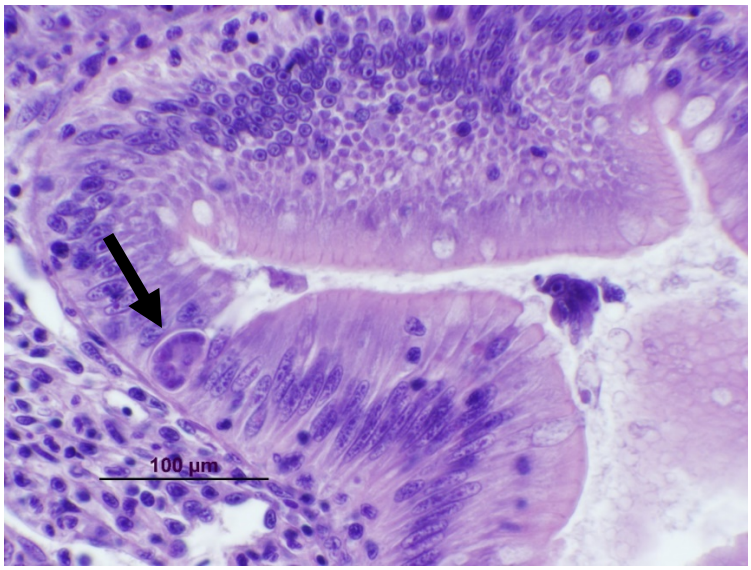
U.S. Fish & Wildlife Service

California Nevada Fish Health Center

FY2018 Technical Report:

Prevalence and severity of *Ceratonova shasta* and *Parvicapsula minibicornis* infection in Feather River Juvenile Chinook Salmon (**January – May 2018**).



J. Scott Foott*[1], Scott R. Freund [1], and Alana Imrie[2]

[1] U.S. Fish and Wildlife Service
California-Nevada Fish Health Center
24411 Coleman Hatchery Road
Anderson, CA 96007

[2] Pacific States Marine Fisheries Commission
California Department of Water Resources
DES Feather River Program
460 Glen Drive, Oroville CA 95966

*direct correspondence

January 2019

Summary: Despite high flows in 2017, *Ceratonova shasta* (CS) infectivity in the Feather River was similar to 2016 and only slightly lower than 2015. Peak prevalence and severity (> 50 % rated as "diseased") occurred in out-migrant Chinook juveniles from mid-March through April. Feather River Fish Hatchery (FRFH) spring-run Chinook, released at Boyd's launch (river mile (rm) 22.3), did not incur the same high level of disease as the natural juveniles collected at Herringer trap (rm 45.7) during this period. CS spore (myxospore and/or actinospore) concentrations showed a bi-modal pattern with peaks of 17 spores/L in early February followed by a second but variable increase beginning in March. Out-migrant CS infection did not correspond to the first "spore" peak in late January – early February suggesting myxospores may comprise some portion of the total spore concentration at this time. Pulse flows beginning in late March through April may have temporally reduced spore concentrations within the infectious zone, however, CS disease was quite prevalent at this time. Affected fry were larger (mean fork length ≥ 48mm for weekly sample groups) than fry captured during this time from the low flow trap. It is possible these larger fry reared in the high flow reach during March and had longer actinospore exposures than previous out-migrant sample groups. In May 2018, *Manayunkia speciosa* was identified from benthic samples within the infectious zone however; densities were lower than typically observed in the Klamath River.

The correct citation for this report is:

JS Foott, Scott R Freund, and Alana Imrie. 2019. Prevalence and severity of *Ceratonova shasta* and *Parvicapsula minibicornis* infection in Feather River Juvenile Chinook Salmon (January – May 2018). U.S. Fish & Wildlife Service California – Nevada Fish Health Center, Anderson, CA. http://www.fws.gov/canvfhc/reports.asp

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.

Introduction:

This is a progress report for 2018 survey efforts. Previous surveys have documented a highly infectious zone for *Ceratonova shasta* that begins at the top of the high flow reach and extends approximately 14 river miles (Foott and Imrie 2016). While infection can occur as early as January, peak prevalence of infection and disease severity tend to occur in March. Adult salmon carcasses produce billions of myxospore in the low flow reach each fall to continue the parasite life cycle (myxospore shed from fish ingested by polychaete worm, multiples and develops actinospore stage within worm, and later actinospores are shed into the river that infect salmon). *Parvicapsula minibicornis*, a myxozoan parasite found in the kidney and sharing the same polychaete host as *C. shasta,* tends to co-infect salmon (Bartholomew et al. 2006).

The objectives of the 2018 study season were:

1. Determine the weekly prevalence and severity of *Ceratonova shasta* (**CS**) and *Parvicapsula minibicornis* (**PM**) infection of natural Chinook juveniles:
    a. Within the "infectious zone" during their freshwater residency period (emergence in January through last out-migrants in late spring). Fish collections occur at Herringer rotary screw trap.

    b. From low flow reach (above the infectious zone at the Eye trap to compare low infectivity trends of previous years.

    c. Both (a) and (b) sample sets referred to as "**river samples**".

2. Determine the CS-associated mortality (prognosis of infection) as well as prevalence of CS infection (CS-**POI**) in a subset of natural Chinook juveniles collected at Herringer and reared in the wet lab for 3 weeks (referred to as "**prognosis"** samples).

3. Estimate the "CS -related mortality": multiply weekly CS disease prevalence in Herringer fish for specific sample types listed below by juvenile salmon passage estimate or actual catch of the same week. CS disease prevalence derived from three sample sets meeting the following criteria:
    1. Rating of CS2 for histological specimen of intestine of either river sample or prognosis survivor.
    2. Mortality of prognosis fish that is also CS-QPCR positive
    3. CS-QPCR river sample result $\geq$ 3 logs of CS DNA (previous work has established this conservative threshold with disease progression into morbidity).

4. Determine prevalence and severity of CS infection in Feather River State Hatchery (FRFH) Spring-run Chinook (SCS) caught in delta.

5. Collate CS “spore/L” data from Feather River water samples collected by DWR and analyzed by Oregon State University.

Methods:

A total of 542 natural Chinook salmon (*Oncorhynchus tshawytscha*) juveniles were collected by California Department of Water Resource (DWR, Scientific Collection Permit 9611 and 13341) biologists from rotary screw trap sites at Eye (river mile [rm] 60.1) and Herringer (rm 45.7) riffles, as well as by beach seine near Star Bend boat launch (rm 18.1) between January and May (Table 1). Tissues fixed in Davidson’s fixative for histological examination or frozen (intestine) for quantitative Polymerase Chain Reaction (QPCR) analysis. Laboratory methods for histology and QPCR analysis are similar to previous surveys (Foott et al. 2016).

