

U.S Fish & Wildlife Service

California-Nevada Fish Health Center

National Wild Fish Health Survey Annual Progress Report FY 2012

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U.S. Fish and Wildlife Service
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National Wild Fish Health Survey Annual Progress Report FY 2012

Center staff conducted the National Wild Fish Health Survey (NWFHS) in 2012 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 (NWFHS) 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. The CA-NV Fish Health Center (Center) has partnered with numerous federal and state agencies, tribal governments, universities, non-profit and educational organizations, private companies and private landowners to collect fish at over 200 collection sites. Over 20,000 fish have been tested for major fish pathogens in the last 15 years. The Center's sampling effort to date comprises a rich diversity of fish species in California, Nevada and Southern Oregon and has provided fish health information that did not exist prior to the NWFHS's inception in 1997.

Each year, the center focuses on specific disease issues that are important to our region such as emerging diseases, health issues in species of special concern, or are important to our partners for managing the fishery resource. Other projects supported by the NWFHS 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 wild fish stocks.

In 2012, the NWFHS focused on health screenings of imperiled stocks of fish with surveys conducted for the Delta Smelt (*Hypomesus transpacificus*), Central Valley Winter-run and Fall-run Chinook Salmon (*Oncorhynchus tshawytscha*). Pathogen surveys were also performed on inland silverside (*Menidia beryllina*), Lost River Sucker (*Deltistes luxatus*), and Lahontan Cutthroat trout (*Oncorhynchus clarki henshawi*). Pathogens detected included the bacteria *Renibacterium salmoninarum*, *Aeromonas salmonicida* and *Yersinia ruckeri*, the Infectious Hematopoietic Necrosis virus, and the parasites *Tetracapsuloides bryosalmonae*, *Ceratomyxa shasta* and *Parvicapsula minibicornis*.

Our survey work would not be possible without the support of numerous partners including: California Department of Fish and Wildlife (**CDFW**), California Department of Water Resources (**CDWR**), U.S. Bureau of Reclamation (**USBOR**), UC Davis (**UCD**), Oregon State University (**OSU**), U.S. Geologic Survey (**USGS**), Oregon Department of Fish and Wildlife (**ODFW**), and U.S. Fish and Wildlife Service Stockton office (**USFWS Stockton**).

Pathogen Survey Summaries

Sacramento River Adult Chinook Salmon

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 and Winter-run adult populations returning to Keswick Dam.

Winter run Chinook salmon were listed as endangered by California Department of Fish and Wildlife (CDFW) 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. In 2012, 63 natural origin Winter Chinook adults were sampled. Infectious Hematopoietic Necrosis virus (IHNV) was detected in 21% (3/14) of males and 47% (16/34) of females. The bacterial pathogens *Aeromonas salmonicida* and *Yersinia ruckeri* were detected in 4% (2/51) and 2% (1/51) of fish tested. The causative agent for Bacterial Kidney Disease, *Renibacterium salmoninarum*, was not detected in 40 fish tested. Additionally, a relatively high proportion of fish were found positive for *Ceratomyxa shasta* and *Parvicapsula minibicornis* with prevalence levels at 83% (20/24) and 88% (22/25) respectively.

Natural origin Late-Fall adults are captured at the base of Keswick Dam and transferred to LSNFH for egg collection. In 2012, 47 naturally produced fish were sampled. IHNV was detected in 88% (14/16) of pooled kidney samples and 100% (6/6) of ovarian fluid samples. *Aeromonas salmonicida* was detected in 2% (1/47) of kidney samples. *Renibacterium salmoninarum* was detected in 17% (1/6) of ovarian fluid and none (0/17) of kidney tissue samples tested by direct fluorescent antibody technique.

Sacramento-San Joaquin River Delta Inland Silversides

The Center partnered with USFWS Stockton to sample inland silverside populations throughout the Sacramento-San Joaquin Delta. Inland silverside is a pelagic species selected as a surrogate for endangered Delta smelt. These efforts were to determine the presence of infectious pathogens and tissue abnormalities (by histopathology). An emerging pathogen of concern is Viral Hemorrhagic Septicemia Virus (VHSV). VHSV is a serious systemic disease of fish and is carried by over 50 species of marine and freshwater fish. The infections are subclinical in some species, but it is associated with severe disease and high mortality rates in others. A



total of 200 inland silversides were screened. No virus was detected in 40 pooled samples, and no tissue abnormalities were detected by histopathology.

