



National Wild Fish
Health Survey



California-Nevada Fish Health Center

Annual Report for Fiscal Year 2011



National Wild Fish Health Survey Annual Progress Report FY 2011

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California-Nevada Fish Health Center

Center staff conducted the National Wild Fish Health Survey (NWFHS) in 2011 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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Notice

The mention of trade names or commercial products in this report does not constitute endorsement or recommendation for use by the Federal government. The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the US Fish and Wildlife Service.

Overview

The National Wild Fish Health 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 19,106 samples have been collected and tested for major fish pathogens over the past 13 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 2011, the Survey focused on health screenings of imperiled stocks of fish with surveys conducted for the Delta Smelt (*Hypomesus transpacificus*), Klamath River Fall-Run Chinook (*Oncorhynchus tshawytscha*), and the Central Valley Fall-Run Chinook (*O. tshawytscha*). In addition, screenings were performed on the Sacramento River Winter-Run (*O. tshawytscha*) and a screening for the emerging pathogen *Nucleospora salmonis* was also conducted in the Tahoe Basin. Pathogens screened include Viral Hemorrhagic Septicemia Virus (VHSV), Infections Hematopoietic Necrosis Virus (IHNV), and Infectious Pancreatic Necrosis Virus (IPNV).

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 recover 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, UC Davis and Oregon State University, USGS, 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 2011 – Pathogen Surveys

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 can 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) for *C. shasta* and *P. minibicornis* for mixed origin Chinook (hatchery and natural fish) during the 2011 monitoring period (April to August) was 17% and 43% respectively by QPCR. Monitoring of natural fish, sampled and tested prior to hatchery releases indicated that *C. shasta* was first detected in the Klamath River by QPCR mid-May in the Shasta River to Scott River reach, Trinity River to Salmon River reach, and in late April in the Trinity River at a POI of 40%, 10%, and 10% respectively. *Parvicapsula minibicornis* was first detected in natural origin Chinook in early May, at POI of 11%, in the Klamath Estuary to Trinity River reach, and reached 100% in the Shasta River to Scott River reach in mid-May. In the lower reaches, *P. minibicornis* POI also reached 100% by June. In the FY2011 sampling season, the incidence of infection indicated that infectivity was very low in comparison to previous years in which monitoring studies were conducted (True et al. 2011).



Examining gills for Columnaris disease on juvenile Chinook salmon.

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 Chinook, 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 natural origin Late-Fall adults returning to Keswick Dam and 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. Samples were taken from 72 natural origin Winter Run Chinook adults. Infectious Hematopoietic Necrosis virus (IHNV) was detected in 24% of males (7/29) and 51% of females (22/43). The causative agent for Bacterial Kidney Disease, *Renibacterium salmoninarum*, was detected in 9 of 12 samples tested by QPCR. Additionally, a relatively high proportion of fish were found positive for *Ceratomyxa shasta* and *Parvicapsula minibicornis* with prevalence levels at 76% (19/25) and 85% (22/26) respectively.

Natural origin Late-Fall adults are captured at the base of Keswick Dam and transferred to LSNFH for egg collection. In 2011, 14 samples were collected from spawned wild fish. IHNV was detected in 5 of 6 (83%) pooled kidney samples and 3 of 3 (100%) ovarian samples. *Renibacterium salmoninarum* was not detected in kidney or ovarian fluid tested by QPCR.

