U.S. Fish & Wildlife Service

California Nevada Fish Health Center

FY2016 Technical Report:

Prevalence and severity of *Ceratonova shasta* and *Parvicapsula minibicornis* infection of natural Feather River Juvenile Chinook Salmon (January – May 2016).

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Summary: A similar pattern of *Ceratonova shasta* and *Parvicapsula minibicornis* infectivity was observed in the 2016 survey (January – May) as 2015. A zone of high *C.shasta* infectivity (top of high flow reach past Boyd's landing (rm 59 – 22)) was demonstrated by water sample *C.shasta* eDNA as well as high prevalence of infection in both sentinel and natural Chinook salmon fry. Initial detection of *C.shasta* in salmon fry within the infectious zone, occurred in late January, peaked in disease severity from late March through April, and had an overall 46% prevalence of infection. It appears that severely infected fish in the infectious zone are unlikely to survive as few such fish were captured 23 – 27 rm downstream. Infectious *C.shasta* actinospore concentration peaked in late February and remained high through April. Sentinel salmon, exposed for 3 days within the infectious zone (at Gridley) in late February and March, had high prevalence of *C.shasta* infection but low disease severity. This pattern did not match observations in natural fry cohorts suggesting a longer exposure period in natural fry.

The correct citation for this report is:

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I**ntroduction**: This is the second season of a 3 year study that has documented a highly infectious zone for *Ceratonova shasta* infection of juvenile Chinook in the first 14+ river miles of the high flow reach. While infection can occur as early as January at 8- 10°C, peak prevalence of infection and disease severity tend to occur in late 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 to polychaete worm, actinospore shed from polychaete to fish). Other background information on *Ceratonova shasta* can be found in Foott et al. (2016).

The objectives of the 2016 study season were:

1. Compare prevalence and severity of *C.shasta* and *P. minibicornis* infection in natural Chinook juveniles in the low and high flow reaches of the Feather River from late January until out-migration tapers off in late spring.
2. Examine juvenile salmon below the confluence of the Yuba R. during the prime infectivity period of March and April. The lower river sample could provide information on prognosis of infections acquired up-river.
3. Determine the level of *C.shasta* infectivity at different locations in the high flow reach from February through early May using sentinel salmon and filtered water eDNA techniques.
    a. One set of sentinel salmon (Coleman Fall-run juveniles) were exposed for 3 days at Gridley, in February and again in March, to represent the documented migration duration of natural fry from the low flow reach to the Herringer trap (pers. comm. J. Kindopp, DWR).
    b. The prognosis of ceratomyxosis in Feather R. SFH Spring-run juveniles released in March and April at either Gridley or Boyd's Landing, and re-captured 4 days later was evaluated after a 20 day rearing period.
    c. Longitudinal set of water samples were collected from rm 59 downriver to rm 18 from late February through May 2, and analyzed for *C.shasta* DNA (estimate actinospore/L). This data helps to define the temporal and spatial aspects of the infectious zone.
4. Survey Fall and Spring-run adult Chinook carcasses for myxospore input into the low flow reach.

Methods:

Natural juveniles were collected by California Department of Water Resource (DWR) biologists by rotary screw traps (Gateway and Herringer) as well as beach seine near the Herringer site (March 7 – May10), Boyd's Landing launch, Live Oak Launch, and Star Bend (Figure 1, Tables 1 and 2). A total of 434 natural juvenile Chinook were collected and sampled under California Department of Fish and Wildlife Scientific Collection Permit (SC-4085, JS Foott). Tissues were fixed in Davidson's fixative for histological examination or frozen (intestine) for QPCR analysis. Ten fry, from the bi-weekly Herringer trap or seine collection, were held for 4 days in a tank supplied with ambient Thermalito afterbay water at the DWR tag shack (at afterbay off Larkin Road). Mortality was monitored daily and dead fish frozen. All survivors were frozen for QPCR analysis on day 4.

Adult fall and spring-run Chinook carcass intestines were sampled within the low flow reach (rm 63 - 67) in October and November 2015. Laboratory assays were similar to 2015 (Foott et al. 2016) and water samples analyzed for *C.shasta* DNA by Oregon State University (Hallett et al. 2012). The *C. shasta* QPCR reference standard curve for intestine samples was obtained using synthesized DNA (Gene Block, IDT, Coralville Iowa) containing the 18S ribosomal DNA target sequence. Specifically, 1 ng of DNA, corresponding to 6.83×10^9copies of *C. shasta* DNA was serially diluted over 8 orders of magnitude in molecular grade water. Using QPCR analysis software, the cycle threshold (C_T) values for each standard concentration were calculated (SDS software 7300 SDS v 1.3.1, Applied Biosystems). The standard curve was used to evaluate PCR amplification efficiency (slope of the standard curve, efficiency was 93%), fit to the curve (R^2 value = 0.997) and the y-intercept (C_T value for a single copy of parasite DNA = 40.1). Histological rankings of 'clinical disease' were based on the presence of multifocal lesions associated with parasite infection: (CS2 rating of intestine = lamina propria hyperplasia with necrotic epithelium and/or necrotic muscularis and PM2 rating of kidney = interstitial hyperplasia, necrotic interstitium or tubule, glomerulonephritis, and/or protein casts within the glomeruli or tubules). The #1 rating required the presence of the parasite in the respective tissue but with minimal inflammatory changes.

Table1. Natural juvenile Chinook sample numbers collected at Gateway (G), Herringer (H), Boyd's Landing (B), and Star Bend (SB) in 2016 during the 18 week study.

Week			G	H	B	SB
1	11-Jan	15-Jan		20		
2	18-Jan	22-Jan				
3	25-Jan	29-Jan	15	22		
4	1-Feb	5-Feb				
5	8-Feb	12-Feb		22		
6	15-Feb	19-Feb			22	
7	22-Feb	26-Feb	15	21		
8	29-Feb	4-Mar			22	22
9	7-Mar	11-Mar		22		
10	14-Mar	18-Mar				
11	21-Mar	25-Mar	15	22	22	22
12	28-Mar	1-Apr				
13	4-Apr	8-Apr		22	22	22
14	11-Apr	15-Apr				
15	18-Apr	22-Apr	6	21	22	22
16	25-Apr	29-Apr				
17	2-May	6-May				
18	9-May	13-May		13		
		totals	51	185	110	88
					season total	434

Figure 1. Base map showing sample sites for natural Chinook collection and sentinel exposure.