QPCR measures the exponential amplification of target DNA sequences using fluorescence dye changes during the reaction (Purcell et al. 2011). Cycle threshold (C_t) refers to the number of amplification cycles when a sample crosses the background threshold. A lower C_t value indicates a higher quantity of target DNA template within the sample (i.e. higher parasite concentration).

Histological rankings of ‘clinical disease’ based on the presence of multifocal lesions associated with parasite infection: (*Ceratonova shasta* 2 (CS2) rating of intestine = lamina propria hyperplasia with necrotic epithelium and/or necrotic muscularis and *Parvicapsula minibicornis* 2 (PM2) rating of kidney = interstitial hyperplasia, necrotic interstitium or tubule, glomerulonephritis, and/or protein casts within the glomeruli or tubules). The #1 rating for these parasite infections required the presence of the parasite in the respective tissue but with minimal inflammatory changes.

Water samples were collected by two methods over the course of the study period; 1) four 1-liter grab samples (majority of “single day longitudinal” water samples), or 2) four 1-liter samples obtained from a 24h composite sample at the Herringer trap (Global Water Sampler WS700, ~600 mL each hour). Filtered (47mm, 5µm nitrocellulose, Millipore) water samples were analyzed for *C. shasta* DNA by Oregon State University (Hallett et al. 2012) and reported as mean site spore/L. Sites for the longitudinal samples are listed in Table 2.

To examine disease progression, eight groups of 13-22 fish were transported from Herringer trap, reared in the California-Nevada Fish Health Center (FHC) wet lab at ambient river temperatures for 21 days post-collection (**dpc**), and survivors sampled for histology. A subset of mortalities that occurred > 3dpc were analyzed for CS DNA by QPCR. Seven days post-release, 16 adipose-clip SCS juveniles were collected by

beach seine near Star Bend and reared for 22dpc at the FHC wet lab prior to sampling (NMFS Section 10d permit 20745). Additionally, Feather R. hatchery SCS coded wire tag carcasses were obtained from the Lodi Fish & Wildlife Office for intestine CS QPCR analysis. Twenty-one fall-run Chinook juveniles were sampled at FRFH on March 29 to examine CS infection status at the hatchery.

Table 1. Natural juvenile Chinook sample numbers and mean (SD) fork length collected from Herringer and Eye traps. Length data obtained from the subsample of frozen fish used for QPCR analysis. Prognosis groups reared for 3 weeks at wet lab prior to sampling. One group collected by beach seine near Star Bend on April 2.

							prognosis	prognosis
	Sample	Herringer	Herringer	Eye	Eye		Herringer	Herringer
week	Date(s)	(n)	FL	(n)	FL		date	(n)
1	16-Jan	21	37 (2)	21	35 (1)			
2	25-Jan	21	35 (1)					
3	30-Jan	20	35 (2)					
4	7-Feb	21	36 (2)				4-Feb	20
5	14-Feb	21	36 (3)	21	35 (3)		12-Feb	16
6	21-Feb	21	35 (2)					
7	26-Feb	21	43 (9)				26-Feb	14
8	6-Mar	21	36 (3)				3-Mar	18
9	13-14-Mar	21	38 (9)	21	34 (2)			
10	21-Mar	20	48 (14)					
11	26-Mar	21	57 (5)				26-Mar	13
12	2-Apr	9	45 (6)					
13	12-Apr	21	65 (7)	7	42 (12)		12-Apr	15
14	18-19-Apr	20	71 (5)					
15	26-Apr	20	73 (6)					
16	30-Apr-1-May	17	74 (12)				30-Apr	14
17	9-May	3	57 (8)				8-May	22
	4/2/2018**	21						
	subtotals	340		70				132
	Natural Juvenile total		542					

** Beach seine collection of unmarked juveniles on April 2, 2018 near Star Bend.

Table 2. Water sample sites and river mile.

	river mile
Eye	60.1
Below TAO	59
Above G-95	57.5
Palm	57
Developing Riffle	52
Gridley	50
24hHerrinnger	45.7
Live Oak	41.5
Shawn's Beach	37.5
Train Bridge	30.5
Boyds	22.3
Star Bend	18.1

Results:

River flow in the high flow reach was below 2500 cfs for most of the survey period with exception of a pulse flow associated with the March 26 hatchery release and a lake level management flow that peaked near 14000 cfs in April (Fig. 1). Between January 1 and May 1, 2018, river temperature at Herringer ranged from 48 - 68°F (Fig. 2). CS infectivity was associated with water temperatures in the 50 - 64°F (10 - 18°C) range.

