



National Wild Fish
Health Survey



California-Nevada Fish Health Center

Annual Report for Fiscal Year 2009



National Wild Fish Health Survey
Annual Progress Report FY 2009
Prepared by Ryan Fogerty

California-Nevada Fish Health Center

Center staff conducted the National Wild Fish Health Survey (NWFHS) in 2009 by working with partners to collect fish tissue samples and performing laboratory tests for major fish pathogens in accordance with standardized procedures (NWFHS Laboratory Procedures Manual – 2009). 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://www.fws.gov/wildfishsurvey/database/>

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Overview

The National Wild Fish Health Survey (Survey) is a program conducted by the U.S. Fish and Wildlife Service's Fish Health Centers to assess the prevalence and distribution of major fish pathogens in wild fish populations. To date, the Center has partnered with numerous federal and state agencies, tribal governments, universities, non-profit and educational organizations and private landowners to collect fish at over 200 collection sites. A total of 16,323 samples have been collected and tested for major fish pathogens over the past 11 years. The sampling effort to date comprises a rich diversity of fish 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.

Each year, the California-Nevada Fish Health Center (Ca-Nv FHC) focuses on specific disease issues that are important in our region such as emerging diseases, health issues in species of special concern, or are important to our partners for management of the fishery resource. Other projects supported by the Survey are reoccurring from year to year in order to provide annual trends in disease prevalence for naturally reproducing broodstock populations, or fish health monitoring of natural juvenile fish, as in the Klamath River basin.

In 2009, the Survey focused on newly emerging pathogens with surveys conducted for Spring Viremia of Carp Virus (SVCV), the amphibian disease caused by the chytrid fungus (*Batrachochytrium dendrobatidis*), and Viral Hemorrhagic Septicemia (VHSV). Both VHSV and SVCV are fish pathogens monitored by Animal Plant Health Inspection Service of the Department of Agriculture as well as international entities such as OIE. A total of 575 samples of Red Shiners (*Cyprinella lutrensis*) and Inland Silverside (*Menidia beryllina*) were taken from the San Joaquin River Delta. Carp from the lower Truckee River were sampled. No virus was isolated from the 2009 samples.

As in previous years, we surveyed natural juvenile chinook in the lower Klamath River for the myxosporean parasites *Ceratomyxa shasta* and *Parvicapsula minibicornis*. The ongoing fish health monitoring program provides annual incidence of infection (IOI) data of the myxosporean parasites, by both Quantitative PCR (QPCR) and histology, and support applied research studies and management objectives to recovery chinook and coho in this basin.

Our survey work would be not possible without the support of numerous partners including: California Department of Fish and Game, U.S. Bureau of Reclamation, several Fish and Wildlife Offices (Arcata, Yreka, Reno, and Stockton). The Karuk, Hoopa, and Yurok tribes of Northern California and Oregon Department of Fish and Wildlife (ODFW).

Accomplishment Report for 2009 – Pathogen Surveys

Spring Viremia of Carp Surveys – Nevada

Spring Viremia of Carp (SVC) is a contagious and potentially fatal viral disease of carp and other cyprinid species. Historically, SVCV has been a problem in Europe, the Middle East, and Russia. Infections have been reported in grass carp (*Ctenopharyngodon idella*), bighead carp (*Aristichthys nobilis*), and silver carp (*Hypophthalmichthys molitrix*) however common carp (*Cyprinus carpio*) are the most susceptible species. SVCV virus outbreaks are temperature dependent and are commonly seen during the spring and early summer months.

The first report of this virus in the United States came from a North Carolina koi farm in the spring of 2002 and was possibly caused by importation of infected fish from another country. The virus is easily spread by contact with infected fish and can stay infectious for long periods of time in water and mud. The only way to prevent SVCV is to avoid introduction of the virus. Since this first outbreak, the USFWS, USDA Animal and Plant Health Inspection Service (APHIS), and local partners have been working together to detect and monitor this emerging pathogen in wild fish populations.

