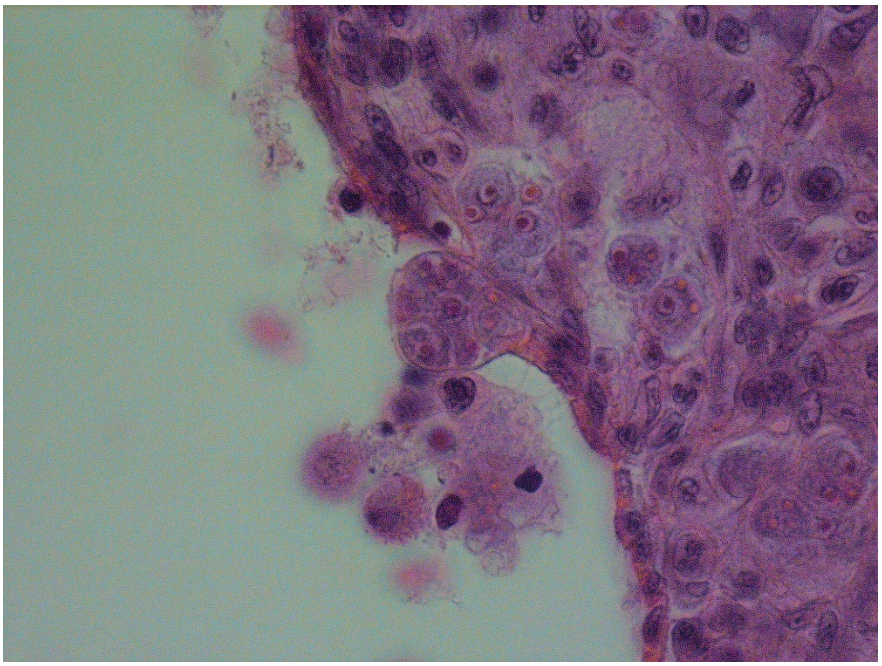
U.S. Fish & Wildlife Service

California Nevada Fish Health Center

FY2018 Technical Report:

Ceratonova shasta and *Parvicapsula minibicornis* (Phylum Cnidaria: Myxosporea) infectivity for juvenile Chinook salmon (*Oncorhynchus tshawytscha*) in the Sacramento River: August – November 2018.



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Summary:

Infectivity for *Ceratonova shasta* in the Sacramento River (river mile 119 - 274) was monitored from August through November 2018. Prevalence and severity of infection was examined from histological samples of both naturally-produced Winter-run Chinook fry (captured at Red Bluff Diversion Dam) and sentinel salmon (exposed monthly throughout the study reach). The *C.shasta* spore concentration of the river was assayed by molecular methods. Relatively low spore concentrations (≤ 5 spore / L) produced a wide range of asymptomatic infections (prevalence 0 – 93%) in sentinel salmon. October sentinel fish had markedly higher prevalence of *C.shasta* infections than other months. Similar to a 2016 survey, naturally-produced Winter-run Chinook fry had a low prevalence of asymptomatic *C.shasta* infection. In water years rated "Below Normal: or wetter, *Ceratonova shasta* appears to represent a low to moderate disease risk for juvenile Winter-run Chinook salmon during their out-migration in the Sacramento River.

The correct citation for this report:

Foott J.S., S Freund, and K. Nichols. 2019. *Ceratonova shasta* and *Parvicapsula minibicornis* (Phylum Cnidaria: Myxosporea) infectivity for juvenile Chinook salmon (*Oncorhynchus tshawytscha*) in the Sacramento River: August – November 2018. U.S. Fish & Wildlife Service California – Nevada Fish Health Center, Anderson, CA. http://www.fws.gov/canvfhc/reports.asp

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Introduction:

Ceratonova (synonym *Ceratomyxa*) *shasta* Noble 1950 (Atkinson et al. 2014) infects freshwater salmonid fishes and is enzootic to anadromous fish tributaries of the Pacific Northwest including the Klamath, Feather, and Sacramento Rivers (Bartholomew 1998, Hendrickson et al. 1989). Ceratomyxosis (enteronecrosis) is a significant mortality factor for juvenile Chinook salmon *Oncorhynchus tshawytscha* in the Klamath River and has been documented in juvenile Chinook salmon from both the Feather and Sacramento Rivers (Foott et al. 2004, Stocking et al. 2006, Fujiwara et al. 2011, Hallett et al. 2012, Foott 2013 & 2014, Foott et al. 2016a, Foott et al. 2017). *C.shasta* has a complex life cycle, involving an invertebrate polychaete host (*Manayunkia speciosa*) as well as the vertebrate salmon host (Bartholomew et al. 1997). *M.speciosa* is a small (3-5 mm) benthic filter feeder that inhabits tubes constructed from silt and sand particles (Mackie and Qadri 1971). Infected polychaetes release actinospores into the water where they can attach to the salmon's gill epithelium, invade into the blood, replicate, and later migrate to the intestinal tract for further multiplication and sporogony (Bjork and Bartholomew 2010). Depending on actinospore genotypes and densities, innate host resistance, and water temperature, infected fish can develop varying degrees of enteritis and associated anemia (Bartholomew 1998, Foott et al. 2004, Bjork and Bartholomew 2009, Ray et al. 2010, Hallett et al. 2012). If the fish survives long enough (approximately 2 weeks at 18ºC, 30 d at 12ºC), sporogony occurs in the intestinal muscularis layer with the production of myxospores (True et al. 2012). Myxospores released from the infected fish after death are ingested by the filter-feeding polychaete and complete the life cycle after invading the worm's gut epithelium (Meaders and Hendrickson 2009). Adult salmon can produce millions of myxospores that are released into the water after death (Foott et al. 2016b). Another myxozoan salmon parasite, *Parvicapsula minibicornis*, shares the same polychaete host (Bartholomew et al. 2006). It infects the salmon kidney and can cause glomerulonephritis. *Ceratonova shasta* myxospores have been observed in the intestines of Sacramento Adult Fall, Late fall, and Winter- run Chinook inspected by the Fish Health Center. As these runs migrate through the river from September through June, it is likely that the Sacramento R. could be infectious for *C. shasta* year round. Naturally- produced Winter-run Chinook fry are reported to pass the Red Bluff Diversion Dam from July through December (Poytress and Carrillo 2012) and could be exposed to the infectious actinospore stage during their entire migration down the Sacramento R. In 2016, we observed asymptomatic

