U.S. Fish & Wildlife Service

California Nevada Fish Health Center

FY2019 Technical Report:

Prevalence and severity of *Ceratonova shasta* and *Parvicapsula minibicornis* infection in Feather River Juvenile Chinook Salmon (**January – May 2019**).

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Summary: Prevalence of *Ceratonova shasta* infection (CS) was 34% by histology and 45% by QPCR in natural Chinook salmon fry captured at the Herringer trap (river mile (rm) 45.7) in 2019. Initial CS infections detected in late January and diseased fry observed in ≥ 50 percent of a given week's sample by mid-March. River temperature ranged from 9 - 11°C during this period. One unique observation in 2019 was the occurrence of diseased fish from the low flow section Eye trap (rm 60) in April. CS spores /L (presumptive actinospores) at Herringer trap was ≥ 20 from late January to mid-March and markedly declined during elevated flows in April. Longitudinal water samples, from Eye trap to Boyd's landing (rm 22.3), demonstrated peak spore/L at Herringer. A sentinel study simulated fry migration rate from the low flow to Herringer in February and early March. Three sentinel groups were exposed for either 2 or 4 days (d) within the infectious zone and reared for a total of 21 days post-exposure prior to histological sampling. The 4 d exposure groups showed a 2.3 – 6.4x greater prevalence of CS infection than the 2 d fry, however, none progressed into a disease state. The majority of diseased fry at the Herringer trap in March were ≥ 40 mm suggesting rearing within the infectious zone prior to capture. These two observations indicate that a significant disease determinant is rearing within the infectious zone for over 4 days. Feather River Hatchery Spring-run salmon smolts captured in the delta were assayed for CS infection by QPCR. Prevalence of CS infection was 39% in the March 31 release fish and 10% for the 2 April releases. Release location (Live Oak or Gridley compared to Boyd's Landing) was not a strong determinate for infection.

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Introduction:

This is a progress report on 2019 survey findings. 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.

The objectives of the 2019 study season were:

1. Determine the prevalence (**POI**) and severity of *Ceratonova shasta* (**CS**) infection in natural Chinook juveniles:
    a. On a weekly basis at the Herringer rotary screw trap (river mile (rm) 45.1) from January through end of April. This sample represents the "infectious zone".
    b. On a monthly basis at the low flow reach Eye trap (rm 60.1). This sample is directly above the "infectious zone".

2. Determine the infection response of Chinook fry sentinels exposed for either 2 or 4 days (d) within the "infectious zone (Big Hole rm 57.6) in February and early March. These exposures represent fry migration from the low flow through the infectious zone in comparison to extended rearing (2- 4 d migration rate estimate J. Kindopp, CDWR, pers. comm.).

3. Determine prevalence and severity of CS infection in Feather River State Hatchery Spring-run Chinook (FRH-SCS) after release. Analyze frozen carcasses of coded wire tagged identified FRH SCS caught in delta sampling by the Lodi Fish & Wildlife Office for CS infection prevalence.

4. Graph mean CS "spore/L" data from Feather River water samples collected by DWR and analyzed by Oregon State University.

Methods: A total of 400 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 (rm 60.1) and Herringer (rm 45.7) riffles, as well as by beach seine near Live Oak (rm 41.5) between January and May (Table 1). Twenty-one juvenile Feather River Hatchery (FRH) Spring-run Chinook were collected directly from the distribution truck on April 5 as a pre-release sample. *C. shasta* infection prevalence and severity (copy number) was evaluated in 86 out- migrant FRH spring or Fall –run Chinook frozen carcasses, captured by the Lodi Fish and Wildlife Office in the lower Sacramento R. and identified by coded wire tag code.

Table 1. Juvenile Chinook salmon sampled for histology (HISTO) or QPCR at the Eye and Herringer Rotary Screw traps, beach seine collection at Live Oak on 10April, Feather River Hatchery (FRH) Spring-run Chinook collected from distribution truck at Boyd's landing on 05April (considered a "pre-liberation" sample), and FRH coded wire tagged (CWT) Fall-run (FCS) and Spring-run (SCS) carcasses provided by the USFWS Lodi Office.

	HISTO	QPCR	
Eye	38	44	
Herringer / Live Oak 10Apr	139	179	
FRH (APR05 SCS)	10	11	
FCS-CWT carcass		4	
SCS-CWT carcass		82	total
	187	320	507

Histology- Tissues fixed in Davidson's fixative for histological examination or frozen (intestine) for QPCR analysis. Laboratory methods for histology are similar to previous surveys (Foott et al. 2016). 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.

C.shasta spore/L -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.

Table 2. Water sample sites and river mile.

Site	river mile
Eye trap	60
Afterbay canal	n/a
Big Hole	57.8
Top of Vance	58.9
Palm	57
Lower McFarland	53
Gridley ramp	50
Herringer trap	45.7
Live Oak	41.5
Shawns Beach	37.5
Train Bridge	30.5
Boyds	22.3

QPCR -Fish necropsy and DNA extraction for *C. shasta* QPCR done according to True et al., 2013. The *C. shasta* standard curve was obtained using synthesized DNA (gBlock Gene Fragments, Integrated DNA Technology, Coralville Iowa) containing the 18S ribosomal DNA target sequence (sequence from Hallett et al., 2006). Specifically, 1 ng of DNA, corresponding to 6.83 x10^9 copies of *C. shasta* DNA was serially diluted over eight orders of magnitude in Tris-EDTA (ethylenediamine tetraacetic acid) buffer. Using QPCR analysis software, the cycle threshold (C_T) values for each standard concentration were calculated (SDS software 7300 SDS v 1.4, Applied Biosystems). The standard curve was used to evaluate PCR amplification efficiency (slope of the standard curve, efficiency was 96%), fit to the curve (R^2 value = 0.998), and the y-intercept (39.6) which is the theoretical C_T value for a single copy of parasite DNA when assays are 100% efficient (Applied Biosystems, 2014). Quantification of fish tissue (DNA copy number) for *C. shasta* were determined using 5 µL of DNA template in a 30 µL reaction. Each assay plate included a standard curve with three concentrations of reference standards (two replicates each) at known DNA copy number, and two negative control wells. Each assay was evaluated for expected C_T values of the reference standards, and assay efficiency. At the end of the season, any plates with more than a 3% decrease in assay efficiency were retested and reevaluated. No plates required retesting in 2019. Criteria for a positive test result required samples to produce a change in normalized fluorescent signal (Δ Rn) greater than or equal to 100,000

florescent units, verifying significant amplification above background levels of the instrument. Samples were also required to have a quantity (DNA copy number) greater than or equal to five copies, as very low copy numbers are not reliable given the limitations of the Poisson distribution (Applied Biosystems, 2016). A “disease” threshold of > 3.0 logs of *C. shasta* DNA was used to segregate data based on the relationship between this value and both histological lesion and pending mortality of experimental salmon (True et al. 2012). This value will be further evaluated for its validity in feral fish.

Sentinels – In an attempt to simulate a relatively rapid fry migration through the infectious zone prior to mid-March, Chinook salmon fry (Coleman NFH) were exposed within the “infectious zone” (Big Hole, 57.8 rm, cover page photo) for 2 or 4 days (d). Groups of 20 fry were held in 0.34 ft^3 live cages containing Onset^{tm} temperature probes that recorded hourly. The second group was moved to tanks at the DWR tag shack until the 4d group was retrieved. All groups were transported to the CA-NV Fish Health Center wet-lab and sampled for histological examination at 21days post-exposure. Mean water temperature at the wet-lab was 2 -3 °C higher than the mean exposure temperature. The tag shack water supply is from the Thermalito Afterbay canal. A group of 10 control fish was held for 4d at the tag shack.

Results:

Flow, temperature, and C. shasta spore/L – Feather River flow at Gridley had two peaks during the January – May 2019 study period (Fig. 1). The first increase occurring in early March to just under 8,000 cfs followed by second increase to 24,683 cfs on April 6. As discussed below, the February 25-March 1 and March 11-15 sentinel groups were exposed during the initial flow increase. Water temperature at Gridley ranged from 8.8 to 15.4°C during the January – May 2019 study period (Fig. 2). Temperature ranged from 9° to 11°C during the onset of increased *C. shasta* infectivity in March.