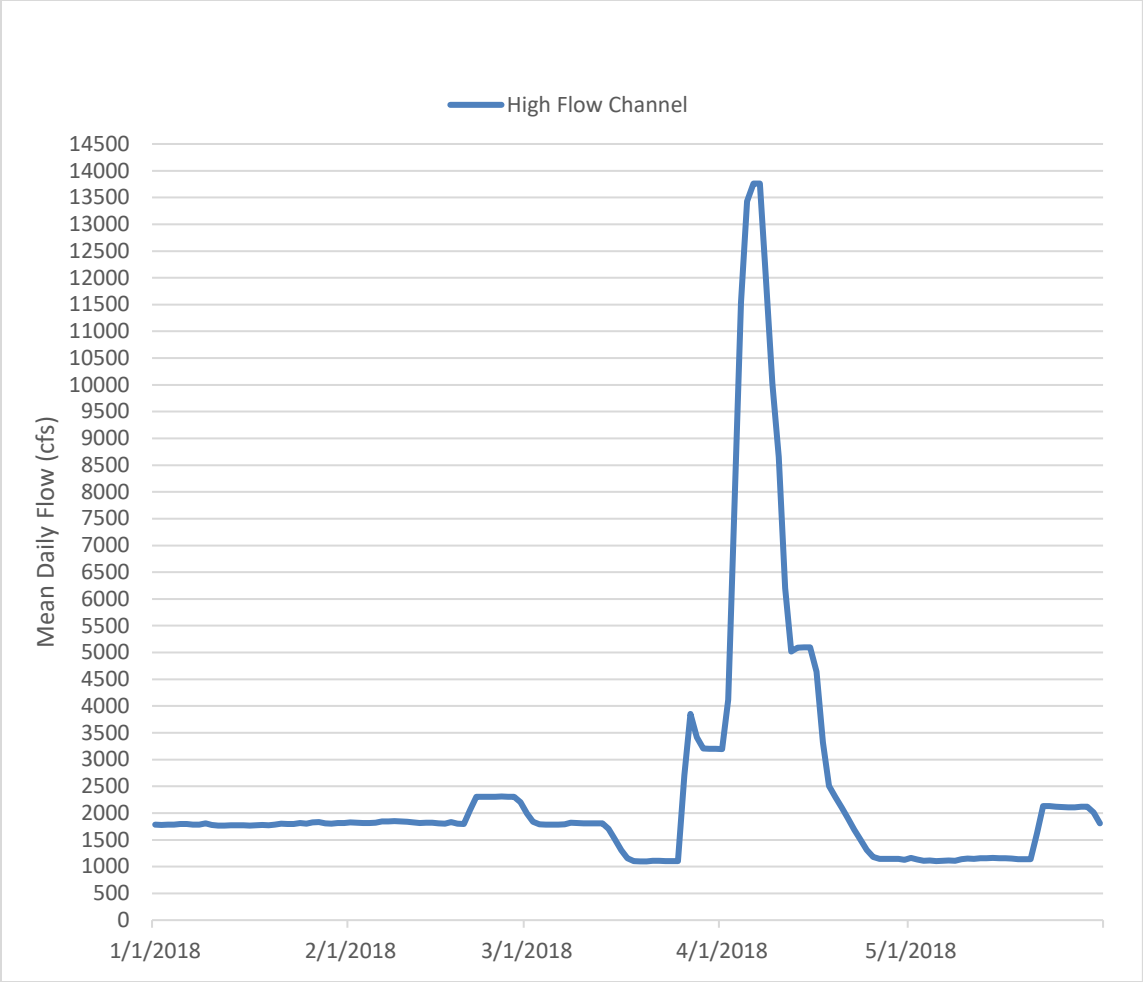


Figure 1. Mean daily river flow (cfs) data for the Feather River at the Thermalito Afterbay Outlet, January 1 to May 31, 2018.

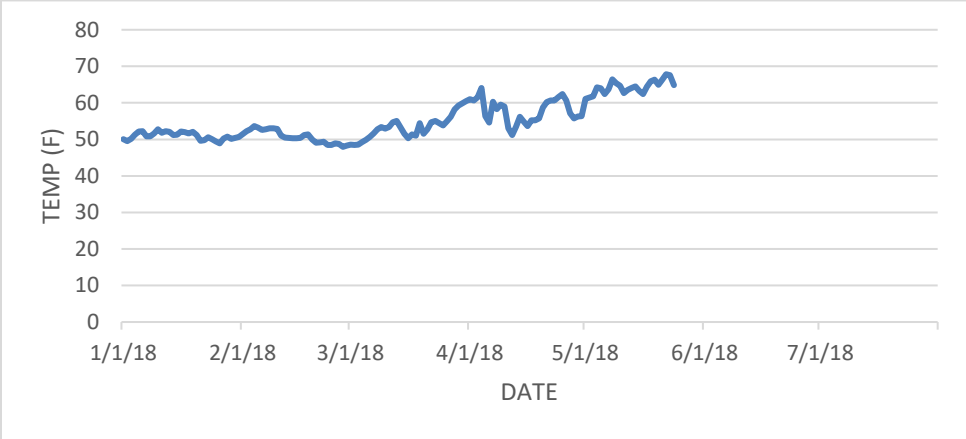


Figure 2. Daily mean water temperature (°F) at Herringer trap between January 1 and May 24, 2018.

River samples from Herringer and Eye traps—Prevalence of CS infection, for natural Chinook salmon juveniles collected from Herringer in 2018, was 40% by histology and 60% by QPCR (Table 3). Prevalence of PM infection by histology was 41%. This prevalence data is similar to 2016 (Table 3).

Table 3. Annual comparison of prevalence of infection (POI%) for *C.shasta* (CS+ / total sample set) and *P. minibicornis* (PM+/total sample set) of natural Chinook salmon collected from the high flow reach (afterbay outlet to Herringer reach). Fish samples analyzed by either histology or QPCR. Sample period reports the first and last sample collected for 2014 – 2018, and likely influences annual POI comparisons.

		Histology			QPCR				Histology		
Year	Sample Period	CS+	total	POI	CS+	total	POI		PM+	total	POI
2014	Mar12-May16	31	44	70%	47	59	80%		36	44	82%
2015	Feb11 - May5	38	65	58%	51	74	69%		34	65	52%
2016	Jan11 - May10	49	106	46%	44	72	61%		47	105	45%
2017	Jan11 - May16	7	73	10%	31	65	48%	*	19	74	26%
2018	Jan16 - Apr30	60	149	40%	100	168	60%		58	142	41%

* 2017 QPCR samples only collected in April and May.

Similar to years past, both prevalence and severity of CS and PM infection from samples collected above the infectious zone was deemed low (0% by histology, 38% CS by QPCR with low parasite DNA concentration, Tables 4 and 5). Fry (mean of ≤ 36mm FL) with yolk were observed through March 6 (Table 1). Herringer trap catch size increased markedly on March 21 through the end of the study (mean FL ≥ 48 mm). *Ichthyophthirius multifiliis* trophonts was observed in 2.7% (5/184) of all gill sections without any associated inflammation. The flagellate *Ichthyoboda* sp. ("Costia") was also seen in a low number of gill specimens without associated inflammation. This parasite is difficult to detect at low numbers which prevents accurate prevalence data.

Similar to previous years, initial CS detection occurred in late February at temperature of approximately 10°C (February 21 histology and QPCR samples, Table 4 and 5) with the peak prevalence and severity of CS occurring between late March and mid- April (Fig. 3). CS infection was also detected in fry collected February 4 and reared in the wet lab for 21d (see prognosis section below). Glomerulonephritis associated with PM infection peaked in the same period (11- 36% PM2 rating, Table 4).

Table 4. Histological examination prevalence of infection (POI) for *Ceratonova shasta* (CS) within intestine and *Parvicapsula minibicornis* (PM) in kidney of natural Chinook salmon juveniles collected at Herringer trap (H), Eye trap (E), or Star Bend (SB) by seine. Data listed includes collection date (c-Date), POI of CS and PM, both the number of specimen (n) and their frequency (%) of positive CS and PM samples rated as (1) infected but no lesion or (2) infected with significant inflammation "diseased".