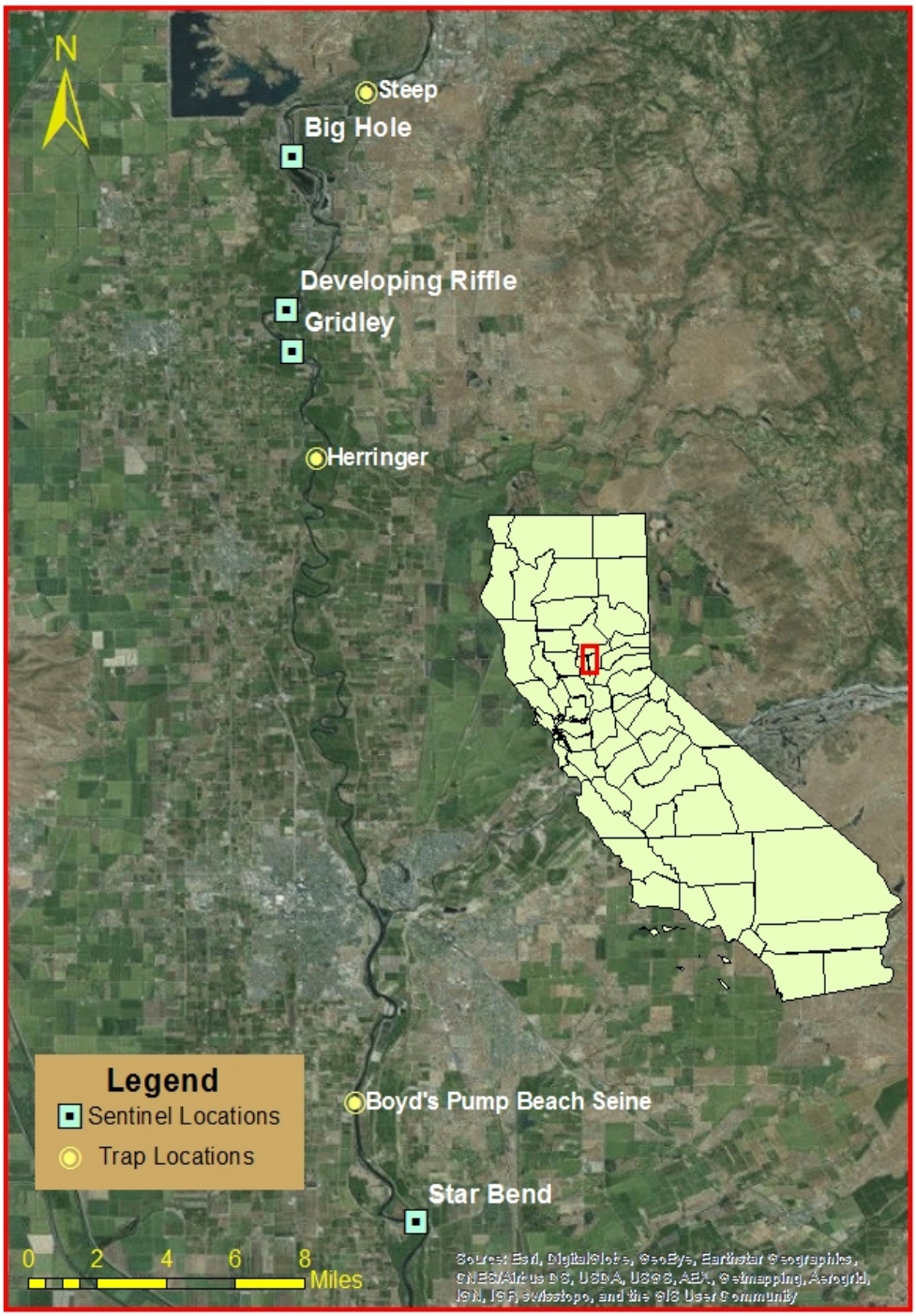


Table 2. Sample site river mile data.

Site	River mile
Riverbend	65
Gateway	59.6
Below TAO	59
above G-95	57.5
Palm	57
Developing Riffle	52
Gridley	50
Herringer	45.7
live Oak	41.5
Shawns Beach	37.5
Train Bridge	30.5
Boyds	22.3
Star Bend	18.1

Sentinel exposures - Coleman National Fish Hatchery Fall-run Chinook fry were held in 0.34 ft^3 live cages 100 yards upriver of the Gridley boat launch for 3 to 4 consecutive days in February (2/16-19) and March (3/25 -29). Hourly temperature was recorded by an Onset temperature probe within the cage. Sentinels were subsequently reared at the CA-NV FHC wetlab, for a total of 24 days post-exposure, and sampled for histological examination. Intestines from mortalities were assayed for *C.shasta* DNA by QPCR.

Concerns about post-release disease impacts on Feather River Hatchery Spring-run Chinook smolt in-river releases, prompted California Department of Fish and Wildlife and DWR to request the CA-NV Fish Health Center evaluate several release groups for ceratomyxosis. These trials were performed “gratis” outside of the annual contract. Releases from either Gridley or Boyd’s Landing launch occurred on March 25 and April 14, 2016. Fish were re-captured by beach seine 4 days post-release near the Gridley launch, and either Boyd’s landing (March) or Star Bend (April). They were transported to the FHC wetlab, reared for a total of 24 or 25 days post-release, and sampled as above. Coded wire tag identity of each individual fish was used to determine its release site.

Results:

Adult carcass myxospores - Adult carcass intestines were sampled in the low flow reach (rm 63-67) on October 5, 19, and November 3, 2015. Spring-run adults were identified by hallprint tags applied in the spring to hatchery ladder returns. Fall-run adults were unmarked and collected several weeks after the peak of spring-run carcass recovery (November 3). The overall prevalence of myxospore detection in all carcass samples was 80% (48 /60), with Spring-run having slightly lower (78%) prevalence than Fall-run (82%). The sample spore concentrations were similar between the runs (Mann-Whitney rank sum test U= 227, P=0.901) with 65% of all positive samples containing >500,000 spores (Table 3). Myxospore concentrations / scraping ranged from the detection threshold of approximately 1000 to 49 million. Mean percent intact ("viable") myxospore ranged from 49 – 79% in the 3 sample groups. No trend in spore concentrations with fork length (as a substitute for age) was observed by linear regression (R^2= 0.052) or sex (Mann-Whitney U = 226.5, P= 0.212). If the median spore concentration for adults with < 500,000 (6,488) and > 500,000 spores (7,500,046) is applied to their prevalence in an hypothetical adult return of 100,000, a total myxospore input of 187.9 billion spores could go into the Feather river during a single season. A similar pattern of high prevalence and high myxospore concentration was observed in carcass samples collected in 2014 (Foott et al. 2016).

Table 3. Carcass myxospore data from both marked Spring-run (SCS) and Fall-run (FCS) Chinook salmon collected in the low flow reach between October 5 and November 3, 2015. Prevalence of myxospore detected from intestine (positive / total (%)), positive samples containing > 500,000 spores, mean percent intact spore of positive samples, and range of estimated myxospore / scraping (M=million).

Date	Run	POI Myxospore Detected	% > 500,000 spore	% intact	Myxospore range
5-Oct-15	SCS	15/20 (75%)	10/15 (67%)	79%	1125 - 17.6M
19-Oct-15	SCS	6/7 (86%)	3/6 (50%)	49%	7438 - 7.1M
3-Nov-15	FCS	27 / 33 (82%)	18/27 (67%)	65%	1150 - 49.0M
	total	48/60 (80%)	31/48 (65%)		

Prevalence and severity in natural Chinook juveniles-

Gateway Trap Collections (low flow reach): Given the low prevalence of infection previously observed from the low flow reach, a total of only 51 fry were sampled on a monthly basis between January and April in 2016. The prevalence of *C.shasta* infection was 28% (10 /36) in QPCR samples with relatively low parasite DNA concentrations (Table 4). The April 6 sample was unique for this site given both its high prevalence and parasite DNA concentration. *C.shasta* or *P.minibicornis* parasites were not observed in 15 histological specimens. Despite the April sample, the low flow reach continues to show low *C.shasta* infectivity. *Ichthyophthirius multifiliis* was observed on one gill section without associated hyperplasia.

Table 4. Gateway trap sample data reported as sample date, mean (SD) fork length, prevalence of infection (POI) for *C.shasta* (CS) and *P. minibicornis* (PM) in histological specimens (1= infection with little disease, 2= disease state), QPCR samples and the C_t range of positive samples. Season prevalence is summation of individual sample dates.

Date	Fork length	CS1	CS2	CS POI (%)	PM1	PM2	PM POI (%)	Cs-QPCR POI	Ct range
Jan 25	33 (1.3)	0/5	0/5	0%	0/4	0/4	0%	1/10	37.4
Feb 22	36 (0.9)	0/5	0/5	0%	0/5	0/5	0%	1/10	35.8
Mar 23	36 (3.8)	0/5	0/5	0%	0/5	0/5	0%	2/10	35.2 - 36.7
Apr 20	67 (6.7	ND	ND	n/a	ND	ND	n/a	**6/6**	30.9 - 35.2
Season POI				0/15 (0%)			0/14 (0%)	10/36 (28%)	

Herringer Trap Collections (high flow reach): Small fry, containing yolk protein within their peritoneum (mean $\leq$ 36mm fork length), were captured in January and February. Fry size increased through the last sample (May10, mean 66mm FL). The initial detection of *C.shasta* occurred in a QPCR sample collected January 25 and one month later by histology (Table 5). Prevalence of infection increased to 100% in late March with the majority having disease signs (CS2 rating, Figure 2). High *C.shasta* DNA quantities (i.e. low C_t values) indicative of severe infections (~ $C_t \leq 26$) were observed beginning in the March 7 collection through April 19 (Table 5). *Parvicapsula minibicornis* infection ranged from 8% in late February to 100% in early April (Table 5).