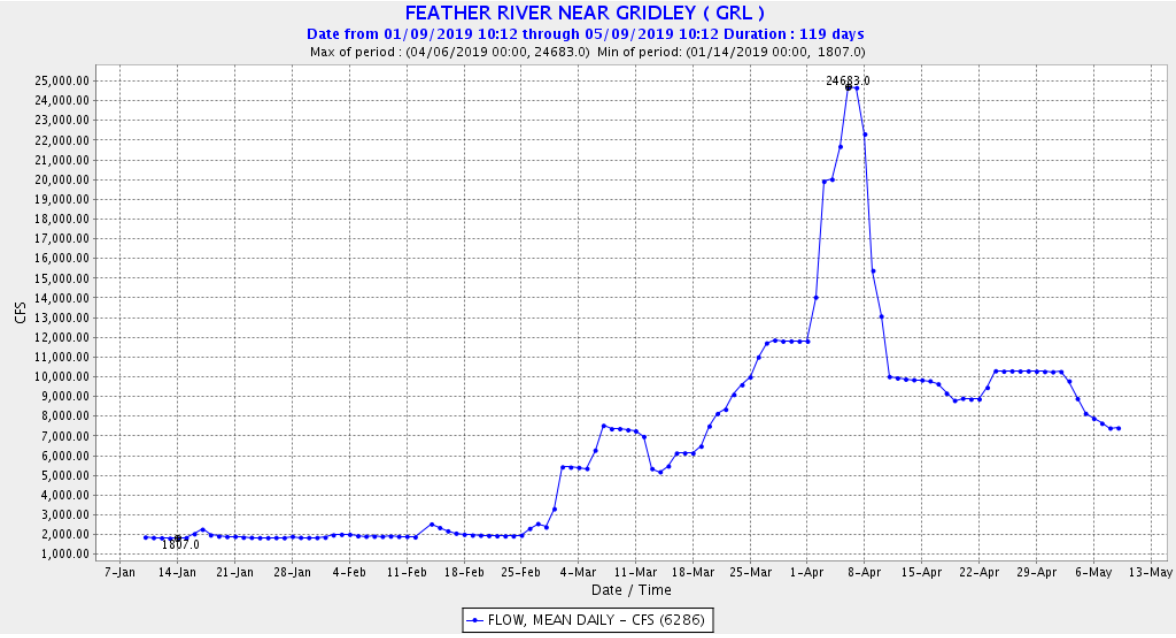


Figure 1. Feather River flow (CFS) at the Gridley gauge January 9 – May 9, 2019 (CDEC GRL gauge).

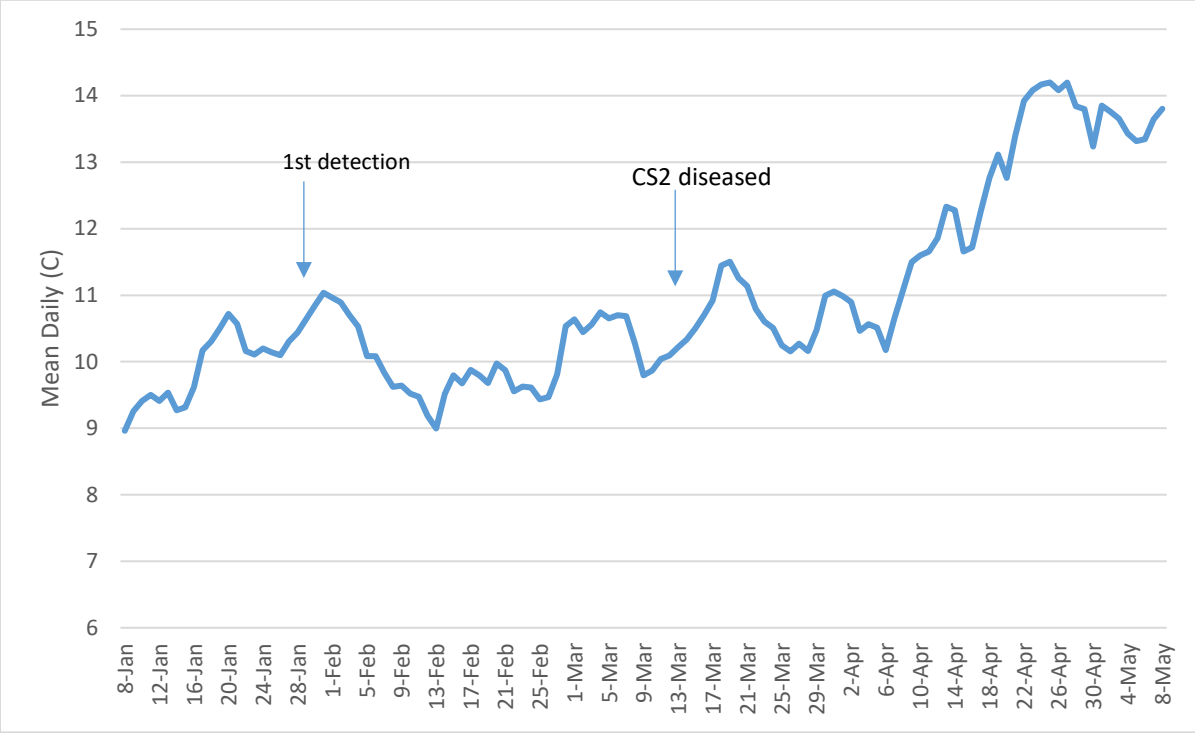


Figure 2. Mean daily river temperature at Gridley (°C) during study period. Arrows denote date of first detection of CS and when ≥ 50 percent of Herringer sample group is classified as diseased (CS2).

C.shasta spore/L (we assume predominately actinospores) measurements at the Herringer trap peaked at 183 on February 11 with values over 20 spore/L occurring from January 25 – March 12 (Fig. 3). As discussed below, initial CS detections occurred at the end of January and diseased fry were observed by mid-March. The spore concentration stayed ≤ 5 spores/L from March 18 through the last sample on May 2. River flow was increasing above 7,000 cfs during this period and may have diluted actinospore concentrations. Longitudinal samples, collected within the Eye to Boyd's reach, showed that peak spore/L concentrations occurred at Gridley or Herringer (Figures 4 and 5). A full set of site collections only occurred on January 29 and February 1. These longitudinal sample sets showed a rapid rise in spore concentration beginning at Big Hole (rm 57.8) through Herringer (rm 45.7) followed downriver by a steady decline. This 12-rm reach warrants further polychaete surveys in multiple habitat types.