California Central Valley Juvenile Fall Run Chinook Salmon

The Sacramento and San Joaquin River systems drain California's Central Valley and converge in the Sacramento-San Joaquin Delta. Fish migrating out of the Central Valley must navigate these waterways before passing through San Francisco Bay to the Pacific Ocean. In partnership with the CDFW, CDWR, USFWS Stockton, and FISHBIO the CA-NV Fish Health Center performed health monitoring of out-migrant Chinook salmon at several locations throughout the Central Valley.

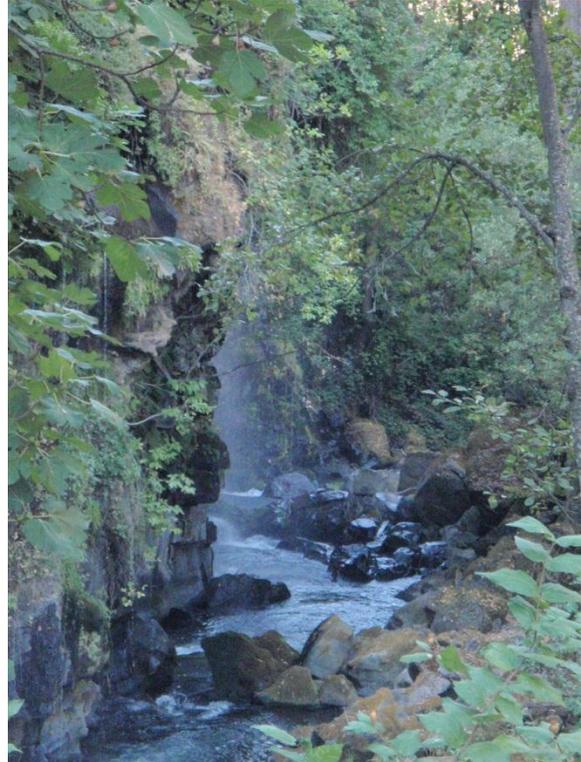
San Joaquin River – Juvenile Chinook salmon were sampled at the Mossdale trawl site near Stockton, CA. A total of 20 fish were sampled. No virus (0/4 pooled samples) or target bacteria (0/20) were detected.

Stanislaus River – Juvenile Chinook salmon were sampled at the Oakdale out-migrant trap site (RM 40) operated by FISHBIO. A total of 90 fish were sampled. No virus (0/18 pooled samples) or target bacteria (0/40) were detected.

Tuolumne River – Juvenile Chinook salmon were sampled at the Waterford trap site (RM 30) operated by FISHBIO. A total of 90 fish were sampled. No virus (0/18 pooled samples) or target bacteria (0/40) were detected.

Merced River – Juvenile Chinook salmon were sampled at the Hopeton trap site (RM 38) operated by FISHBIO. A total of 173 fish were sampled. No virus (0/11 pooled samples) or target bacteria (0/70) were detected. Infections with *Tetracapsuloides bryosalmonae*, the myxozoan parasite that causes proliferative kidney disease, was detected in 45% (41/91) fish sampled.

Sacramento River – Juvenile Chinook salmon were sampled at the Knight's Landing trap site (RM 90) operated by CDFW. A total of 74 fish were sampled. No virus (0/16 pooled samples) or target bacteria



(0/15) were detected. *Ceratomyxa shasta* and *Parvicapsula minibicornis* infections were observed in 60% (9/15) and 100% (15/15) of fish respectively.

Feather River – Juvenile Chinook salmon were sampled at the Sunset Pumps trap site (RM 45) operated by the CDWR. A total of 50 fish were sampled. No virus (0/50) or target bacteria (0/35) were detected. *Ceratomyxa shasta* and *Parvicapsula minibicornis* infections were observed in 77% (34/44) and 100% (47/47) of fish respectively.

Upper Klamath Lake Adult Lost River Suckers

Lack of recruitment into Lost River Sucker spawning populations in Upper Klamath Lake is a primary factor preventing their recovery. Poor water quality, disease, algal toxins (microcystin), and predation have been suggested as causes of high juvenile sucker mortality. The CA-NV Fish Health Center conducted pathogen screening on adult Lost River Suckers which were spawned to produce juvenile suckers for laboratory investigations. The fish health center partnered with the USGS Klamath Falls Field Station to capture adult suckers from Upper Klamath Lake, OR. A total of 10 suckers were screened for viral pathogens, with no virus detected.