Nucleospora screening in Central Sierra Nevada Salmonids

The FHC was asked to assist in a screening for *Nucleospora salmonis* in four Trout species in the Sierra Central Nevada basin. *N. salmonis* is a microsporidian parasite of salmonids fishes that has caused disease in California hatchery populations (Fogerty, 2011). This pathogen is considered a health concern for the California Department of Fish and Game (W. Cox, Sep 14, 2010 interagency meeting). Sample sites included Fallen Leaf Lake (Lake Trout, *Salvelinus namaycush*), Independence Lake (Brook Trout, *Salvelinus fontinalis*, and Cutthroat Trout, *Oncorhynchus clarkii*), and Taylor Creek (Kokanee, *Oncorhynchus nerka*). Samples from Independence Lake and Taylor Creek were kindly provided by the USGS and CDFG respectively. The Kokanee, Brook Trout, and Cutthroat Trout were tested for *N. salmonis* only. Lake Trout samples were examined for viral and cultured bacterial fish pathogens under the standardized procedures of the National Wild fish health Survey in addition to the screening for *N. salmonis* and were collected with the assistance of the Reno Fish and Wildlife. All samples tested by SSU QPCR for *N. salmonis* were negative. Additionally, no viral/bacterial pathogens were detected nor were parasites observed by histology in the Lake Trout samples from Fallen Leaf Lake.



Stanislaus River

Fish were surveyed from two out-migrant monitoring sites (RM's 6 and 40) along the Stanislaus River to screen for fish pathogens and disease. Liver, kidney and gill sections were examined for abnormalities indicative of environmental toxicity. No insult or tissue abnormalities were detected in the 194 fish sampled. No viral pathogens were detected in 76 fry or parr samples and only a few (12/70) were found to have bacterial or parasitic infection. When assayed by direct fluorescent antibody test, a low prevalence (12/112, 11%) of kidney samples were found; however, none of these were confirmed by PCR (Foott, 2011). No evidence of *Tetracapsuloides bryosalmonae*, the causative agent of Proliferative Kidney Disease, was



| observed in 62 samples compared to a 7% POI in 2010 (Nichols, 2010).

Lower Sacramento River Kodiak Surveys

The Ca-Nv Fish Health Center partnered with California Fish and Game and the Stockton Fish and Wildlife Office to obtain samples of Delta Smelt and Inland Silverside populations from several sites in the Lower Sacramento River. These efforts were to determine the presence of infectious pathogens (virus, bacteria, or parasite) and tissue abnormalities. An emerging pathogen of concern is Viral Hemorrhagic Septicemia Virus (VHSV). 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 55 Delta Smelt and 158 Inland Silversides were captured and screened. No reportable viral or bacterial pathogens were detected. The Delta Smelt were apparently healthy as demonstrated by a lack of clinical signs or morbidity (Foott & Bigelow, 2010). No viral pathogens were detected in Inland Silversides and few tissue abnormalities or overt infectious diseases were detected by histology.

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), Infectious Pancreatic Necrosis virus (IPNV), Infectious Salmon Anemia Virus (ISAV), and Viral Nervous Necrosis (VNNV) using accepted cell culture techniques. Kidney and spleen tissues were tested individually, or from 3-5 fish pooled into one sample. The World Organization for Animal Health accepts the use of SSN-1 cell lines for the detection of VNNV (http://www.oie.int/eng/normes.fmanual/A_00024.htm)

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), Striped Snakehead (SSN-1), or Bluegill Fry (BF-2) cell lines in replicate onto 48-well plates. The SSN-1 cell line was selected to screen for nodavirus in Delta fish samples and requires L-15 media. 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.

Quantative Polymerase Chain Reaction (QPCR) for *C. shasta* and *P. minibicornis*

Combined intestine and kidney tissues were digested in 1ml NucPrep Digest Buffer containing 1.25 mg/ml proteinase K (Applied Biosystems, Foster City, CA) at 55°C for 2 hours with constant shaking. A subsample of digested tissue homogenate was diluted 1:33 in molecular grade water and extracted in a 96 well vacuum filter plate system. Extracted DNA was stored at -20°C until the QPCR assays were performed.

Samples were assayed in Real Time PCR Sequence Detection Systems (SDS) using probes and primers specific to each parasite. The combined tissues were tested for *C. shasta* 18S rDNA using TaqMan Fam-Tamra probe and primers (Hallett and Bartholomew 2006) on the 7300 Sequence Detection System (Applied Biosystems, Foster City, CA). Separately, the combined tissues were tested for *P. minibicornis* 18S rDNA utilizing TaqMan Minor-Grove-Binding (MGB) probe and primers (True et al. 2009) on the StepOne Plus Sequence Detection System (Applied Biosystems Foster City, CA). Reaction volumes of 30µL, containing 5µL DNA template, were used for both assays under the following amplification conditions: 50°C for 2 min.; 95°C for 10 min; 40 cycles of 95°C for 15s and 60°C for 1 min. Plasmid standards, extraction control and no template control (NTC) wells were included on each assay plate.