The California-Nevada Fish Health Center (FHC) collected common carp (*Cyprinus carpio*) as part of the Survey to determine whether the disease is present in the lower Truckee River and Pyramid Lake. Sample collection would not have been possible without the collaboration from our local partners; Reno Fish and Wildlife Office. A crew from the Reno Fish and Wildlife Office participated in sample collection training, and collection of carp from Pyramid Lake, just outside Reno, NV. Seventy-eight fish were sampled for this effort; no virus or reportable bacteria were detected.

Klamath River Fish Health Monitoring Program

Concerns in the Klamath River basin regarding flow allocations and the relationship to disease incidence were heightened during the 2002 adult Chinook salmon (*O. tshawytscha*) fish kill. Many federal, state, local and tribal biologists are conducting research to better understand what biological factors influence the incidence of disease in this river system. Two parasites, *Ceratomyxa shasta* and *Parvicapsula minibicornis*, which occur as dual infections in a large proportion of juvenile salmon, are of special concern. Fish health monitoring studies address the potential disease impacts on survival of natural and hatchery produced out-migrating juvenile Chinook, and provides annual trend data.

Ceratomyxosis 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 2008 for *C. shasta* and *P. minibicornis* for mixed origin Chinook (hatchery and natural fish) during the 2009 monitoring period (May to August) was 43% and 82% respectively by QPCR. Monitoring of natural fish, sampled and tested prior to hatchery releases indicated that *C. shasta* was first



Examining gills for Columnaris disease on juvenile chinook salmon.

detected by QPCR during April in the Shasta River to Scott River reach at a POI of 10%. By May, the POI had peaked at 97% by QPCR. Further downriver, between the Salmon River and Trinity River confluence, the *C. shasta* POI was lower (60%) in May. *Parvicapsula minibicornis* was first detected in natural origin Chinook in late April, at POI of 77%, in the upper reach (Shasta River to Scott River), and reached 100% by early May. In the lower reach, *P. minibicornis* POI also reached 100% by June. As in previous years, *P. minibicornis* prevalence rose rapidly, and the majority of natural fish infected with *C. shasta* were also infected with *P. minibicornis*.

In support of the current effort to re-introduce anadromous fishes above the Klamath dams, the FHC partnered with the Oregon Department of Wildlife to survey redband trout in Klamath river above JC Boyle dam. As seen below the dams, both *Parvicapsula minibicornis* 9/12 (75%) and *Ceratomyxa shasta* 4/12 (33%) were detected as well as *Yersinia ruckeri* 1/43 (2%). It is likely that the myxosporean parasites are different genotypes than in the lower river.

San Joaquin Delta

The Ca-Nv Fish Health Center partnered with the Stockton Fish and Wildlife Office to obtain samples of baitfish populations from several sites in the San Joaquin Delta. These efforts were to screen for Viral Hemorrhagic Septicemia virus. Viral hemorrhagic septicemia virus (VHSV) is a serious systemic disease of fish. The VHS virus is carried by at least 50 species of marine and freshwater fish. The infection is subclinical in some species, but it is associated with severe disease and high mortality rates in others. Clinical infections are economically important in farmed fish, particularly rainbow trout, turbot and Japanese flounder. Outbreaks have also been reported in some wild populations, including Pacific herring and pilchard along the Pacific coast of North America. A total of 575 fish, including Red Shiners (*Cyprinella lutrensis*) and Inland Silversides (*Menidia beryllina*) were captured and screened. No reportable viral or bacterial pathogens were detected.

Surveys of Spawning 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 and Livingston Stone National Fish Hatcheries. Returning Fall Chinook Salmon, Steelhead, Late Fall, and Winter Chinook adults are monitored each year to determine the disease status of adult salmonid populations in the upper Sacramento basin. This report focuses on Winter run populations.

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 population levels.

Natural origin Winter run adults are captured at the base of Keswick Dam and transferred to LSNFH for egg collection. In 2009, 73 samples were collected from spawned wild fish. Infectious Hematopoietic Necrosis virus (IHNV) was detected in 11 of 29 (38%) kidney samples and 24 of 44(55%) ovarian fluid samples. *Renibacterium salmoninarum* was not detected in kidney or ovarian fluid tested by QPCR. Sections of intestine and kidney had parasites *C. shasta* 25/30 (83%) and *P. minibicornis* 20/30 (67%) respectively. The bacterium *Aeromonas salmonicida*, which causes furunculosis disease, was detected in 2/57 (4%) of the adults.