Significant inflammatory changes in the kidney (PM2) were observed in April through the last sample on May 10. Three gill sections out of 100 had mild *Ichthyophthirius multifiliis* infection without associated hyperplasia. These trends were also observed in previous years (2013 – 2015). River temperature during the documented *C.shasta* infection period (late January – May10) ranged from approximately 10° - 18°C (Figure 3). Cumulative mortality, in the 10 fry groups held at the tag shack, ranged from 0 to 60% (Table 5). The highest mortalities occurred to groups captured on March25 through April 19. This period corresponds closely to high prevalence of histological disease CS2 rating (Figure 2). This data suggests that the prognosis for severe infection is death in a short period of time. A conservative estimate of disease mortality, for fry migrating through the infectious zone in March and April, is likely to be over 50%. The impact on natural production may be limited as this time period corresponds to less than 6% of the total fry passage estimates at the Herringer trap between December and April.

Table 5. Data from Herringer rotary screw trap and seine collection of juvenile Chinook. Prevalence of infection (POI) for of *C.shasta* (CS) and *P. minibicornis* (PM) in histological specimens (1= infection with little disease, 2= disease state). Percent mortality of captured fish held in tag shack tank for 3-4d post-collection, their mean (SD) fork length, *C.shasta* POI by QPCR is reported as well as POI of the entire sample group over season for both histology and QPCR assays and C_t range of positive samples.

Date	Fork length	CS1	CS2	CS POI (%)	PM1	PM2	PM POI (%)	MORT(%)	QPCR(%)	Ct range
Jan 11 - 15	34 (1.1)	0/12	0/12	0	0/12	0/12	0	0	0/8	n/a
Jan 25 - 29	34 (1.3)	0/12	0/12	0	0/10	0/10	0	0	1/10	35.7
Feb 9 - 13	36 (1.7)	0/12	0/12	0	0/12	0/12	0	0	4/10	30.1 - 35.2
Feb 23 - 25	36 (1.5)	1/11	0/11	8	1/11	0/11	8	0	5/10	28.0 - 32.0
Mar 7 - 11	43 (2.2)	6/12	1/12	58	7/12	0/12	64	10	10/10	20.8 - 35.9
Mar 21 - 25	39 (5.8)	1/12	8/12	75	9/12	0/12	75	60	10/10	20.0 - 33.7
Apr 4 - 8	42 (6.6)	1/12	9/12	91	9/12	3/12	100	30	4/4	20.9 - 35.2
Apr19 - 20	54 (10.3)	1/10	9/10	100	4/11	6/11	91	60	10/10	17.7 - 24.2
10-May	66 (8.1)**	9/13	3/13	40	1/13	7/13	62	n/a	n/a	n/a
Season POI				49 / 106 (46%)			47 / 105 (45%)		44/72 (61%)	

** Fork length data from histology sample set

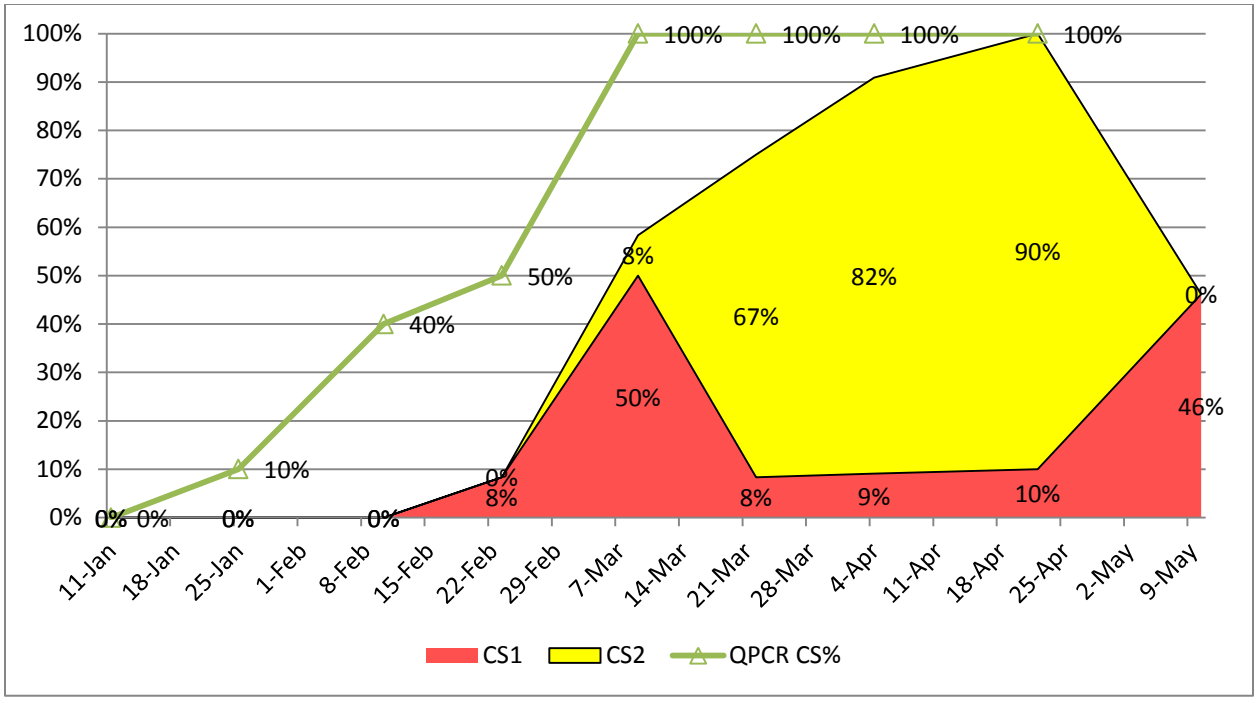


Figure 2. Prevalence of *C.shasta* infection severity categories (1= infection with little disease, 2= disease state) and by QPCR in juvenile Chinook salmon captured at Herringer trap between January 11 – May 9, 2016.

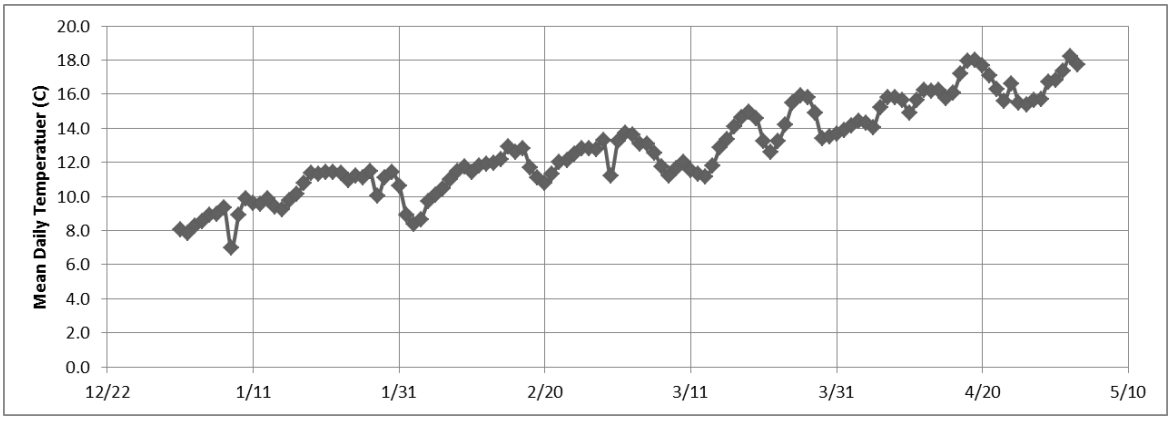


Figure 3. Mean daily temperature (°C) at the Herringer trap site.

Boyd's Landing Launch collection- *C.shasta* and *P. minibicornis* were detected in fry from the initial February 19 sample through the final April 19 sample (Table 6). Prevalence of *C.shasta* infection ranged from 25 – 75% by histology and ≥ 70% by QPCR (Figure 4). Despite the high prevalence, few infections (2 of 60, 3%) were rated as "diseased" (CS2) or had QPCR C_t values ≤ 26 (6 of 50, 12%). *Parvicapsula* prevalence increased from 33% to 100% with only 3% rated as diseased (Table 6). Pre-sporogonic forms were seen in kidney tubules beginning with the March 22 sample but were not associated with significant inflammation. It would appear that *Parvicapsula* infection was not a significant health impairment for this sample group. No ectoparasites were seen in 47 gill sections. Elevated flows in March were not associated with decreased transmission of either *C.shasta* or *P. minibicornis* but may have influenced severity (Figure 5).