infections in sentinel salmon held in the upper Sacramento above the Red Bluff Diversion Dam from July through October (Foott et al. 2017).

The objectives of this study were:

a. Determine the temporal and spatial pattern of *Ceratonova shasta* (**CS**) and *Parvicapsula minibicornis* (**PM**) infectivity in the Sacramento River (river mile (rm) 274 – 119) during Winter-run Chinook fry out-migration period of August – November. Methods used include:
    i. Monitor development of CS and PM infection and disease in sentinel juvenile salmon (hatchery Late-fall run Chinook) exposed in the river for 4 days.
    ii. Assay river water for CS spore concentration (QPCR of filtered water samples).
b. Examine by histology, naturally-produced Winter-run fry captured at Red Bluff Diversion Dam to determine the prevalence of CS and PM infection and disease.

Methods:

Sentinels- Groups of 20-24 juvenile Late-fall run Chinook salmon (*Oncorhynchus tshawytscha*) from Coleman National Fish Hatchery were exposed for 4 d to the Sacramento River at five locations ranging from river mile (rm) 274- 119 (Table 1, Figure 1). Mean fork length (standard deviation) of sentinel salmon used in the four exposures was 84 mm (10), 91 mm (16), 91 mm (11), and 91mm (16), respectively. In an attempt to manage columnaris disease (*Flavobacterium columnare),* exposure groups were treated with a 30 min, 2 mg/L nitrofuran bath upon return to the wet lab. Columnaris in several sentinel groups was further treated with additional nitrofuran baths and tetracycline top-coated feed (0.08 mg/g fish).

Four exposures were conducted every 4 weeks between August 13 and November 1, 2018 (Table 2). At each site, two cages (0.34 ft^3) containing 10- 12 fish each were deployed with an Onset temperature probe that recorded hourly river temperature. Rearing temperature for sentinel salmon was similar to lower river sites except for the November exposure (Table 4). A rapid decline in ambient water temperature affected the mean rearing temperature value for the November group. Sentinels were fed 1%

body weight/d salmon diet during the weekdays and mortalities frozen or fixed. Histology was performed if the mortality was deemed fresh (some color to gill). Previous prognosis work has demonstrated that mortality due to ceratomyxosis typically occurs 2 weeks post-exposure if the actinospore challenge is sufficiently high. QPCR analysis of mortalities were censored to identify fish that likely died of ceratomyxosis (> 14dpe, hemorrhagic intestine, no significant external lesions associated with *Flavobacterium columnare* or fungal infection). After 19 to 23 days post-exposure (**dpe**) survivors were euthanized in an overdose of MS222, examined for clinical signs of infection (pale gill, hemorrhage or catarrhal exudate within the intestine, and swollen kidney), and placed in Davidson's fixative for histological analysis.

Intestinal tract (small and large intestine) and kidney were processed for 5 µm paraffin sections stained with hematoxylin and eosin. All tissues for a given fish placed on one slide and identified by a unique sample code. Each slide examined at 40X to 400X magnification. Histological rankings of 'clinical disease' were based on the presence of multifocal lesions associated with the parasite infection (CS2 rating of intestine = lamina propria hyperplasia, necrotic epithelium and or sloughing, necrotic muscularis; and PM2 rating of kidney = interstitial hyperplasia, necrotic interstitium or tubule, interstitial granuloma, glomerulonephritis, and protein casts within the glomeruli or tubules). The #1 rating for both parasites required the presence of the parasite in the respective tissue but with minimal inflammatory changes. Histological analysis is less sensitive than molecular methods in detecting asymptomatic infections.

Table 1. Sentinel exposure and water sample sites (site abbreviation).

	RM	Latitude	Longitude
Reading Island Park (R ISL)	274	40.387527	-122.196543
Red Bluff Diversion Dam (RBDD)	242	40.152297	-122.202068
River Road (RV RD)	194	39.709675	-121.943806
Highway 162 Bridge (SR162)	168	39.457351	-121.995998
Tisdale ramp (TSDL)	119	39.026838	-121.822331

