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 2006-2007

Prepared by Lisa Ratcliff

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California-Nevada Fish Health Center

Center staff conducted the National Wild Fish Health Survey (NWFHS) in the 2006/2007 fiscal years by collecting fish tissue samples and performing laboratory tests for major fish pathogens in accordance with standardized procedures (NWFHS Laboratory Procedures Manual – 2006). 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/

Survey staff consists of:

Kimberly True, Assistant Project Leader
Lisa Ratcliff, Microbiologist

Additional assistance with field collection and laboratory work by:
Scott Foott, Project leader
Ken Nichols, Fishery Biologist
Ryan Fogerty, Fishery Biologist
Ron Stone, Fishery Biologist

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 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 aeromonads), and two myxozoan parasites (*Parvicapsula minibicornis* and *Ceratomyxa shasta*). The incidence and often dual infections with *Ceratomyxa shasta* and *Parvicapsula minibicornis,* in juvenile Chinook salmon is of special concern.

Another focus of the Survey in 2006-2007 collaborated with California Department of Fish and Game (CDFG) located in Yreka, California. Rainbow trout (*Onchorhynchus mykiss*) and Yellow perch (*Perca flavescens)* were sampled in June, 2007 from Iron Gate Reservoir to determine if *Ceratomyxa shasta* and *Parvicapsula minibicornis* myxozoan parasites occur in resident trout populations in the upper Klamath Basin. *C. shasta* and *P. minibicornis* were detected in 6/14 (43%) and 2/14 (4%) of the fish tested by QPCR, respectively. *Renibacterium salmoninarum* (Rs) was confirmed by QPCR in Iron Gate Reservoir rainbow trout. No other significant fish pathogens were detected in the rainbow trout or perch sampled.

An additional project conducted with CDFG occurred in the Eel River in August, 2007. Pike minnow (*Ptychocheilus grandis*) and California Roach (*Hesperoleucus symmetricus*) were collected from the Eel River near Fortuna, CA. Culturable bacteria and viruses were not detected. *C. shasta* and *P. minibicornis* were detected in 7/16 (44%) and 15/16 (94%) respectively, by QPCR, however this finding has not be confirmed by histology. Additional sampling is planned for 2008.

In the Sacramento-San Joaquin Delta, the Delta POD (Pelagic Organisms in Decline) project was conducted for a second year. In partnership with California Department of Fish and Game, University of California at Davis, and the Stockton FWO, a trawl was used to collect 597 fish in 2007 and 453 fish in 2006 during numerous sampling dates from April to October of each year. The Center's contribution to this program consisted of monitoring populations of Longfin smelt (*Spirinchus thaleichthys*) and Threadfin shad (*Dorosoma petenense*) for viruses and abnormalities noted by histological examination. Specifically fish were visually examined in the field for any abnormalities, fixed for histological examinations, and subsets of fish were brought back to the laboratory for virology processing. To date, no virus or notable infectious organisms have been detected that might explain the decline of these two pelagic species. A full pathology report for the 2006 histological findings are included in the Sample Summary appendix. Histology results for 2007 are still pending at the time of this publication but can be accessed via the NWFHS website in the near future.

A continued focus of the survey in 2006-2007 consists of partnerships with Lahontan National Fish Hatchery and UC Davis to monitor *Renibacterium salmoninarum* (Rs) in wild trout populations in Utah and California. Doudy Ponds, Utah is the location of a donor

broodstock population of the Pilot Peak strain of Lahontan cutthroat trout (LCT). Eggs are taken from this population and subsequently reared at Lahontan NFH and then re-stocked in LCT recovery watersheds. Sagehen Creek and Fallen Leaf Lake, CA are part of the Lake Tahoe basin and are potential stocking sites for recovery of LCT's in California. *Renibacterium salmoninarum* antigen was detected in Saghen Creek Rainbow trout (*Oncorhynchus mykiss*) and Brown trout (*Salmo trutta*), indicating exposure but not active infection with this bacterium. When Rs antigen is detected by ELISA, PCR confirmation of Rs DNA is required to confirm the presence of viable Rs bacteria. *Renibacterium salmoninarum* was confirmed by PCR in Lake Trout (*Salvelinus namaycush*) but not Kokanee (*Onchorhynchus nerka*) sampled from Fallen Leaf Lake in 2006.

In February 2006, the Center provided NWFHS support to the Arizona Game and Fish Department (AGFD) while the regional USFWS fish health center was undergoing a move of their laboratory facility to Dexter, New Mexico. Rainbow trout (*Onchorhynchus mykiss*), Brown trout (*Salmo trutta*), Specked dace (*Rhinichthys osculus*) and Desert Sucker (*Catostomus clarkii*) were collected by backpack electro-shocking for a general assessment of fish health in the Black River and Colorado River, AZ fish populations. Tissues were submitted to the Center for laboratory testing. One viral sample was suspect for what appeared to be an aquatic reovirus, and was submitted to UC Davis for confirmation. Electron Microscopy (EM) studies indicated the isolate was a nidovirus (Order Nidovirales) but different from other known members of this order (e.g., Coronviridae, Ateriviridae, Roniviridae) (personal communication, Ronald Hedrick, UCD). *Yersinia ruckeri* was detected in 1 speckled dace and *Renibacterium salmoninarum* was detected by ELISA and confirmed QPCR in Rainbow trout.

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 with numerous federal, state, and universities as well as NGOs and private landowners to collect fish at over 200 collection sites. A total of 15,024 samples have been collected. The sampling effort to date comprises a rich diversity of species in California and Nevada and has provided fish health information that did not exist prior to the National Wild Fish Health Survey's inception in 1997.

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 (Puzach, 2006) 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 2006-2007 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 to immunologically confirm the identity of bacterial pathogens.

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 and 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 antigen, 0.500-.999 moderate level, and values of 1.00 or higher were considered high antigen levels. Corroborative testing of ELISA antigen positive test results is required to

confirm the presence of *Renibacterium salmoninarum* DNA, and is performed with standard or quantitative Polymerase Chain Reaction (PCR).

Virology

Samples of kidney and spleen, or visceral tissue in the case of smaller fish, were removed from each fish and assayed 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 individually, or from 3-5 fish pooled into one sample.

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 onto 48-well plates. Samples were incubated on a platform rocker for 30-60 minutes at 15°C. Wells were supplemented with 0.5ml of liquid overlay which contained Minimum Essential Media with 10% Fetal Bovine Serum (MEM10) or MEM10 with methylcellulose (EPC cell line), and incubated at 15°C for 21 d.
Plates were examined bi-weekly for evidence of viral cytopathic effects (CPE), and re-inoculated onto fresh cells if generalized toxicity or suspect CPE was noted. 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 sagitally halved, to provide an archive set for PCR confirmation testing. The remaining set of halved heads were heated in a 60°C water bath for 60 minutes, 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 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 minutes. The larger remaining particles were filtered through cheesecloth or large-pore filters, 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 TPD positive results was done by PCR.