Small Subunit gene (SSU) Polymerase Chain Reaction (PCR) for *Nucleospora salmonis*

A protocol was adopted from Badil et al. (in press) that targets the ribosomal SSU gene and has been reported superior to PCR methodology included in the 2010 AFS-FHS Blue Book and currently no methodology exists within the laboratory protocols in the National Wild Fish Health Survey Procedures Manual (<http://fisheries.fws.gov/FHC/FHCNational.htm>).

N. salmonis SSU QPCR protocol:

Ribosomal Small Subunit Gene (SSU) Quantitative PCR to identify *Nucleospora (Enterocytozoon) salmonis* DNA within Fish Tissue USGS. Western Fisheries Research Center, 6505 NE 65th St. Seattle, WA 98115. Maureen Purcell.

References and Additional Reading

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Appendix I - NWFHS SUMMARY TABLE FOR FY 2011

Case #	Date Collected	Location	Species	Partners	Number of Sites	Total Fish	Significant Findings
11-005	10/22/2010	Fallen Leaf Lake, Ca	Lake Trout	Reno FWO	1	38	
11-006, 11-013	10/26/2010	Independence Lake, Ca	Brook Trout	USGS	1	134	
11-007	10/26/2010	Taylor Creek, Ca	Kokanee	CDFG	1	52	
11-010	10/26/2010	Independence Lake, Ca	Cutthroat Trout	USGS	1	1	
11-014 11-020 11-026 11-043	11/18/2010 through 3/01/2011	Liberty Island, Ca Sacramento San Joaquin River Delta	Inland Silverside Delta Smelt	Stockton FWO CDFG	Multiple	213	See Appendix II for results
Multiple cases	6/13/2011 through 8/24/2011	Sacramento River Livingston Stone NFH	Winter Chinook Salmon	LSNFH	1	72	IHNV 29/72 (40%) <i>R. salmoninarum</i> 9/12 (75%) <i>Ceratomyxa shasta</i> 19/25 (76%) <i>P. minibicornis</i> 22/2 (85%)
11-019 11-032 11-033 11-037	4-14-2010 (4 sample dates)	Keswick Dam Livingston Stone NFH	Late Fall Salmon	LSNFH	1	14	IHNV 8/9 (89%)
11-040	2/25/2011	Red Bluff Research Pumping Station	Green Sturgeon	BOR	1	2	

Case #	Date Collected	Location	Species	Partners	Number of Sites	Total Fish	Significant Findings
Multiple cases	3/12/2011 through 5/03/2011	Stanislaus River, Ca	FCS	FISHBIO Cramer Fish Sciences	2	194	<i>R. salmoninarum</i> 12/112 (11%)
11-105	6/29/2011	Bucktail Flats, Ca	BullFrog	Jamie Betasso	1	19	<i>B. dendrobatidis</i> 17/19 (89%)
11-127	9/07/2011	Big Tujunga Creek	Santa Ana Sucker	USGS	2	6	
Multiple cases	Multiple sample dates	Trinity River, Ca	Chinook Salmon	Hoopa Tribal Fisheries	2	61	<i>P. minibicornis</i> 0/21 <i>Ceratomyxa shasta</i> 1/21 (5%)
Multiple cases	Multiple sample dates	Klamath River, Ca	Chinook Salmon	Arcata FWO, Hoopa/Yurok/ Karuk Tribal Fisheries	4	260	<i>P. minibicornis</i> 80/209 (38%) <i>Ceratomyxa shasta</i> 42/248 (17%)