c-Date	SITE	POI Cs	CS1 (n)	CS1 %	CS2 (n)	CS2 %	POI PM	PM1 (n)	PM1 %	PM2 (n)	PM2 %
16-Jan	H	0/10	0	0%	0	0%	0/10	0	0%	0	0%
25-Jan	H	0/9	0	0%	0	0%	0/9	0	0%	0	0%
30-Jan	H	0/10	0	0%	0	0%	0/9	0	0%	0	0%
7-Feb	H	0/10	0	0%	0	0%	0/9	0	0%	0	0%
14-Feb	H	0/10	0	0%	0	0%	0/8	0	0%	0	0%
21-Feb	H	2/10 (20)	1	10%	1	10%	1/10	1	10%	0	0%
26-Feb	H	0/10	0	0%	0	0%	0/10	0	0%	0	0%
6-Mar	H	3/10 (30)	3	30%	0	0%	0/10	0	0%	0	0%
14-Mar	H	2/9 (22)	0	0%	2	22%	6/6 (100)	4	67%	2	33%
21-Mar	H	8/9 (89)	2	22%	6	67%	6/9 (67)	5	56%	1	11%
26-Mar	H	10/10 (100)	5	50%	5	50%	9/10 (90)	6	60%	2	20%
2-Apr	H	5/5 (100)	1	20%	4	80%	4/5 (80)	3	60%	1	20%
12-Apr	H	10/10 (100)	5	50%	5	50%	10/10 (100)	7	70%	3	30%
18-Apr	H	8/11 (73)	4	36%	4	36%	9/11 (82)	5	45%	4	36%
26-Apr	H	7/9 (78)	6	67%	1	11%	8/9 (89)	6	67%	2	22%
30-Apr	H	5/7 (71)	4	57%	1	14%	5/7 (71)	5	71%	0	0%
total	H	60 / 149 (40)					58 / 142 (41)				
16-Jan	E	0/10	0	0%	0	0%	0/10	0	0%	0	0%
12-Feb	E	0/10	0	0%	0	0%	0/10	0	0%	0	0%
12-Mar	E	0/9	0	0%	0	0%	0/9	0	0%	0	0%
11-Apr	E	0/3	0	0%	0	0%	0/3	0	0%	0	0%
total	E	0 / 32					0 / 32				
2-Apr	SB	5/10 (50)	3	30%	2	20%	1/9 (11)	1	11%	0	0%

April 18 sample includes fish collected 4/19.

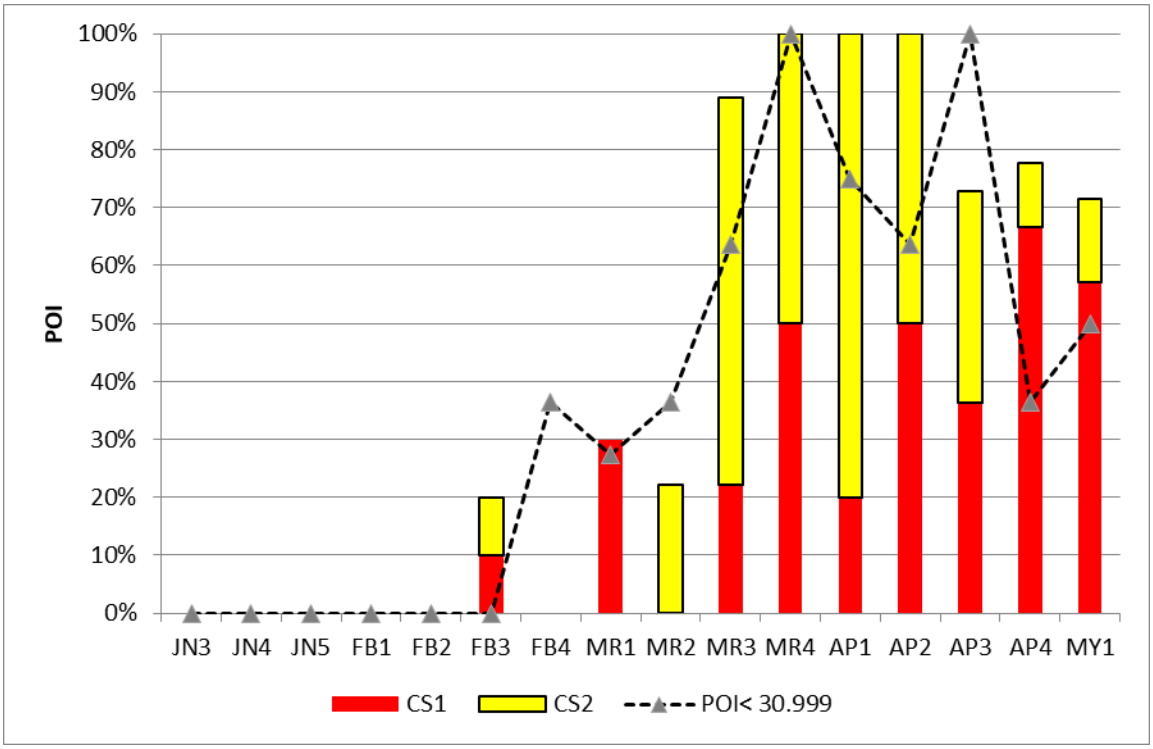


Figure 3. Prevalence of CS infection in histological specimens (CS1 = infection without disease, CS2= infection and disease state) and QPCR samples at ≥ 3 logs of parasite DNA (indicative of disease progression). Y-axis is week number in January (JN), February (FB), March (MR), April (AP) or first week in May (MY1).

Table 5. CS QPCR data from river sampled juveniles (H=Herringer, E=Eye, SB = Star Bend). Listed below is date of collection (c-DATE), prevalence of CS-QPCR infection (positive/total (%)), mean, minimum, and maximum Cycle threshold (Ct) of positives.

c-DATE	SITE	CS PCR POI		Ct MEAN	Ct min (High DNA)	Ct max (low DNA)
16-Jan	H	0/11		N/A	N/A	N/A
25-Jan	H	0/11		N/A	N/A	N/A
30-Jan	H	0/10		N/A	N/A	N/A
7-Feb	H	0/11		N/A	N/A	N/A
14-Feb	H	0/11		N/A	N/A	N/A
21-Feb	H	1/11 (9)		N/A	N/A	37.02
26-Feb	H	11/11 (100)		32.32	25.48	37.46
6-Mar	H	11/11 (100)		31.52	28.35	33.78
13-Mar	H	11/11 (100)		32.72	26.66	37.76
21-Mar	H	10/11 (91)		28.04	22.09	35.06
26-Mar	H	11/11 (100)		24.21	19.05	28.81
2-Apr	H	4/4 (100)		28.31	21.22	36.71
14-Apr	H	11/11 (100)		29.73	26.51	33.35
18-Apr	H	9/9 (100)		29.19	23.90	30.99
26-Apr	H	10/11 (91)		31.571	22.54	37.33
1-May	H	8/10 (80)		29.31	20.41	33.98
9-May	H	3/3 (100)		21.67	20.22	22.88
total	H	100 / 168 (60)				
16-Jan	E	0/11		N/A	N/A	N/A
12-Feb	E	0/11		N/A	N/A	N/A
12-Mar	E	11/11 (100)		31.96	29.41	37.35
11-Apr	E	3/4 (75)		33.76	33.46	33.99
total	E	14 / 37 (38)				
2-Apr	SB	7/11 (64)		32.89	23.44	39.2

N/A not applicable as no CS-QPCR samples in group

Prognosis Herringer trap juveniles- Eight groups of juvenile salmon were collected from the Herringer trap between February 4 and May 8. They were reared for 19 – 22 days at the wet lab prior to histological sampling (Table 6). The May 8 collection group incurred a columnaris outbreak that prompted us to sample them at only 6 dpc. Mortality ranged from 0 – 85% (Figure 4). Majority of dead fish tested positive for *C.shasta* DNA at > 3 log (22 of 27 QPCR sample values indicative of clinical disease). The initial CS infected mortality occurred in less than 10 dpc beginning in late March (Table 7). Prognosis survivors had CS POI ranging from 26 – 100% with both peak prevalence and severity (CS2 rating) occurring in the March groups (Table 7). *Parvicapsula minibicornis* POI ranged from 0 – 100% with peaks in prevalence beginning in March. Few PM infections (4%) rated as diseased (Table 7). As judged by CS–associated mortality and clinical diseased prevalence, severe infections occurred in March through the last May 8 sample.