Table 6. Boyd's landing seine collection of juvenile Chinook data. Date of collection, mean (SD) fork length, prevalence of infection (POI) for of *C.shasta* (CS) and *P. minibicornis* (PM) in histological specimens (1= infection with little disease, 2= disease state), *C.shasta* by QPCR and C_t range of positive samples. POI of the entire sample group over season for both histology and QPCR assays also reported.

Date	Fork length	CS1	CS2	CS POI (%)	PM1	PM2	PM POI (%)	Cs-QPCR(%)	Ct range
Feb 19	38 (5.2)	3/12	0/12	25%	3/9	0/9	33%	7/10	30.5 - 36.9
Mar 1	47 (9.8)	6/12	1/12	58%	4/12	0/12	33%	10/10	27.0 - 34.9
Mar 22	42 (1.4)	7/12	1/12	67%	6/10	1/10	70%	10/10	22.7 - 37.1
Apr 5	43 (4.3)	9/12	0/12	75%	7/12	1/12	67%	9/10	23.3 - 35.5
Apr 19	60 (4.1)	6/12	0/12	50%	12/12	0/12	100%	10/10	25.4 - 30.4
Season POI				33/50 (66%)			34/55 (62%)	46/50 (92%)	

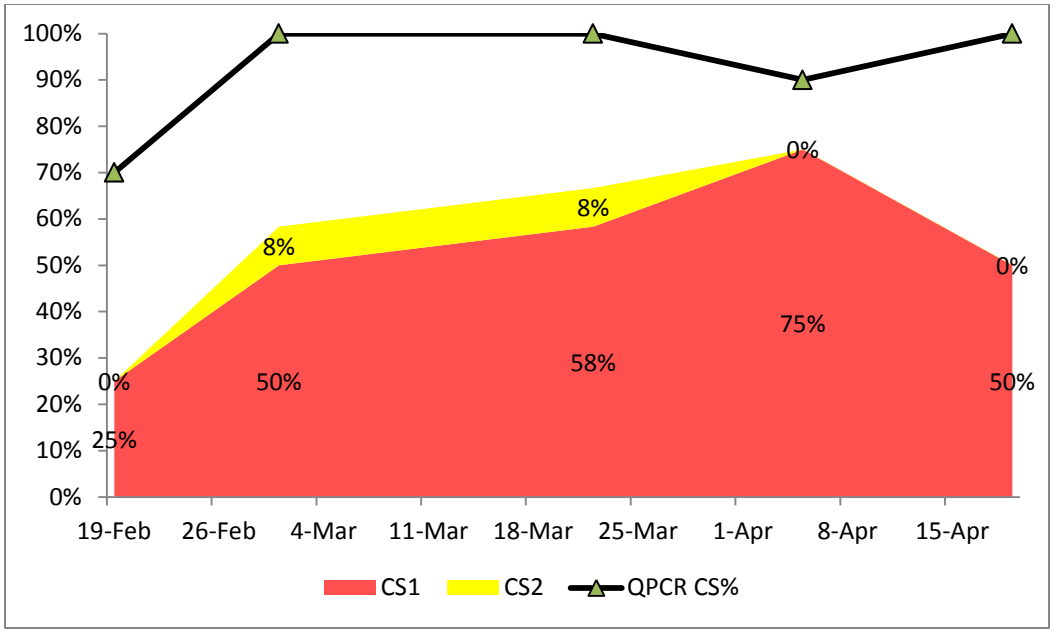


Figure 4. Prevalence of *C.shasta* infection severity categories (1= infection with little disease, 2= disease state) and by QPCR in juvenile Chinook salmon captured at Boyd's Landing between February 19 – April 19, 2016.

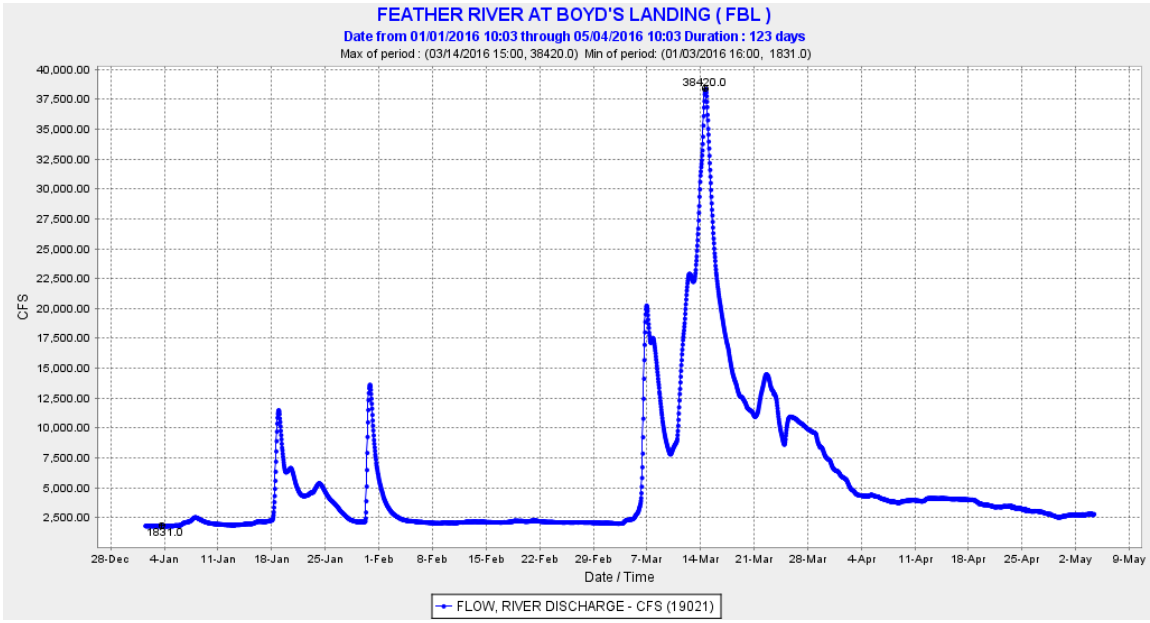


Figure 5. Feather river flow at Boyd's landing (cfs) between January 1 and May 4, 2016.

Star Bend collection – Both the prevalence (67 – 100% by histology, ≥ 70% by QPCR) and severity (25 – 33% CS2 rating for 3 of 4 collections) of *C.shasta* infection was deemed high at this sample site (Table 7, Figure 6). . *Parvicapsula* prevalence increased from 36% to 92% with only 4% rated as diseased (Table 7). No external parasites were observed in 47 gill sections.

Table 7. Star Bend seine collection of juvenile Chinook data. Date of collection, mean (SD) fork length, prevalence of infection (POI) for of *C.shasta* (CS) and *P. minibicornis* (PM) in histological specimens (1= infection with little disease, 2= disease state) and for *C.shasta* by QPCR and C_t range of positive samples. POI of the entire sample group over season for both histology and QPCR assays also reported.