Upper Klamath Lake (Link River dam) The FHC performed a health evaluation of juvenile suckers (including endangered Lost River and Shortnose suckers), blue chubs and Fathead minnows captured by the Bureau of Reclamation as part of a salvage program. Histological evaluation of 154 fish demonstrated a high incidence of parasite infection (*Trichodina sp.*, *Myxobolus sp.* and various helminths).

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 (Heil, 2009) at the following websites:

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

CANV Fish Health Center <http://www.fws.gov/canvfhc/nwfhsman.htm>

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) 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 halved, to provide an archive set for PCR confirmation testing and for PTD testing. Cranial elements were heated in a 60°C water bath for 60 minutes to remove 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.

References and Additional Reading

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Appendix I - NWFHS SUMMARY TABLE FOR FY 2008

Case #	Date Collected	Location	Species	Partners	Number of Sites	Total Fish	Significant Findings
09-005	9-15-2008	Carmel River, Ca	Steelhead	Monterey Bay Peninsula Water District	1	41	Metacercaria 1/11 (9%) <i>Tetracapsula bryosalmonae</i> (PKX) 1/11 (9%)
09-091 (8 sample dates)	05-09 through 07-09	Livingston Stone NFH	Winter Chinook	LSNFH	1	73	IHNV 48% <i>Aeromonas salmonicida</i> 4% <i>Ceratomyxa shasta</i> 83% <i>Parvicapsula minibicornis</i> 67%
09-033	2-10-2009	San Joaquin River, Ca	Inland Silverside	Stockton FWO	1	150	
09-034	2-10-2009	San Joaquin River, Ca	Red Shiner	Stockton FWO	1	125	
09-046	3-31-2009	Pyramid Lake, Nev	Common Carp	Reno FWO	2	78	
09-047	3-20-2009	Stanislaus River	Chinook Salmon	Cramer Fish Sciences	1	7	
09-050	4-14-2009	San Joaquin River, Ca	Inland Silverside	Stockton FWO	1	300	
09-093	5-12-2009	Klamath River, Ca	Chinook Salmon	Arcata FWO, Hoopa/Yurok/ Karuk Tribal Fisheries	5	89	<i>Parvicapsula minibicornis</i> 64/89 (72%) <i>Ceratomyxa shasta</i> 32/89 (36%)
09-095	5-30-2009	Trinity River, Ca	Chinook Salmon	Hoopa Tribal Fisheries	2	124	<i>Parvicapsula minibicornis</i> 6/124 (5%) <i>Ceratomyxa shasta</i> 16/124 (13%)
09-097	8-11-2009	Upper Klamath Lake, Or	Lost River/ Shortnose Sucker, Blue Chub, Fathead Minnow	United States Bureau of Reclamation	2	154	See Appendix II for per species results.

Case #	Date Collected	Location	Species	Partners	Number of Sites	Total Fish	Significant Findings
09-126	9-14-2009	Klamath River, Ca	Rainbow Trout-Interior Redband	Bill Tinniswood-ODFW	1	43	<i>Yersinia ruckeri</i> 1/43 (2%) <i>Parvicapsula minibicornis</i> 9/12 (75%) <i>Ceratomyxa shasta</i> 4/12 (33%)

Appendix 2 – Pathology and Sample Summary Reports to Partners

Case #09-005 (Carmel River, Ca Steelhead)

Tetracapsuloides bryosalmonae, the causative agent for Proliferative Kidney Disease (PKX), was observed by Histology in 1/11 (9%) Kidney samples. Metacercaria was observed in 1/11 (9%) eye samples.