Table 6. Prognosis group data (dates collected, moved to wet lab, and final sample of survivors). The total days post-exposure and mean (standard deviation) fork length of sampled survivors as well as average (standard deviation) water temperature (°C).

Group	Date Collected	Date to wetlab	Survivor sampled	Days post collection	Survivor FL	Wetlab temp
1	4-Feb	5-Feb	23-Feb	19	37 (3)	11.0 (1.7)
2	12-Feb	13-Feb	5-Mar	21	37 (3)	11.2 (1.3)
3	26-Feb	27-Feb	19-Mar	21	38 (4)	12.3 (0.9)
4	13-Mar	13-Mar	3-Apr	21	46 (8)	13.6 (0.8)
5	26-Mar	28-Mar	16-Apr	21	58 (4)	14.0 (0.9)
6	12-Apr	13-Apr	4-May	22	68 (5)	15.1 (1.7)
7	30-Apr	1-May	21-May	21	76 (6)	16.8 (1.3)
8	8-May	9-May	14-May	6	79 (4)	17.1 (0.7)

Table 7. Prognosis group data (mortality (%) during wet lab rearing, prevalence of CS-QPCR positive mortalities (CS+ mort %), initial CS+ mortality day post collection (1st Cs mort dpc), prevalence of CS and Pm infection in survivor histological samples (1 rating = parasite present without disease signs, 2= parasite present with disease signs).

Group	Date Collected	wetlab mortality (%)	Cs+ Mort (%)	1st Cs mort (dpc)	survivor Cs1 (%)	survivor Cs2 (%)	survivor Pm(%)
1	4-Feb	1/20 (5)	1/1 (100)	10	5/19 (26)	0/19(0)	0/19(0)
2	12-Feb	2/16 (16)	1/1 (100)*	14	5/14 (36)	0/14	4/14 (29)
3	26-Feb	0/14	na	na	5/12 (42)	1/12 (8)	1/13 (8)
4	13-Mar	7/18 (39)	7/7 (100)	15	4/11 (36)	7/11 (64)	11/11 (100)
5	26-Mar	11/ 13 (85)	10/10 (100)*	4	0/2	2/2 (100)	2/2 (100)
6	12-Apr	7/15 (47)	7/7 (100)	1	3/8 (38)	2/8 (25)	8/8 (100)
7	30-Apr	3/14 (21)	3/3 (100)	3	4/11 (36)	2/11 (18)	11/11 (100)
8	8-May	8/22 (36)	5/6 (83)*	3	5/14 (36)	1/14 (7)	9/11 (82)

*decomposition prevented QPCR testing of all mortalities

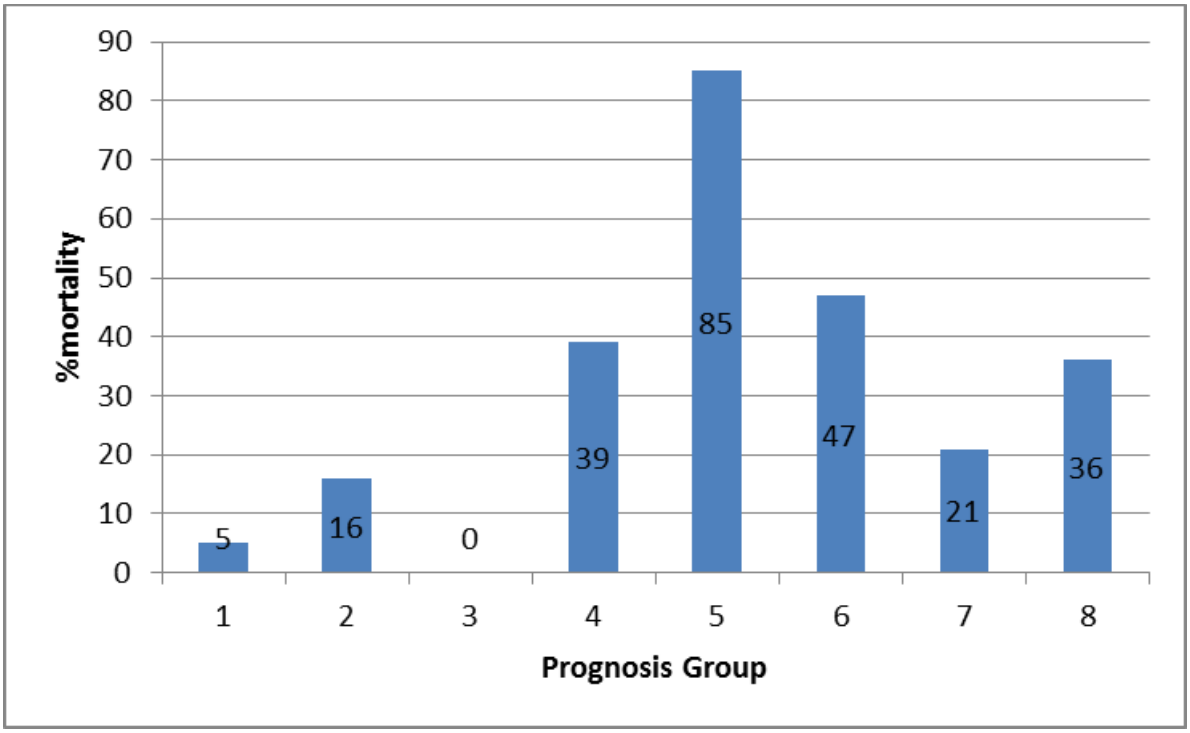


Figure 4. Percent mortality in the eight prognosis groups (see table 5 and 6 for group number).

CS related disease mortality estimates - Estimated weekly CS related mortality of the out-migrant population peaked in mid-March through April period ranging from 25 to 100% (Fig. 5). The high and low estimates are based on percentage of fry identified as "diseased" during that week from three sources of data (1.histological rating of CS2 in river sample or prognosis survivor, 2. Prognosis mortality that is CS-QPCR positive, or 3. River sample with CS-QPCR values indicative of 3 logs or greater of CS DNA). When applied to the weekly trap catch (Table 8), the disease loss ranged from 345 (5%) to 1091 (15%) fry of the 7243 total catch during the study period. Approximately 77% of the catch had occurred prior to 4th week of February when the percentage of diseased fish reached > 25%. The 4th week in February also coincides with an increase in fish size indicative of rearing behavior.