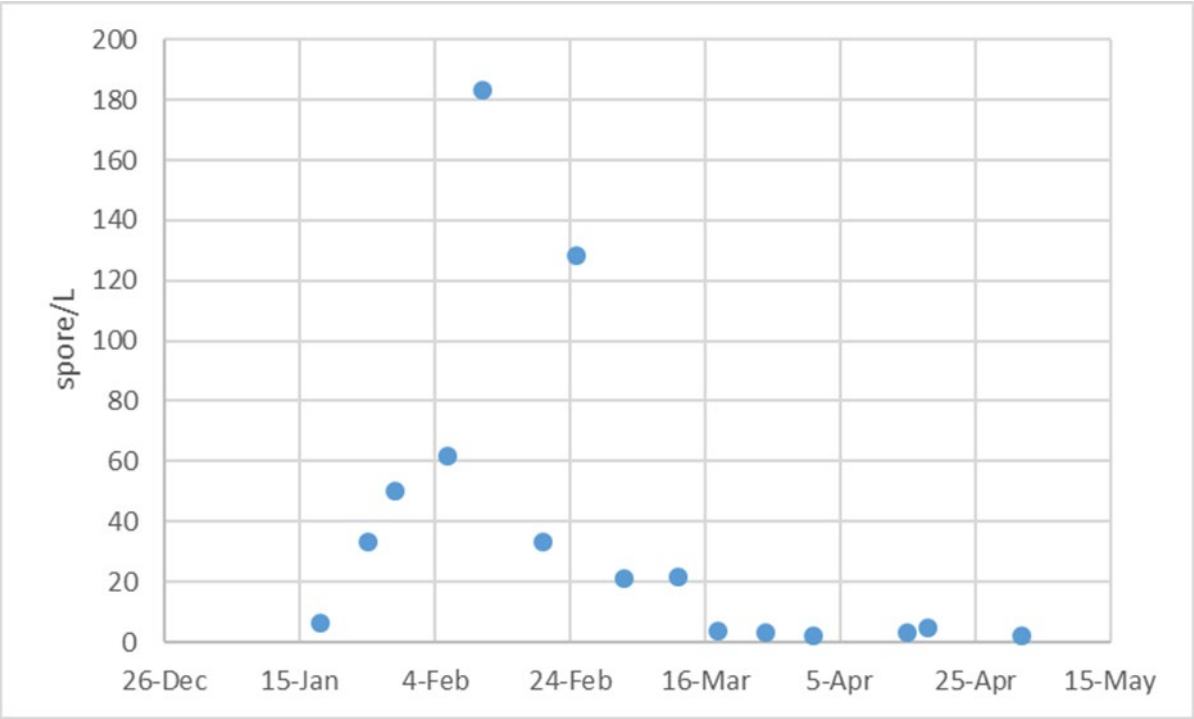
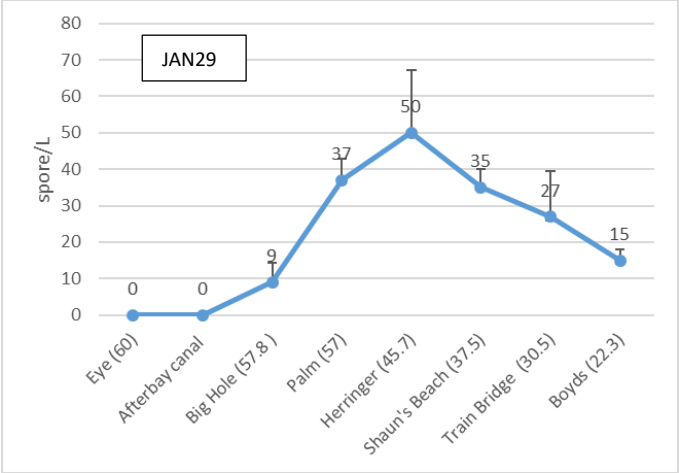
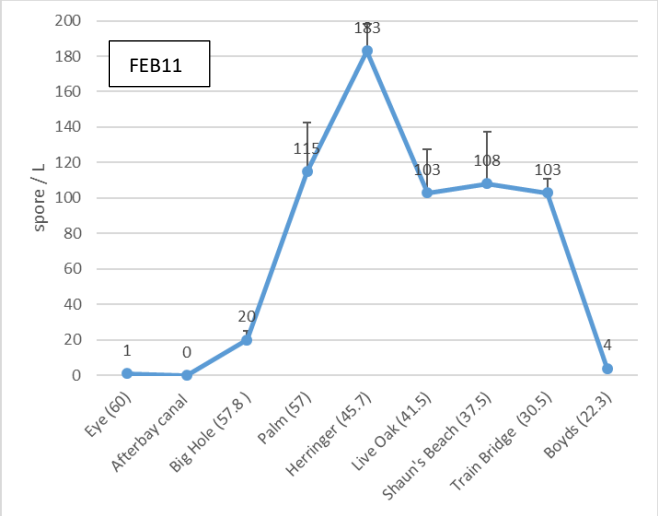


Figure 3. Mean *C.shasta* spore/L from 24h composite samples taken at Herringer trap from Jan. 18 to May 2, 2019.





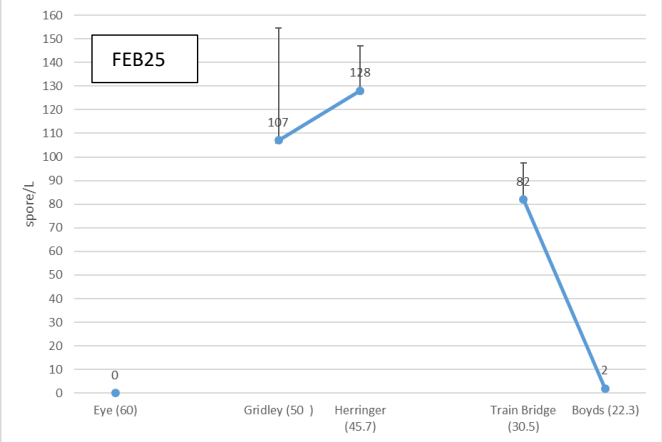


Figure 4. Mean *C.shasta spore/L* for Feather R. sites (rm 22 – 60) collected January 29, February 11 and 25. Standard deviation indicated by error bar. Note that Y-axis has different scales.

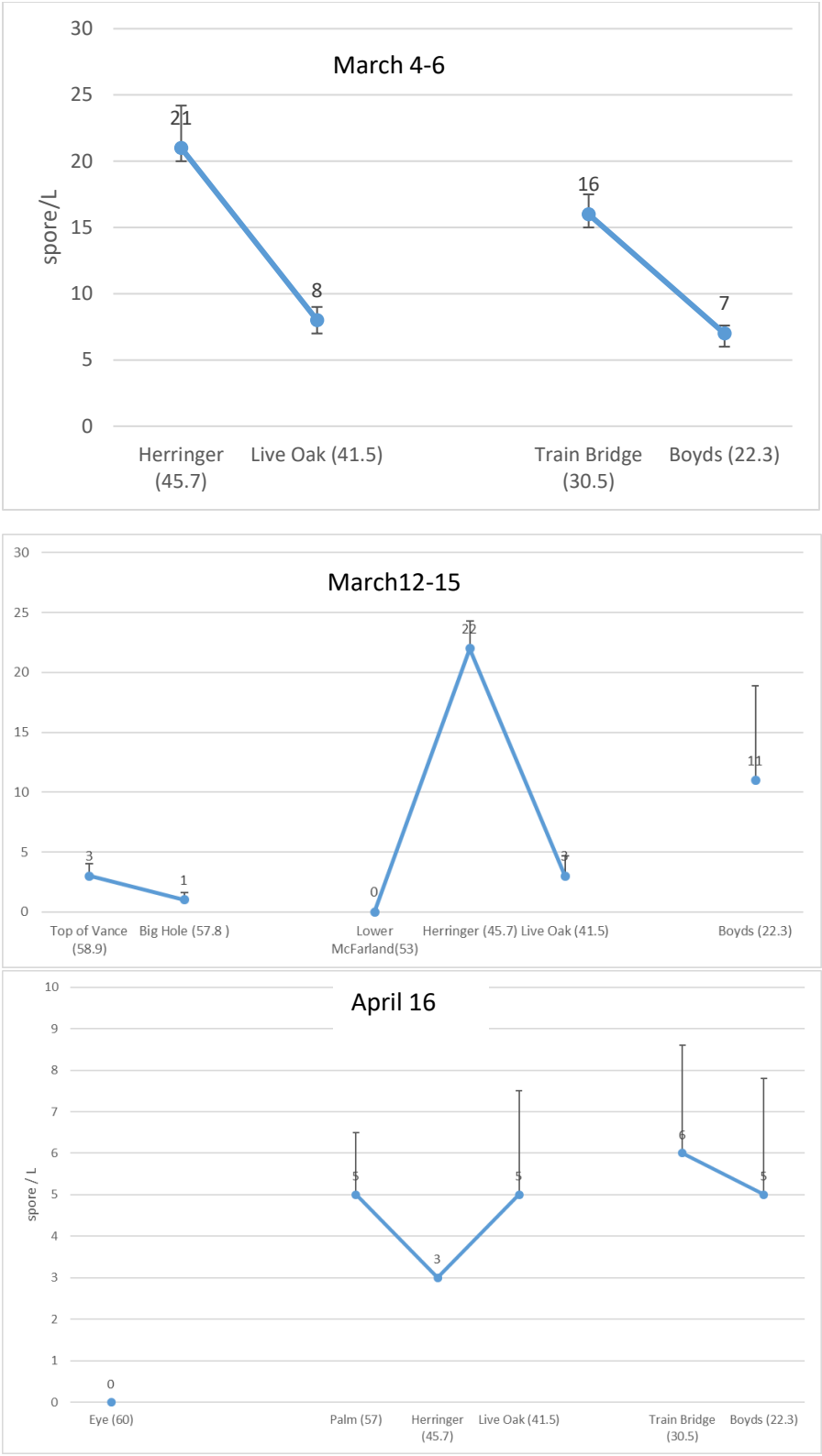


Figure 5. Mean *C.shasta spore/L* for Feather R. sites (rm 22 – 60) collected March 4-6, March 12 -15, and April 16. Standard deviation indicated by error bar. Note that Y-axis has different scales.