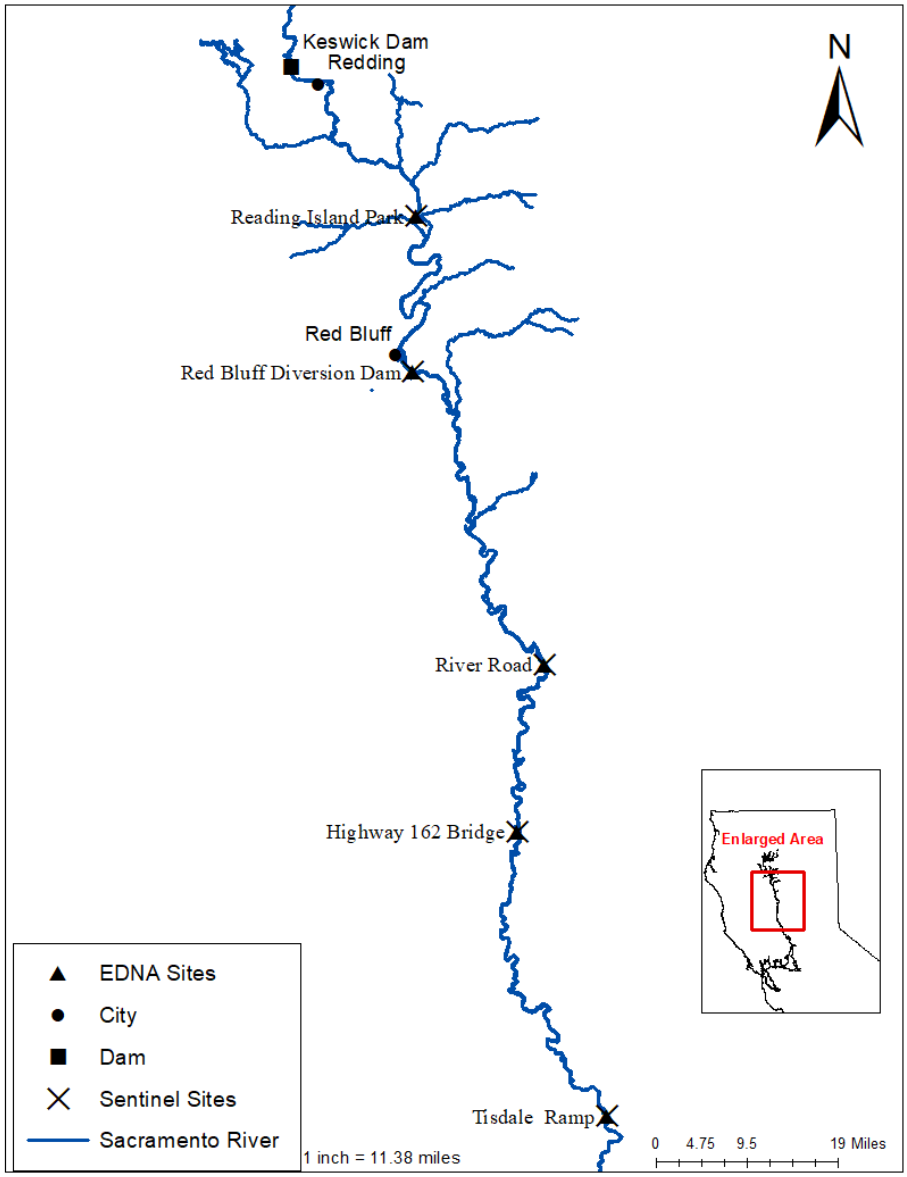

Figure 1. Map of water sample (EDNA) and sentinel cage sites.

River C.shasta spore / L- River water was collected from the five sentinel sites (Fig. 1) between August 13 and November 19, 2018. Collections occurred when sentinel fish were placed in the river and again 4d later at the time of pick up. The mean spore/L value for these two collections are reported as exposure concentration. Additional collections occurred on a two week basis. Four 1-liter samples were removed from a 19L bucket of water taken from a zone of flowing water, held on ice, and filtered within 5 h of collection. Under vacuum, the sample was filtered (MF-Millipore cellulose 47 mm diameter, 5 µm pore) with both the sample bottle and filter assembly rinsed with distilled water and rinse run through the filter. The filter was folded with forceps and placed into labeled 2 mL microfuge tube. It was stored at -20°C until shipment on dry ice

to the Bartholomew laboratory at Oregon State University for extraction and QPCR analysis (Hallett & Bartholomew 2006, as modified in Hallett et al. 2012). The filter assembly and forceps were wiped with DNA away (Molecular BioProducts, San Diego) and rinsed in distilled water before filtering samples from a new site. Inhibition was assessed for each sample using an internal positive control (Applied Biosystems). Data is reported as mean spore/L from the triplicate samples. A censor protocol dictated that 2 of the 3 samples must obtain a positive signal to calculate this mean value (Appendix 7). If DNA concentration less than one spore but greater than the cutoff threshold is measured, a value of < 1spore/L is assigned to the sample.

Winter-run fry - Between September 19 and November 7, 2018, ten fry were sampled each week from the USFWS rotary screw traps below the Red Bluff Diversion Dam (section 10(a)(1)(A) permit (#1415-3M) for direct take of listed species for scientific research and enhancement purposes). Fork length was recorded and then a caudal fin clip was taken for genetic identification (no genetic data at time of report, pers. comm. Scott Voss, USFWS). Gill tissue was placed into RNA later solution for Dr. Richard Connon (University of California, Davis), an incision was made into the peritoneum, and the carcass placed into Davidson's fixative for 48 h. Fry smaller than 50 mm were de-calcified and processed as sagittal sections. In larger fry, the gill, kidney and visceral mass was dissected and sectioned.