Pathogen Surveys

Klamath River, CA

Concerns exist in the Klamath River basin regarding flow allocations and the relationship to disease incidence; these concerns were heightened during the 2002 adult chinook fish kill. Many regional, state, local and tribal biologists are conducting research to better understand what biological factors influence the incidence of disease in this river system. The incidence of the two parasites, *C. Shasta* and *P. minibicornis*, occurring as dual infections in a large proportion of infected fish, is of concern because of potential impacts for survival of out-migrating juvenile Chinook.

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). The prevalence of infection (POI) in 2006 for *C. shasta* was 22% by histology and 48% by PCR. For *P. minibicornis*, POI was 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% six weeks later. Nearly all fish infected with *C. shasta* were also infected with *P. minibicornis*. The overall prevalence of infection (POI) in 2007 for *C. shasta* was 8% by histology and 17% by PCR. For *P. minibicornis*, POI was 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.

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 Iron Gate Fish hatchery is located below the dam. Iron Gate Reservoir is home to an abundance of yellow perch (*Perca flavescens*) and Rainbow trout (*Oncorhynchus mykiss*). These species were collected and tested for *C. shasta* and *P. minibicornis* in 2007 to determine if these fish potentially harbor these myxozoan parasites.

In June 2007, at coordinates N 41 58.356, W 122 22.088, fourteen Rainbow trout and forty Yellow perch were collected by Jim Whelan in collaboration with the Ca-Nv FHC. Fish tissues were tested for Survey target pathogens in addition to *C. shasta* and *P. minibicornis*. Rs antigen was detected in 3/3 (100%) of Rainbow Trout samples and 1/3 (33%) were confirmed by QPCR. *P. minibicornis* was found in 2/14 (14.2%) of samples, and *C. shasta* was detected in 6/14 (42.8%) of samples. There were no culturable bacteria, viruses, or whirling disease (*Myxobolus cerebralis*) detected.

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*), Pike minnow (*Ptychocheilus grandis*), and California roach (*Hesperoleucus symmetricus*). Both Pike minnow and California roach were collected to determine the general health of non-salmonid fish in the Eel River.

In August 2007, at coordinates N 40' 16 10.08, W 123' 43 40.31, forty Pike minnow and seven California Roach were collected by CDFG fisheries biologists in collaboration with the Ca-Nv FHC. Select tissues were tested for major fish pathogens and myxozoan parasites. Rs antigen was detected in 9/16 (56%) of samples, but presence of viable cells (DNA) was not confirmed by QPCR.
P. minibicornis was detected in 15/16 (94%) of samples and *C. shasta* was detected in 7/16 (44%) of samples. Additional confirmation of these parasites is planned for 2008, using histologically examination. No culturable bacteria or viruses were detected in these fish species.

Delta POD, San Francisco, CA

Significant declines in pelagic fish abundance in the Sacramento-San Joaquin River Delta over the last decade has prompted the Interagency Ecological Program (IEP) to augment its monitoring program. The disease survey focused on virus isolation and histological examination. Several sampling sites were used along the Delta which included Stockton, Suisun Bay, and San Pablo Bay. In 2006, one hundred and forty-seven Longfin smelt (*Spirinchus thaleichthys*) were collected for histological inspection and four hundred and four were collected for virology testing. Cestodes were found in the intestinal tract of 16/147 (10%), there was no associated inflammation or necrosis present. There were no viral isolates obtained from this sample set. Forty three Threadfin shad (*Dorosoma petenense*) were collected, fifteen were examined histologically, and eleven were processed for viral. Epitheliocyctis (Chlamydia) infection was observed in the gill lamellae, 11/15 (73%) There were no viral isolates detected from this sample set. For more information, see Foott, et al 2006 report in Appendix 2.

In 2007, sixty-five Longfin smelt and eighty-three Threadfin shad have been examined by histology and virology. Preliminary findings to date have shown that one Threadfin shad 1/65 (1.5%) was found to have an intestinal cestode and 1/65 (1.5%) was found to have a trematode. Epitheliocyctis was found in 14/83 (16%) examined Threadfin shad. No viral isolates have been detected.

Spawning Surveys of Adult Salmonids

The completion of Shasta dam in 1945 had an inevitable impact on Chinook salmon and steelhead access to historic spawning habitat. The significant loss of natural spawning areas above the dam was mitigated through the completion of Coleman National Fish Hatchery. Returning FCS, STT and LFS adults are monitored each year to determine the disease status of each run in the upper Sacramento basin.

Winter Chinook Adults – Livingston Stone National Fish Hatchery

Winter run Chinook salmon were listed as endangered by California Fish and Game in 1989 and the National Marine Fisheries Service in 1994. Attempts to imprint juveniles reared at CNFH to the upper main-stem Sacramento River were unsuccessful, and in 1997, the Bureau of Reclamation developed a main-stem rearing facility, Livingston Stone NFH, at the base of Shasta Dam. This facility was successful in producing captive and natural production goals, and ensuring winter run adults returned to the upper Sacramento River. The hatchery's ultimate goal is to recover Winter run chinook populations to self sustaining levels.

Wild origin Winter run adults are captured at the base of Keswick Dam and transferred to LSNFH for egg collection. In 2006, 92 samples were collected from spawned wild fish (39 males, 53 females). Eighty-four fish were sampled for viral testing with 21/33 (64%) kidney samples and 38/51(73%) ovarian fluid samples positive for IHNV. 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 *P. minibicornis* 80/88 (91%) and *C. shasta* 88/92 (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 37/39 (94%) kidney samples and 26/27 (96%) ovarian fluid samples were positive for IHNV. Rs and culturable bacteria were not detected.

Steelhead Adult Spawning – Coleman National Fish Hatchery

The Steelhead propagation program began in 1952 following the Central Valley Project which reduced steelhead spawning habitat. 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 upper basin watersheds.

Natural spawning fish are collected and spawned 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*. 3/10 (30%) of the natural population tested (10 pooled samples) were positive for IHNV and 7/9 (78%) were positive for Rs 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% of 2 pool samples tested positive for Rs by DFAT.

Tahoe Basin, CA

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 were conducted in Fallen Leaf Lake (Lake trout and Kokanee) and Sagehen Creek near Stampede Reservoir (Rainbow trout and Brown trout) to determine if *R. salmoninarum* was present in resident salmonid species.

Sagehen Creek 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. This spring-fed stream supports a healthy native fishery that includes Lahontan residents (*Richardsonius egregius*), speckled dace (*Rhinichthys osculus*), Tahoe and mountain sucker (*Catostomus tahoensis*, *Catostomus platyrhynchus*), and the Paiute sculpin (*Cottus beldingii*). In September 2006, Rainbow trout (*Onchorhynchus mykiss*) and Brown trout (*Salmo trutta trutta*) were collected by field crews from Lahontan NFH and UC Davis, and submitted for laboratory testing. *Renibacterium salmoninarum* antigen was detected, but no viable bacteria were confirmed by PCR.