Date	Fork length	CS1	CS2	CS POI (%)	PM1	PM2	PM POI (%)	Cs-QPCR POI	Ct range
Mar 1	47 (6.5)	9/12	3/12	100%	4/11	0/11	36%	7/10	23.3 - 33.9
Mar 22	42 (2.8)	7/12	4/12	92%	10/12	0/12	83%	8/10	26.4 - 35.5
Apr 5	53 (8.4)	6/12	4/12	83%	7/12	1/12	67%	7/10	27.2 - 33.7
Apr 19	48 (4.8)	8/12	0/12	67%	10/12	1/12	92%	11/11	21.8 - 34.8
Season POI				41/48 (85%)			33/47 (70%)	33/41 (80%)	

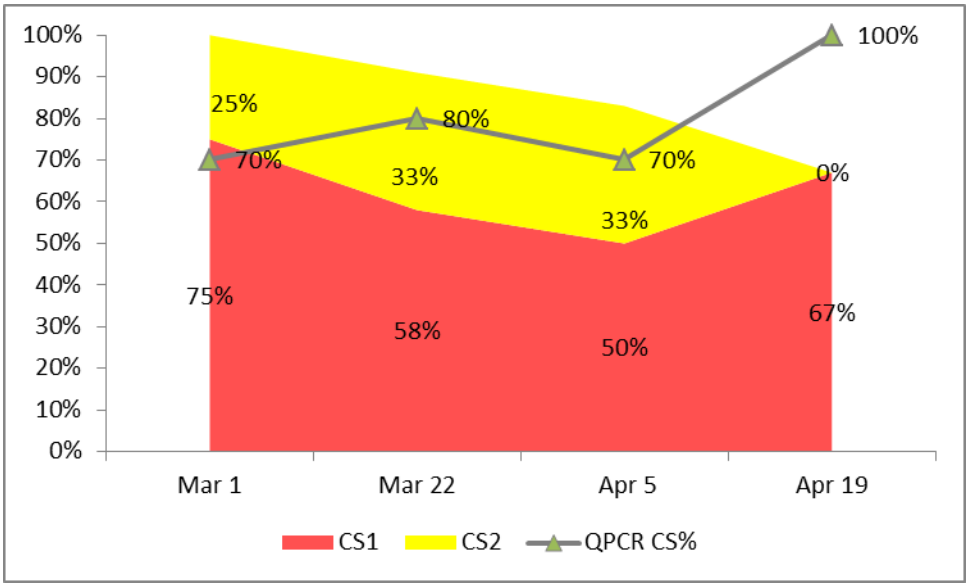


Figure 6. Prevalence of *C.shasta* infection severity categories (1= infection with little disease, 2= disease state) and by QPCR in juvenile Chinook salmon captured at Star Bend between February 19 – April 19, 2016.

Comparison of collection groups - Similar to previous surveys, fry in the low flow reach (Gateway) were largely uninfected. In March and April, fork length was not significantly different between Herringer, Boyd's, and Star Bend sample groups suggesting similar out-migrant populations were sampled (Kruskal-Wallis ANOVA on ranks H= 3.14, 2 df, P= 0.208).

Chi-square analysis, of histological *C.shasta* rating prevalence from the Herringer, Boyd's, and Star Bend groups during the same collection periods, showed the following trends:

1) Significance difference between Herringer and Boyd's
   Chi-sq=34.247, 2df, P<0.01 Herringer having >13x more CS2 "diseased" fish

2) Significance difference between Boyd's and Star Bend
   Chi-sq=10.094, 2df, P=0.006 Star Bend having >2x more CS2 "diseased" fish
   It is unclear what factors (e.g. history of sampled fish, proportion of Yuba origin fry, sample size bias, seining habitat difference in velocity) are associated with this difference given the short distance (~4 rm) between these sites.

QPCR data showed a slightly different trend from the histology data. *C.shasta* DNA concentration within the intestine increased over time at Herringer (decline in C_t values = increased *C.shasta* DNA content) while there was little change in DNA concentration at both Boyd's and Star Bend (Figure 7). Median C_t values of *C.shasta* positive samples demonstrate the same trend: Gateway 34.6, Herringer 25.5, Boyd's 31.9, and Star Bend 31.5 (again, C_t values are inverse to parasite DNA concentration).

It appears that severely infected fish in the infectious zone are unlikely to survive. Between late March and late April, 67 – 90% of the Herringer sample was classified as "diseased" compared to $\leq$8% at Boyd's landing (approximately 23 rm downstream) or $\leq$ 33% at Star Bend (~ 28 rm distance). These same Herringer fry samples also had significant kidney inflammation (PM2 rating) that was rarely observed at either Boyd's or Star Bend. The elevated flows in March were not associated with decreased transmission of either *C.shasta* or *P. minibicornis* but may have influenced severity at Boyd's but not at Herringer.

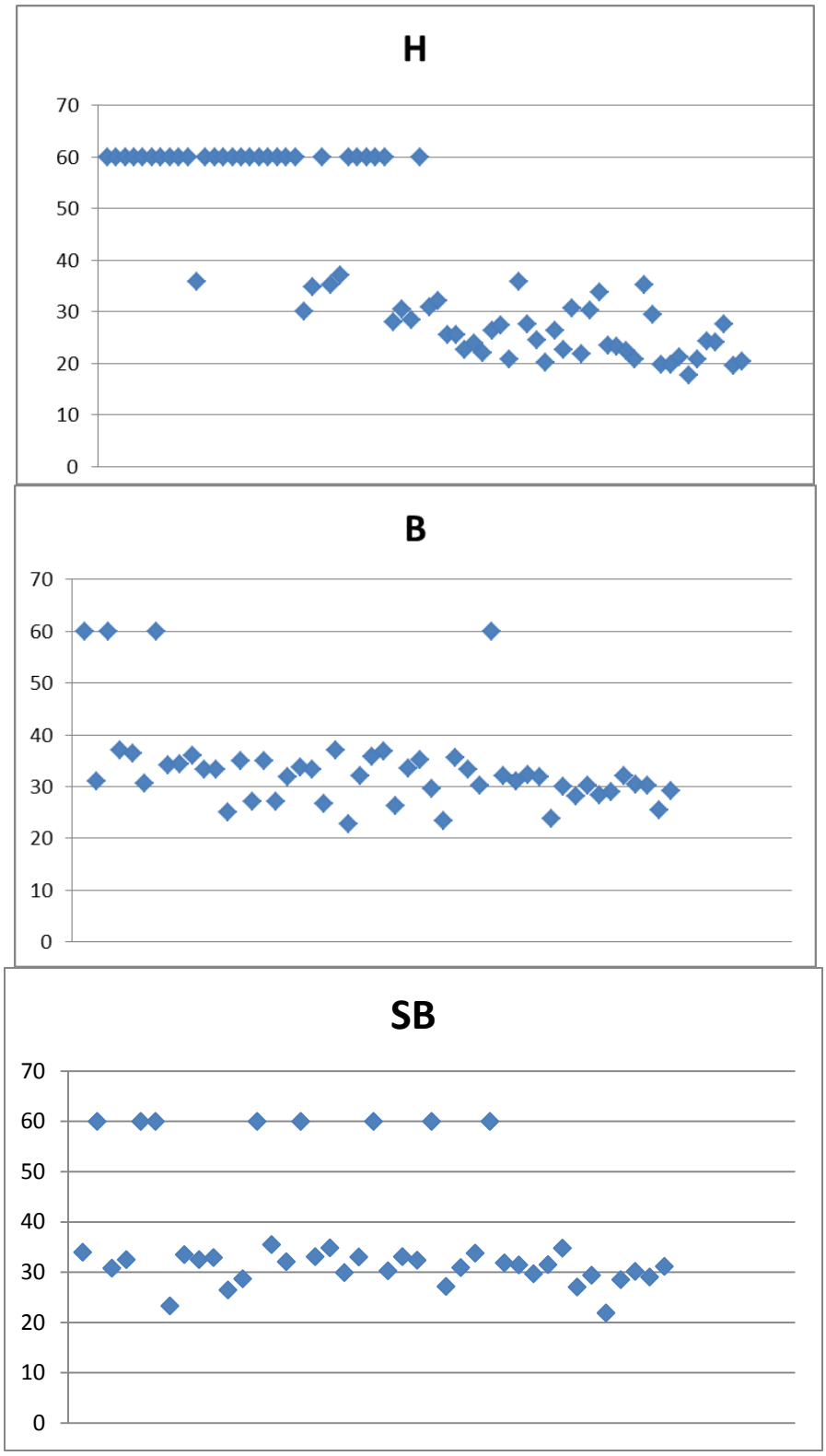


Figure 7. Cycle Threshold (C_t) data from Cs-QPCR assay of intestines from Herringer (H),Boyd's Landing (B), and Star Bend (SB) shown in sample collection date order (early left – later right). Note C_t = 60 is designated as undetected.