Pyramid Lake Lahontan Cutthroat

The Lahontan Cutthroat trout is a threatened species native to the drainages that were once part of Lake Lahontan in Northwestern Nevada and parts of California and Oregon. Pyramid Lake is the largest remnant of Lake Lahontan and is entirely within the Pyramid lake Indian Reservation governed by the



Pyramid Lake Paiute Tribe. The Lahontan Cutthroat, extirpated from the Pyramid Lake due to water diversions in the early 20th century, has been reintroduced and sustained by tribal hatcheries. The fish health center monitors the health of the feral adult broodstock. A total of 60 fish were sampled from adult fish returning to the egg collection facility near Sutcliffe. No virus or *R. salmoninarum* was detected (0/60). The bacterial pathogen *Aeromonas salmonicida* was detected in 6% (2/32) of fish sampled.

Laboratory Methods

The methods used in the NWFHS to collect, process and test fish tissues are standardized throughout the labs participating in the survey. The detailed procedures and laboratory protocols can be found in [The National Wild Fish Health Survey Procedures Manual](#) (Heil 2009).

Bacteriology

A sample of kidney tissue from each fish was streaked onto petri plates or test tube slants of brain heart infusion agar (BHIA) and incubated at room temperature. If growth appeared on the BHIA media within 72 hours, isolated colonies were cultured on fresh BHIA plates to supply pure cultures of bacteria for phenotypic characterization and presumptive identification. Subcultures isolated were screened for bacterial fish pathogens by standard microscopic and biochemical testing (e.g., morphology, Gram stain, motility, cytochrome oxidase). Bacterial isolates that are ubiquitous in freshwater and without associated clinical signs were identified to a general group, while the identity of potential fish pathogens such as *Aeromonas salmonicida* or *Yersinia ruckeri* were resolved 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

Tissue samples were screened for the *R. salmoninarum* bacteria by enzyme linked immunosorbent assay (ELISA) or FAT. For the ELISA assay, kidney tissue from each fish was removed and diluted 1/8 with phosphate buffered saline (PBS) with tween-20, homogenized and centrifuged to separate the supernatant. The sample supernatant was then loaded onto replicate wells of a 96-well plate and assayed. The optical density (OD) values for each sample were averaged. The antigen levels in individual fish were classified according to the samples ELISA OD values. Values greater than two standard deviations above the negative reference control tissue to less than 0.500 were “low”, 0.500 to less than 1.000 were “moderate”, and values of 1.000 or more were considered “high”. For the FAT assay kidney or ovarian fluid pellet smears were created on a glass slide and fixed. Polyclonal antisera to *R. salmoninarum* (FITC labeled) was applied to the slide and allowed to react for 1 hour. The slide was then washed, counterstained and a coverslip was applied with fluorescence compatible mounting media. The slides were then examined on a fluorescence microscope. Corroborative testing of and positive samples (by ELISA or FAT) were performed with quantitative polymerase chain reaction (QPCR).

Virology

Samples of ovarian fluid, kidney and spleen or whole viscera 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 virus (VNNV) using accepted cell culture techniques (Heil 2009). Kidney and spleen tissues were tested individually, or in up to 5 fish pooled samples. Final sample dilutions of 1/20 or 1/100 were inoculated onto appropriate confluent cells lines (Table 1). Plates were examined bi-weekly for evidence of viral cytopathic effect (CPE), and re-inoculated onto fresh cells if generalized toxicity or suspect CPE was noted. Corroborative testing, if positive was done by immunohistochemistry (modified indirect FAT).

Table 1. Cell lines used in viral screening.

Fish	Cell line 1	Cell line 2	Cell line 3
Chinook salmon	CHSE-214 (15°C)	EPC (15°C)	
Inland silversides	EPC (15°C)	CHSE-214 (15°C)	
Lost River Suckers	EPC (15°C)	CHSE-214 (15°C)	FHM (25°C)
Cutthroat trout	CHSE-214 (15°C)	EPC (15°C)	

Parasites

Ceratomyxa shasta and *Parvicapsula minibicornis* were screened by histopathology and QPCR. 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.