	SAMPLE NAME /TISSUE TYPE	NO. SAMPLES (POOL SIZE)	NO. POSITIVE /TOTAL	PERCENT POSITIVE	TOTAL FISH SAMPLED
Parasitology:					
Pepsin/Trypsin Digest. Used to test for presence of <i>Myxobolus cerebralis</i>	Head	4(5)p	0/4	0%	20
HISTOLOGY:					
Microscopic examination of organs fixed in Davidson's and stained with Hematoxylin and eosin.	Kidney	11 (1p)	1/11	9%	11
	Gill	11 (1p)	0/11	0%	
	Eye	11 (1p)	1/11	9%	
	Heart	11 (1p)	0/11	0%	
	Liver	11 (1p)	0/11	0%	
BACTERIOLOGY:					
Non-Culturable BACTERIA					
<i>Renibacterium salmoninarum</i> (Bacterial Kidney Disease)	Kidney	21(1p)	0/21	0%	21
Rs-QPCR: Confirmation by PCR is required for Rs-positive status					

Case # 09-091 (Keswick, CA) Winter Chinook Adults

This table summarized results from 8 separate sample dates. 35/73 samples were

positive for IHNV. *Aeromonas salmonicida*, the causative agent for Furunculosis, was detected in 2/57 samples on culturable media. *Ceratomyxa shasta* (Cs) and *Parvicapsula minibicornis* (Pm) was detected by QPCR in 83% (25/30) and 67% (20/30) respectively.

	SAMPLE NAME /TISSUE TYPE	NO. SAMPLES (POOL SIZE)	NO. POSITIVE /TOTAL	PERCENT POSITIVE	TOTAL FISH SAMPLED
VIROLOGY:					
Specific cell lines used: EPC and CHSE-214	Kidney/Ovarian Fluid	73(1)p	35/73	48%	73
BACTERIOLOGY:					
Non-Culturable Bacteria					
<i>Renibacterium salmoninarum</i> (FAT)	Kidney	73(1)p	0/73	0%	73
Culturable bacteria on BHIA pure plates	Kidney	57(1)p	2/57	4%	57
PARASITOLOGY:					
CS-QPCR: Detects CS 18s DNA, presumably viable <i>Ceratomyxa shasta</i> trophozoites in intestinal tissue	Intestine	30(1)p	25/30	83%	30
PM-QPCR: Detects PM 18s DNA, presumably viable <i>Parvicapsula minibicornis</i> trophozoites in kidney	Kidney	30(1)p	20/30	67%	30

Case# 09-033 (San Joaquin River, Ca)

No viral isolates were observed in either the primary EPC or secondary CHSE-214 cell lines. No culturable bacteria were seen on BHIA plates.

	SAMPLE NAME /TISSUE TYPE	NO. SAMPLES (POOL SIZE)	NO. POSITIVE /TOTAL	PERCENT POSITIVE	TOTAL FISH SAMPLED
VIROLOGY:					
Specific cell lines used: EPC and CHSE-214	Inland Silverside-KD	30(5)p	0/30	0%	150
BACTERIOLOGY:					
Culturable bacteria on BHIA pure plates	Inland Silverside-KD	30 (1)p	0/30	0%	30

Case# 09-034(San Joaquin River, Ca)

No viral isolates were observed in either the primary EPC or secondary CHSE-214 cell lines. No culturable bacteria were seen on BHIA plates.

	SAMPLE NAME /TISSUE TYPE	NO. SAMPLES (POOL SIZE)	NO. POSITIVE /TOTAL	PERCENT POSITIVE	TOTAL FISH SAMPLED
VIROLOGY:					
Specific cell lines used: EPC and CHSE-214	Red Shiner-KD	25 (5)p	0/25	0%	125
BACTERIOLOGY:					
Culturable bacteria on BHIA pure plates	Red Shiner-KD	30 (1)p	0/30	0%	30

Case# 09-046 (Pyramid Lake, NV)

No viral CPE was observed in either the KF-1 (Koi Fin) or EPC cell lines. No reportable bacteria were seen on culturable media.

	SAMPLE NAME /TISSUE TYPE	NO. SAMPLES (POOL SIZE)	NO. POSITIVE /TOTAL	PERCENT POSITIVE	TOTAL FISH SAMPLED
VIROLOGY:					
Specific cell lines used: KF-1 and EPC	Carp-KD	26(3)p	0/26	0%	78
BACTERIOLOGY:					
Culturable bacteria on BHIA pure slants:	Carp-KD	30(1)p	0/30	0%	78

Case# 09-047 (Stanislaus River, Ca)

No parasites or tissue inflammation observed. All fish were juvenile Chinook salmon.

