Sacramento and Feather River Juvenile Chinook Pathogen Survey
Spring 2014

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Summary: *Ceratomyxa shasta* infection was detected in juvenile Chinook salmon collected from the lower Sacramento R. (74%) and Feather R. (35%). Lower Sacramento salmon were held at 18°C in the FHC wetlab for 9 – 14d prior to sampling. Both columnaris and Ich infection were associated with the 18 – 69% mortality. Prevalence of *C.shasta* infection in mortalities was ≥ 95% and 33 – 69% in survivor groups. Clinical ceratomyxosis was observed in 9 – 25% of the 9 -14 d survivors. *Parvicapsula minibicornis* was observed in 97% of the kidney sections with 24% showing clinical disease.

The Feather R. appears to have a yet undefined "*C.shasta* infectious zone" in the higher flow reach. Prevalence and severity of both *C.shasta* and *Parvicapsula minibicornis* infection was much higher in Feather R. salmon collected from the high flow reach (Herringer trap, 68%*C.shasta* and 82% *P. minibicornis*) than the low flow (Gateway trap, 5%*C.shasta* and 3% *P. minibicornis*). Severe ceratomyxosis occurred in high flow fish collected between 12March and 10April. Reduced prevalence in later high flow reach Feather R. collections may be related to out-migration of low flow juveniles or seasonal changes in actinospore concentrations. In contrast to the Klamath R., ceratomyxosis occurred in Feather R. salmon when water temperature was ≤ 12°C (12March).

No other significant bacterial or viral infection was detected in salmon from the two rivers. Sacramento R. Chinook juveniles need to be sampled later in their outmigration to assess whether ceratomyxosis is a significant pathogen to the population. It is likely that *C.shasta* infection is exerting a strong negative impact on Feather R. natural Chinook production.

Introduction: 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. 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 (http://www.fws.gov/wildfishsurvey/database/nwfhs/, http://www.fws.gov/wildfishsurvey/related.htm, http://www.fws.gov/canvfhc/reports.asp.).

In 2014, one focus of the CA-NV Fish Health Center's NWFS efforts was on juvenile fall run Chinook pathogen infection (particularly *Ceratomyxa shasta* and *Parvicapsula minibicornis* in the Sacramento and Feather Rivers.

Methods: All fish were collected in March, April and May 2014 from cooperators, under Scientific Collecting Permit SC-4085. Cooperators include:

California Department of Fish & Wildlife at Lower Sacramento rotary screw traps (RST)
Rm 119 Tisdale
Rm 90 Knights
California Department of Water Resources Feather R. RST
Rm 61 Gateway,
Rm 46 Herrington

In general, the following measurements and samples were collected from each fish: Fork length (mm), weight (0.1g), bacterial (cultured and *R. salmoninarum* DFAT) and viral assay inoculum (anterior kidney and spleen), and either the entire fish (< 65mm) or specific organs (GI tract, liver, gill, and kidney) were placed into Davidson's fixative for histology. The appearance of pale gill (anemia), swollen kidney, pale or hemorrhagic intestine was recorded for each fish.

Lab methods- Bacterial and viral assays were performed as per NWFS protocols Histological sections were stained with hematoxylin and eosin. QPCR analysis was performed as per the FHC's Klamath R. Juvenile Chinook Myxosporian survey (Bolick et al. 2013). Disease rating for QPCR samples was based on the amount of DNA present (Cq ≤ 34).

Results:

Lower Sacramento Juvenile Chinook- A total of 110 salmon were obtained from the RSTs at Knight's Landing (K) and above Tisdale ramp (T) between March 21 and April 4, 2014. Mean (SD) fork length (mm) of captivity survivors was 45 (8), 54 (9), 59 (9), and 56 (11) for the four collection groups respectively. These dates were targeted to maximize collection of larger natural juveniles, as late in spring as possible, prior to Coleman NFH release smolts. Salmon were transported to the FHC wetlab and held for 9 – 14d in in 350L circular tanks at 18ºC and fed frozen tubifex worms. The original intent was to hold fish for 21d so as to allow for the development of ceratomyxosis. Mortality was frozen and later assayed for *C.shasta* DNA by QPCR. Mortality ranged from 18 – 69% and the rapid onset dictated early sampling of the survivors at 9 – 14d post-capture (Table 1). QPCR analysis determined that 91 – 100% of the mortalities had *C.shasta* DNA in the intestine suggestive of clinical disease (C_q ≤ 34). The exact cause of death was not determined and a low percentage of fish showed columnaris lesions. Additionally, *Ichthyophthirius multifiliis* was present in the capture groups as evident in gill histology of 2 survivor fish (2 of 68 sections, 3%).