Feral Chinook fry – First detection of CS occurred in fry at both Eye and Herringer traps during late January (Tables 3 and 5). At Herringer trap, low prevalence of CS1 rating infections followed the late January positive until March 13 when CS2 diseased rated fry were observed through the end of the survey (Table 3). Mid-March diseased fry occurred approximately 5-6 weeks after elevated spore/L concentrations. There was moderate correlation between infection severity (CS DNA quantity as C_t value) and fish size at Herringer trap (Fork length = 56.972 – (0.512*Ct, N=53, R^2 = 0.515, P<0.01, Fig. 7). The majority of diseased fry in this period were ≥ 40mm suggesting they reared within the infectious zone prior to capture. Remnants of yolk were observed within ≥ 60 percent of each histology sample group through March at both Eye and Herringer traps. Atypical of previous surveys, CS infection reached 100% at the Eye trap on April 18 and included diseased fry (Tables 3 and 5). CS was also detected by QPCR in Eye trap fry the month prior (March 29). *Parvicapsula minibicornis* infection followed a similar temporal trend as CS (Fig 6), however observations of kidney inflammation (PM2) was about a month later than CS2 diseased occurrence. External parasites (*Ichthyoboda, Ichthyophthirius multifiliis) w*ere seen in 5% of the gill sections of all sampled fry; however, they were not associated with hyperplasia.

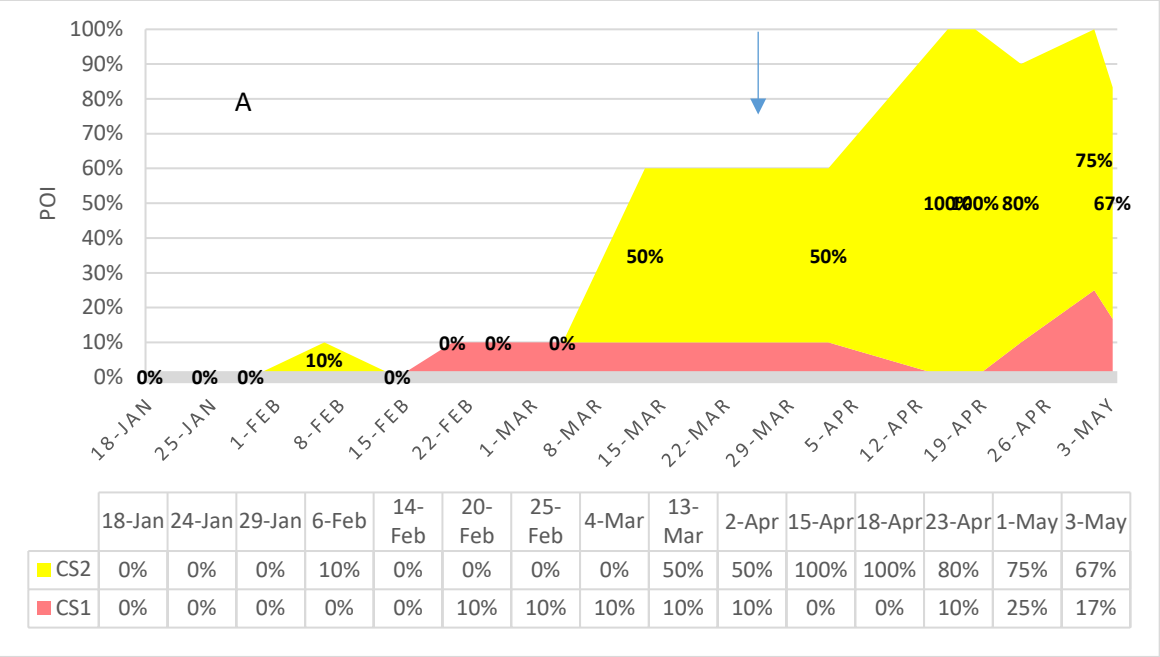
Inter-annual comparisons for both CS and PM between 2015 and 2019 (excluding 2017 flood event) show a trend of declining prevalence of infection from 2015 drought conditions with similar levels of disease (#2 rating) for each year (Figures 8 and 9). The initial CS detection occurred in Julian week 3 (January15-21) to Julian week 6 (February 5-11) during these survey years.

Table 3. Prevalence of infection (%POI) for ***C.shasta*** histology rating CS1(infected no inflammation) and CS2 (infected and inflammation "diseased") and overall histology POI in salmon fry collected at Live Oak (LO) by seine, Eye (E) and Herringer (H) traps between January 18 – May 3, 2019.

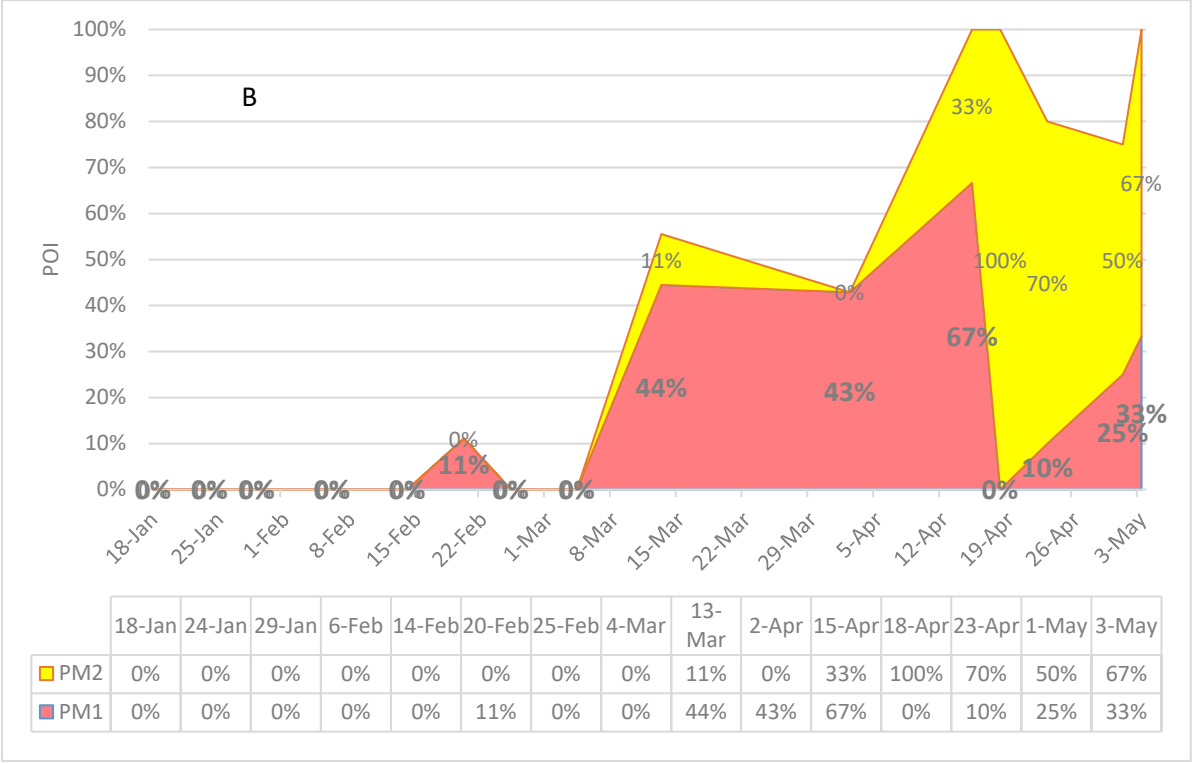
SITE	Date	N	CS1 +	CS1 %POI	CS2 +	CS2 %POI	Histology POI Cs
E	27-Jan	10	0	0%	**1**	10%	10%
E	25-Feb	8	0	0%	0	0%	0%
E	29-Mar	10	0	0%	0	0%	0%
E	15-Apr	4	0	0%	0	0%	0%
E	18-Apr	6	1	17%	**5**	83%	100%
H	18-Jan	10	0	0%	0	0%	0%
H	24-Jan	10	0	0%	0	0%	0%
H	29-Jan	10	0	0%	0	0%	0%
H	6-Feb	10	0	0%	**1**	10%	10%
H	14-Feb	9	0	0%	0	0%	0%
H	20-Feb	10	1	10%	0	0%	10%
H	25-Feb	10	1	10%	0	0%	10%
H	4-Mar	10	1	10%	0	0%	10%
H	13-Mar	10	1	10%	**5**	50%	60%
H	2-Apr	10	1	10%	**5**	50%	60%
H	15-Apr	9	0	0%	**9**	100%	100%
H	18-Apr	1	0	0%	**1**	100%	100%
H	23-Apr	10	1	10%	**8**	80%	90%
H	1-May	4	1	25%	**3**	75%	100%
H	3-May	6	1	17%	**4**	67%	83%
LO	10-Apr	10	3	30%	**5**	50%	80%