Fallen Leaf Lake is located southwest of Lake Tahoe, and is connected by its outlet Taylor Creek into Lake Tahoe. Fallen Leaf Lake is similar in elevation at 6377 feet and reaches depths of 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 primary game fish in the lake. Recreational fishing is supplemented by stocking of brown trout (*Salmo trutta trutta)*, rainbow trout (*Onchorhynchus mykiss*) and kokanee salmon (*Oncorhynchus nerka*) in Fallen Leaf Lake.

At various collection dates in the months of July through August, forty-five mackinaws and thirteen Kokanee salmon were collected by John Stead of UC Davis. Intact fish were kept frozen and to the Ca-Nv Fish Health Center for necropsy and laboratory testing. *Renibacterium salmoninarum* was detected by ELISA and confirmed using QPCR in 31/45 (69%) of Lake trout population.
Kokanee had no detectable Rs antigen present. No whirling disease or other significant fish pathogens were detected in the Fallen Leaf Lake species that were sampled.

Black River, AZ

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. In February 2006, thirty-six Speckled dace (*Rhinichthys osculus*), forty-eight desert suckers (*Catostomus clarki*), thirty-nine rainbow trout (*Onchorhyncus mykiss*) were collected by Jimmy Fulmer, Arizona Game and Fish. Tissue samples were collected in the field and sent to Ca-Nv FHC for routine Survey testing. *Renibacterium salmoninarum* antigen was detected by ELISA in Rainbow trout 3/39 (8%), but no viable bacteria were confirmed by PCR. A single viral sample from speckled dace produced CPE similar to the type observed with aquatic reoviruses. Later EM studies by UCD identified the isolate as a Nirovirus. No culturable bacteria were detected.

Little Colorado River, AZ

The Little Colorado River is located in Arizona and is a tributary of the Colorado River. The Little Colorado River is the principal drainage for the Painted Desert. In April 2006, at UTM coordinates 12S0642737 3770806 and 12S0642751 3770182, sixty Speckled dace (*Rhinichthys osculus*), twenty-one Brown trout (*Salmo trutta*), and thirty-nine Rainbow trout (*Oncorhynchus mykiss*) were collected. *Yersinia Ruckeri* was detected in one Speckled dace and was confirmed using IFAT. *Renibacterium salmoninarum* was confirmed in Rainbow trout. There were no viruses detected.

References

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.

Puzach, C (Ed) 2006. National Wild Fish Health Survey - Laboratory Procedure Manual. 4.0 Edition. U. S. Fish and Wildlife Service, Onalaska, WI.

True K, MP Purcell, and JS Foott. Development and validation of a QPCR to detect *Parvicapsula minibicornis* and comparison to histologically ranked juvenile Chinook salmon (*Oncorhynchus tshawytscha) from the Klamath River, USA. Manuscript submitted to Journal of Fish Disease, Nov 2007.*

Appendix I - NWFHS SUMMARY TABLES FOR FY 2006 and 2007

NWFHS SUMMARY TABLE: FY 2006

Case #	Date Collect	Location	Species	Number of Fish	Significant Findings
06-025	2-28-06	Black River, AZ	Speckled dace	36	Viral sample sent to UC Davis for confirmation, Nidovirus
06-026	2-28-06	Black River, AZ	Desert sucker	48	
06-027	2-28-06	Black River, AZ	BNT RBT	16 23	
06-036	3-29-06	Delta POD	Longfin smelt	70	
06-040	4-05-06	Little Colorado River	BNT RBT Speckled dace	21 39 60	SpDace-*Yersinia ruckeri* (2%)
06-046	5-1-06	Delta POD	Longfin smelt	96	
06-055	4-26-06	Delta POD	Longfin smelt	3	
06-062	4-27-06	Delta POD	Longfin smelt	17	
06-066	5-10-06	Delta POD	Longfin smelt	130	
06-083	5-24-06	Delta POD	Longfin smelt	70	
06-100	6-21-06	Delta POD	Longfin smelt	7	
06-116	7-17-06	Delta POD	Longfin smelt	60	
06-134	8-29-06	Delta POD	Longfin smelt	23	
06-136	9-13-06	Sagehen Creek Stampede Reservoir	RBT Brown Trout	12 18	
Various Cases	Various Dates	Livingston Stone NFH	Winter Chinook-AD	92	IHNV (70%) *C. shasta* (96%) *P. minibicornis* (91%) *R. salmoninarum* (6%)
06-139	Various Dates	Fallen Leaf Lake	Lake Trout Kokanee Salmon	45 13	LKT-*R. salmoninarum* (33%)

Total Fish = 1318

NWFHS SUMMARY TABLE: FY 2007

Case #	Date Collected	Location	Species	Number of Fish	Significant Findings
07-002	10/18/2006	IGH	FCS	30	*P.minibicornis* (100%)
07-004	10/26/2006	Suisun Bay Delta Pod	LFS TFS	22 11	
07-006	11/9/2006	IGH	FCS	30	*P.minibicornis* (100%)
07-007	10/27/2006	Delta Pod	LFS	12	
07-043	3/28/2007	Stanislaus River	FCS	5	
07-053	4/19/2007	Klamath River	FCS COHO	1284 85	*P. minibicornis* (21%) *C. shasta* (71%)
07-055	4/24/2007	Doudy Pond	CUT	19	*R. salmoninarum* (6%)
Various Dates	Various Dates	Livingston Stone NFH	Winter Chinook AD	39	IHN virus (94%)
07-080	6/8/2007	Iron Gate R	RBT	14	*R. salmoninarum* (33%) *P. minibicornis* (4%) *C. shasta* (42%)
07-081	6/8/2007	Iron Gate R.	Yellow Perch	40	
07-117	8/23/2007	Eel River	Pike Minnow CA. Roach	41 7	*P. minibicornis* (94%) *C. shasta* (44%)

Total Fish= 1635

DELTA POD SUMMARY FY2006

Case #	Date	Species	Virology Results	Histology	Total Fish/Site	Coversheet Notes / Site Information	Significant Findings
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	5-1-06	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	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.	
06-116	7-18-06	LFS	0/12 (5-p) n=60		60	SF collected: Viral 60; Histo 26,1-p	