Appendix 2 – Pathology and Sample Summary Reports to Partners

Case# 11-127 (Big Tujunga Creek, Ca) Santa Ana Sucker, *Catostomus santaanae*

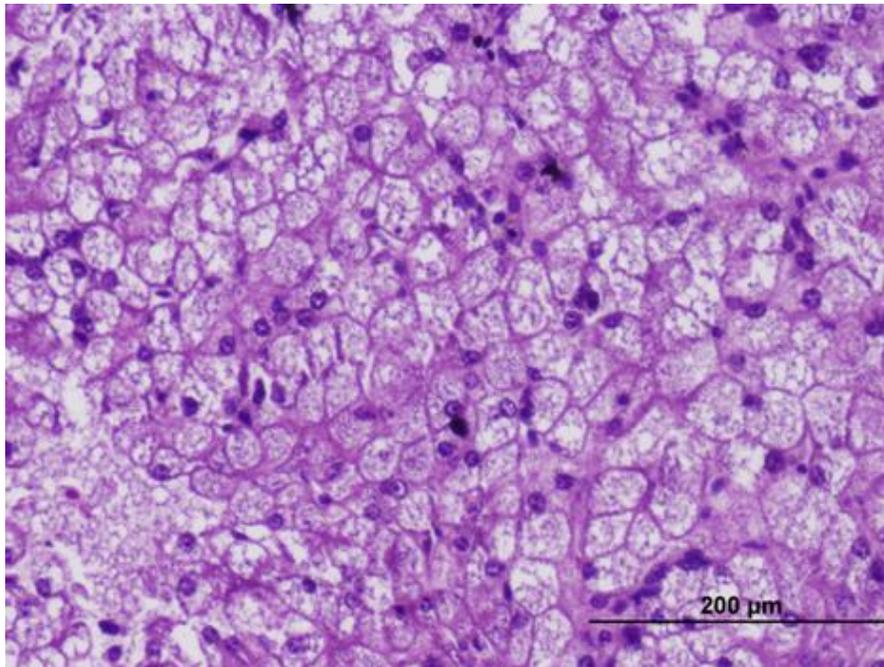
A total of 6 Santa Ana Suckers were examined for parasitic or bacterial infection by histology from Big Tujunga Creek. No significant abnormalities or infections observed in any tissues.

	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	5 (1p)	0/5	0%	6
	Liver	5 (1p)	0/5	0%	6
	Intestine	1 (1p)	0/1	0%	6
	Muscle Skin	1 (1p)	0/1	0%	6
	Gill	1 (1p)	0/1	0%	6
	Spleen	2 (1p)	0/2	0%	6

Case# 11-040 (Red Bluff Research Pumping Station, Ca) Green Sturgeon, *Acipenser medirostris*

A total of 2 juvenile Green Sturgeon were examined for parasitic or bacterial infection by histology from the Sacramento River. No significant abnormalities or infections observed in any tissues. No bacteria seen in gram stains from gill stained imprint.

	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	2 (1p)	0/2	0%	2
	Liver	2 (1p)	0/2	0%	2
	Intestine	2 (1p)	0/2	0%	2
	Gill	2 (1p)	0/2	0%	2



Liver with vacuolated hepatocytes. Likely normal fat and glycogen storage

Case #11-005 (Fallen Leaf Lake, Ca) Adult Lake Trout-*Salvelinus namaycush*

No parasites were observed in any tissues assayed by histology. No reportable bacteria were seen on culturable media.

	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	20 (1p)	0/20	0%	20
	Intestine	20 (1p)	0/20	0%	20
VIROLOGY:					
Specific cell lines used: EPC, CHSE-214	Kidney	8(3-5p)	0/8	0%	38
PARASITOLOGY:					
Ns-PCR. Used to detect SSU of rDNA of <i>Nucleospora salmonis</i> in kidney tissue	Kidney	38 (1p)	0/38	0%	38
BACTERIOLOGY:					
Non-Culturable Bacteria <i>Renibacterium salmoninarum</i> (ELISA)	Kidney	38(1p)	0/38	0%	38
Culturable bacteria on BHIA pure plates	Kidney	30(1p)	0/30	0%	30

Case #'s 11-006, 11-007, 11-010, 11-013
Testing for *Nucleospora salmonis*

A total of 187 Brook Trout (*Salvelinus fontinalis*), Kokanee (*Oncorhynchus nerka*), and Cutthroat Trout (*Oncorhynchus clarkia*) were examined by PCR to screen for the parasite, *Nucleospora salmonis*. Fish samples were taken from Independence Lake and Taylor Creek.