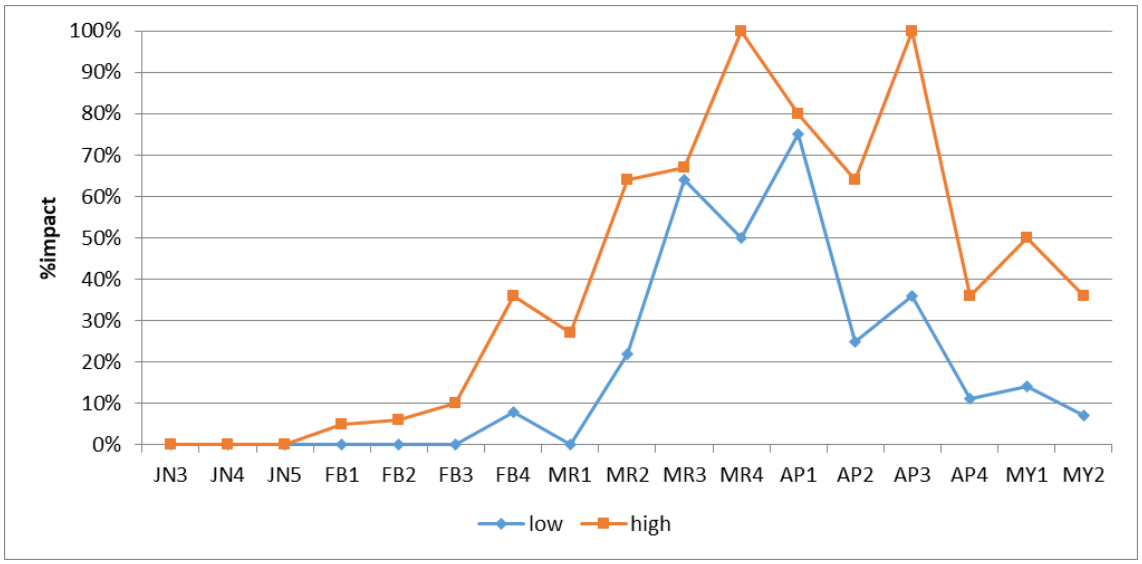


Figure 5. Range of weekly CS related disease (%impact) on juvenile Chinook collected over the 17-week study (Jan16 – May9) at the Herringer trap.

Table 8. Estimated direct disease loss in Herringer trap salmon catch during study period of January 16 – April 30, 2018 (sample date). Week identity (ID) ranges from 3rd week of January (JN3) to 1st week in May (MY1) with estimated catch that occurred during the week as well as percent of total catch over study period (%total catch). See methods for definition of low and high weekly CS disease prevalence. Affected fish is the product of weekly catch and disease prevalence. Dashed line indicates when diseased percentage was > 25%.

sample date	Week ID	weekly Estimated Catch	% total catch	lowCS disease%	fish afffected low CS disease	highCS disease%	fish affected high CS disease
16-Jan	JN3	511	7.1%	0%	0	0%	0
25-Jan	JN4	536	7.4%	0%	0	0%	0
30-Jan	JN5	1298	17.9%	0%	0	0%	0
7-Feb	FB1	1465	20.2%	0%	0	5%	73
14-Feb	FB2	1069	14.8%	0%	0	6%	64
21-Feb	FB3	690	9.5%	0%	0	10%	69
26-Feb	FB4	468	6.5%	8%	37	36%	168
6-Mar	MR1	197	2.7%	0%	0	27%	53
14-Mar	MR2	198	2.7%	22%	44	64%	127
21-Mar	MR3	159	2.2%	64%	102	67%	107
26-Mar	MR4	118	1.6%	50%	59	100%	118
2-Apr	AP1	n/a	n/a	75%	n/a	80%	n/a
12-Apr	AP2	112	1.5%	25%	28	64%	72
18-Apr	AP3	89	1.2%	36%	32	100%	89
26-Apr	AP4	111	1.5%	11%	12	36%	40
30-Apr	MY1	222	3.1%	14%	31	50%	111
				total loss	345	total loss	1091

Feather River Fish Hatchery Spring-run (SCS) – On March 26, 2018, CDFW released approximately 494,000 coded wire tagged smolts (codes 06-14-54 and 06-14-05) into the Feather River at Boyd's pump (J. Rowan, CDFW release notification 3/26/2018). No pre-release SCS samples were obtained, however, 21 FRFH fall-run Chinook juveniles were sampled at the hatchery for histology and QPCR on March 29. On April 2, sixteen adipose fin marked smolts collected by beach seine near Star Bend (rm 18.1) were transported to the FHC wet lab, reared at 13°C for a total of 22 days post-release, and sampled for histological examination. No mortalities occurred over the rearing period and mean fork length was 84 (6) mm. Developing testes were noted at sampling. Fifty-five SCS CWT carcasses (smolts) were graciously supplied by the Lodi Fish & Wildlife Office for intestine CS QPCR analysis. These smolts were captured in the Chipps Island trawl, Liberty Island beach seine, and Kodiak trawl near Sherwood Harbor (SR055M) between April 9 and May 1 (14 – 36 "days at large").

The histology and QPCR data for FRFH Fall-run Chinook fry did not correspond (Table 9). No parasites observed in kidney or intestine histological sections however, low levels of CS DNA was detected in 73% of the sample set. We suspect that a processing error (necropsy or DNA extraction) introduced cross contamination from infected natural juvenile sample processed at the same time. In 2017*, C.shasta* was not detected from FRFH pre-release samples (Foott et al. 2017). The April 2 re-capture group had a high prevalence of both *C.shasta* (94%) and *P. minibicornis* (100%) infections (Table 9). The majority of these infections deemed light as no pathology was associated with the observed parasites (rating 1). These fish were exposed to the river for 7 d and reared in the wet lab for an additional 15 d. The CS-POI in QPCR assayed frozen SCS CWT carcasses was 29% with only 2 fish having *C.shasta* DNA concentrations indicative of disease state ($\geq$ 3 log CS DNA). The difference in CS POI observed between fish captured outside of the Feather R. and the April 2 Star Bend capture group may reflect *C.shasta* actinospore exposure differences in rapid verses slower out-migration smolts.

Table 9. Prevalence of *C.shasta* and *P. minibicornis* infection in Feather River Fish Hatchery (FRFH) Chinook juveniles (Fall run =FCS, coded wire tagged Spring-run = SCS CWT) collections at Star Bend (April 2, SB) or frozen carcasses at the Lodi FWO representing fish captured 14 – 36 days at large (DAL). Data includes number of positive histology samples for *P.minibicornis* (PM) and *C.shasta* (CS) rated as (1) infected no lesion or (2) infected with lesions as well as intestine CS DNA assayed by QPCR. Samples containing three or more logs of parasite DNA were considered to have clinical infections.