Sentinel infection - The February Gridley exposure, of 47 Coleman NFH fry, had a 21% cumulative mortality over the rearing period with a 78% prevalence of *C.shasta* infection (histology and QPCR) and 89% prevalence of *P. minibicornis* infection by histology (Table 8). *Ichthyophthirius multifiliis* was observed in this exposure group on day 10 post-exposure and the group was treated with formalin (100mg/L, 30min.) and 1% salt bath (20min.) for 3 days. Two histological samples were lost during processing. The CS histology ratings were 29 of 40 (73%) for CS1 and 2 of 40 (5%) for CS2. This indicates that sentinels had relatively moderate disease response after 25 dpe and would likely recover from the infection. Many of the *C.shasta* trophozoites were observed within serosal vessel granulomas indicating a robust host immune response against the parasite. Similarly, *Parvicapsula* infections of the kidney were rated as mild (all PM1 rating). Mean river temperature during the exposure was 12.1°C. No mortality or observation of either myxozoan occurred in the 12 fish control group.

The March Gridley exposure group showed a similar infection and disease response as the February fish (Table 8). While prevalence of infection for both parasites was high (*C.shasta* 82%, *P. minibicornis* 93%), the majority were of a moderate disease (CS1) rating. No mortality occurred to the exposure group. No mortality or observation of either myxozoan occurred in the 10 fish control group.

Table 8. Sentinel Fall-run fry (Coleman NFH) data for both February and March 3-4 day exposures at Gridley reported as mean (SD) temperature (°C) during river exposure and post-exposure rearing, mean fork length (mm), cumulative mortality over post-exposure rearing (total mortality/total population(%)), prevalence of histological disease (CS and PM) ratings (1= infection with little disease, 2= disease state) and *C.shasta* in mortalities by QPCR (Cs PCRmort), and overall prevalence of infection (POI) of the entire sample group for both histology and QPCR assays.

	Feb16 - 19	March 25 - 29
Rivertemp	12.1 (0.8)	14.9 (1.2)
wetlabtemp	13.2 (0.6)	14.4 (0.7)
Fork lgth	53	61
Cummort	10 / 47 (21%)	0 / 28 (0%)
CS1	29/40	21 / 28
CS2	2/40	2 / 28
CS PCRmort	4 / 5	N/A
CS POI(%)	35 / 45 (78%)*	24 / 28 (82%)
PM1	33/37	21 / 28
PM2	0/37	5 / 28
PM POI (%)	33/37 (89%)	27 / 28 (93%)

* both QPCR and histology sample set

Re-captured FRH SCS – The March 25 release Spring-run Chinook, recaptured 4 days later at either Gridley (n = 13) or Boyd's launch (n = 7) reach, had both a high prevalence (19 /20 (95%)) and severity of *C.shasta* infection (14 / 20 (70%) rated as CS2 "diseased" (Table 9). No difference in *C.shasta* severity was observed between the 2 release site groups (Chi-square 3, 2df, P=0.223) however sample size limits trend analysis. No mortalities occurred over the 20d observation period for a total of 24d post-release. Mean fork length of the 24dpe sample group was 78mm. Most of the SCS were recaptured in their release area with the exception of 3 Gridley release salmon captured at Boyd's launch. It should be noted that Feather R. hatchery SCS showed a more severe disease response (43 and 85% CS2 rating compared to 6%) for their 4d exposure compared to the Coleman NFH Fall-run fry sentinel group exposed for 3d at Gridley (Figure 8). It is unclear whether specific exposure history (actinospore concentrations within the same reach), physiological state (transport stress, smoltification) and/or genetic resistance could have influenced this different response. Kidney inflammatory changes (PM2), indicative of a disease state, were seen in 5 of 20 spring-run (25%).

Table 9. Prevalence of *C.shasta* (CS) and *P. minibicornis* (PM) infection in coded wire tagged Feather R. SFH Spring-run Chinook released March 25 at Gridley and Boyd's ramp and re-captured on March 29. Histological disease ratings (CS or PM1 = infection with little disease, CS or PM2= disease state).

	Gridley	Boyds
CS 1	1/13 (8%)	4/7 (57%)
CS 2	11 / 13 (85%)	3/7 (43%)
PM 1	7/13 (54%)	4/7 (57%)
PM 2	4/13 (31%)	1/7 (14%)

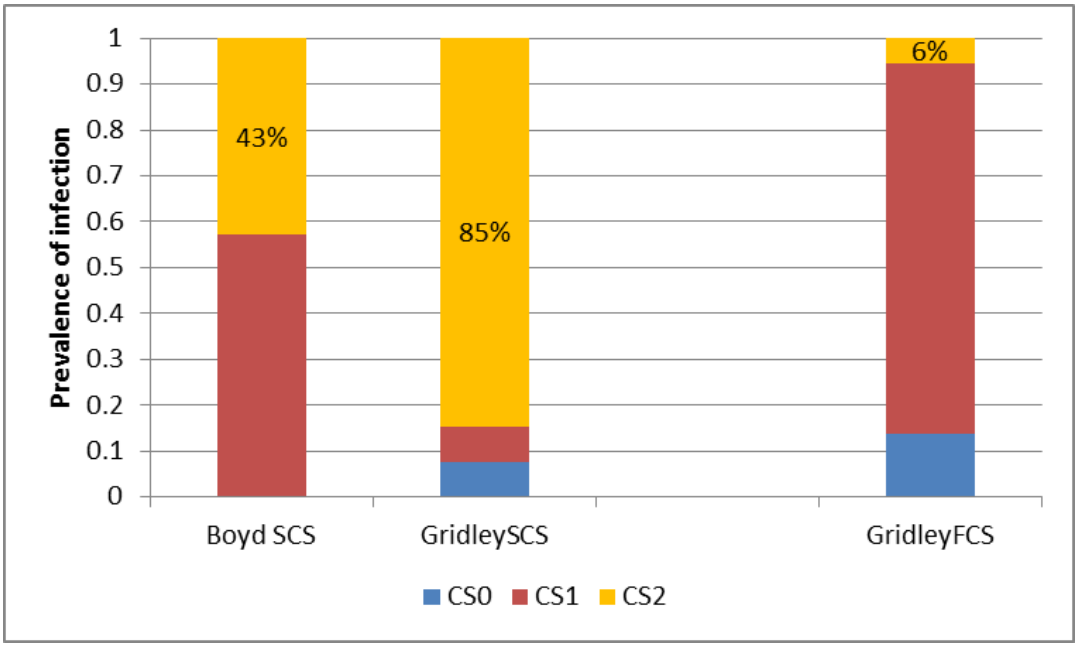


Figure 8. Prevalence of *C.shasta* infection severity (CS0= no parasite observed, CS1 = infection with little disease, CS2= disease state) in histological tissue sections of intestine from coded wire tagged Feather River SFH Spring-run Chinook salmon (SCS) released at Gridley and Boyd's ramp on March 25 and collected on March 29, and sentinel Coleman NFH Fall-run Chinook (FCS) salmon held at Gridley ramp (3/25 – 29).

A second release of FRH SCS occurred on April 14 at both Gridley and Boyd's landing launch. River temperature was between 14 - 16°C (Herringer trap, figure 3). On April 18, adipose fin clipped juvenile Chinook were captured alive by beach seine at both Live Oak (10 fish) and Star Bend (9 fish). The Live Oak site is approximately 9.5 rm downriver of Gridley. These salmon (mean fork length of 88mm) were held overnight at the DWR tag shack and transported to the CA-NV FHC wet lab (mean rearing temperature 14 °C) on April19. One salmon, from the 4/18 Live oak capture group, was dead upon arrival to the wetlab on April 19. Histological examination of its kidney and intestine identified high numbers of both *P. minibicornis* and *C.shasta,* respectively. This observation is notable given the short 5 dpe status of this fish. In error, the head was misplaced so no release group identification could be done. It is possible that this fish could have been part of the March 25 release. No other fish died during the 20 d rearing period of the April release group. Coded wire tag analysis of the 18 fish sample group revealed that 15 were released from Gridley and 3 from Boyd's. Only 20% of the Gridley release fish had enteronecrosis at 20dpe (Table 10). No *C.shasta* trophozoites were seen in 40% of the sample while low number of parasites, without significant host

inflammation, was observed in the remaining 40% of the sample group. The low number of Boyd's release site fish precludes any comparison of the release site in April. All fish were infected with *P. minibicornis* with only one showing significant kidney inflammation (PM1). The March release group experienced a greater degree of enteronecrosis (70%) than the April group (17%).