References

- Hallett S.L. and J.L. Bartholomew. 2006. Application of real-time PCR assay to detect and quantify the myxozoan parasite *Ceratomyxa shasta* in water samples. *Diseases of Aquatic Organisms* 71:109-118.
- Heil, N. (Ed) 2009. National Wild Fish Health Survey - Laboratory Procedures Manual. 5.0 Edition. U.S. Fish and Wildlife Service, Warm Springs, GA.
- True K., M.K. Purcell and J.S. Foott. 2009. Development and validation of a quantitative PCR to detect *Parvicapsula minibicornis* and comparison to histologically ranked juvenile Chinook salmon (*Oncorhynchus tshawytscha*) from the Klamath River, USA. *Journal of Fish Disease*. 32: 183-192.

Appendix I. NWFHS Summary Table for FY 2012

NWFHS Case#	Collection Date(s)	Location	Species	Partners	Number of Fish	Significant Findings
CN12-097	May-July 2012	Sacramento River, Keswick Dam	Chinook salmon	USFWS	63	IHNV, <i>A. salmonicida</i> , <i>Y. ruckeri</i> , <i>C. shasta</i> , <i>P. minibicornis</i>
CN12-21	Jan-Feb 2012	Sacramento River, Keswick Dam	Chinook salmon	USFWS	47	IHNV, <i>A. salmonicida</i> , <i>R. salmoninarum</i>
CN12-25, 26, 29, 38, 40, 62, 91	Jan-May 2012	Sacramento-San Joaquin Delta, various locations	Inland silverside	USFWS Stockton	200	
CN12-69	April 9, 2012	San Joaquin River, Mossdale Trawl	Chinook salmon	CDFW, USFWS	20	
CN12-31, 52, 66	Feb-Apr 2012	Stanislaus River, Oakdale trap	Chinook salmon	FISHBIO	90	
CN12-31, 52, 57	Feb-Apr 2012	Tuolumne River, Waterford trap	Chinook salmon	FISHBIO	90	
CN12-50, 64, 71, 83, 89, 94	Mar-May 2012	Merced River, Hopeton trap	Chinook salmon	FISHBIO	173	<i>T. bryosalmonae</i>
CN12-56, 63, 76	Mar-Apr 2012	Sacramento River, Knight's Landing trap	Chinook salmon	CDFW	74	<i>C. shasta</i> , <i>P. minibicornis</i>
CN12-75, 84	Apr-May 2012	Feather River, Sunset pumps trap	Chinook salmon	CDRW	50	<i>C. shasta</i> , <i>P. minibicornis</i>
CN12-80	Apr 2012	Pyramid Lake, Sutcliffe	Lahontan Cutthroat Trout	Pyramid Lake Paiute Tribe	60	<i>A. salmonicida</i>
CN12-79	Apr 2012	Upper Klamath Lake, sucker springs	Lost River Sucker	USGS	10	
Total Fish					877	

Appendix 2 – Sample Summary Reports

Adult winter-run Chinook salmon (*Oncorhynchus tshawytscha*)
 Sacramento River, Keswick Dam
 NWFHS case: CN12-97

ASSAY	TISSUE TYPE	NO. SAMPLES (POOL)	RESULTS	PERCENT POSITIVE	FISH SAMPLED
Virus (tissue culture)	Kidney	14(1-2p)	3/14 IHNV ^a	21%	17
	Ovarian Fluid	34(1-2p)	16/34 IHNV ^a	47%	35
Bacteria (cultured)	Kidney	51(1p)	1/51 <i>Y. ruckeri</i> ^a	2%	51
			2/51 <i>A. salmonicida</i> ^a	4%	
			6/51 Aeromonas / Pseudomonas sp.	12%	
<i>R. salmoninarum</i> (DFAT)	Ovarian Fluid	40(1p)	0/40	0	40
Parasites (histology)	Kidney	25(1p)	22/25 <i>P. minibicornis</i>	88%	25
	Intestine	24(1p)	20/24 <i>C. shasta</i>	83%	24
Total Fish					63

a. Confirmed by immunohistochemistry (IHC)

Adult Late Fall-run Chinook salmon (*Oncorhynchus tshawytscha*)
 Sacramento River, Keswick Dam
 NWFHS case: CN12-21