Results:

Sentinels- Prevalence of *C.shasta* infection ranged from zero to 93% in the 19 – 23 dpe survivor fish from the four exposure groups (Table 2, Fig. 2) with the majority of infected fish rated as asymptomatic (CS1 "no disease"). Only 3 salmon from exposure 1 showed significant intestinal inflammation associated with the parasite (CS2 "diseased" rating, Table 2). The October exposure produced markedly higher prevalence of CS infection (56 – 93%) in comparison to August, September, and November (Fig. 2). Month of exposure was significant at all sites if October is included in the analysis (Chi-square analysis by month for each individual site produced scores ranging from 10.062 – 30.240 and P values 0.018 to < 0.01). With the exception of the November exposure, spatial differences were not detected by Chi-square tests on individual exposures. The zero detections at Highway 162 Bridge and Tisdale in November influenced this finding. Asymptomatic *Parvicapsula minibicornis* (**PM**)

infections ranged from zero to 67% with RBDD sentinel groups having the highest prevalence of infection (Table 2, Fig 3). Low numbers of PM trophozoites were only observed in the kidney glomeruli and there was no associated inflammation. September and October exposures had greater prevalence of PM infection than August or November.

Despite post-exposure treatments, sentinel mortality was largely associated with severe columnaris lesions particularly in groups exposed at mean temperatures ≥ 17°C (River Road to Tisdale). Most of the mortality occurred after return to the wet lab (>5dpe) with the exception of the August and September Tisdale exposure groups (Table 4). Post – exposure mortality ranged from 0 – 39% with the highest mortality occurring in the August exposures. Histological examination was conducted on 11 of the 41 post-exposure mortalities deemed "fresh" with only one sample having a CS trophozoite within the intestine (October Reading Island 19dpe mortality). No QPCR analysis was conducted on the 30 frozen samples as they were either: 1) < 14 dpe, 2) showed no signs of enteritis (catarrhal or hemorrhagic exudate), and/ or 3) had severe external lesions (columnaris or fungus lesions).

Mean river flow during the 4d exposures, measured at gauges located in the upper (Bend Bridge) and lower study reach (below Wilken slough), ranged from 4,206 to 10,941 cfs (Table 3). The Bend bridge gauge is approximately 16 rm upstream of Red Bluff Diversion Dam and had flows 1179 to 4204 cfs higher than the lower gauge yet these flows were associated with higher CS prevalence of infection than the lower river Tisdale groups. It does not appear that exposure temperature had an obvious influence in CS infection as upper river sites had similar temperatures for all four exposures but different prevalence of CS infection (Table 2). Similarly, there was only a 1 °C difference in the October exposures at River road (93% CS) and highway 162 bridge (63%CS).

Table 2. Prevalence of *C. shasta* (CS) and *P.minibicornis* (PM) infection (positive / total sample (%)) in sentinel salmon exposed for 4d at Reading Island (R ISL), Red Bluff Diversion Dam (RBDD), River road (RV RD), Highway 162 bridge (SR162), and Tisdale ramp (TSDL). CS1 and PM1= parasite present with little to no tissue inflammation, or CS2 = parasite present with significant inflammation ("disease state").

	Exp. 1	Exp. 2	Exp. 3	Exp. 4
	AUG13 -17	SEP6-9	OCT5-10	NOV1-5
R ISL				
CS1	n/a	5 / 16 (31)	10 /17 (59)	1 / 16 (6)
CS2	n/a	0 / 16	0 / 17	0 / 16
PM1	n/a	3 / 16 (19)	5 / 17 (29)	0 / 16
RBDD				
CS1	5 / 16 (31)	2 / 16 (13)	10 / 15 (67)	5 / 16 (31)
CS2	1 / 16 (6)	0 / 16	0 / 15	0 / 16
PM1	4 / 16 (25)	7 / 15 (47)	10 / 15 (67)	1 / 16 (6)
RV RD				
CS1	1 / 15 (7)	4 / 16 (25)	14 / 15 (93)	1 / 16 (6)
CS2	2 / 15 (13)	0 / 16	0 / 15	0 / 16
PM1	2 / 16 (13)	2 / 15 (13)	2 / 15 (13)	2 / 16 (13)
SR162				
CS1	3 / 14 (21)	4 / 16 (25)	10 / 16 (63)	0 / 16
CS2	0 / 14	0 / 16	0 / 15	0 / 16
PM1	0 / 14	4 / 16 (25)	5 / 16 (31)	0 / 16
TSDL				
CS1	2 / 12 (17)	2 / 15 (13)	9 / 16 (56)	0 / 16
CS2	0 / 12	0 / 15	0 / 16	0 / 16
PM1	0 / 12	1 / 15 (7)	0 / 16	0 / 16