	Location	Species	Total Samples Assayed	Results	
PARASITOLOGY:	Taylor Creek	Kokanee	52	0/52	0%
Ns-PCR. Used to detect SSU of rDNA of <i>Nucleospora salmonis</i> in kidney tissue	Independence Lake	Brook Trout	134	0/134	0%
	Independence Lake	Cutthroat Trout	1	0/1	0%

**Case #'s 11-019, 11-032, 11-033, 111-037 (Keswick, CA) Adult Late-Fall Chinook-
*Oncorhynchus tshawytscha***

This table summarized results from 4 separate sample dates. 12/14 fish were positive for IHNV. 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: EPC and CHSE-214 Screening for IHNV	Kidney/Ovarian Fluid	9(1-2p)	8/9	89%	14
ImmunoHisto Chemistry (IHC) confirmation of IHNV	Kidney/Ovarian Fluid	8 (1-2p)	8/8	100%	12
BACTERIOLOGY: Non-Culturable Bacteria					
<i>Renibacterium salmoninarum</i> (FAT)	Kidney	12(1p)	0/12	0%	12
Culturable bacteria on BHIA pure plates	Kidney	39(1p)	0/39	0%	39

**Case#'s 11-014, 11-020, 11-026, 11-043 (Sacramento/San Joaquin River Delta, Ca) Delta smelt-
Hypomesmus transpacificus, (Liberty Island, Ca) Inland Silversides *Menidia beryllina***

This table summarized results from 4 separate sample dates. No viral isolates were observed in the EPC, CHSE-214, Bluegill BF-2, or Snakehead SSN-1 cell lines. Few tissue abnormalities and no overt infectious disease was observed in specimens assayed by histology.

	SAMPLE NAME /TISSUE TYPE	NO. SAMPLES (POOL SIZE)	NO. POSITIVE /TOTAL	PERCENT POSITIVE	TOTAL FISH SAMPLED
VIROLOGY: Specific cell lines used: EPC, CHSE-214, BF-2, SSN-1	In. Silversides-	28 (5p)	0/28	0%	140
	Whole Body	6 (3p)	0/6	0%	18
	Delta Smelt- Whole Body	11 (5p)	0/11	0%	55
<hr/>					
HISTOLOGY: Microscopic examination of organs fixed in Davidson's and stained with Hematoxylin and eosin.	<u>Inland Silverside</u>				
	Gill	40 (1p)	1,1/40 ^{1,2}	2.5%	42
	Skin	41 (1p)	0/41	0%	42
	Muscle	41 (1p)	0/41	0%	42
	Brain	28 (1p)	0/28	0%	42
	Eye	37 (1p)	0/37	2%	42
	Intestine	40 (1p)	1/40 ³	2.5%	42
	Liver	40 (1p)	0/40	0%	42
	Kidney	28 (1p)	0/28	0%	42
	Gonads	29 (1p)	0/29	0%	42
	<u>Delta Smelt</u>				
	Intestine	39 (1p)	0/39	0%	55
	Stomach	34 (1p)	0/34	0%	55
	Gill	26 (1p)	0/26	0%	55
	Acinar	23 (1p)	0/23	0%	55
	Adipose	23 (1p)	0/23	0%	55
	Heart	14 (1p)	0/14	0%	55
	Liver	24 (1p)	0/24	0%	55

1=suctorian, 2=epitheliocystis, 3=trematode

**Case #'s 11-091, 11-092, 11-096, 11-098, 11-101, 11-102, 11-104, 11-108, 11-110, 11-111, 11-113, 11-114, 11-115, 11-116, 11-117, 11-123 (Sacramento River, CA) Winter Chinook-
*Oncorhynchus tshawytscha***

This table summarized results from 16 separate sample dates. 29/72 fish were positive for IHNV. No reportable bacteria were seen on culturable media. *Renibacterium salmoninarum* was detected in 9 of 12 samples assayed by PCR; however, those were low-level infections near the detection limit of the assay.