ASSAY	TISSUE TYPE	NO. SAMPLES (POOL)	RESULTS	PERCENT POSITIVE	FISH SAMPLED
Virus (tissue culture)	Ovarian Fluid	6 (1-3p)	6/6 IHNV ^a	100%	11
	Kidney	16 (1-3p)	14/16 IHNV ^a	88%	35
Bacteria (cultured)	Kidney	47 (1p)	1/47 <i>A. salmonicida</i>	2%	47
			9/47 <i>Aeromonas</i> / <i>Pseudomonas</i> sp.	19%	
<i>R. salmoninarum</i> (DFAT)	Ovarian Fluid	6 (1-2p)	1/6 <i>R. salmoninarum</i> ^b	17%	11
	Kidney	17 (1p)	0/17	0	17
Total Fish					47

a. Confirmed by immunohistochemistry (IHC)

b. Confirmed by QPCR

Inland Silversides *Menidia beryllina*

Liberty island, Dad's, Knights Landing, Sacramento-San Joaquin delta

Jan18-19-26, Feb14-16, March29, May10, 2012

NWFHS case: CN12-25, 26, 29, 38, 40, 62 and 91

ASSAY	TISSUE TYPE	NO. SAMPLES (POOL)	RESULTS	PERCENT POSITIVE	FISH SAMPLED
Virus (tissue culture)	Anterior Body	40(5p)	0/40	0	200
Total Fish					200

Histological sample narrative: Twenty four sagittal sections were examined and 2 fish from Knight's Landing had a single cestode in their intestine. No lesion was associated with this parasite.

Biomarker assay: Acetylcholinesterase activity was assayed in 53 brain samples collected in January and February (Wheelock et al. 2005). Similar mean (SD) activities were seen in all samples (range of means 0.370 – 0.429). We do not have published records to compare these values but given the similarity of the values and the normal behavior of the silverside, it is unlikely that these fish were recently exposed to an inhibitory contaminate such as organophosphate pesticides.

Note: No virus has been isolated from ~ 600 silversides sampled in 2009 and 2010.

Reference:

Wheelock CE, Eder KJ, Werner I, Huang H, Jones PD, Brammell BF, Elskus AA and Hammock BD. 2005. Individual variability in esterase activity and CYP1A levels in Chinook salmon (*Oncorhynchus tshawytscha*) exposed to esfenvalerate and chlorpyrifos. *Aquatic Toxicology* 74:172 – 192.

Juvenile Fall Chinook Salmon Smolts (*Oncorhynchus tshawytscha*)
San Joaquin River, Mossdale trawl (Rm 54)
April 9, 2012
2012 Case (69)

ASSAY	TISSUE TYPE	NO. SAMPLES (POOL)	RESULTS	PERCENT POSITIVE	FISH SAMPLED
Virus (tissue culture)	Kidney	4 (5p)	0/4	0	20
Bacteria (cultured)	Kidney	20 (1p)	4/20 <i>Aeromonas</i> / <i>Pseudomonas</i> sp.	20%	20
<i>R. salmoninarum</i> (DFAT)	Kidney	20 (1p)	0/20	0	20
Total Fish					20

Histological sample narrative: No parasites or abnormalities seen in 6 multiple organ histological samples. .

Biomarker assay: Acetylcholinesterase activity was assayed in 8 brain (Wheelock et al. 2005). Mean (SD) activity was 0.221 (0.031) μ moles substrate/min/mg protein. This activity was considered normal for Chinook smolts and was similar to smolts tested in 2012 from the Knight's landing trap (Sacramento R.) and San Joaquin R. basin (mean values 0.221 – 0.271). Given this acetylcholinesterase activity, it is unlikely that these fish were recently exposed to an inhibitory contaminate such as organophosphate pesticides.

Reference:

Wheelock CE, Eder KJ, Werner I, Huang H, Jones PD, Brammell BF, Elskus AA and Hammock BD. 2005. Individual variability in esterase activity and CYP1A levels in Chinook salmon (*Oncorhynchus tshawytscha*) exposed to esfenvalerate and chlorpyrifos. *Aquatic Toxicology* 74:172 – 192.

Juvenile Fall Chinook Salmon Smolts (*Oncorhynchus tshawytscha*)
Stanislaus River, Oakdale Rotary Screw Trap (Rm 40)
Feb 1, March 6, and April 5, 2012
2012 Cases (31, 52, and 66)

ASSAY	TISSUE TYPE	NO. SAMPLES (POOL)	RESULTS	PERCENT POSITIVE	FISH SAMPLED
Virus (tissue culture)	Whole body	6(5p)	0/6	0	90
	Kidney	12 (5p)	0/12	0	
Bacteria (cultured)	Kidney	40 (1p)	5/40 <i>Aeromonas</i> / <i>Pseudomonas</i> sp.	13%	40
<i>R. salmoninarum</i> (DFAT)	Kidney	40 (1p)	0/40	0	40
Total Fish					90

Histological sample narrative: No parasites or abnormalities seen in 12 sagittal sections of fry collected on 1 Feb. A total of 20 multiple organ histological samples were collected from either parr or smolts on 6 March and 5 April. A single parasite (cestode) was seen in the intestine from 1 of 20 histological samples. No lesion was associated with the cestode.