n/a sample not available as cage missing at pickup

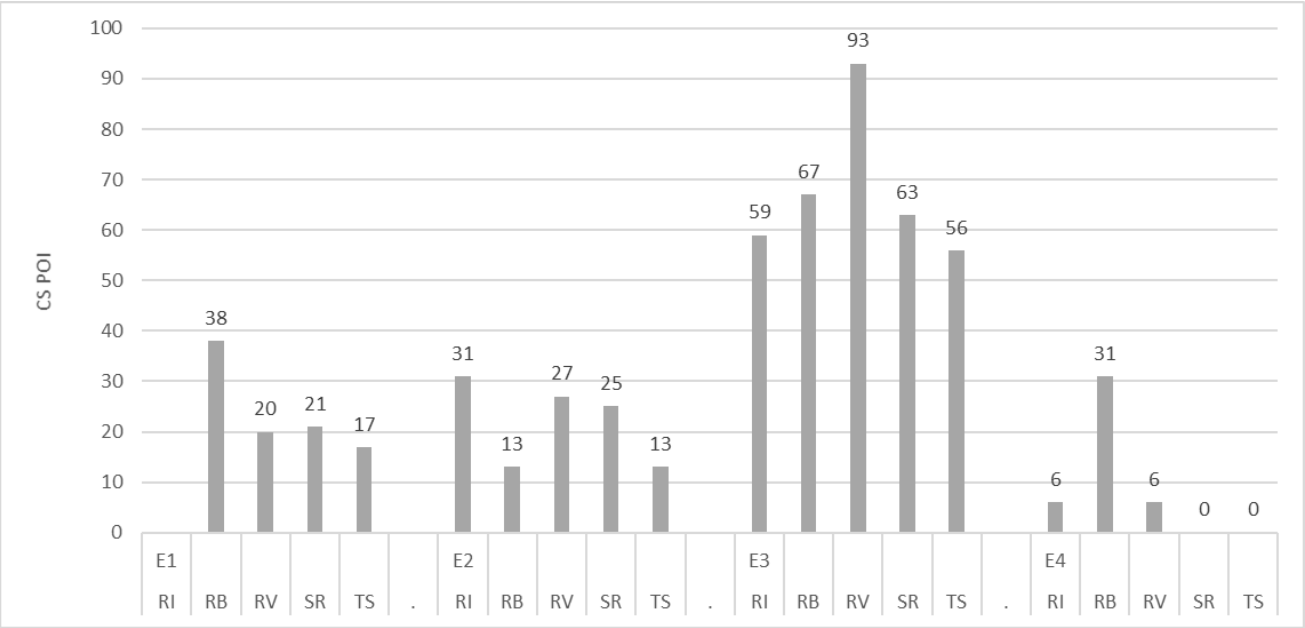

Figure 2. Prevalence of *C.shasta* infection (CS POI) in sentinel salmon exposed in the Sacramento River at Reading Island (RI), Red Bluff Division Dam (RB), River Road (RV), Hwy 162 Bridge (SR), and Tisdale weir (TS) for 4d in August (E1),September (E2), October (E3), and November 2018 (E4).

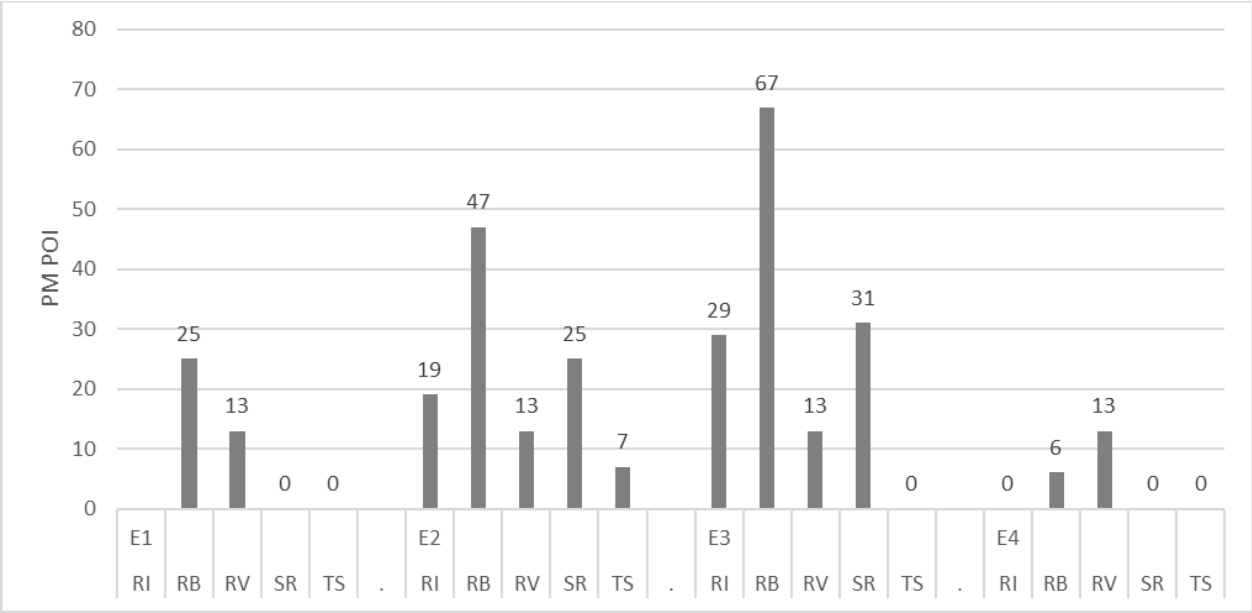

Figure 3. Prevalence of *P. minibicornis* infection (PM POI) in sentinel salmon exposed in the Sacramento River at Reading Island (RI), Red Bluff Division Dam (RB), River Road (RV), Hwy 162 Bridge (SR), and Tisdale weir (TS) for 4d in August (E1), September (E2), October (E3), and November 2018 (E4).

Table 3. Mean flow (cfs) during sentinel exposures near top (Bend Bridge, CDEC “BND”) and bottom (Below Wilken Slough, CDEC “WLK”) of study reach.

Exposure No.	Exposure dates	Mean flow Bend Bridge	Mean flow Below Wilken Slough
1	AUG 13-17	10,941	6,737
2	SEP 6-10	9,208	7,258
3	OCT 5-9	7,937	6,758
4	NOV 1 - 5	6,278	4,206

Table 4. Mean (standard deviation) water temperature (°C) for 19 – 23 d rearing (wetlab temp.), 4d river exposure (Exp. Temp.), as well as exposure group mortality at end of 4d exposure (Exp. Mortality), during post-exposure rearing (>5dpe Mort.), and cumulative mortality (Total loss %). Exposures 1-4 dates listed for sentinel groups held at Reading Island (R ISL), Red Bluff Division Dam (RBDD), River Road (RV RD), Hwy 162 Bridge (SR162), and Tisdale weir (TISDL).