Table 10. Prevalence of *C.shasta* (CS) and *P.minibicornis* (PM) infection in coded wire tag Feather R. SFH Spring-run Chinook released April14 at Gridley and Boyd's landing launch and re-captured on April 18 at Live Oak or Star Bend. Histological disease ratings (CS1 or PM1 = infection with little disease, CS2 or PM2= disease state).

	CWT	CWT
	Gridley	Boyds
CS 1	6 / 15 (40%)	1 / 3 (33%)
CS 2	3 / 15 (20%)	0 / 3
PM 1	14 / 15 (93%)	3 / 3 (100%)
PM 2	1 / 15 (7%)	0 / 3

C.shasta DNA in river water - During autumn of 2015, *C.shasta* DNA was detected at relatively low levels (2 – 5 spore/L) in the low flow reach (Fig.9). While this assay cannot distinguish between actinospore and myxospore, we assume it is measuring myxospores as carcasses in the low flow reach had high concentrations of this life stage. After November 20, *C.shasta* DNA was undetectable at Gateway (just above the high flow reach's infectious zone) but remained stable at Riverbend (spawning grounds). One 1 liter samples may not be sufficient volume to detect low myxospore concentrations flowing pass the Gateway site or myxospores could be settling to the river bottom in late November. No DNA was detected in Gateway samples collected 12/30/2015, 1/22/2016, or 2/22/2016.

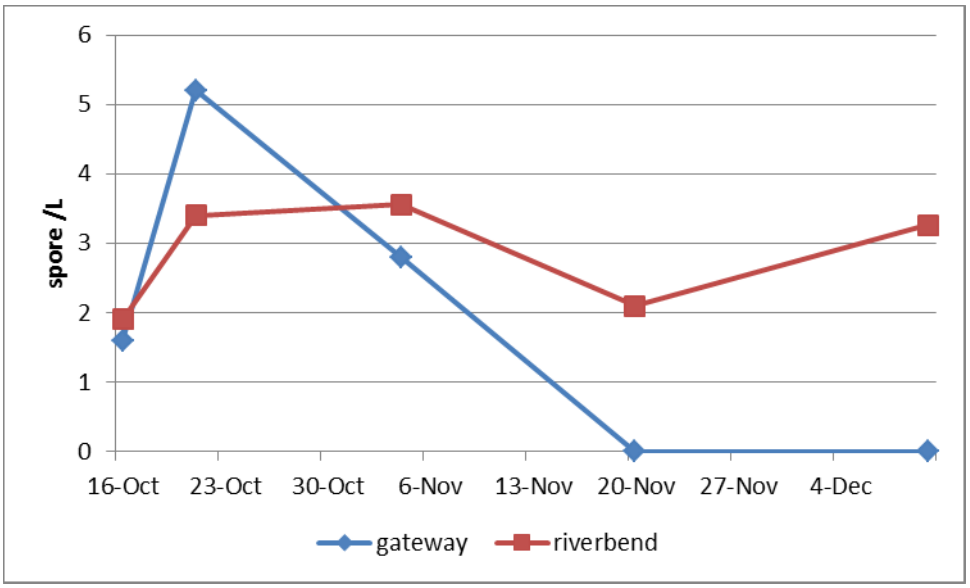


Figure 9. *C.shasta* DNA concentration (spore/L) in river water collected from October 16 – December 10, 2015 at Gateway (rm 59.6) and Riverbend (rm 65).

In comparison, mean spore /liter at Herringer (rm 45.7) on 12/30/2015, 1/11/2016, and 1/25/2016 was 10, 25, and 26 spores/L (Fig 10). It is uncertain what spore stage or mixture of spore stages is represented by these infectious zone samples, however, the near absence of adult Chinook carcasses in this reach, zero DNA detection upstream at Gateway, and the January 25, 2016 detection of *C.shasta* infection (QPCR) in fry points toward actinospores as the source of the DNA. By February 9, spore/L concentration had reached 90 and peaked at 168 on February23. By May 10, there was a steady decline to 5 spores / L. This data indicates that polychaetes in the infectious zone are releasing relatively high levels of actinospores in the winter. This time corresponds with

salmon fry emergence and early rearing. If fry are rearing within the infectious zone, the late February actinospore peak corresponds well with the late March peak prevalence and severity of *C.shasta* infection observed in fry (Table 5). At these river temperatures, clinical infection post-exposure would occur after 2-3 weeks post-exposure. Alternately, March fry samples could represent fish that changed their migratory behavior and had increased rearing in the infectious zone.

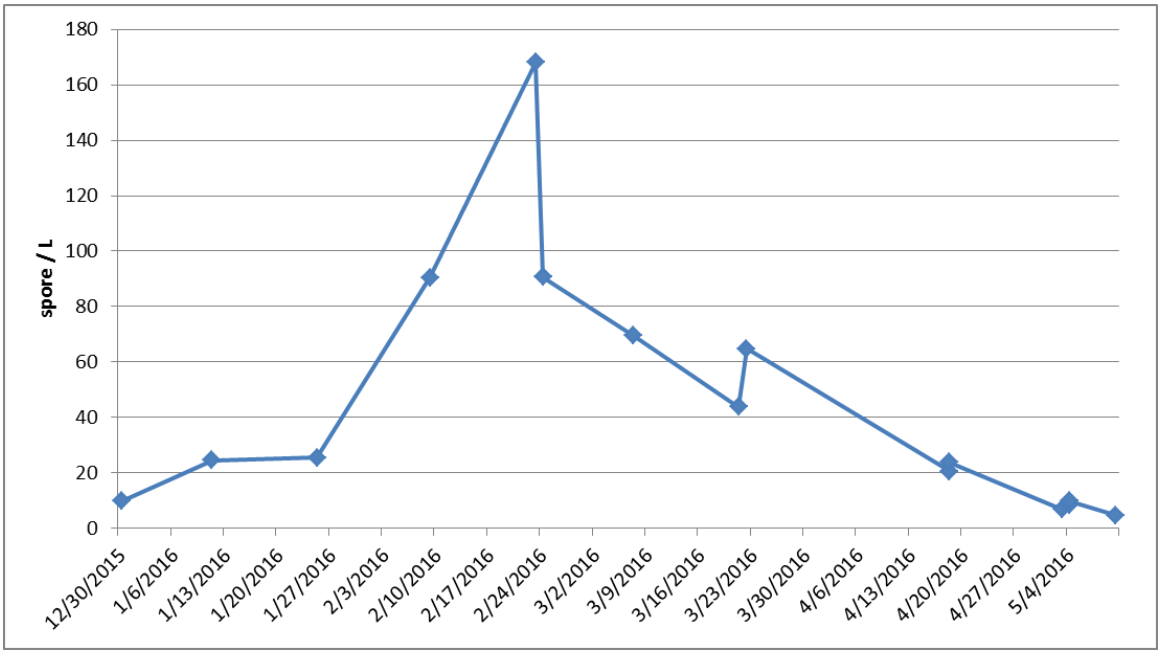
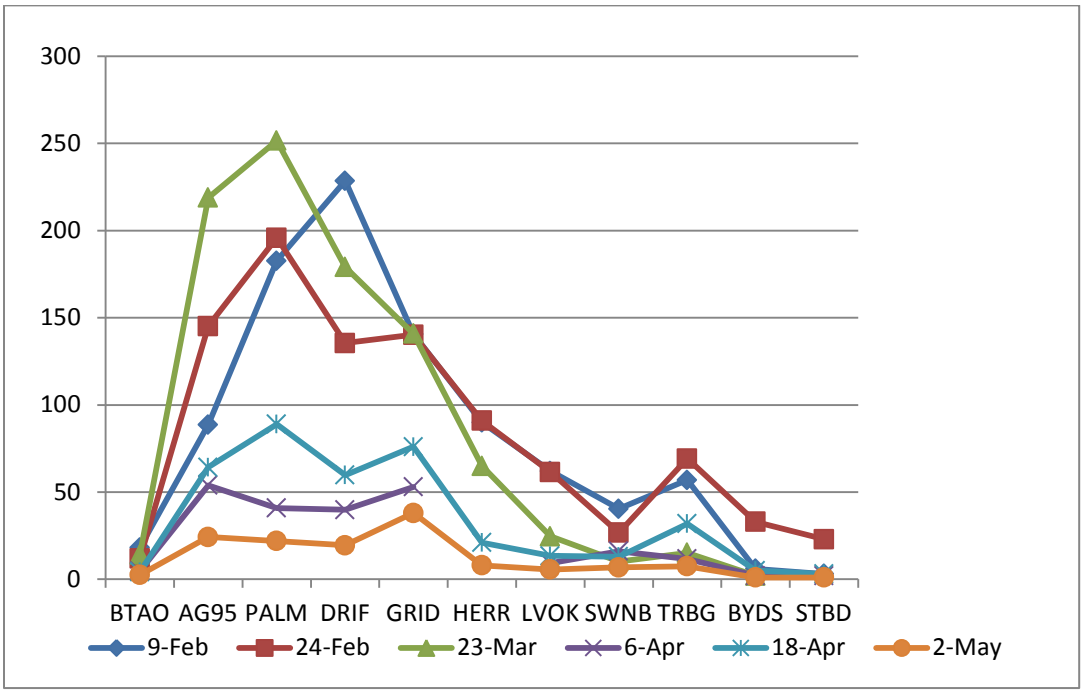