	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 screening for IHNV	Kidney/Ovarian Fluid	72 (1p)	29/72	40%	72
ImmunoHisto Chemistry (IHC) confirmation of IHNV	Kidney/Ovarian Fluid	29 (1p)	29/29	100%	29
HISTOLOGY:					
Microscopic examination of organs fixed in Davidson's and stained with Hematoxylin and eosin. Screening for <i>Ceratomyxa shasta</i> in Intestine and <i>Parvicapsula minibicornis</i> in kidney tissue.	Intestine	25 (1p)	19/25	76%	26
	Kidney	26 (1p)	22/26	85%	26
BACTERIOLOGY:					
Non-Culturable Bacteria					
<i>Renibacterium salmoninarum</i> (PCR)	Kidney	12(1p)	9/12	75%	12
Culturable bacteria on BHIA pure plates	Kidney	72(1p)	0/72	0%	72

Case#'s 11-029, 11-050, 11-054, 11-055, 11-060, 11-075, 11-076 (Stanislaus River, Ca)
Juvenile Fall Chinook Salmon-*Oncorhynchus tshawytscha*

This table summarized results from multiple sample dates. No viral isolates were observed in the EPC or CHSE-214 cell lines. No reportable bacteria were seen on BHIA plates. *Renibacterium salmoninarum* was observed in 12/112 samples observed by dFAT but none were confirmed by PCR. Few tissue abnormalities and no overt infectious disease was observed in 62 specimens assayed by histology.

	SAMPLE NAME /TISSUE TYPE	NO. SAMPLES (POOL SIZE)	NO. POSITIVE /TOTAL	PERCENT POSITIVE	TOTAL FISH SAMPLED
VIROLOGY:					
Specific cell lines used: EPC, CHSE-214,	Kidney	17 (1-5p)	0/22	0%	76
BACTERIOLOGY:					
Culturable bacteria on – BHIA pure plates	Kidney	70 (1p)	0/70	0%	70
Non-Culturable bacteria (Rs) assayed by FAT	Kidney	112 (1p)	12/112	11%	112
Non-Culturable bacteria (Rs) assayed by PCR	Kidney	6 (1p)	0/6	0%	6
HISTOLOGY:					
Microscopic examination of organs fixed in Davidson's and stained with Hematoxylin and eosin.	Kidney	62 (1p)	0/62	0%	62
	Intestine	62 (1p)	6/62 ¹	15%	62

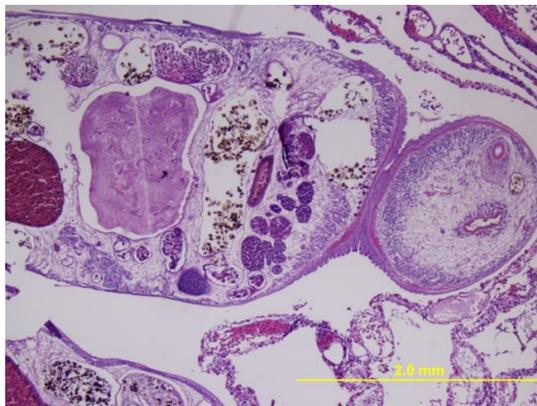
¹=Trematode/Nematode

Case# 11-105 (Lewiston, Ca) American Bullfrog- *Rana catesbeiana*

No viral isolates were observed in either the EPC cell lines. No *Batrachochytrium dendrobatidis* was observed tissue samples histologically. *B. dendrobatidis* was detected in 16/18 samples assayed by QPCR.