Biomarker assays: Acetylcholinesterase activity was assayed in 47 brain samples collected on 3 March and 5 April (Wheelock et al. 2005). Similar mean (SD) activities of 0.271 (0.031) and 0.238 (0.044) μ moles substrate/min/mg protein were measured in the 2 sample groups. These activity are considered normal for Chinook smolts and was similar to smolts tested in 2012 from the Knight's landing trap (Sacramento R.) and San Joaquin R. basin (mean values 0.221 – 0.271). Given this acetylcholinesterase activity, it is unlikely that these fish were recently exposed to an inhibitory contaminate such as organophosphate pesticides.

Similarly, liver tissue was assayed for lipid peroxidation (malondialdehyde formation) with an Oxis Research LPO-586 kit. Lipid peroxidation occurs when cells are exposed to oxidative stresses such as pesticides and heavy metals. We have not located published salmon liver malondialdehyde using this method for comparison. Six (6 March) and 12 livers (5 April) were tested and malondialdehyde levels were generally below the background levels. We observed 3 samples that were markedly higher (>2x of median value) which could indicate exposure to oxidative stress for these fish (Table 2).

(Continued from previous page)

Juvenile Fall Chinook Salmon Smolts (*Oncorhynchus tshawytscha*)

Stanislaus River, Oakdale Rotary Screw Trap (Rm 40)

Feb 1, March 6, and April 5, 2012

2012 Cases (31, 52, and 66)

Table 2. Lipid peroxidation (nM MDA/mg protein) values of liver tissue

	<u>No.</u>	<u>Mean(SD)</u>	<u>median</u>	<u>no. sample > 2xmedian</u>
3 March	6	17.5 (12.0)	13.9	1
5 April	12	27.9 (34.4)	16.5	2

Reference:

Wheelock CE, Eder KJ, Werner I, Huang H, Jones PD, Brammell BF, Elskus AA and Hammock BD. 2005. Individual variability in esterase activity and CYP1A levels in Chinook salmon (*Oncorhynchus tshawytscha*) exposed to esfenvalerate and chlorpyrifos. *Aquatic Toxicology* 74:172 – 192.

**Juvenile Fall Chinook Salmon Smolts (*Oncorhynchus tshawytscha*)
 Tuolumne River, Waterford Rotary Screw Trap (Rm 30)
 Feb 1, March 6, and April 5, 2012
 2012 Cases (31, 52, and 67)**

ASSAY	TISSUE TYPE	NO. SAMPLES (POOL)	RESULTS	PERCENT POSITIVE	FISH SAMPLED
Virus (tissue culture)	Whole Body	6 (5p)	0/6	0	90
	Kidney	12 (5p)	0/12	0	
Bacteria (cultured)	Kidney	40 (1p)	11/40 <i>Aeromonas</i> / <i>Pseudomonas</i> sp.	28%	40
			3/40 <i>Serratia liquenfaciens</i>	8%	
<i>R. salmoninarum</i> (DFAT)	Kidney	40 (1p)	0/40	0	40
Total Fish					90

Histological sample narrative: No parasites or abnormalities seen in 9 sagittal sections of fry collected on 1 Feb. A total of 18 multiple organ histological samples were collected from either parr or smolts on 6 March and 5 April. A single parasite (trematode) was seen in the intestine from 1 of 18 histological samples. No lesion was associated with this parasite. No parasites were seen in the kidney.

Biomarker assay: Acetylcholinesterase activity was assayed in 47 brain samples collected on 3 March and 5 April (Wheelock et al. 2005). Similar mean (SD) activities of 0.267 (0.035) and 0.231 (0.025) μ moles substrate/min/mg protein were measured in the 2 sample groups. These activity are considered normal for Chinook smolts and was similar to smolts tested in 2012 from the Knight's landing trap (Sacramento R.) and San Joaquin R. basin (mean values 0.221 – 0.271). Given this acetylcholinesterase activity, it is unlikely that these fish were recently exposed to an inhibitory contaminate such as organophosphate pesticides.