	Histology						QPCR	
	CS1	CS2	POI CS	Pm1	Pm2	POI Pm	POI CS	> 3log DNA
FCS-FRH	0	0	0/10	0	0	0/10	8/11 (73%)*	0/11
SCS CWT (SB)	11	4	15/16 (94%)	16	0	16/16 (100%)	ND	ND
SCS CWT 14-36 DAL	ND	ND	ND	ND	ND	ND	16/55 (29%)	2 /55 (4%)

ND not done
*Suspect processing artifact

C.shasta spores in river water- Three longitudinal collections conducted on January 31, March 12, and May 7, 2018 (Fig. 6). Concentrations ≥ 10 spores/L were observed in the Gridley to Train Bridge reach in January while the maximum concentration in March was 8.3 spore/L at Herringer. May 7 samples showed a pattern of ≥ 10 spores/L throughout the study reach below the outlet (Above G-95 to Star Bend). An April 2 Star Bend sample, taken during beach seining, was quite low at 1 spores / L. Similarly, an Eye trap sample taken on April 10 was zero spores/L.

The weekly water sample data from Herringer trap showed multiple peaks during the study period (Fig.7). The first peak occurring on February 8 (17 spores / L) with the increase in March interrupted by a sharp decline on March 26 until levels > 15 spores / L were reached by the next week. It is possible that flow increases beginning on March 26 may have influenced the spore concentration decline, however, similar spore /L values occurred 4d prior and 5d after the April 7 peak flow(13761 cfs).Alternately, the March 26 (5 spores / L) decline could be a sample variability issue. Grab water samples tended to have high coefficient of variation.

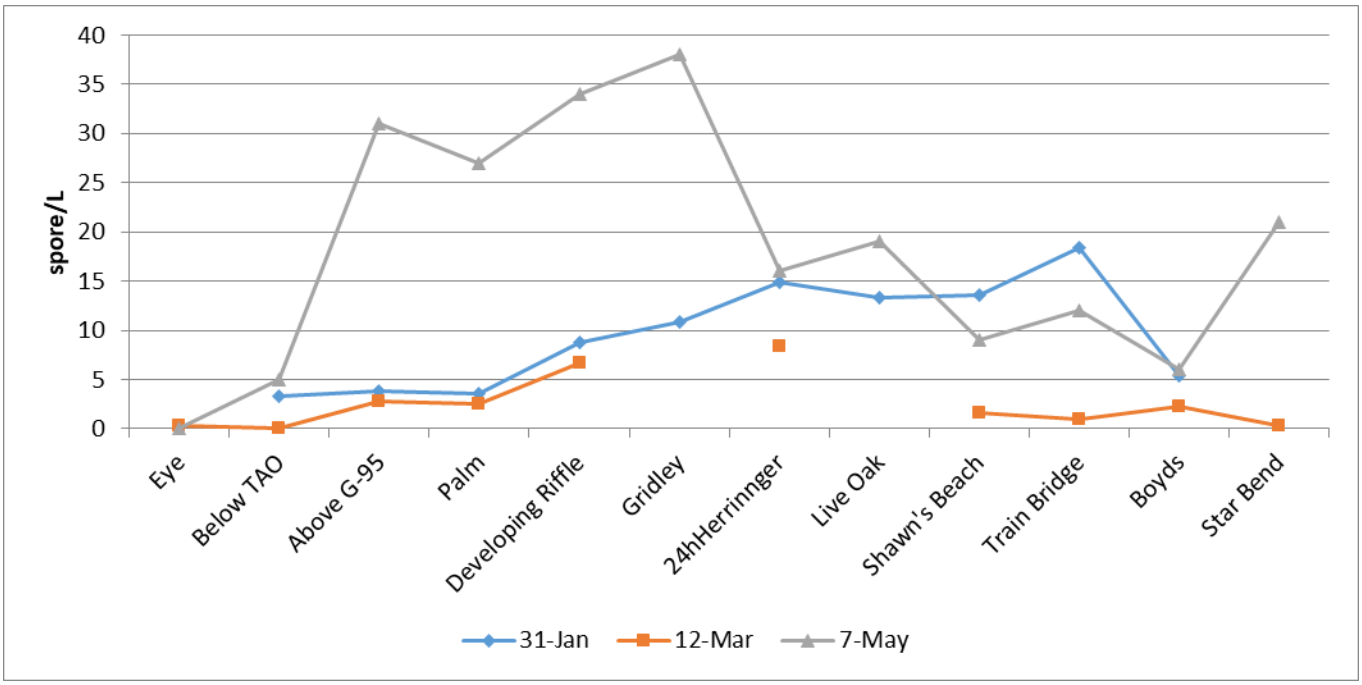


Figure 6. Mean *C.shasta* spore /L in grab and 24h composite (Herringer only) water samples collected from Eye (rm 60.1) to Star Bend (rm 18.1) on Jan 31, March 12, and May 7, 2018.