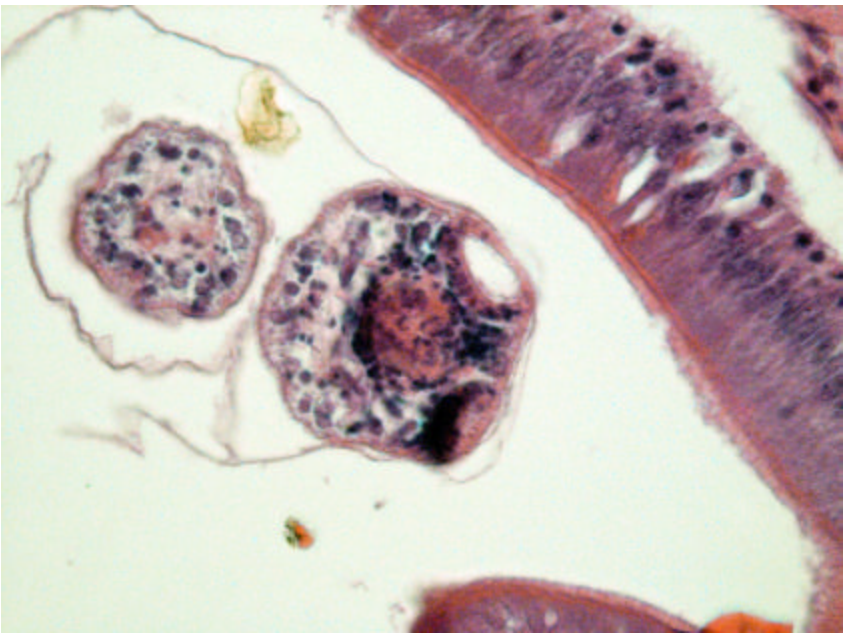
Appendix 2 – Pathology Reports and Sample Summary Tables:

U.S. Fish & Wildlife Service

California Nevada Fish Health Center
FY2006 Investigational Report:

Histological Evaluation and Viral Survey of Juvenile Longfin Smelt (*Spirinchus thaleichthys*) and Threadfin Shad (*Dorosoma petenense*) collected from the Sacramento – San Joaquin R. Delta, April – October 2006.

J.Scott Foott*, K. True, and R. Stone



U.S. Fish and Wildlife Service
California-Nevada Fish Health Center
24411 Coleman Hatchery Road
Anderson, CA 96007
PH: (530) 365-4271 FAX: (530) 365-7150
December 2006

*direct correspondence

Introduction:

Significant declines in pelagic fish abundance in the Sacramento – San Joaquin River Delta over the last decade has prompted the Interagency Ecological Program (IEP) to augment its monitoring program with a disease screening effort in 2006 for juvenile Longfin smelt, Delta smelt, and Striped bass. The disease survey focused on virus isolation and histological examination. Delta smelt and Striped bass were examined by two UC Davis research teams headed by Drs Swee Teh and David Ostrach, respectively. The California-Nevada Fish Health Center (FHC) was tasked to examined Longfin smelt (**LFS**, *Spirinchus thaleichthys)* and Threadfin shad (**TFS**, *Dorosoma petenense*) juveniles collected between April and October 2006.

Methods:

Fish were collected by the Department of Fish and Game's Bay-Delta monitoring program using sampling gear and methods specific for the spring 20mm, Summer Townet , Bay Study and Fall Midwater Trawl surveys. Locations sampled are referenced to the survey sampling gear in use at the time; Summer Townet stations represent a subset of those for the 20mm Survey, so Townet stations are not referenced in text or documented on a separate map (Table 1, Figure1). As soon as LFS or TFS from a given tow were sorted and measured for fork length, a subset was placed in either 10% buffered formalin (Z-fix, Anatech) or Davidson's fixative, transferred to 70% ethanol after 24 – 48 h, processed for 5 µm paraffin sections (sagittal whole body or dissected organs in fish >75mm) and stained with hematoxylin and eosin (Humason 1979). Specimens from the September and October collection were de-calcified for 24h prior to processing.Initial collections were placed into 10% buffered formalin, however, poor tissue morphology resulting from shrinkage artifacts necessitated changing to Davidson's fixative starting with the 10May collection. The general sectioning protocol was to place a "shallow" (~ 50 - 100µm from the epidermis) and "deeper" (midline of fish) section onto each slide. All tissues for a given fish were placed on one slide and identified by a unique code number. Each slide was examined at both low (40X) and high magnification (400X) without knowledge of collection group.

Fish selected for virological assays were placed into cold antibiotic-mycotic solution in pools of 5 or less fish and a 40 and 100x dilution of whole body homogenate was inoculated onto both Epithelioma Papulosum Cyprini (EPC) and Chinook Salmon Embryo (CHSE214) cell lines. Cultures were held at 15°C for 18 – 21 d and examined for cytopathic effects. Suspect cultures were subsequently filtered (0.2µm) and re-inoculated on new cell cultures.

The RNA:DNA ratio growth index was determined from 20 LFS caudal sections (including tail) frozen on dry ice after collection and held at -70°C until assayed by a modification of the method of Kaplan et al. (2001). Briefly, the sample was digested with proteinase K (45°C, 50min in 10µg PK /mL TE buffer) and the supernatant assayed for total RNA and DNA with Quant- iT ™ RNA and dsDNA fluorometric kits by Molecular Probes (Eugene OR).

Results and Discussion:

Longfin smelt histology - A total of 142 of the 147 LFS processed for histology were examined for parasites and abnormalities (Table 1). Despite the inherent difficulties of obtaining sagittal sections containing all organs, four targeted organs (liver, kidney, intestine, and gill) were observed in the majority of sections (Figure 2).

Table. 1. Longfin smelt (*Spirinchus thaleichthys*) collection dates, monitoring survey station numbers and their referenced survey and estuary location, fork length (FL), and histological sample numbers examined in 2006.

Date	Station No.	FL (mm)	Sample No.
29March	336,340 20mm Survey, eastern San Pablo Bay and lower Napa River	13-32 Avg=21	10
12April	320,336,340 20mm Survey, eastern San Pablo Bay and lower Napa River	12-34 Avg=22	20
26April	340 20mm Survey, lower Napa River	16 – 27 Avg=23	1
10May	328,329,334,335, 336 20mm Survey, eastern San Pablo Bay	18 – 39 Avg=25	25
24May	334,335,336 20mm Survey, eastern San Pablo Bay	16 – 30 Avg=25	10
18July	318 – 323,325,346 Bay Study Survey, throughout San Pablo Bay	27 – 80 Avg=42	27
29August	418,504,602 Fall Midwater Trawl Survey, Suisun and Grizzly bays	29 – 95 Avg=50	19
26-27October	517,519,504,601,609,610 20mm Survey and Fall Midwater Trawl Survey, Honker Bay and Montezuma Slough	45 – 98 Avg=60	35
	total		147