Reference:

Wheelock CE, Eder KJ, Werner I, Huang H, Jones PD, Brammell BF, Elskus AA and Hammock BD. 2005. Individual variability in esterase activity and CYP1A levels in Chinook salmon (*Oncorhynchus tshawytscha*) exposed to esfenvalerate and chlorpyrifos. *Aquatic Toxicology* 74:172 – 192.

Juvenile Fall Chinook Salmon (*Oncorhynchus tshawytscha*)

Merced River, Hopeton Rotary Screw Trap (Rm 38)

1 Mar – 15 May

CA-NV FHC case #'s: 12-050, 12-064, 12-071, 12-083, 12-089, 12-094

ASSAY	TISSUE TYPE	NO. SAMPLES (POOL)	RESULTS	PERCENT POSITIVE	FISH SAMPLED
Virus (tissue culture)	Whole Fry	11 (5p)	0/11	0	53
Bacteria (cultured)	Kidney	65 (1p)	0/65	0	65
<i>R. salmoninarum</i> (DFAT)	Kidney	70 (1p)	0/70	0	70
Parasites (Histopathology)	Kidney	91 (1p)	41/91 <i>Tetracapsuloides bryosalmonae</i>	45%	91
Total Fish					173

**Juvenile Fall Chinook Salmon (*Oncorhynchus tshawytscha*)
 Sacramento River, Knight's Landing Rotary Screw Trap (Rm 90)
 2012 Cases (56, 63, and 76)**

ASSAY	TISSUE TYPE	NO. SAMPLES (POOL)	RESULTS	PERCENT POSITIVE	FISH SAMPLED
Virus (tissue culture)	Whole Fry	6 (5p)	0/6	0	30
	Kidney	10 (4-5p)	0/10	0	44
Bacteria (cultured)	Kidney	15 (1p)	3/15 <i>Aeromonas</i> / <i>Pseudomonas</i> sp.	20%	15
<i>R. salmoninarum</i> (DFAT)	Kidney	15 (1p)	0/15	0	15
Total Fish					74

Histological sample narrative: No abnormalities or parasites observed in 16 sagittal sections of fry collected in March. *Ceratomyxa shasta* trophozoites observed 60% of intestine sections (9 of 15) and *Parvicapsula minibicornis* in 100% of kidney sections (15 of 15) from smolts collected on 19 April. Multifocal regions of inflammation and necrosis were seen in 6 of the 9 *C.shasta*-positive intestines and were characterized as early, moderate infections. No kidney abnormality was associated with the *P.minibicornis* infection. No PCR confirmation was performed.

Biomarker assays: Acetylcholinesterase activity was assayed in 23 brain samples collected from smolts on 9April (Wheelock et al. 2005). Mean (SD) activity was 0.228 (0.049) µmoles/min/mg protein. This activity was considered normal for Chinook smolts and was similar to smolts tested in 2012 from the Feather R. and San Joaquin R. basin (mean values 0.221 – 0.271). Given this acetylcholinesterase activity, it is unlikely that these fish were recently exposed to an inhibitory contaminant such as organophosphate pesticides.

Similarly, liver tissue was assayed for lipid peroxidation (malondialdehyde formation) with an Oxis Research LPO-586 kit. Lipid peroxidation occurs when cells are exposed to oxidative stresses such as pesticides and heavy metals. Twelve fry (29March) and 12 smolts (19April) were tested and malondialdehyde levels were below the background levels indicating no overt oxidative stress.

Reference:

Wheelock CE, Eder KJ, Werner I, Huang H, Jones PD, Brammell BF, Elskus AA and Hammock BD. 2005. Individual variability in esterase activity and CYP1A levels in Chinook salmon (*Oncorhynchus tshawytscha*) exposed to esfenvalerate and chlorpyrifos. *Aquatic Toxicology* 74:172 – 192.