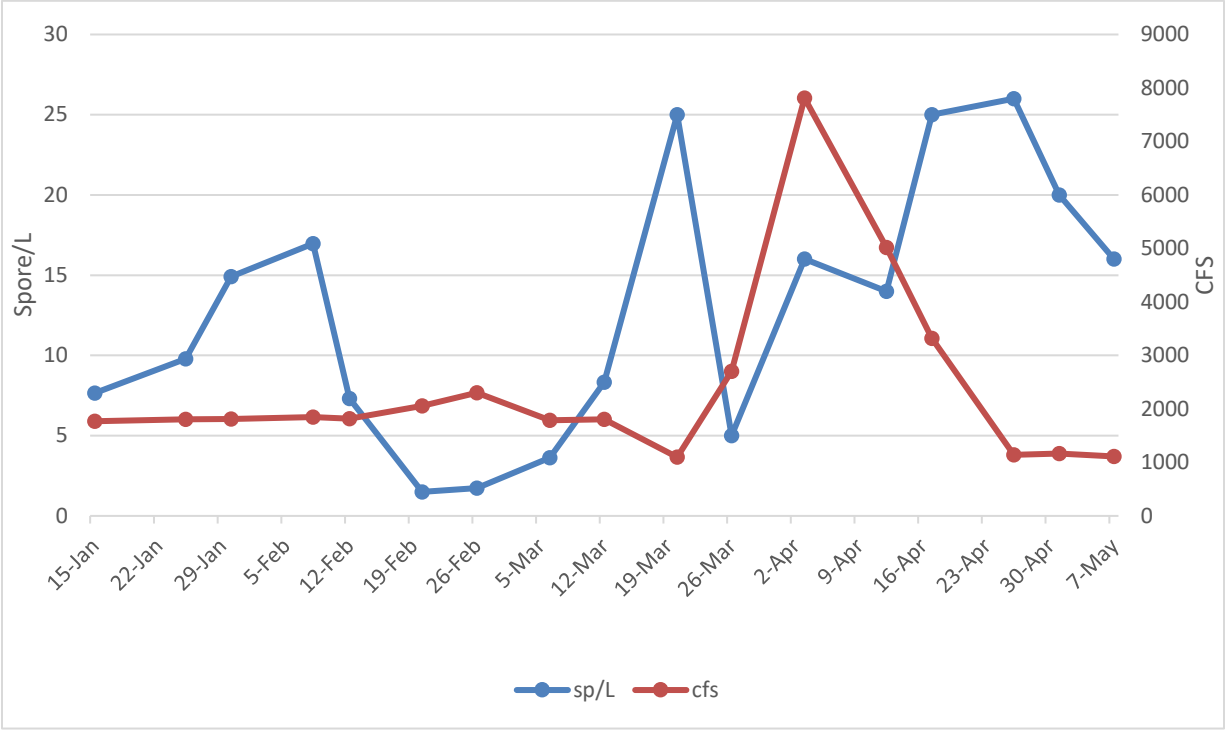


Figure 7. Mean *C.shasta* spore /L in 24h composite water samples collected from Herringer site and flow (cfs).

Polychaete collection – On May 30, 2018, Dr. Julie Alexander (Oregon State University) surveyed various sites within the infectious zone for the alternative polychaete host, *Manayunkia speciosa* (Table 109). While at lower densities (based on surface area sampled) than observed in the Lower Klamath River, the polychaete was identified in the Feather River (pers. Comm. J. Alexander, Oregon State University).

Table 10. Polychaete count and estimated density data from May 30 benthic collection from five sites within the infectious zone.

site	name	count	density
1	Keister	1	44.1
2	Lower McFarland	5	220.5
3	Gridley Ramp US	1	44.1
4	Swampy Bend	4	176.4
5	Big Hole East	122	5380.2
6	Top O Vance East	18	793.8

Discussion: Despite the 2017 flood flows, CS infectivity in the study reach was similar to 2016 and only slightly lower than 2015. It appears the polychaete population was not significantly reduced in 2017. Despite increased flows in late March – April 2018, actinospore production (5-27 spore/L at Herringer trap) was sufficient to incur high prevalence of severe infections beginning in March. Prognosis of fry CS mortality corresponds with peak prevalence of diseased (CS2 or > 3 log DNA) infections in Herringer trap samples from late March through the last May sample. Our estimate of CS related disease suggests that 50% or more of the out-migrants, moving through or rearing in the infectious zone between mid-March through mid-April, were in a disease state and likely died due to CS. This assertion is based on previous prognosis studies where morbidity was associated with CS2 histological rating.

The late March increase, in both prevalence and severity of CS infection, corresponded to the occurrence of larger fry at Herringer (mean fork length 35 – 43mm until February 21 when weekly sample mean increased to 45 – 74mm). In comparison, mean fork length of fry at Eye trap ranged from 34 – 42 mm during March and April. This shift in size of the Herringer trap samples and their difference from the low flow sample group could indicate rearing and greater exposure to CS actinospores within the high flow "infectious zone" beginning in March. Screw trap and beach seine data demonstrate that juvenile CS rear in the high flow channel throughout the spring (pers. Comm. J.

Kindopp, CDWR). Despite elevated flows in late March through the third week of April, extended exposure within the infectious zone, due to rearing vs migratory behavior, may explain the observed increase in disease severity. Previous exposure history and changes in migratory behavior may limit the benefits of enhanced flows through the infectious zone in March. One possible management action could be the use of pulse flow(s) in the low flow reach prior to March. The objective would be to induce out-migration of additional fry through the infectious zone prior to the increase in actinospore concentrations.

FRFH SCS likely experience only a moderate CS disease effect. This assertion is based on the moderate CS-POI detected in SCS captured from the delta and lack of disease progression seen in the 7d post-release FRFH SCS.

QPCR analysis of filtered water does not distinguish between actinospore and myxospore. It is unclear why high spore concentrations at the Herringer trap in late January – early February was not associated with higher out-migrant CS infection prevalence. It is possible that myxospores, from adult carcasses (infectious for polychaetes not fish), may have comprised some portion of the total spore estimate in January. This hypothesis would indicate that myxospores, from adult carcasses in the low flow reach, can be transported through the infectious zone for months after carcass breakdown. As discussed above, rapid migration through the infectious zone in January and February would limit fry exposure to CS actinospores and thereby influence prevalence and severity of infection. It is unclear why the March 12 longitudinal samples had such low spore/L values. Increased river flows did not begin until March 26. The increased river flow beginning in late March through mid-April may have reduced spore concentrations for several weeks but did not result in a prolong reduction. Similarly, increased flow was not associated with lower prevalence of CS infection in Herringer trap samples during this period.

Acknowledgements: We thank Anne Voss for assistance with the PCR assay, DWR fishery staff for fish collection and boat operations, Oregon State University (Drs. Sascha Hallett and Stephen Atkinson) for water sample DNA analysis, Jason Kindopp for high flow reach “cfs” graph and report review, and Lodi FWO (Jack Ingram and Cory Graham) for providing FRFH SCS carcasses. Partial funding for this project provided by DWR agreement 4600010798.

Author Responsibilities:

JSF lead, SCS collection, histological evaluation, report
SRF histological processing, assist with necropsy, wet lab rearing and sampling, fish transport
AI primary DWR contact, collector frozen& fix samples, Boat operations for seining, Water collection

References:

Bartholomew J.L., S.D. Atkinson, and S.L.Hallett.2006. Involvement of *Manayunkia speciosa* (Annedlida: Polychaeta: Sabellidae) in the life cycle of *Parvicapsula minibicornis*, a myxozoan parasite of Pacific salmon. J. Parasitology 92(4):742 – 748.

Foott J.S., J. Jacobs and A. Imrie. 2016. Prevalence and severity of *Ceratonova shasta* and *Parvicapsula minibicornis* infection of natural Feather River Juvenile Chinook Salmon (January – May 2015). U.S. Fish & Wildlife Service California – Nevada Fish Health Center, Anderson, CA. http://www.fws.gov/canvfhc/reports.asp

Hallett S.L., R.A. Ray, C.N. Hurst, R. A. Holt, G.R. Buckles, S.D. Atkinson, and J.L. Bartholomew. 2012. Density of the waterborne parasite *Ceratomyxa shasta* and its biological effects on salmon. Applied and Environmental Microbiology 78(10):3724 – 3731.

Purcell M.K., R.G. Getchell, C.A. McClure, and K.A. Garver.2011. Quantitative Polymerase Chain Reaction (PCR) for detection of aquatic animal pathogens in a diagnostic laboratory setting. J. Aquatic Animal Health 23:148 – 161.