Table 4. Prevalence of infection (%POI) for ***P. minibicornis*** histology rating PM1(infected no inflammation) and PM2 (infected and inflammation "diseased")and overall histology POI in salmon fry collected at Live Oak (LO) by seine, Eye (E) and Herringer (H) traps between January 18 – May 3, 2019.

SITE	Date	N	PM1 +	PM1 %POI	PM2 +	PM2 %POI	Histology POI Cs
E	27-Jan	10	0	0%	0	0%	0%
E	25-Feb	8	0	0%	0	0%	0%
E	29-Mar	8	0	0%	0	0%	0%
E	15-Apr	4	1	25%	0	0%	25%
E	18-Apr	5	2	40%	0	0%	40%
H	18-Jan	10	0	0%	0	0%	0%
H	24-Jan	10	0	0%	0	0%	0%
H	29-Jan	10	0	0%	0	0%	0%
H	6-Feb	10	0	0%	0	0%	0%
H	14-Feb	6	0	0%	0	0%	0%
H	20-Feb	9	1	11%	0	0%	11%
H	25-Feb	10	0	0%	0	0%	0%
H	4-Mar	9	0	0%	0	0%	0%
H	13-Mar	9	4	44%	1	11%	56%
H	2-Apr	7	3	43%	0	0%	43%
H	15-Apr	9	6	67%	3	33%	100%
H	18-Apr	1	0	0%	1	100%	100%
H	23-Apr	10	1	10%	7	70%	80%
H	1-May	4	1	25%	2	50%	75%
H	3-May	6	2	33%	4	67%	100%
LO	10-Apr	10	3	30%	5	50%	80%



	18-Jan	24-Jan	29-Jan	6-Feb	14-Feb	20-Feb	25-Feb	4-Mar	13-Mar	2-Apr	15-Apr	18-Apr	23-Apr	1-May	3-May
CS2	0%	0%	0%	10%	0%	0%	0%	0%	50%	50%	100%	100%	80%	75%	67%
CS1	0%	0%	0%	0%	0%	10%	10%	10%	10%	10%	0%	0%	10%	25%	17%



	18-Jan	24-Jan	29-Jan	6-Feb	14-Feb	20-Feb	25-Feb	4-Mar	13-Mar	2-Apr	15-Apr	18-Apr	23-Apr	1-May	3-May
PM2	0%	0%	0%	0%	0%	0%	0%	0%	11%	0%	33%	100%	70%	50%	67%
PM1	0%	0%	0%	0%	0%	11%	0%	0%	44%	43%	67%	0%	10%	25%	33%

Figure 6. Prevalence of infection for *C.shasta* (A) and *P. minibicornis* (B) infection rated 1 (infected but no inflammation) or 2 (infected with inflammation "diseased") of salmon fry collected from Herringer trap between January18 – May 3. Arrow in (A) denotes when CS QPCR > 3 logs occurred.

Table 5. Prevalence of infection for ***C.shasta*** by QPCR (CS qPCR POI) in salmon fry collected at Live Oak (LO) by seine, Eye (E) and Herringer (H) traps between January 18 and May 3, 2019. Data for sample groups includes mean and standard deviation fork length (mm), sample number (n), number of samples positive (+), POI 3 logs or greater of CS DNA (>3log POI), mean C_t , range of CS DNA including minimum positive C_t value that equates with highest CS DNA (C_t min), and maximum positive C_t value for lowest CS DNA value (C_t max).

DATE	SITE	Mean FL	Std Dev FL	qPCR (n)	CS QPCR +	CS qPCR POI	CS qPCR > 3log	CS qPCR >3log POI	Ct MEAN	Ct min (HIGHEST)	Ct max (lowest)
27-Jan	E	35	2	11	0	0	0	0	N/A	N/A	N/A
25-Feb	E	36	2	11	0	0	0	0	N/A	N/A	N/A
29-Mar	E	41	8	11	3	27%	0	0%	34.80	35.90	33.40
18-Apr	E	44	7	11	11	100%	4	36%	30.37	35.90	24.14
			TOTAL	44	14	32%	4	9%			
18-Jan	H	34	1	10	0	0	0	0	N/A	N/A	N/A
24-Jan	H	37	2	11	0	0	0	0	N/A	N/A	N/A
30-Jan	H	35	1	11	2	18%	0	0	32.06	32.82	31.29
6-Feb	H	34	2	11	0	0	0	0	N/A	N/A	N/A
14-Feb	H	35	1	11	0	0	0	0	N/A	N/A	N/A
22-Feb	H	36	2	11	1	9%	0	0	N/A	32.16	N/A
25-Feb	H	36	2	11	0	0	0	0	N/A	N/A	N/A
4-Mar	H	35	4	14	4	29%	0	0	34.09	35.05	32.43
13-Mar	H	36	5	11	3	27%	1	9%	32.30	36.49	24.03
25-Mar	H	45	5	9	9	100%	9	100%	25.54	28.46	22.47
2-Apr	H	46	6	19	19	100%	19	100%	22.49	27.31	19.41
15-Apr	H	51	4	4	4	100%	4	100%	22.15	24.89	
18-Apr	H	47	7	7	7	100%	6	86%	24.96	30.76	18.88
23-Apr	H	55	7	4	4	100%	4	100%	24.09	28.70	20.03
26-Apr	H	49	3	8	8	100%	8	100%	19.20	22.56	17.19
1-May	H	67	14	4	4	100%	3	75%	26.79	30.78	19.51
3-May	H	58	6	8	8	100%	8	100%	23.55	29.37	20.22
			TOTAL	164	73	45%	62	38%			