Figure 1. Map of 20mm Survey sample station numbers

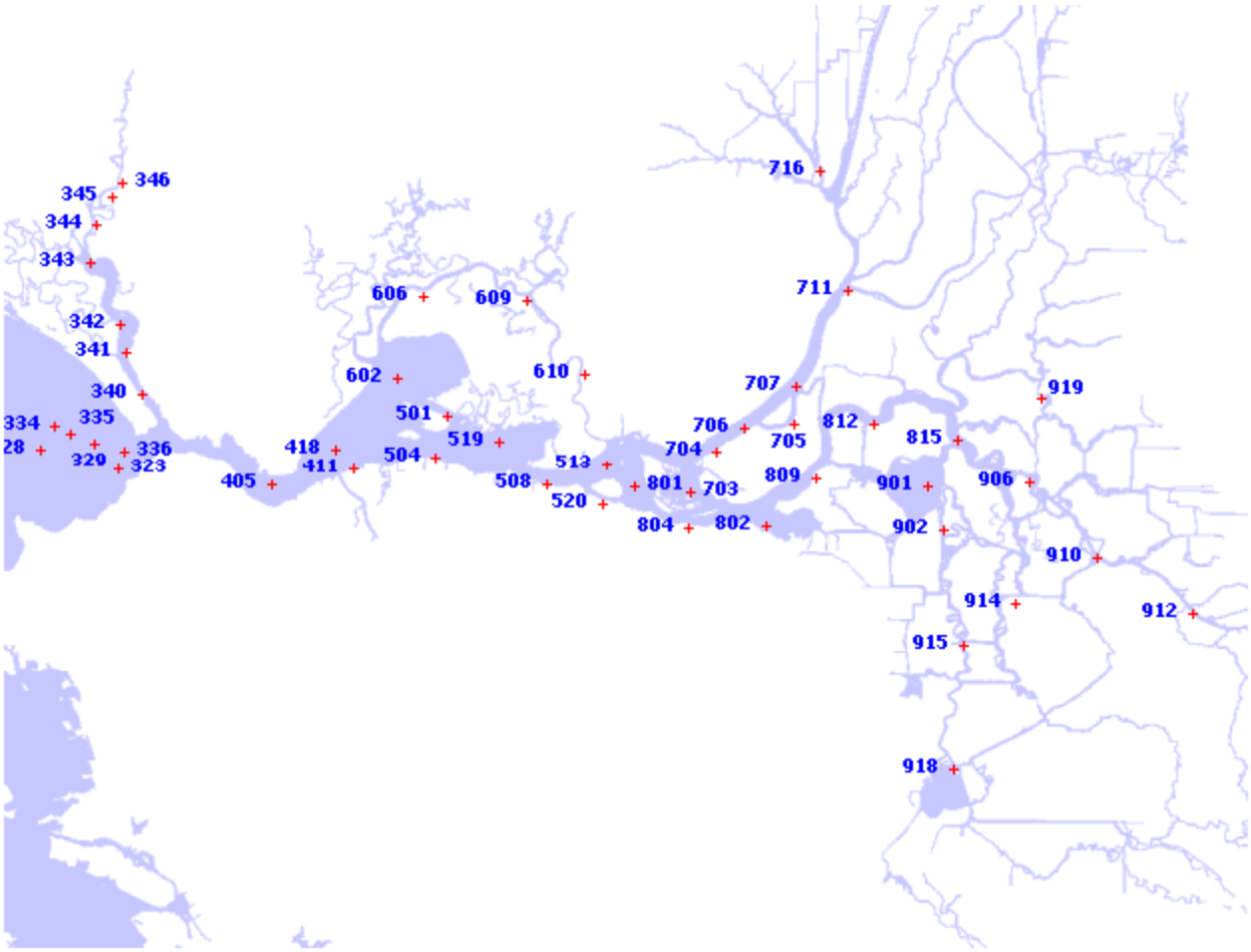
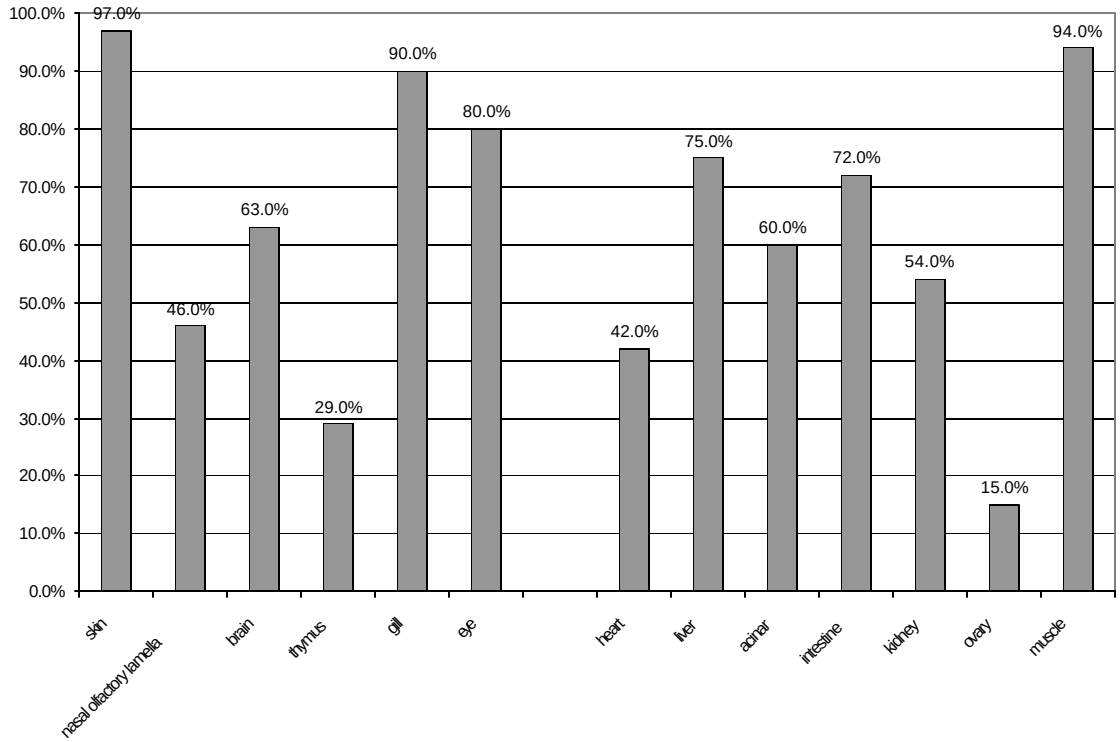
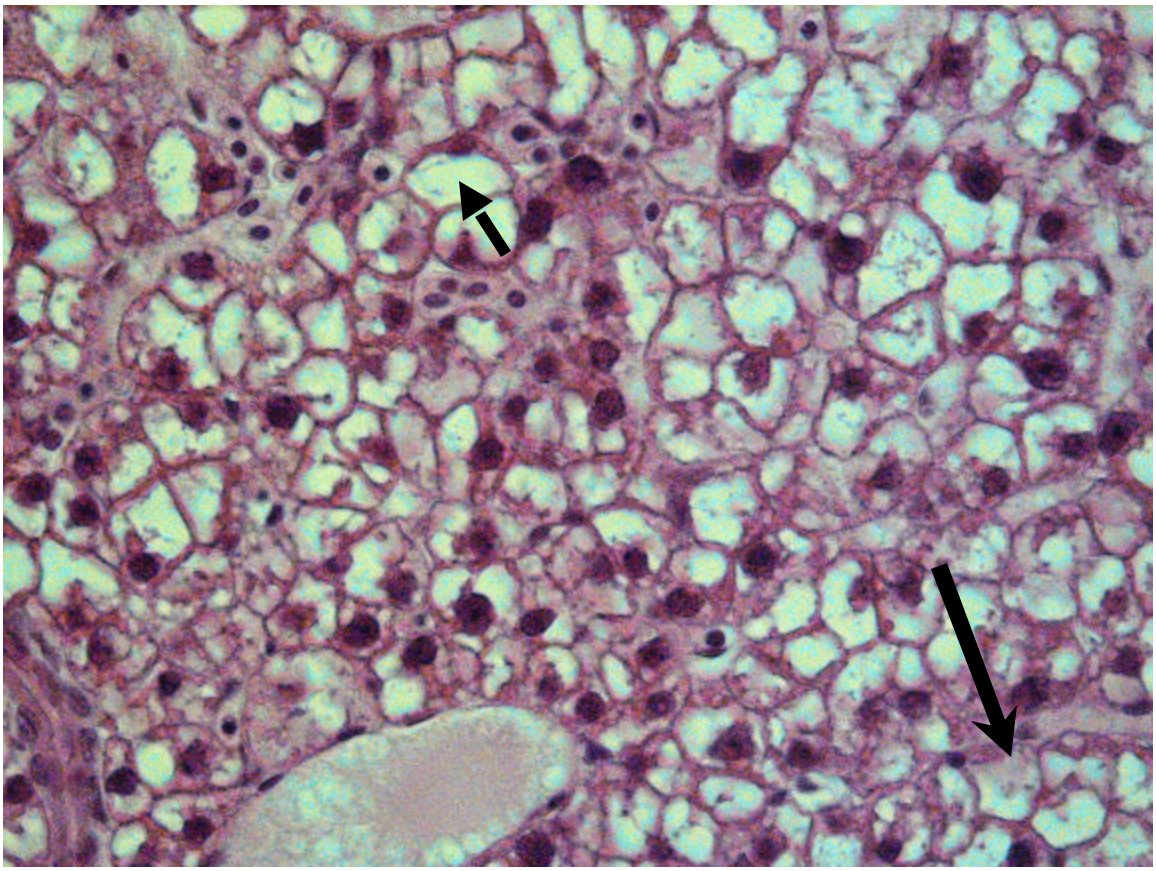