Figure10. *C.shasta* DNA concentration (spore/L) in river water collected from Herringer (rm 45.7) between December 30, 2015 and May 10, 2016. This data represents the infectious challenge within the infectious zone during 2016.

Spatial and temporal trends in actinospore/L concentrations were consistent with 2015 data (Fig. 11). Peak spore concentrations occurred on March 23 at 4 sites (above G-95, Palm, Developing Riffle, and Gridley) and in February at sites below Gridley (Herringer, Live Oak, Shawn's beach, and Train Bridge). An infectious zone was apparent as spore/L concentration was arranged as follows: Palm>Above G-95>Developing Riffle>Gridley> Herringer>Live Oak "=" Train Bridge< Shawn's Beach << Below the Afterbay outlet (BTAO). Concentration ranged from 2 – 18 spores/L at BTAO. At the same time, no detection occurred 0.6 rm above BTAO at Gateway. This data indicates that BTAO (top of high flow reach) is the start of the *C.shasta* infectious zone. Despite being 7 rm downstream, the Train bridge site (rm 30.5) had 5 – 42

spore/L higher concentrations than Shawn's beach (rm 37.5) on 4 of 6 sample dates and was quite similar to Live Oak (rm 41.5).



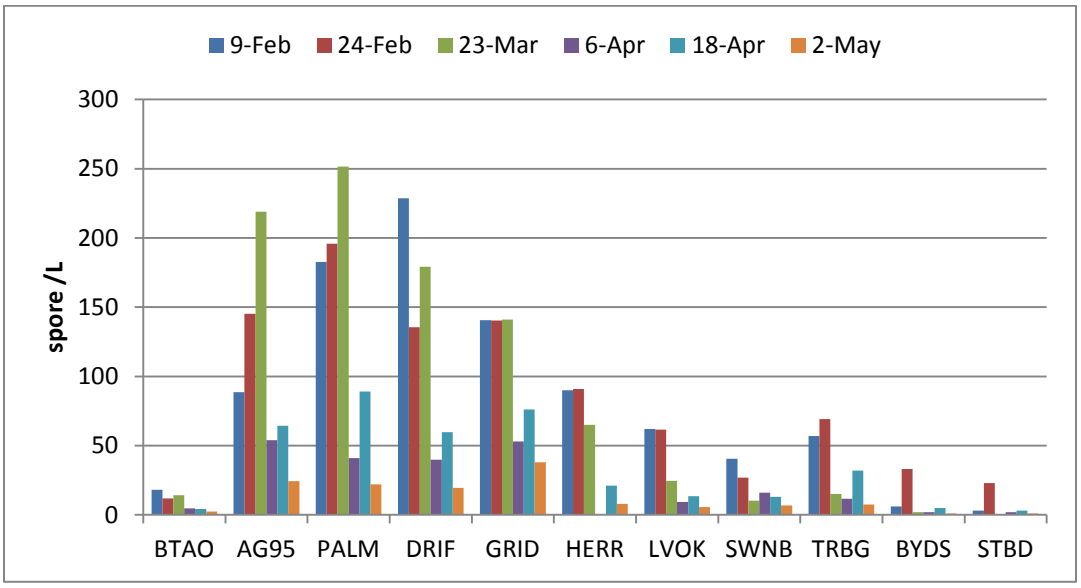


Figure 11. Downstream profile of *C.shasta* DNA concentration (spore/L) in river water collected from the Feather River immediately Below The Afterbay Outlet (BTAO, rm59), above G-95 (AG95, rm 57.5), Palm (rm 57), Developing Riffle (DRIF, rm 52), Gridley (GRID, rm 50), Herringer (HERR, rm 45.7), Live Oak (LVOK, rm 41.5), Shawn's beach (SWNB, rm 37.5), Train Bridge (TRBG, rm 30.5), Boyd's Landing (BYDS, rm 22.3), and Star Bend (STBD, rm 18.1) between February 9 – May 2, 2016. Top graph depicts temporal trend while bottom graph of same data shows spatial trends.

Discussion:

We observed similar spatial and temporal occurrence of infection for both *C.shasta* and *P. minibicornis* as in 2013- 2015 (Foott 2013 and 2014, Foott et al. 2016). If 10 actinospores / L is used as criteria for delineating *C. shasta* infectious river (Bjork and Bartholomew 2009, Hallett et al. 2012), the infectious zone begins at the top of the "high flow" reach at rm 59 and extends at least to the reach near Boyd's landing (rm 22.3). Extremely high actinospore concentrations (> 50 spores/L) were detected in the infectious zone from early February through late April 2016. The observed peak in ceratomyxosis (severity of infection) at Herringer occurred about one month later (late March through the end of April). Several factors could influence this difference in timing; 1) migration rates of sampled fry is reduced (rearing behavior) in March compared to February thus extending their exposure to actinospores, 2) disease progression is greater at temperatures ≥ 14°C or, 3) enteronecrosis tends to occurs about 2+ weeks post-exposure. *Parvicapsula minibicornis* infection of the kidney was very common among all sample groups however severe kidney inflammation was only observed in the late April sample groups (≥ 53%) from Herringer. This myxozoan shares the same polychaete host as *C.shasta* and tends to have a higher prevalence of infection than *C.shasta*. Its role in fry survival is not understood but is likely an added stressor to co-infected fry.

High flows in March did not seem to influence infection and disease prevalence at the Herringer site. It appears that significant dilution of actinospores or scouring of polychaetes did not occur in March. Another observation similar to previous surveys is the high prevalence and intensity of myxospores found in both spring and fall-run carcasses in the low flow spawning reach. Water samples in this reach were positive for *C.shasta* DNA through the end of December (last sample) indicating a continuous transport of myxospores downriver. Evidence of myxospore transport into the infectious zone is limited as the current 1 liter water samples from Gateway did not detect *C.shasta* DNA after early November. Future sampling efforts may use a larger volume to enhance detection of low spore concentrations.

We employed sentinel salmon to model the potential disease response of natural fry migrating and / or rearing within the infectious zone. The February sentinels displayed

a high prevalence but low severity of *C.shasta* infection. This observation suggests a low to moderate disease response for migrating fry in mid-February despite the high actinospore concentrations. The 3 day exposure at Gridley was based on DWR observations of similar fry migration rate through the infectious zone (J. Kindopp, pers. comm.). Three day exposures in late March resulted in a similar response in the Coleman fall-run sentinels. In contrast, FRH spring-run released at Gridley and re-captured within 4 days had a much higher prevalence of enteronecrosis than the sentinel salmon caged at Gridley during the same period. It is unclear why this difference in disease response occurred between these 2 salmon groups but exposure conditions were likely different. Spring-run smolt recaptured 4d after the April 14 FRH release had a lower prevalence of enteronecrosis than the March release (20% vs 85%). It is important to note that the low numbers of FRH spring run examined limits a generalization about the entire release population. Future work should attempt to sample the out-migrants in the lower river for several weeks post-release to assess disease impacts. Another emphasis for future survey work should apply weekly fry abundance within the infectious zone to weekly prevalence and severity of *C.shasta* infection (i.e. what percent of the fry population experience disease). This information could provide insight into *C.shasta's* impact on the natural fry population survival within the Feather River.

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