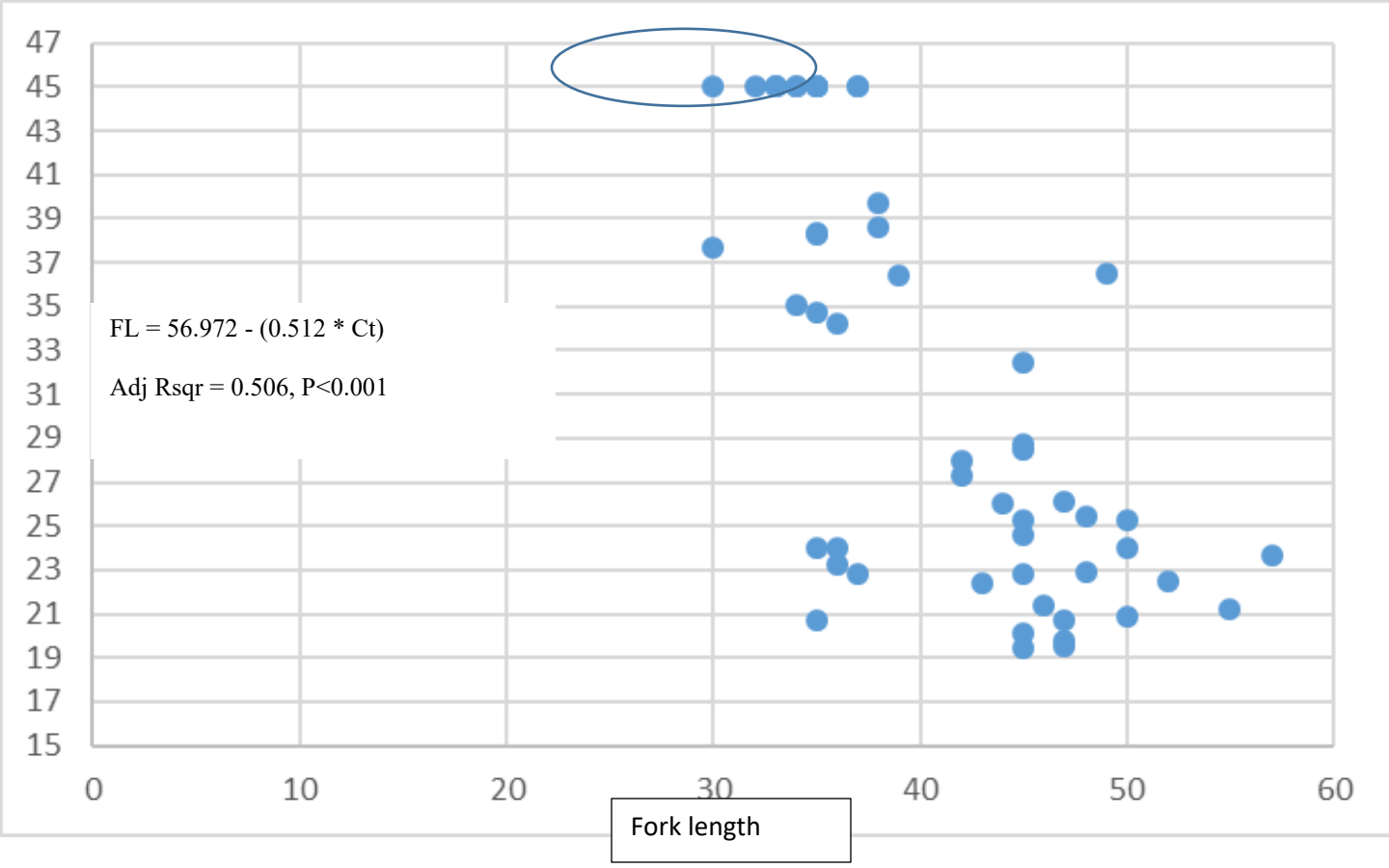


Figure 7. Relationship between fork length (mm) and CS C_t value for 53 salmon fry collected at Herringer between March 4 and April 1, 2019. Undetectable CS DNA assign a C_t of 45 (circled).

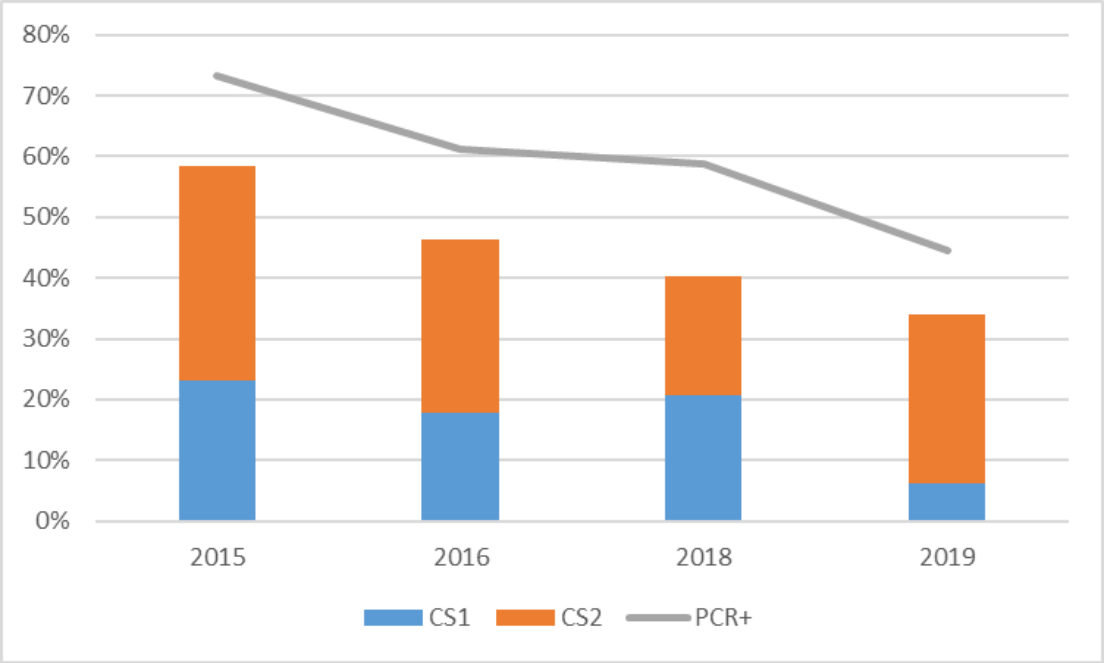


Figure 8. Annual comparison for prevalence of CS infection in Chinook fry captured within the infectious zone (Afterbay – Herringer trap) by histology (CS1 and CS2 rating) and QPCR (line).

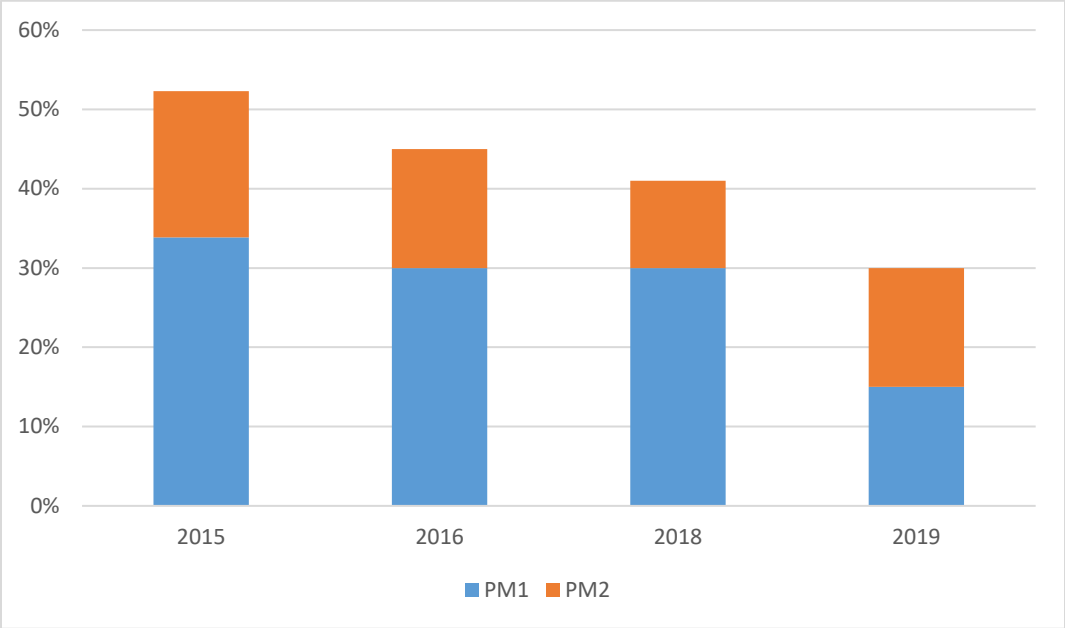


Figure 9. Annual comparison for prevalence of PM infection in Chinook fry captured within the infectious zone (Afterbay – Herringer trap) by histology (PM1 and PM2 rating).