Aeromonas-Pseudomonas bacteria were isolated from the kidney of 23% (10 of 43) of the survivors with prevalence increasing with each collection group. No virus was detected in 11 pooled samples representing 32 fish. *Renibacterium salmoninarum* was not observed by direct FAT in 5 pooled kidneys sample (20 fish).A common histological observation in survivors was inflammation of the visceral adipose tissues. *Parvicapsula*

minibicornis was seen in the kidneys of 91%- 100% of each collection group (overall prevalence of 97%). Glomerulonephritis and interstitial hyperplasia (inflammatory response) occurred in 24% (17 of 70) sections. *Ceratomyxa shasta* in survivor fish was seen in 33 – 69% of the sample groups with 9 – 25% of the intestines showing clinical signs of disease (Table 2). A gross clinical sign, of intestine hemorrhage or catarrhal exudate, was observed in 25 and 33% of the later 2 collection groups. Combining mortality QPCR and survivor histology results the overall prevalence of *C.shasta* infection was 74%. On 17April, the Red Bluff FWO submitted 5 juvenile Chinook histology samples to the FHC in response to a local mortality event (later found to be associated with CDFW fish salvage). Asymptomatic infections of both *Parvicapsula* and *C.shasta* were observed in 2 of 5 fish.

Table 1. Lower Sacramento Chinook collection dates from the RSTs at Knight's Landing (K) and Tisdale (T) combined and held at the wetlab for 9 – 14d. The number of fish collected, mortality (mort), and prevalence of *C.shasta* infection by QPCR in the mortality subdivided into low DNA (lowPOS) and high DNA (Cl POS).

Collect date	Site	fish collected	days captivity	mort	%mort	MORT CS POI	(N) PCR	lowPOS	Cl POS
21-Mar	T	20							
	K	20	14	15	38%	100%	16	1	15
26-Mar	T	12							
	K	20	13	22	69%	95%	21	0	20
3-Apr	T	20							
	K	0	12	9	45%	100%	4	0	4
4-Apr	T	18							
	K	20	9	7	18%	100%	7	0	7

Table 2. Lower Sacramento Chinook captivity survivor histological examination of Gastrointestinal tract (GI (N)) samples for *C. shasta* (CS+). Data reported as prevalence of infection (POI), number and POI of sections demonstrating clinical disease, the overall prevalence of CS infection of both QPCR mortality and histological survivor samples.

						Histo+PCR
Collect date	GI (N)	CS +	CS POI	CS disease	disPOI	CS POI
21-Mar	24	9	38%	4	17%	63%
26-Mar	9	3	33%	1	11%	77%
3-Apr	16	11	69%	4	25%	75%
4-Apr	19	10	45%	2	9%	70%

Feather R. Juvenile Chinook- A total of 226 fall-run Chinook salmon were sampled from the Gateway and Herringer RSTs between March 12 and May 16, 2014 (Table 3). Mean fork length and condition factor ranged from 33 – 71 mm and 0.772 – 1.05,7, respectively (Table 3). *Aeromonas*-Pseudomonas bacteria were isolated from the kidney of 19% (19 of 100). No virus was detected in 29 pooled samples representing 106 fish. *Renibacterium salmoninarum* was not observed by direct FAT in 24 pooled kidneys sample (48 fish). *Parvicapsula minibicornis* was seen in 82% of the Herringer kidney samples and only 3% of the Gateway fish (overall prevalence of 37%). Glomerulonephritis and interstitial hyperplasia (inflammatory response) occurred in 28% of the Herringer kidneys only. *Parvicapsula minibicornis* infection was confirmed by QPCR in a subsample of the May 16 sample set (9/12 Herringer, 0/12 Gateway). *Ceratomyxa shasta* was seen in 7% of the Gateway (low flow section) and 70% of the Herringer (high flow below afterbay) histological sample groups (Fig. 1). Herringer RSTsalmon showed gross clinical signs of ceratomyxosis (pale gill and/or hemorrhage or catarrhal intestine) in 38 – 58% of the sample groups while only 3 Gateway fish had similar signs. Combining QPCR and histology results the overall prevalence of *C.shasta* infection was 68%(70/103) at Herringer and 5% (6/117) at Gateway. Similar levels of C.shasta infection were observed in high flow Chinook in 2013 (82%) and 2012 (77%).