Figure 2 Percent of Longfin smelt histological sections containing specific organs.



No abnormalities were observed in the LFS tissues. Hepatocyte vacuoles (vacuolated hepatocytes in $\geq$ 30% of the liver section) were seen in 14 of 107 liver sections (13%) beginning with the 18JULY collection. It is the lead author's opinion that this condition indicates that the smelt were storing excess energy reserves during the summer months and is not an abnormality. This opinion is based on previous work done on fatty liver diagnosis in juvenile hatchery salmon (Free and Foott, 2000). The 2 types of vacuoles observed in the LFS livers contained either fat (defined vacuole wall with cleared interior that is PAS negative and cryosections from the same liver stain positive with Oil Red-O) or glycogen (microvesicular or with poorly defined walls containing eosinophilic granular material that is PAS positive). The LFS livers contained varying percentages of both types of vacuoles and no nuclear abnormalities or necrotic changes were associated with these cytoplasmic vacuoles (Figure 3).

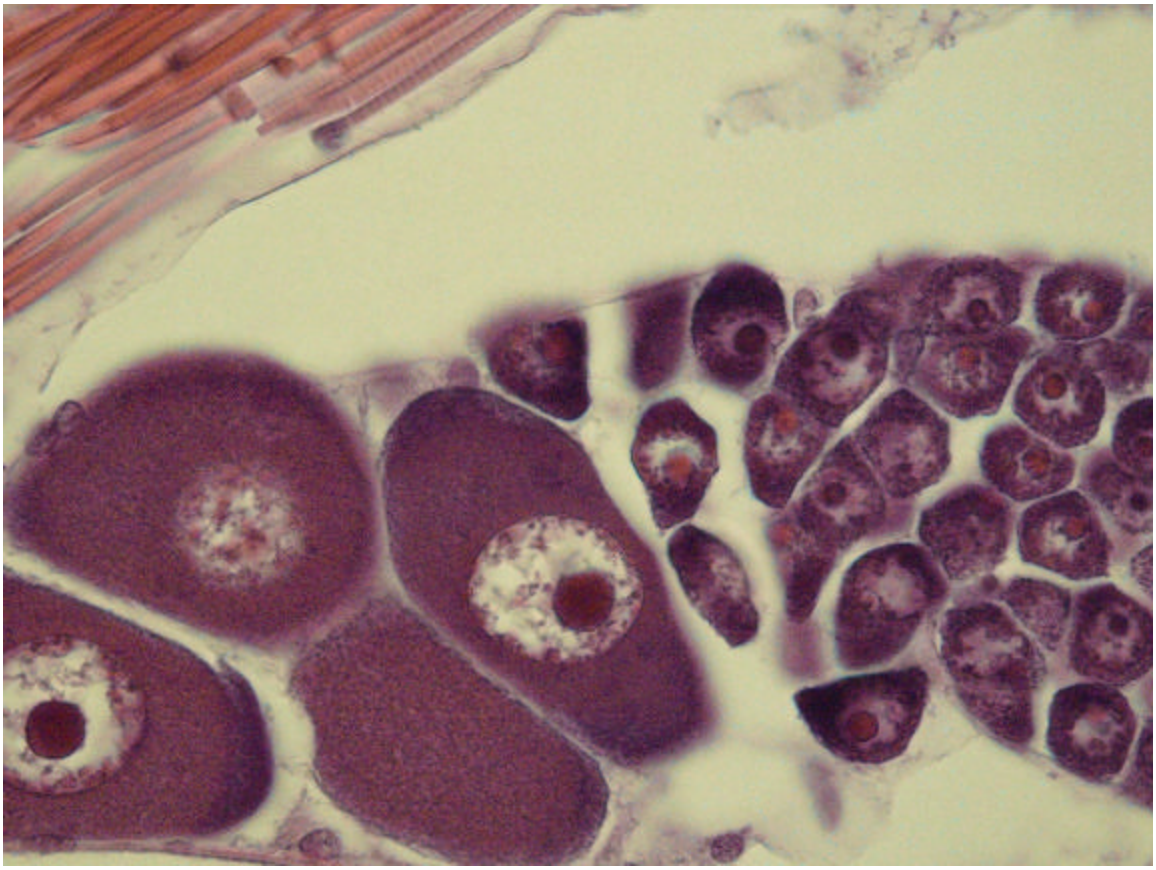
Figure 3 LFS liver section containing vacuolated hepatocytes. Presumptive fat-containing (short arrow) and glycogen (long arrowhead) containing vacuoles (400x total magnification, Davidson's fixative H&E stain).