	Exp. 1	Exp. 2	Exo. 3	Exp. 4
	AUG13 -17	SEP6-9	OCT5-10	NOV1-5
wetlab temp.	18.0*	16.9 (0.6)	14.3 (0.8)	11.0 (1.5)
R ISL				
Exp. Temp.	n/a	12.5 (0.7)	13.1 (0.6)	13.0 (0.4)
Exp. Mortality	n/a	0	0	0
>5dpe Mort.	n/a	2	2	0
Total loss(%)	100%**	9%	10%	0%
RBDD				
Exp. Temp.	14.6 (0.5)	14.0 (0.3)	14.0 (0.2)	13.5 (0.3)
Exp. Mortality	0	0	0	0
>5dpe Mort.	1	0	1	1
Total loss(%)	4%	0%	5%	5%
RV RD				
Exp. Temp.	16.9 (0.3)	16.3 (0.5)	15.1 (0.3)	14.0 (0.3)
Exp. Mortality	1	0	1	0
>5dpe Mort.	7	1	5	0
Total loss(%)	33%	5%	25%	0%
SR162				
Exp. Temp.	18.4 (0.1)	17.7 (0.4)	15.9 (0.3)	14.4 (0.3)
Exp. Mortality	1	1	0	1
>5dpe Mort.	9	3	0	1
Total loss(%)	42%	25%	0%	10%
TSDL				
Exp. Temp.	19.9 (0.3)	19.1 (0.2)	16.7 (0.3)	14.7 (0.2)
Exp. Mortality	5	6	0	0
>5dpe Mort.	6	1	2	0
Total loss(%)	48%	32%	10%	0%

* Error in onset probe launch, data from daily wetlab check records
** 100% loss as Reading Island cages taken on exposure 1.

River C.shasta spore / L. The data set was characterized by low spore concentrations throughout the study reach over the 4 month period ranging from zero to 15 spores / L (Appendix 2 - 6). The October 5 RBDD sample was not analyzed due to its high ash content causing inhibition. This sample was collected after the first significant rain event following the Carr fire. Of the 177 samples analyzed 17% (30) were rated at zero and 29% (51) as greater than zero but less than one spore. Red Bluff Diversion dam tended to have the highest mean concentrations (9 of the 11 sample dates) followed River Ranch Road (second highest for 7 of 12 sample dates). Except for November 1, mean spore concentrations $\geq$ 7 spores / L occurred in RBDD samples collected between September 17 and November 19 (Fig. 4). The QPCR assay cannot distinguish between the actinospore (infectious to fish) or myxospore (shed from infected fish especially adult carcasses). Given the sentinel salmon infections throughout the study period, we believe the majority of the spores detected in the water samples were actinospores. There was a poor correlation between mean exposure spore / L values (mean of sample taken at deployment and retrieval) and prevalence of CS infection in the sentinel group (Fig. 5). Pearson product moment correlation did not detect a strong relationship between spore/L and CS prevalence (R=0.328, P=0.170, no. = 19).

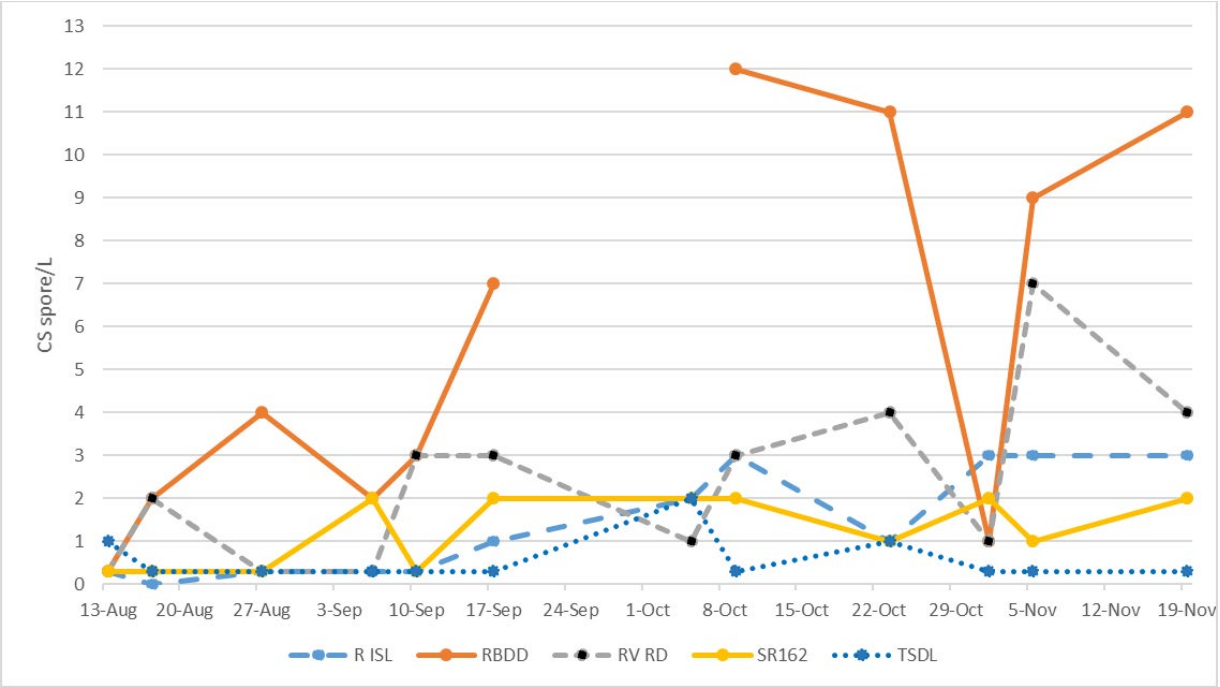

Figure 4. Mean spore/L data from triplicate 1-Liter samples collected from Sacramento River at Reading Island (R ISL), Red Bluff Division Dam (RBDD), River Road (RV RD), Hwy 162 Bridge (SR162), and Tisdale weir (TISDL) between August 13 and November 19, 2018. Data rated as greater than zero but less than 1 spore / L was assigned a value of 0.3 for graphing purposes.