	SAMPLE NAME /TISSUE TYPE	NO. SAMPLES (POOL SIZE)	NO. POSITIVE /TOTAL	PERCENT POSITIVE	TOTAL FISH SAMPLED
Histology:					
Microscopic examination of organs fixed in Davidson's and stained with Hematoxylin and eosin.	Kidney	7(1)p	0/7	0%	7
	Gill	7(1)p	0/7	0%	7
	Intestine	7(1)p	0/7	0%	7

Case# 09-050 (San Joaquin River, Ca)

No viral CPE or significant fish pathogens were observed.

	SAMPLE NAME /TISSUE TYPE	NO. SAMPLES (POOL SIZE)	NO. POSITIVE /TOTAL	PERCENT POSITIVE	TOTAL FISH SAMPLED
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VIROLOGY:					
Specific cell lines used: EPC, CHSE-214	Inland Silverside- Kidney/Spleen/Gut	60(5)p	0/60	0%	300
BACTERIOLOGY:					
Culturable bacteria on BHIA pure slants:	Inland Silverside-KD	30(1)p	0/30	0%	30

Case#09-093 (Klamath River, Ca)

Ceratomyxa shasta was detected in 42% (8/19) samples assayed by Histology and 36% (32/89) by QPCR. *Parvicapsula minibicornis* was detected in 84% (16/19) samples assayed by Histology and 72% (64/89) by QPCR.

	SAMPLE NAME /TISSUE TYPE	NO. SAMPLES (POOL SIZE)	NO. POSITIVE /TOTAL	PERCENT POSITIVE	TOTAL FISH SAMPLED
HISTOLOGY:					
Microscopic examination of organs fixed in Davidson's and stained with Hematoxylin and eosin.	Kidney	19(1)p	16/19	84%	19
	Intestine	19(1)p	8/19	42%	19
PARASITOLOGY:					
CS-QPCR: Detects CS 18s DNA, presumably viable <i>Ceratomyxa shasta</i> trophozoites in intestinal tissue	Intestine	89(1)p	32/89	36%	89
PM-QPCR: Detects PM 18s DNA, presumably viable <i>Parvicapsula minibicornis</i> trophozoites in kidney	Kidney	89(1)p	64/89	72%	89

Case#09-094 (Devils Hole, NV)

A total of 9 Devils Hole Pupfish were examined for parasites by histology. No reportable Pathogens or tissue inflammation was observed.

	SAMPLE NAME /TISSUE TYPE	NO. SAMPLES (POOL SIZE)	NO. POSITIVE /TOTAL	PERCENT POSITIVE	TOTAL FISH SAMPLED
Histology:					
Microscopic examination of organs fixed in Davidson's and stained with Hematoxylin and eosin.	Kidney	9(1)p	0/9	0%	9
	Gill	9(1)p	0/9	0%	9
	Gonads	9(1)p	0/9	0%	9

Case#09-095 (Trinity River, Ca)

Ceratomyxa shasta was detected in none of the samples (0/39) assayed by histology and 5% (6/124) by QPCR. *Parvicapsula minibicornis* was not detected in any of the samples assayed by histology (0/39) and was found in 13% (16/124) of samples assayed by QPCR.

	SAMPLE NAME /TISSUE TYPE	NO. SAMPLES (POOL SIZE)	NO. POSITIVE /TOTAL	PERCENT POSITIVE	TOTAL FISH SAMPLED
HISTOLOGY:					

Microscopic examination of organs fixed in Davidson's and stained with Hematoxylin and eosin.	Kidney	39(1)p	0/39	0%	39
	Intestine	39(1)p	0/39	0%	39
PARASITOLOGY:					
CS-QPCR: Detects CS 18s DNA, presumably viable <i>Ceratomyxa shasta</i> trophozoites in intestinal tissue	Intestine	124(1)p	6/124	5%	124
PM-QPCR: Detects PM 18s DNA, presumably viable <i>Parvicapsula minibicornis</i> trophozoites in kidney	Kidney	124 (1)p	16/124	13%	124

Case#09-097 (Link River/J-Canal OR)

A total of 118 fish (LRS-Lost River Sucker, BCB-Blue Chub, FHM-Fathead Minnow) were examined by histology. Not all tissues were examined from every animal.