	SAMPLE NAME /TISSUE TYPE	NO. SAMPLES (POOL SIZE)	NO. POSITIVE /TOTAL	PERCENT POSITIVE	TOTAL FISH SAMPLED
VIROLOGY:					
Specific cell lines used: EPC	Liver	5 (1-5p)	0/5	0%	19
HISTOLOGY:					
Microscopic examination of organs fixed in Davidson's and stained with Hematoxylin and eosin	Skin	1 (1p)	0/1	0%	1
	Testes	1 (1p)	0/1	0%	1
	Liver	1 (1p)	0/1	0%	1
	Lung	1 (1p)	0/1 ¹	0%	1
QPCR:					
Molecular examination Of DNA extracted From swabs	Mouthpart	17 (1p)	15/17	88%	17
	Skin	1 (1p)	1/1	100%	1

1=Trematode



Trinity River, Ca Juvenile Fall Chinook Salmon-*Oncorhynchus tshawytscha*

This table summarized results from multiple sample dates. *Ceratomyxa shasta* was detected in 3% (1/30) of the samples assayed by histology and 3% (1/31) by QPCR. *Parvicapsula minibicornis* was detected in 10% (3/30) of the samples assayed by histology and was found in 0% (0/31) of samples assayed by QPCR.

	SAMPLE NAME /TISSUE TYPE	NO. SAMPLES (POOL SIZE)	NO. POSITIVE /TOTAL	PERCENT POSITIVE	TOTAL FISH SAMPLED
HISTOLOGY:					
	Kidney	30 (1p)	3/30	10%	30
Microscopic examination of organs fixed in Davidson's and stained with Hematoxylin and eosin.	Intestine	30 (1p)	1/30	3%	30
PARASITOLOGY:					
CS-QPCR: Detects CS 18s DNA, presumably viable <i>Ceratomyxa shasta</i> trophozoites in intestinal tissue	Intestine	31 (1p)	1/31	3%	31
PM-QPCR: Detects PM 18s DNA, presumably viable <i>Parvicapsula minibicornis</i> trophozoites in kidney	Kidney	31 (1p)	0/31	0%	31

Case #'s 10-081 (Klamath River, Ca) Juvenile Fall Chinook Salmon- *Oncorhynchus tshawytscha*

This table summarized results from multiple sample dates. *Ceratomyxa shasta* was detected in 2 % (2/98) samples assayed by Histology and 17% (42/248) by QPCR. *Parvicapsula minibicornis* was detected in 27% (27/100) samples assayed by Histology and 38% (80/209) 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	100 (1p)	27/100	27%	100
	Intestine	98 (1p)	2/98	2%	100
PARASITOLOGY:					
CS-QPCR: Detects CS 18s DNA, presumably viable <i>Ceratomyxa shasta</i> trophozoites in intestinal tissue	Intestine	248 (1p)	42/248	17%	248
PM-QPCR: Detects PM 18s DNA, presumably viable <i>Parvicapsula minibicornis</i> trophozoites in kidney	Kidney	209 (1p)	80/209	38%	248

Appendix 3 – Partnerships and Sample Sites

Sample Location	Partner	Map
1. Sacramento River, Ca	Livingston Stone NFH-USFWS	1
2. Keswick Dam, Ca	Livingston Stone NFH-USFWS	1
3. Independence Lake, Ca	Reno-United States Geologic Survey	2
4. Taylor Creek, Ca	California Department of Fish and Game	2
5. Stanislaus River, Ca	Cramer Fish Sciences/FishBio	2
6. Trinity River, Ca	Hoopa Tribal Fisheries, Arcata FWO-USFWS	1
7. Trinity River, Ca	Hoopa Tribal Fisheries, Arcata FWO-USFWS	
8. Klamath River, Ca	Arcata FWO-USFWS, Karuk & Yurok Tribal Fisheries	1
9. Fallen Leaf Lake, Nv	Reno FWO-USWFS	2
10. Red Bluff Diversion Dam, Ca	Red Bluff Bureau of Reclamation	1
11. Big Tujunga Creek, Ca	United States Geologic Survey	3
12. Bucktail Flats, Ca	Jamie Betasso	1
13. Liberty Island, Ca/Sacramento San Joaquin River Delta	Stockton FWO-USFWS, CDFG	2