Salmon from the two sample sites differed in the occurrence of gill parasites and degree of visceral adipose inflammation. *Ichthyophthirius multifiliis* was seen *in* 4 Gateway gill sections (7%) with no associated hyperplasia. One Gateway fish (April10) showed gill hyperplasia that was associated with an amoeba infection. Single *Ichthyophthirius multifiliis* trophozoites were seen in *2* Herringer gill samples collected on May16. Conversely, visceral adipose inflammation was quite prevalent in Herringer fish (>50%) compared to Gateway fish (11%).

March-April flows, at the Gridley Feather R. gauge, was variable but generally 400 – 500 cfs. Water temperature at Herringer RST on 3/12 was 12-13ºC and was associated with severe ceratomyxosis. This temperature is lower than the onset of ceratomyxosis in the Klamath R. and could indicate a higher loss of natural Chinook that rear in the high flow reach. Ceratomyxosis in the Klamath R. is typically associated with water temperatures in excess of 15ºC. Future Feather R. survey should begin earlier in the season to examine infection in fry.
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Table 3. Feather R. Chinook collection dates from Gateway (G) and Herringer (H) rotary screw traps, sample number (collect), mean and standard deviation (sd) fork length (FL,mm), weight (WT,0.1g), and condition factor (KFL = $wt/FL^3 x10^5$).

Date	Site	collect	FL	sd	WT	sd	KFL	sd
12-Mar	H	24	34	3	0.4	0.1	0.909	0.13
12-Mar	G	24	33	6	ND		ND	
26-Mar	H	24	42	4	0.6	0.2	0.753	0.13
26-Mar	G	24	35	6	0.4	0.3	0.772	0.16
10-Apr	G	22	52	7	1.5	0.6	1.034	0.11
10-Apr	H	11	ND		ND		ND	
24-Apr	H	24	65	12	3.1	1.7	0.989	0.14
24-Apr	G	19	68	8	3.5	1.2	1.057	0.1
16-May	H	26	71	10	3.7	1.4	1.011	0.1
16-May	G	28	65	8	3	1	1.054	0.05

ND not done. Herringer 4/10 sample frozen for PCR by DWR

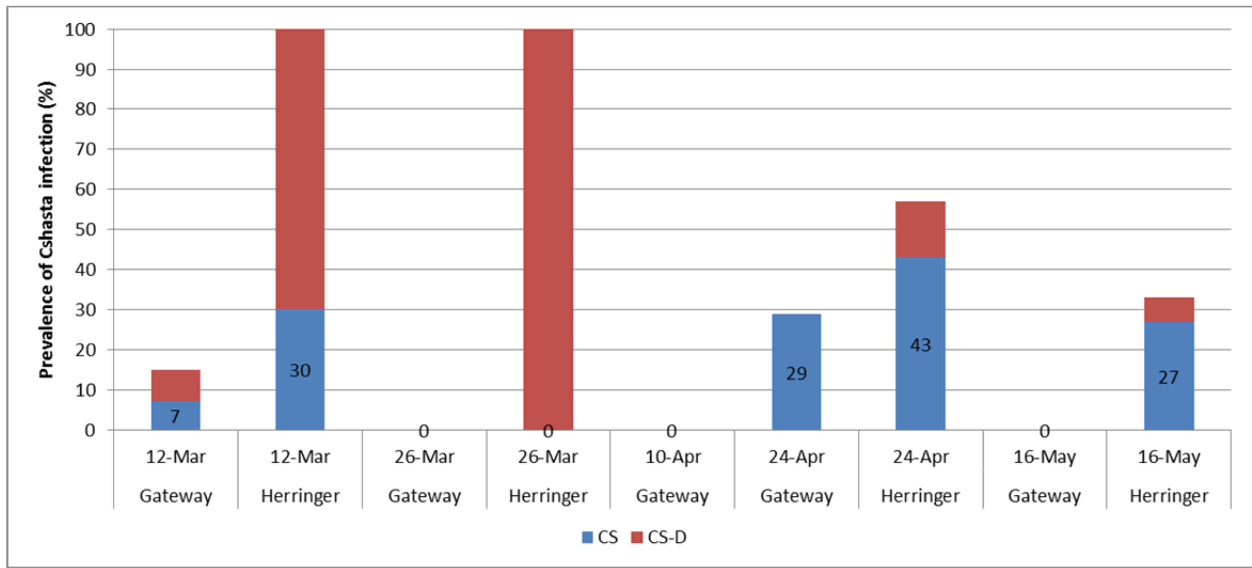

Figure 1. Prevalence of *Ceratomyxa shasta* infection (CS) and disease state (CS-D) in juvenile Chinook collected from Gateway and Herringer RSTs from March12 – May16. No histology sample collected from Herringer on 10April. Number within bar graph represent subcategory prevalence including no detections (0).