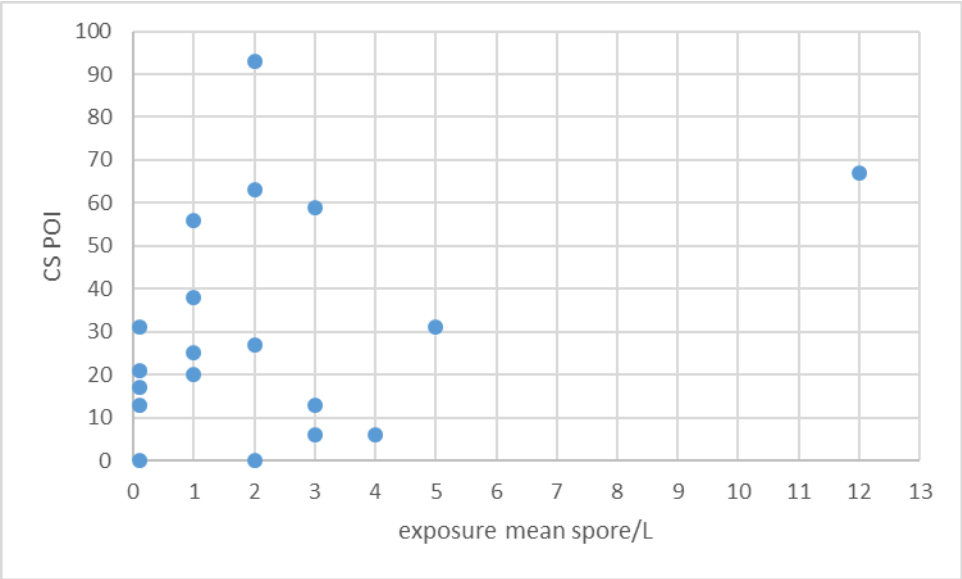

Figure 5. Relationship between mean CS spore/L of exposure group and its CS prevalence of infection (CS-POI).

Naturally-produced Winter-run fry – A total of 80 fry were collected at the Red Bluff Diversion Dam rotary screw traps for histological examination between September 19 and November 7, 2018. Fork length of the collection group ranged from 33 to 69mm (Fig. 6). The increase in length beginning on October 12 suggests fry were rearing above the trap site. Not all tissues were examined in each fish due to the nature of sagittal sectioning. The prevalence of *C.shasta* and *P. minibicornis* infection was 10% (8 / 80) and 26% (18/70), respectively (Table 5). CS and PM infections were first observed in the October 10 sample. Infections were rated as asymptomatic (rating #1). CS infected fry tended to be larger and ranged from 51 – 69mm FL. No gill parasites or abnormalities were observed in the sample set.

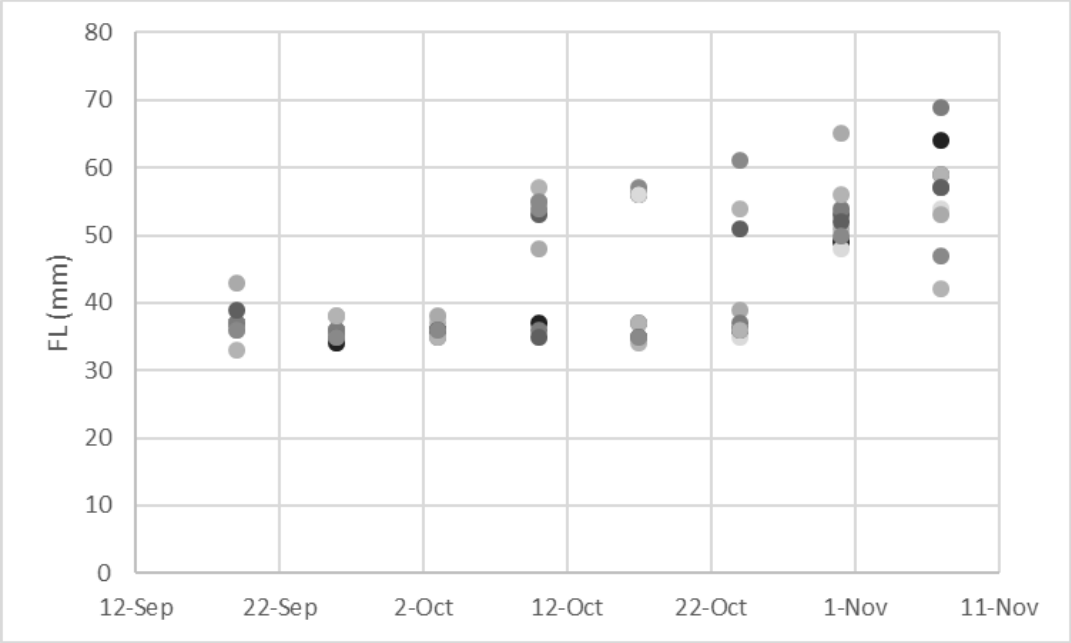

Figure 6. Fork length (mm) of naturally-produced Winter-run fry sampled at Red Bluff Diversion Dam rotary screw traps between September19 and November 7, 2018.