Map-1 Northern California



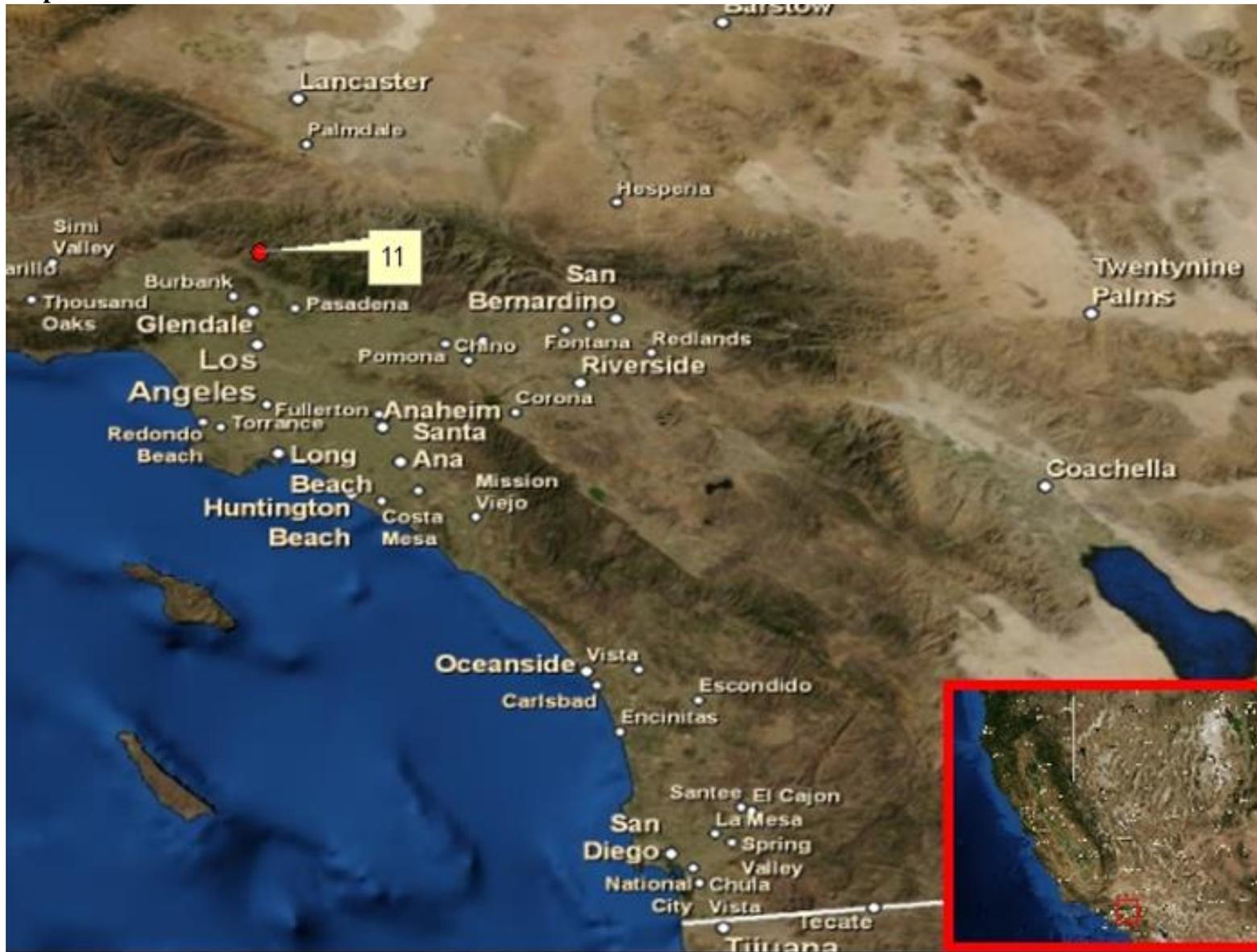
Corresponding partners and sample sites

Map-2 Central California



Corresponding partners and sample sites

Map-3 Southern California



Corresponding partners and sample sites