Sentinel Chinook exposures- Mean (standard deviation) water temperature during the three 4d exposures was 9.2° (0.3), 9.5° (0.6), and 9.9°C (0.4). The corresponding rearing temperature at the wetlab was 11.4° (1.7), 12.7° (0.5), and 13.3°C (0.8). No mortalities occurred during exposure or rearing. No infection was observed in the controls held at the tag shack and exposed to afterbay water. We consider this data to support the assertion that no additional CS actinospore exposure occurred to 2d sentinels held for another 2 d at the tag shack. Longer exposure resulted in higher POI for both *C.shasta* and *P. minibicornis*, however no fish showed signs of disease after 21days post-exposure (i.e. only CS1and PM1 rating). The 4d exposure groups showed a 2.3 – 6.4x greater prevalence of *C.shasta* infection than the 2d group (Fig. 10). *P. minibicornis* appears to be more efficient at transmission as there was only a 1.3 – 1.6x greater prevalence of infection difference between 2 and 4d (Fig. 10). Average *C.shasta* spore/L values, from triplicate 1 liter grab samples taken when cages were first inserted at exposure site and at the 4 d retrieval, ranged from 1 – 20 spore/L (Table 6). No water sample was taken for Feb.25 insert date on exposure 2, however, a Feb. 26 Gridley sample (1.2 rm downstream) was quite high at 107 spores/L. The relatively low spore/L concentrations were associated with 32 – 80% POI of low intensity *C.shasta* infections after 4d of exposures.

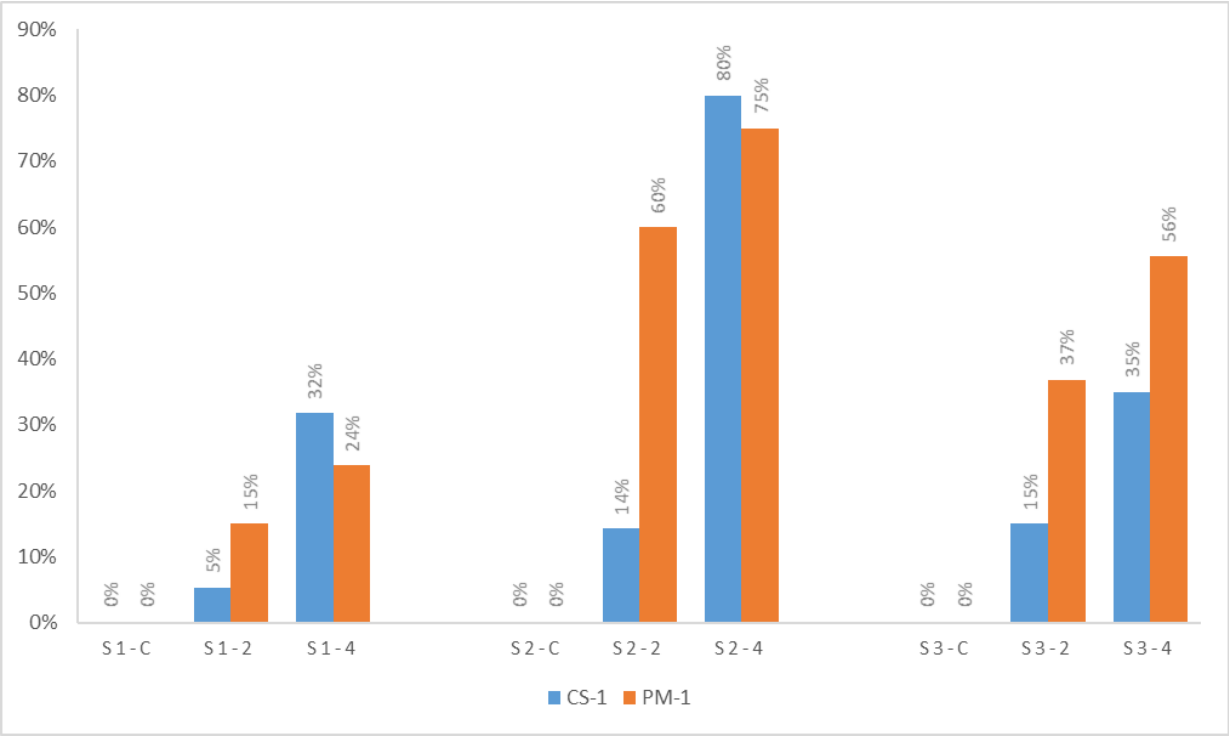


Figure 10. Prevalence of *C. shasta* (CS-1) and *P. minibicornis* (PM-1) infection (histology rating 1) in sentinel salmon held for 2 or 4 days at Big Hole (rm 57.8). Sentinel group 1 (S1) exposed between 11-15Feb., group S2 between 25Feb- 01 Mar., and group S3 between 11-15Mar.

Table 6. River water *C.shasta* spore/L data at Big Hole exposure site for input date and 4 day cage retrieval for exposure (E) 1 to 3. Spore per liter is the mean of triplicate 1-liter grab samples.

		spore/L
E1	11-Feb	20
	15-Feb	5
E2	25-Feb	nd
	1-Mar	9
E3	11-Mar	2
	15-Mar	1

[nd] not done

FRH Spring-run – The April 5 "pre-release" sample of Spring-run Chinook (SCS) collected at Boyd's launch had a low prevalence of *C.shasta* infection by QPCR (2 of 11 fish, Table 7). Neither *C.shasta* nor *P. minibicornis* was observed in 10 histological sections from this same group. This limited data, on FRH salmon sampled prior to river exposure, suggests a low level CS actinospore exposure at the hatchery. Coded wire tag identified carcasses from all three in-river releases were recovered in the lower Sacramento River (majority of samples from Chipps trawl at rm18). QPCR analysis was conducted on SCS that were ≥ 13d post-release to allow for parasite invasion into the intestinal tract and associated QPCR detection in this tissue. In general, few SCS carcasses tested positive by QPCR with even fewer considered "diseased" (≥ 3 logs of parasite DNA).

The highest prevalence of CS infection occurred to the March 31 release sample groups. CS infection was detected in 9 of 31 (29%) fish from the Live Oak release site and 12 of 23 (52%) from Boyd's (Table 7). Four fish (3 Live Oak, 1 Boyd's) were considered "diseased" with ≥ 3 copies of CS DNA. The mean days at large (DAL) was 27 and 29d for the CS infected Live Oak and Boyd's fish. Average (SD) fork length for the March 13 Live Oak and Boyd's sample fish was 84(5) and 86 (8), respectively. No CS DNA was detected in seven samples from the April 5 release groups (Table 7). Similarly, only three fish (1 of 9 Gridley release and 2 of 7 Boyd's release) were CS positive from the April 22 release (Table 7). The DAL for the two April release group samples ranged from 14 – 22d and the mean fork length for both releases was 90 mm (SD= 6). Four Fall-run Chinook released at Boyd's on May 5 were assayed by QPCR.

These fish represented the longest DAL (5-8 days) for the Fall-run group in the carcass collection and no CS was detected.

Table 7. *C.shasta* QPCR analysis of Brood year 2018 Feather River Hatchery (FRH) coded wire tagged Spring-run (SCS) and Fall-run (FCS) Chinook salmon juveniles captured in lower Sacramento- Delta by USFWS Lodi office. Data include release date (R-date) and site (includes sample from hatchery on 05April), tag code (CWT), prevalence of *C.shasta* infection (Cs POI), range of minimum and maximum Cycle threshold (Ct) of positives, parasite DNA copy number (log), days at large (DAL), and fork length (FL mm).