Small immature cestodes, resembling the pleurocercoid stage of *Phyllobothrium salmonis,* were observed in the intestinal tracts of 16 smelt (16% incidence of infection). The first detection occurred in a 10May sample and infected fish occurred through the final 26OCT sample. This presumptive identification is based on the observation of at least 3 suckers on the scolex without an apical sucker (see photomicrograph on title page) and the common occurrence of this cestode in adult Sacramento River salmon. No inflammation or necrosis was associated with these cestodes. Copepods, an element in the LFS's diet, are an intermediate host for the cestode's procercoid stage that is infectious to fish. No external parasites were observed on the skin or gills with the exception of 2 fish collected on 27OCT. Several "ciliate / ameba-like" parasites were seen on gill lamellae without any associated inflammation. Histological processing can result in the loss of unattached external parasites however, inflammatory signs of external parasite infection was not observed in the sample set.

Ovary was observed in 15 LFS (11%) while testis was identified in only one fish. The first observation of ovary occurred in a 10MAY fish. The most mature ova stage seen was the primary oocyte with little to no yolk vesicles (Fig. 4).

Figure 4. Ovary section from LFS female collected 26OCT06 (400x total magnification, Davidson's fixative H&E stain).



Longfin smelt viral samples – A total of 404 whole fish homogenate samples were assayed for virus (Table 2). No viral isolates were obtained from the sample set. Cytopathic (CPE) changes, such as syncytium formation, in EPC cell cultures were observed in several samples from the 29March, 29August, and 26October collection but did not replicate further when a 0.22µm filtrate of the original culture was passed onto fresh EPC cultures. It is unclear whether the initial CPE was due to toxicity or a virus.

Table 2. Longfin smelt (*Spirinchus thaleichthys*) collection dates, monitoring survey station numbers and their referenced survey and estuary location, and viral sample numbers in 2006. Sample collections that showed suspected Cytopathic Effects (CPE) in the initial EPC culture are identified.

Date	Station No.	Sample No.	CPE
29March	336,340 20mm Survey, eastern San Pablo Bay and lower Napa River	60	+
12April	320,336,340 20mm Survey, eastern San Pablo Bay and lower Napa River	76	
26-27April	328,336,340 20mm Survey, eastern San Pablo Bay and lower Napa River	19	
10May	328,329,334,335,336 20mm Survey, eastern San Pablo Bay	80	
24May	334,335,336 20mm Survey, eastern San Pablo Bay	60	
21June	335, 341 20mm Survey, eastern San Pablo Bay and lower Napa River	7	
18July	318 – 323,325,346 Bay Study Survey, throughout San Pablo Bay	60	
29August	418,501,602 Fall Midwater Trawl Survey, Suisun and Grizzly bays	20	+
26-October	504,517,519,601,606,609,610 20mm Survey and Fall Midwater Trawl Survey, Honker Bay and Montezuma Slough	22	+
	total	404	

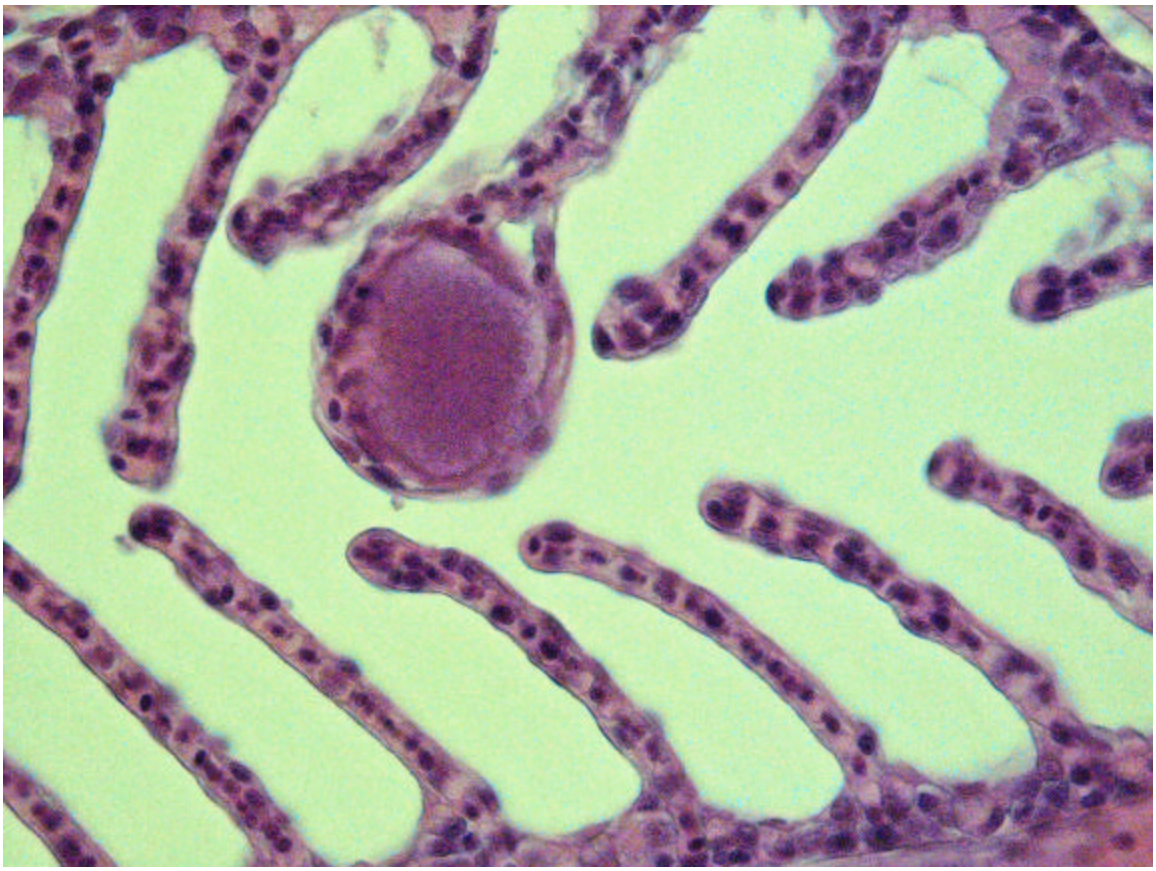
Pilot effort in RNA:DNA ratio analysis - The total quantity of DNA per cell is relatively constant while RNA content tends to reflect protein synthesis activity. The basis of the R:D ratio as a growth measurement is that growing tissue will contain cells with high RNA to DNA ratios. The mean R:D ratios obtained from the 16 caudal sections was within the values reported for juvenile mummichog white muscle samples (Kaplan et al. 2001) although the authors used a different RNA assay (Table 3). The coefficient of variation was extremely high for the sample set. We observed poor proteinase K digestion of the fin tissue and speculate that fin would contribute a different R:D ratio than white muscle. If this growth index is desired for future surveys, only caudal muscle should be included in the sample.