**Juvenile Fall Chinook Salmon Smolts (*Oncorhynchus tshawytscha*)
Feather River, Sunset pumps Rotary Screw Trap (Rm 45)
2012 Cases (75 and 84)**

ASSAY	TISSUE TYPE	NO. SAMPLES (POOL)	RESULTS	PERCENT POSITIVE	FISH SAMPLED
Virus (tissue culture)	Kidney	10 (5p)	0/10	0	50
Bacteria (cultured)	Kidney	35 (1p)	4/35 <i>Aeromonas</i> / <i>Pseudomonas</i> sp.	11%	35
<i>R. salmoninarum</i> (DFAT)	Kidney	35 (1p)	0/35	0	35
Parasites (Histopathology)	Kidney	47 (1p)	47/47 <i>P. minibicornis</i>	100%	47
	Intestine	44 (1p)	34/44 <i>C. shasta</i>	77%	44
Total Fish					50

Histological sample narrative: Intestine and kidney tissue sections were obtained from 47 smolts. *Ceratomyxa shasta* trophozoites observed 77% of intestine sections (34 of 44) and *Parvicapsula minibicornis* in 100% of kidney sections (47/47) from smolts collected on 19 April and 2 May. Multifocal regions of inflammation and necrosis were seen in 9 of the 34 *C.shasta*-positive intestines with the majority of sections characterized as early, moderate infections. Glomerulonephritis and interstitial hyperplasia was associated with 51% (24 of 47) *P.minibicornis* infections. No PCR confirmation was performed.

Biomarker assays: Acetylcholinesterase activity was assayed in 12 brain samples collected from smolts on 19 April (Wheelock et al. 2005). Mean (SD) activity was 0.261 (0.041) µmoles/min/mg protein. This activity was considered normal for Chinook smolts and was similar to smolts tested in 2012 from the Knight's landing trap (Sacramento R.) and San Joaquin R. basin (mean values 0.221 – 0.271). Given this acetylcholinesterase activity, it is unlikely that these fish were recently exposed to an inhibitory contaminant such as organophosphate pesticides.

Similarly, liver tissue was assayed for lipid peroxidation (malondialdehyde formation) with an Oxis Research LPO-586 kit. Lipid peroxidation occurs when cells are exposed to oxidative stresses such as pesticides and heavy metals. Eleven (19 April) and 20 smolts (2 May) were tested and malondialdehyde levels were below the background levels indicating no overt oxidative stress.

Reference:

Wheelock CE, Eder KJ, Werner I, Huang H, Jones PD, Brammell BF, Elskus AA and Hammock BD. 2005. Individual variability in esterase activity and CYP1A levels in Chinook salmon (*Oncorhynchus tshawytscha*) exposed to esfenvalerate and chlorpyrifos. *Aquatic Toxicology* 74:172 – 192.

**Adult Lahontan Cutthroat Trout (*Oncorhynchus tshawytscha*)
 Pyramid Lake, NV, Sutcliffe brood stock collection facility
 2012 Case (12-080)**

ASSAY	TISSUE TYPE	NO. SAMPLES (POOL)	RESULTS	PERCENT POSITIVE	FISH SAMPLED
Virus (tissue culture)	Kidney	30 (1p)	0/30	0	60
	Ovarian Fluid	30 (1p)	0/30		
Bacteria (cultured)	Kidney	33 (1p)	2/32 <i>A. salmonicida</i>	6%	32
<i>R. salmoninarum</i> (DFAT)	Kidney	30 (1p)	0/30	0	60
	Ovarian Fluid	30 (1p)	0/30		
Total Fish					60

Adult Lost River Suckers
Upper Klamath Lake, Sucker Springs
CA-NV FHC case number: 12-079

ASSAY	TISSUE TYPE	NO. SAMPLES (POOL)	RESULTS	PERCENT POSITIVE	FISH SAMPLED
Virus (tissue culture)	Ovarian Fluid	10 (1p)	0/10	0	10
Total Fish					10

Appendix 3 Partners and Sample Sites

Map ID	Location	Water Body	Partner
1	Sucker Springs	Upper Klamath Lake	USGS
2	Keswick Dam trap	Sacramento River	USFWS
3	Sutcliffe trap	Pyramid Lake	Pyramid Lake Paiute Tribe
4	Sunset Pumps trap	Feather River	CDWR
5	Knights Landing	Sacramento River	CDFW & USFWS
6	Liberty Island seine	Sacramento/San Joaquin Delta	USFWS
7	Louis Park seine	San Joaquin River	USFWS
8	Mossdale trawl	San Joaquin River	CDFW & USFWS
9	Oakdale trap	Stanislaus River	FISHBIO
10	Waterford trap	Tuolumne River	FISHBIO
11	Hopeton trap	Merced River	FISHBIO