R-date	Site	Run	CWT	Cs POI	range Ct positives	range copy#(log)	range DAL	range FL(mm)
N/A	FRH- 05APR	SCS	N/A	2 / 11 (18%)	34.94 - 36.04	0.1 - 1.0	N/A	72 -81
13-Mar	Live Oak	SCS	061972	9 / 31 (29%)	27.54 - 34.63	1-3	16 41	72 - 94
	Boyds		061973	12 / 23 (52%)	26.86 - 36.94	0.1 - 4	16 -39	70 - 101
5-Apr	Gridley	SCS	061974	0 / 7	N/A	N/A	16-18	82 - 93
	Boyds		061975	0 / 5	N/A	N/A	16-18	75 - 96
22-Apr	Gridley#	SCS	061534, 061607	1 / 9 (11%)	31.73	0.1	14 - 17	91 - 105
	Boyds		061976	2 / 7 (29%)	27.40 - 35.56	1 - 3	14 - 22	84 - 97
5-May	Boyds	*FCS*	061979	0 / 4	N/A	N/A	5 - 8	88 - 95

two of four CWT codes for 22April Gridley release assayed.

Discussion: Within the "infectious zone" (Herringer trap), prevalence of CS infection was 34% by histology and 45% by QPCR in 2019. There has been steady decline in CS POI since 2015 however; over half of infected fry are categorized as diseased in each of the study years (2017 excluded due to unique flood conditions). Timing of initial CS infection, as well as timing of diseased fry, was similar to 2015 – 2018. Initial infection occurring in late January to early February and diseased fry observed in ≥ 50 percent of a given week's sample by mid-March. One unique observation in 2019 was the occurrence of diseased fish from the Eye trap in April. While CS infection has been detected from the low flow trap, it is typically at both low prevalence and severity. Given the increased flow down this section in April, it seems unlikely that infected fry from the high flow section would move upstream and later be captured at Eye trap. CS infected polychaete abundance within the low flow reach likely increased in 2019 compared to previous years.

Low river temperature slows disease progression in exposed salmonids. Udey et al. (1975) reported ceratomyxosis mean day to death (MDD) for rainbow trout was 83 d at 9.4° C compared to 19 d at 20.5°C. Ratliff (1983) describes a similar response in the Deschutes River with MDD ranging from 45 to 90 days at temperatures of 7° to 10°C. Ray et al. (2012) model of CS transmission and mortality describe 2.2X difference in disease progression between CS infected juvenile Chinook reared at 13° compared to 15°C. Elevated CS spore/L conditions at Herringer began in late January and continued through early March. Given the >20 spore/L concentrations, we assume that fish-infective actinospores were the dominant parasite stage in the water samples as previous work examining post-spawning river samples were magnitudes of DNA lower (Foott et al. 2016 and 2017). Temporal difference between infectivity of the high flow section and observation of disease was likely influenced by the 9 - 11°C during this period.

Exposure duration ("dose") influences transmission and disease progression (Ray et al. 2012). In March, larger fry at Herringer trap tended to incur greater disease (CS2 rating) than smaller cohorts captured at the same time. This observation suggests that rearing within the infectious zone is a ceratomyoxsis determinant for Feather River juvenile Chinook. The increased prevalence of CS infection in 4 d exposure compared to the 2 d sentinels also supports this rearing (duration) hypothesis. The lack of disease progression observed in the 4 d sentinels is likely due to moderate actinospore concentration at Big Hole in February and early March. These sentinels demonstrate that migratory fry within the infectious zone do not face the same disease risk as rearing fry. Rate of migration seems to have an inverse relationship on Feather River hatchery juvenile salmon release CS infection. In 2019, the first release group (March 31) had the highest CS POI with infected fish having greater days at large compared to the larger

smolts from the two April releases. We hypothesize that larger smolts (~90mm FL) will migrate rapidly from the Feather River and thereby avoid exposure to lethal actinospore concentrations regardless of release site (Gridley or Boyd's).

Passage estimates at the Herringer trap indicated that over 99% of fry had moved past it by the time diseased fry were observed in mid-March (pers. comm. J.Kindopp December 2019). Given this passage data, it is not clear whether *C.shasta* exerts a population level effect on Feather River natural chinook juveniles. There is little data on CS infection of fry that move through infectious zone prior to March and reared lower in the river system. Similarly, it is unclear whether the larger fry sampled in March would recruit at a higher rate than early out-migrant fry had they not incurred such a high disease rate?

Future study should consider these questions:

1. **Health of out-migrant fry in the lower Feather** -What is the CS infection profile of fry that move through infectious zone prior to March and rear in the lower Feather and Sacramento rivers? Does latent CS infection progress and negatively influence survival? Natural recruitment has been relatively low yet >90% move past the infectious zone prior to March.

2. **Spatial distribution and abundance of CS infected polychaetes within the infectious zone** – To date, the few survey efforts in the Feather River have not identified habitats with abundant polychaete worms yet high concentrations of actinospores are shed within the infectious zone.
    a. Do these potential polychaete habitats share a common set of features (substrate, flow exposure, etc.)?
    b. What is the annual abundance and prevalence of CS infection in these polychaetes?
    c. Can they be monitored for myxospore transmission in the fall and winter?

Lack of such knowledge impedes potential management actions for this part of the parasite's life cycle.

3. **Why does the infectious zone start below the afterbay canal outlet? A**ctinospores are not detected in afterbay water.
    a. Does the afterbay provide polychaetes with a food source (planktonic input)?

4. **What is the duration and extent of myxospore dispersal from adult salmon carcasses?** Determine myxospore transport pattern and transmission efficiency throughout winter and early spring. Could flushing winter flows transport them past polychaete sites within the infectious zone?

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References:

Applied Biosystems. (2016). Application Note: Real-time PCR: Understanding Ct. Publication CO019879 0116. Retrieved September 2018 from Thermo Fisher Scientific: https://www.thermofisher.com/content/dam/LifeTech/Documents/PDFs/PG1503-PJ9169-CO019879-Re-brand-Real-Time-PCR-Understanding-Ct-Value-Americas-FHR.pdf

Applied Biosystems. (2014). Real-time PCR handbook. Publication CO010759 0914. Retrieved December 2018 from Thermo Fisher Scientific: https://www.thermofisher.com/content/dam/LifeTech/global/Forms/PDF/real-time-pcr-handbook.pdf

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.

Foott J.S. and A. Imrie. 2017. Prevalence and severity of *Ceratonova shasta* and *Parvicapsula minibicornis* infection in Feather River Juvenile Chinook Salmon (January – May 2017). 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.

Ratliff D.E. 1983. *Ceratomyxa shasta*: Longevity, distribution, timing, and abundance of the infectious stage in Central Oregon. J. Fisheries Research Board of Canada 40:1622 - 1632.

Ray R.A., R.A. Holt, and J.L. Bartholomew. 2012. Relationship between temperature and *Ceratomyxa shasta*-induced mortality in Klamath River salmonids. J. Parasitology 98(3): 520 -526.

True, K, A. Bolick and JS Foott. 2012. FY2008 Investigational Study: Prognosis of *Ceratomyxa shasta* and *Parvicapsula minibicornis* infections in Klamath River Coho and Trinity River Chinook. U.S. Fish & Wildlife Service California – Nevada Fish Health Center, Anderson, CA. http://www.fws.gov/canvfhc/reports.asp

True, K., Bolick, A., & Foott, J. (2013). Myxosporean Parasite (*Ceratomyxa shasta* and *Parvicapsula minibicornis*) Annual Prevalence of Infection in Klamath River Basin Juvenile Chinook Salmon, April-August 2012. Anderson, CA: US Fish and Wildlife Service. California-Nevada Fish Health Center.

Udey LR, JL Fryer, and KS Pilcher. 1975. Relation of water temperature to Ceratomyxosis in Rainbow trout (*Salmo gairdneri*) and Coho salmon (*Oncorhynchus kisutch*). J. Fisheries Research Board of Canada 32: 1545 – 1551.