donor populations for reintroduction. This population has been found to have no introgression from non-native salmonids (Crookshanks, 2005). A total of 37 fish were collected on July 13, 2005 from Mill creek and held on ice until sampled. The fish appeared fairly healthy externally although fins were a bit more eroded than those in Hendry's and stomach contents significantly less. No viruses or parasites were detected that were tested for, however, three samples sent from ELISA were confirmed positive for *Renibacterium salmoninarum* by QPCR (3/37, 8%).

Oregon

Pathogen Survey

Upper Klamath Lake, OR

Upper Klamath Lake has long been a contention between agricultural and environmental groups vying for limited water supplies. Currently, Upper Klamath Lake is home to two species of endangered suckers, the Shortnose Sucker (*Chasmistes brevirostris*) and Lost River Sucker (*Deltistes luxatus*) which are the focus of several ongoing research projects. For the second year in a row the Center partnered with USGS in August of 2005, to perform a fish health assessment on two large populated species in Klamath Lake, the Tui Chub (*Gila bicolor*) and Fathead minnow (*Pimephales promelus*). Ideally, fish health assessments of these populations may provide a window into the health of the environment *and* the shared habitat of endangered suckers.

The conditions of the lake are extreme. In a collaborative research effort between the Center and USGS-Klamath Lake in the summer months of 2004, pH levels averaged 9.5 and dissolved oxygen levels dropped to less than 2 ppm following an algal bloom crash (Foott, 2004). Continued surveying under the NWFHS may be helpful in determining what kind of health repercussions these extreme environmental conditions pose.

A total of 45 Tui chubs and 60 Fathead minnows were sampled from one sample site in the north lake. Fish were collected using modified fyke nets and held in buckets until Center staff could process. All fish sampled were negative for major viral and bacterial fish pathogens under the NWFHS procedures.

Bibliography

Crookshanks, Chris 2005. Nevada Division of Wildlife. Personal communication

Elliot, John 1997. *1997 Field Trip Report*. Nevada Division of Wildlife

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.

Jenne, Alan. 2005. Nevada Division of Wildlife. Personal communication.

Knutson, C. 2001. California's Wild Trout Program. Tracks. Lorna Bernard, editor. Spring 2001. California Department of Fish and Game, Sacramento, CA. pp.8-10

Hedrick, R.P., A Plan for Basin-wide Research on Infectious Hematopoietic Necrosis Virus (IHNV) in Salmon and Trout in California. Progress Report June 2004, UC-Davis

National Wild Fish Health Survey Laboratory Procedures Manual. Kimberly True, editor. 2nd Ed. June, 2004. United States Fish and Wildlife Service. Division of Fish Hatcheries. Washington, D.C. 321 pp.

True, K. 2004. Fish Health Monitoring of Fall Chinook and Steelhead in the Yuba and Feather Rivers (2002-2003). Draft Report April 2004, USFWS.

Appendix I - NWFHS SUMMARY TABLE for FY 2005

Case #	Date Collect	Location	Species	Number of Fish	Total Fish from Site
04-167	11/16/04	Yuba River	FCS (carcass)	44	44
various	12/14/04 -2/23/05	CNFH	STT AD	19	19
various	4/27/05- 7/19/05	LSNFH	WCS-AD	113	113
various	3/9/05 - 5/11/05	Klamath River	FCS	803	803
05-111	7/12/05	Andorno Creek, NV	LCT	7	7
05-112	7/12/05	N.F. Battle Creek, NV	LCT	20	20
05-113	7/12/05	Hendry's Creek, NV	BCT	37	37
05-114	7/13/05	Mill Creek, NV	BCT	37	37
05-115	7/13/05	Santa Fe Creek, NV	LCT	29	29
05-109	7/6/05	Sagehen Creek	BKT	60	60
05-147	8/10/05	Fallen Leaf	MAK	3	6
05-156	9/29/05		Kok	1	
			MAK	2	
05-129 05-128	8/23/05	Upper Klamath Lake	Tui Chubs FHM	45 60	105
05-132 05-133 05-134	9/13/05	Hat Creek	RBT BNT SS	30 4 30	64
Total Samples					1338

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 # 04-167 (Yuba River, CA – FCS carcass)

	No. SAMPLES (POOL SIZE)	No. POS/ TOTAL	Number to IHC conf.	Number POS. confirmed	Percent Positive
VIROLOGY:					
<i>Tissue Culture on EPC and CHSE cell lines (IHNV, IPNV, VHSV, OMV)</i>					
KIDNEY	44 (1p)	27/44	15	15	61%

Case – various (AD WCS – LSNFH)

	# Samples (pool size)	Total Fish	Results	# Positive (% Positive)	Notes
Virus					
Tissue culture (Kd)	18(1-2)	20	IHNV	6 (33%)	
Tissue culture (OF)	46(1-2)	50	IHNV	20 (43%)	
Bacteria					
BHIA culture (Kd)	52 (1)	52	<i>Aeromonas/Pseudomonas</i>	16 (31%)	
			<i>Aeromonas salmonicida</i>	7 (13%)	
			<i>Yersinia ruckeri</i>	0 (0%)*	
<i>Rs</i> -QPCR (Kd)	79 (1)	79	<i>Renibacterium salmoninarum</i>	39 (49%)	
<i>Rs</i> -QPCR (OF)	24 (1)	24	<i>Renibacterium salmoninarum</i>	16 (67%)	
Parasites					
Histo. (It)	22 (1)	22	<i>Ceratomyxa shasta</i>	15 (68%)	