	SAMPLE NAME /TISSUE TYPE	NO. SAMPLES (POOL SIZE)	NO. POSITIVE /TOTAL	PERCENT POSITIVE	TOTAL FISH SAMPLED
Histology:					
Microscopic examination of organs fixed in Davidson's and stained with Hematoxylin and eosin.	LRS-Gill	40(1p)	25/40 ¹	63%	42
	LRS-Muscle	18(1p)	5/18 ³	28%	
	LRS-Kidney	41(1p)	15/41 ⁷	37%	
	LRS-Intestine	41(1p)	9/41 ²	22%	
	LRS-Intestine	41(1p)	9/41 ⁵	22%	

BCB-Gill	37(1p)	32/37 ¹	86%	37
BCB-Gill	37(1p)	2/37 ²	5%	
BCB-Gill	37(1p)	7/37 ⁶	19%	
BCB-Muscle	34(1p)	6/34 ³	18%	
BCB-Muscle	34(1p)	5/34 ²	15%	
BCB-Intestine	37(1p)	17/37 ⁵	46%	
BCB-Eye	33(1p)	1/33 ⁴	3%	
FHM-Gill	51(1p)	16/51 ¹	31%	51
FHM-Gill	51(1p)	19/51 ²	37%	
FHM-Muscle	50(1p)	12/50 ³	24%	
FHM-Eye	49(1p)	3/49 ⁴	6%	
FHM-Intestine	50(1p)	7/50 ⁵	14%	

1=Trichodina, 2=Myxozoan cyst, 3=Metacercaria (blackspot), 4=Trematode, 5=Helminth
6=Epitheliocystis, 7=Myxozoan trophozoite

Case# 09-126 (Klamath River, OR)

	SAMPLE NAME /TISSUE TYPE	NO. SAMPLES (POOL SIZE)	NO. POSITIVE /TOTAL	PERCENT POSITIVE	TOTAL FISH SAMPLED
VIROLOGY: Specific cell lines used: EPC and CHSE-214	Kidney	8(5)p,1(3)p	0/9	0%	43

BACTERIOLOGY:					
Non-Culturable Bacteria					
<i>Renibacterium salmoninarum</i> (FAT) Rs-QPCR: Confirmation by PCR is required for Rs-positive status	Kidney	43(1)p	0/43	0%	43
Culturable bacteria on BHIA pure plates FAT confirmation required for <i>Yersinia ruckeri</i> positive status	Kidney	36(1)p	1/36	3%	36
PARASITOLOGY:					
CS-QPCR: Detects CS 18s DNA, presumably viable <i>Ceratomyxa shasta</i> trophozoites in intestinal tissue	Intestine	43(1)p	6/43	14%	43
PM-QPCR: Detects PM 18s DNA, presumably viable <i>Parvicapsula minibicornis</i> trophozoites in kidney	Kidney	43(1)p	9/43	21%	43
Pepsin/Trypsin Digest. Used to test for presence of <i>Myxobolus cerebralis</i>	Head	8(5)p, 1(3)p	0/9	0%	43

Appendix 3 – Partnerships and Sample Sites

Sample Location	Partner
1. Carmel River, CA	Monterey Bay Peninsula Water District
2. Shasta Reservoir, CA	Livingston Stone NFH-USFWS
3. Dads Point, San Joaquin River, CA	Stockton FWO-USFWS
4. Dos Reis County Park, San Joaquin River, Ca	Stockton FWO-USFWS
5. Pyramid Lake, NV	Reno FWO-USFWS Marble Bluff Fish Facility
6. Stanislaus River, Ca	Cramer Fish Sciences
7. Sherman Island, San Joaquin River, Ca	Stockton FWO-USFWS
8. Klamath River, Ca	Arcata FWO-USFWS, Karuk & Yurok Tribal Fisheries
9. Trinity River, Ca	Hoopa Tribal Fisheries
10. Devils Hole, Ca	United States Bureau of Reclamation
11. Upper Klamath Lake, Or	United States Bureau of Reclamation
12. Klamath River, Or	Oregon Department of Fish and Wildlife