Table 4. Prevalence of infection (positive/total) for *C.shasta* (Cs) and *P. minibicornis* (Pm) by histology (histo) and QPCR (PCR) as well fish identified as clinical disease (disease).

		H	G	H	G	H	G	H	G	H	G
		12-Mar		26-Mar		10-Apr		24-Apr		16-May	
Cs histo	POI	10/10	2/13	12/12	0/10	ND	0/14	4/7	2/7	5/15	0/15
Cs histo	disease	7/10	1/13	12/12	0/10	ND	0/14	1/7	0/7	1/15	0/15
Cs QPCR	POI	12/12	0/12	0/10	1/12	11/11	0/10	6/12	0/12	6/12	1/12
Cs QPCR	disease	11/12	0/12	0/10	0/12	11/11	0/10	1/12	0/12	3/12	1/12
Pm histo	POI	10/10	2/13	12/12	0/10	ND	0/14	4/7	0/7	10/15	0/15
Pm histo	disease	4/10	0/13	2/12	0/10	ND	0/14	3/7	0/7	2/15	0/15

ND not done

Nineteen adult Wakasagi (*Hypomesus japonicus*) were collected from the Herringer RST between March12 and April10, 2014. They ranged in fork length from 52 -90 mm. No virus was detected in a 10 fish sample collected on March12. *Aeromonas-Pseudomonas* bacteria were isolated from the kidney of 86% (6 of 7). Histological examination of 7 fish showed severe gill hyperplasia associated with amoeba and *Chilodonella sp.* (presumptive) infections in 4 of 5 fish collected on March12 (Fig. 2).

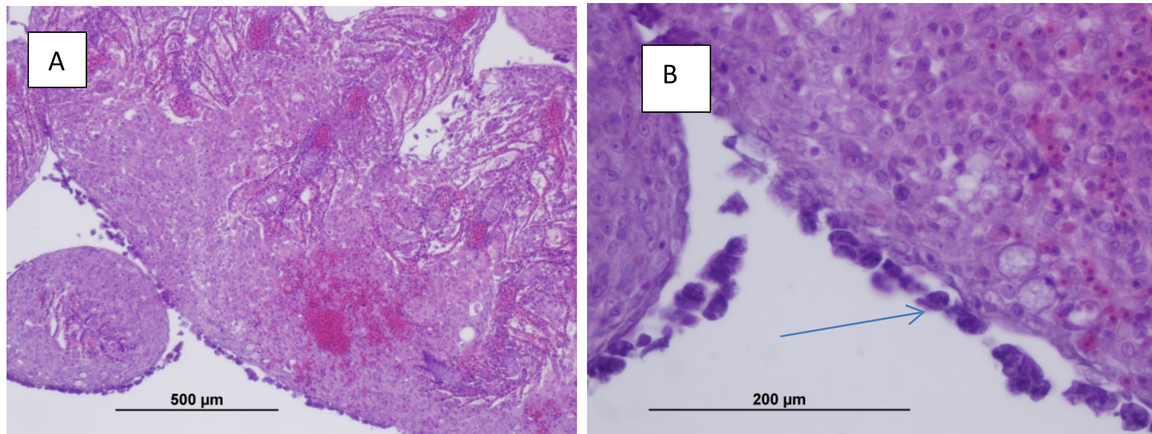

Figure 2. Hyperplastic Wakasagi gill (A) and higher magnification showing amoeba on epithelial surface (B).

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References:

Bolick, A., True, K., & Foott, J. (2013). Myxosporean Parasite (Ceratomyxa shasta and Parvicapsula minibicornis) Annual Prevalence of Infection in Klamath River Basin Juvenile Chinook Salmon, April-August 2013. U.S. Fish & Wildlife Service California – Nevada Fish Health Center, Anderson, CA. http://www.fws.gov/canvfhc/reports.asp.