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%)
<u>ELISA Rsa/ Positive</u> 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%)
<u>BACTE</u> 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:					
<u>Rs-ELISA*</u> (<i>Renibacterium salmoninarum</i>) 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.					
<u>Rs-QPCR**</u> (<i>Renibacterium salmoninarum</i>) 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)					
<u>Culturable Bacteria</u>					
A – KD	7 (1p)	0/7	(0)	7	

B –KD	20 (1p)	0/20	(0)	20
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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 # 05-113 and 05-114 (Hendry's Creek A and Mill Creek B, NV – BCT)

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

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

A – K/S	2 (3-4p)	0/2	(0)	37
B – K/S	7 (2-3p)	0/7	(0)	37

BACTERIOLOGY:

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

A – KD	7 (1p)	1/37	(14)	37	Antigen pos. only
B – KD	20 (1p)	1/37	(5)	37	Antigen pos. only

*Highest Optical Density (OD) value at 405nm = 0.424 for Hendry's Creek and 0.736 for Mill 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	11 (1p)	1/11	(9)	11
B – EP	7 (1p)	3/7	(43)	7

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

Culturable Bacteria

A – KD	37 (1p)	0/37	(0)	37
B –KD	37 (1p)	0/37	(0)	37

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	7 (4-5p)	0/7	(0)	37
B - Heads	7 (4-5p)	0/7	(0)	37

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

Case # 05-115 (Santa Fe Creek, NV – LCT)

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

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

K/S	10 (2-3p)	0/10	(0)	29	
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BACTERIOLOGY:

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

KD	29 (1p)	2/29	(7)	29	Antigen pos. only
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*Highest Optical Density (OD) value at 405nm = 0.245 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.

EP	3 (1p)	1/3	(33)	3	
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** Corroborative testing by Quantitative Polymerase Chain Reaction confirmed one sample positive for Rs (EP – ELISA pellet)

Culturable Bacteria

KD	29 (1p)	0/29	(0)	29	
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No obligate fish pathogens (*Yersinia ruckeri* or *Aeromonas salmonicida*) were detected.

PARASITOLOGY:

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

Heads	6 (4-5p)	0/6	(0)	29	
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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
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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)

Case # 05-128, 05-129 (Upper Klamath Lake, OR – FHM and Tui)

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

Tissue culture on CHSE and FHM cell lines (IPNV)

Tui – K/S	9 (5p)	0/9	0	45
FHM – VISC	12 (5p)	0/12	0	60

BACTERIOLOGY:

Culturable bacteria from Kidney's (on Brain Heart Infusion Agar):

Tui – KD	30 (1p)	0/30	0	30
FHM – KD	30 (1p)	0/30	0	30

Rs-ELISA* (*Renibacterium salmoninarum*. Assay detects a specific bacterial P57 protein)

Tui – KD	31 (1p)	0/31	0	31	Antigen pos. only
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*Highest Optical Density (OD) value at 405nm = 0.110, indicating suspect levels of the *Rs* antigen. Threshold for the assay is 2 STD above the Negative Control Tissue (NCT) or 0.077 OD.

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

Tui – EP	3 (1p)	0/3	0	3
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No target bacterial fish pathogens (*Yersinia ruckeri* or *Aeromonas salmonicida*) were detected.

Case # 05-132, 05-133, 05-134 (Hat Creek, CA – RBT, BNT, SS)

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

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

A – K/S	10 (3p)	0/10	0	30	
B – K/S	1 (4p)	0/1	0	4	
C – K/S	6 (5p)	0/6	0	30	

BACTERIOLOGY:

Rs-ELISA* (*Renibacterium salmoninarum*. Assay detects a specific bacterial P57 protein)

A – K	30 (1p)	1/30	3	30	Antigen pos. only
B – K	4 (1p)	0/4	0	4	

*Highest Optical Density (OD) value at 405nm = 0.241 (05-132) and 0.136 (05-133) indicating positive and suspect levels, respectively, of the Rs antigen. Threshold for the assay is 2 STD above the Negative Control Tissue (NCT) of 0.080 OD.

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

A – EP	9 (1p)	8/9	89	9	
B – EP	4 (1p)	1/4	25	4	

** Corroborative testing by Quantitative Polymerase Chain Reaction confirmed all samples negative for Rs. (EP – ELISA pellet)

Culturable Bacteria

A – K	30 (1p)	0/30	0	30	
B – K	4 (1p)	0/4	0	4	

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	10 (3p)	0/10	0	30	
B – Heads	1 (4p)	0/1	0	4	

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

Appendix 3 –

Partnerships

List of partners corresponds to sample sites on map

Map Site	Partners
1. Yuba River, CA	Jones and Stokes, YCWA
2. Coleman NFH, CA	USFWS – CNFH
3. Sagehen Creek, CA	UC Davis
4. Upper Klamath Lake, OR	USGS – Klamath Falls
5. N.F. Battle Creek, NV Andorno Creek, NV Hendry's creek, NV Mill Creek, NV Santa Fe Creek, NV	NDOW – Winnemucca NDOW – Ely NDOW – Elko
6. Hat Creek, CA	CDFG – Redding
7. Fallen Leaf Lake, CA	USFWS – Reno FRO
8. Klamath River, CA	Karuk Tribe, Yurok Tribe, USFWS – Arcata FRO, USGS
9. Livingston Stone NFH, CA	USFWS – LSNFH