Table 3. Mean RNA: DNA ratio (Std. Dev).

Collection	R:D ratio
26April, n = 1	0.83
21June, n = 7	1.67 (1.58) high 95% CV
18July, n= 8	1.68 (0.74) 44%CV

Threadfin shad - One viral sample group (n=11) was collected on 26OCT and no viral CPE was observed in the cultures. A total of 15 juvenile TFS were processed for histological examination from the 46 collected. These fish were collected by CDFG on 29AUG (n = 3, site 602) and 19SEP (n =43, sites 511,512,609, and 703). Only 12 of the 43 TFS in the 19SEP collection were processed due to poor fixation issues (too many fish in a given tube of fixative). Epitheliocystis (chlamydia) infection was observed in the gill lamellae of 11 TFS (73%). The few cysts observed per section appear to be relatively benign and did not elicit an inflammatory response (Fig. 5). Five TFS sagittal sections contained immature ovaries and 2 fish had livers with vacuolated hepatocytes similar to the LFS. No other abnormalities were observed in sections.

Figure 5. Epitheliocystis cyst in TFS gill lamellar cell (400x total magnification, Davidson's fixative H&E stain).



Summary- LFS and TFS juveniles collected in 2006 appeared to be relatively healthy and did not demonstrate significant parasitic infections or tissue abnormalities. We did not isolate virus in the tissue cultures (EPC and CHSE214 cell lines) employed for the survey but this area could benefit from addition of other fish cell lines such as Fathead Minnow.. The growth index, RNA:DNA ratio, can be measured from larval LFS under the current collection protocols however fin tissue should be excluded from the sample. Given the labor required for

the histological analysis (>60 h for processing and another 30+ h for microscopic examination) and lack of observed abnormalities, we do not recommend that future health LFS surveys employ this same level of histological sampling.

Acknowledgements: Partial funding for this project was obtained from the Interagency Ecological Program. We thank the following cooperators for their assistance with field collection and logistics: CDFG Bay-Delta Program biologists (in particular Julio Samii and Steve Slater), Gouhua Zhang (UC Davis, T Sweh Laboratory), and Dr. David Ostrach. , Both L. Rosten and J. Grey helped with viral assays.

Reference:

Free D and JS Foott. 2000. FY97 investigational report: Effects of diet composition, feeding rate, and water temperature on liver lipoid disease, growth, smolt development, and adult return in Coleman NFH Late-Fall Chinoook Salmon (broodyear 1996). USFWS Ca-NV Fish Health Center, Anderson CA (http://www.fws.gov/canvfhc , Activities / reports).

Humason GL. 1979. Animal tissue techniques. 4th ed., WH Freeman and Co., San Francisco.

Kaplan LA, J Leamon, and JF Crivello. 2001. The development of a rapid and sensitive, high-through-put protocol for RNA:DNA ratio analysis. Journal of Aquatic Animal Health 13:276 – 279.

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
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
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
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 Myxobolid 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
Pm QPCR - Detects Pm 18s DNA, presumably viable *Parvicapsula minibicornis* trophozoites in kidney tissue				
RBT-KD	14(1p)	2/14	(4.2%)	14
Cs QPCR - Detects Cs 18 s DNA, presumably viable *Ceratomyxa shasta* trophozoites in intestine				
RBT-IT	14(1p)	6/14	(42.8%)	14
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%)	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
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
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
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

Steelhead Adults (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 *Rsal* 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 *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%)

Steelhead Adults (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	6/120 (5%)
-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-*R.sal* #PCR confirmed	9(3)p	5(3)p	6(3)p	10(3)p	3(3)p 2 poss.	0	4(3)p	111	2/111(0.2%) 2/6(0.33%)
Natural-*Rsal* DFAT	0	1(1)P	0	0	1(1)P	0	0	2	
BACTE *A. salmonicida* *Yersinia ruckeri* *Pseudomonas/ Aeromonas*	0/13 0/13 0/13	NT	0/4 0/4 2/4	1/12 0/12 1/12	NT	0/5 0/5 0/5	NT	34 34 34	1/34 (3%) 0/34 (0%) 3/34 (9%)

Winter Chinook Adults (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	*Aeromonas/Pseudomonas*	0 (0%)	
			Aeromonas salmonicida	0 (0%)	
			Yersinia ruckeri	0 (0%)	
Rs-QPCR (Kd-male)	33 (1)	33	*Renibacterium salmoninarum*	3 (9%)	
Rs-QPCR (Kd-female)	51 (1)	51	*Renibacterium salmoninarum*	2 (4%)	
Parasites					
Cs-QPCR	92 (1)	92	*Ceratomyxa shasta*	88 (96%)	
Pm-QPCR	88(1)	88	*Parvicapsula minibicornis*	80 (91%)	

Winter Chinook Adults (LSNFH) – 2007

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

Case #06-139, (Fallen Leaf Lake, CA LKT -KOK)

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

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 (1p)	31/45	(69%)	45
KOK-KD	13 (1p)	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 (1p)	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 Myxobolid spores. Further testing by PCR is required to confirm the species identity of observed spores as *Myxobolus cerebralis*.

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

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

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

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	3
BNT-KD	3 (1p)	0/3	Negative	3

PARASITOLOGY:

Para-*Mc*-TPD – Screening test for presence of Myxobolid 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, AZ SDC-DSK-RBT)

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

BACTERIOLOGY:

Culturable bacteria on BHIA pure slants:

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

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

RBT – KD	39 (1p)	3/39	(8%)	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.

*** Confirmation by PCR required for a positive test result; RBT samples were negative for *Rs* DNA by QPCR**.

VIROLOGY:

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

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

***Cytopathic effects (CPE) observed on EPC cell line, indicative of a replicating virus. Sample submitted to UC Davis for further confirmation. EM studies indicate this is Nirovirus.**

Case #06-040 (Little Colorado River, AZ SDC-BNT-RBT)

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

BACTERIOLOGY:

Culturable bacteria on BHIA pure slants:

SDC – KD	60 (1p)	**1/60** ***Y.ruck***	(2)	60
BNT – KD	21 (1p)	0/21	(0)	21
RBT – KD	39 (1p)	0/39	(0)	39

Indirect Flourescent 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 (1p)	s20/21	(0)	21
RBT – KD	39 (1p)	s3/39	(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 and Sample Sites

List of partners corresponding 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.	Delta POD	CDFG, Stockton FWO, UCD
7.	Sagehen Creek	LNFH, UCD
8.	Fallen Leaf Lake	LNFH, UCD
9.	Lahonton National Fish Hatchery	USFWS

Map of Sample Sites 2006-2007