Table 5. Naturally-produced Winter-run Chinook fry prevalence of infection (number positive / total sample) for *C.shasta* (CS), *P. minibicornis* (PM), and gill parasites in histological specimens (1= infection with little disease, 2= disease state). Data reported includes sample date. Season prevalence is summation of individual sample dates.

	CS1	CS2	PM1	Gill Parasite
19-Sep	0/10	0/10	0/10	0/10
26-Sep	0/10	0/10	0/8	0/10
3-Oct	0/10	0/10	0/9	0/8
10-Oct	1/10	0/10	2/9	0/10
17-Oct	0/10	0/10	1/8	0/9
24-Oct	1/10	0/10	3/7	0/10
31-Oct	2/10	0/10	6/9	0/10
7-Nov	4/10	0/10	6/10	0/2
	8/80 (10%)	0/80	18/70 (26%)	0/69

Discussion:

Relatively low actinospore concentrations (≤ 5 spore / L) produced a wide range of infection prevalence (0 – 93% for 4 d exposures). Even trace spore concentrations (< 1 spore / L) produced an average of 16% CS infection (range 0 – 31%). It appears that *Ceratonova shasta* in the Sacramento River is efficient at transmission. The marked increase in CS infection during October is particularly interesting as spore concentrations were not consistently higher than other exposures, temperatures were similar, and flow was only 7-16% lower than September's exposure. The QPCR assay of filtered water samples cannot distinguish between the actinospore (infectious to fish) and myxospore (infectious to polychaete) stages. The ratio of actinospore to myxospore could help explain the increased CS infections during October however most myxospores are generally produced by infected adult carcasses (Foott et al. 2016b). Adult Fall-run Chinook spawning is just beginning in early October and carcasses would likely be limited. From an evolutionary perspective, increased transmission during the early fall would benefit the parasite as it needs to infect migrating adults to complete its life cycle. Both the water sample and sentinel infection data suggests the reach above Red Bluff Diversion dam has the highest infectivity of the system and likely contains a greater concentration of infected polychaetes. The wider spatial range of sentinel infection can be explained as actinospores remain infectious for at least 7d and can move a great distance downstream from the infected polychaete (Foott et al. 2007).

As observed in 2016, Upper Sacramento River (Reading Island (6- 59%) and Red Bluff Diversion Dam (13 – 67%) sentinels had higher prevalence of CS infection than observed in naturally-produced fry (10%) captured at Red Bluff Diversion dam. The first CS detection in naturally-produced fry occurred in an October 1, 2018 sample with the majority occurring in late October and early November. In comparison, October 1 – 5 Reading Island and Red Bluff Diversion Dam exposures produced 59 and 67% prevalence of CS infection, respectively. This difference in response could be influenced by their respective exposure history (specific micro environment and duration of actinospore challenge), impaired immune function of sentinels during cage exposure, or potential host (Winter-run vs Late fall) genetic resistance. An open question is whether the low prevalence of clinical disease observed in the 4d exposure sentinel fish is representative of naturally-produced Winter-run juveniles. Naturally-produced parr migrate and rear in the Sacramento River for much longer than 4 days. Our exposure

time was limited by columnaris outbreaks in warm water conditions. A related health question is the effect of *Flavobacterium columnare* infection and disease on juvenile salmon populations in the Sacramento-San Joaquin River system when water temperature is above 16 - 17°C. Both questions could be examined from sufficient samples of feral juvenile salmon in the lower river and delta during late spring.

In water years rated "Below Normal: or wetter, *Ceratonova shasta* appears to represent a low to moderate disease risk for juvenile Winter-run Chinook salmon during their out-migration. This assertion is based on the low prevalence of clinical disease and mortality observed in sentinel fish exposed to the river in late summer and fall of 2016 and 2018. Under these limited challenge conditions, the sentinel fish's immune system appear to effectively limit CS initial infection, migration to the intestine, and subsequent multiplication. Both of these water years were classified as "Below Normal" in the Sacramento basin by California Department of Water Resources. In contrast, sentinel fish exposures in September of 2015 ("Critical" water year) in the upper Sacramento River developed clinical disease (Foott 2016). Similarly, the low prevalence of asymptomatic CS infection observed in naturally-produced Winter-run fry captured at RBDD indicates that the upper Sacramento River, during water years rated as "Below Normal: or wetter, is not a significant health threat to juvenile Winter-run Chinook.

Acknowledgements: Scott Voss, Casey Collins, Robert Larson, Bradley Stokes, and Kathryn Sykes for collection of naturally-produced Winter-run Chinook fry at RBDD traps, Anne Voss for transporting sample supplies between the FHC and Red Bluff FWO, Leonard Cheskiewicz (Red Bluff FWO) for figure 1, Oregon State University Bartholomew laboratory for eDNA qPCR analysis (Jamie Graen, Stephen Atkinson, Sascha Hallett), and both Joe Silveria (Sacramento NWR) and Bill Poytress (RBFWO) for their coordination efforts. Partial funding for this work came from CVPIA (488330835FRO).

Responsibilities:

JSF- study design, field & lab aspects of sentinel fish, histological evaluation, and report

SF – sentinel field deployment, wetlab rearing and sampling, histological sample preparation, temperature and flow data.

KN – filtration, coordination of sample shipments for eDNA

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Appendix 1.
Legend:

RBFWO ID 18-#	Fish ID# from RBFWO
block RNAlater number	Fish ID# for RNAlater gill sample
block#	histology block number
Collect date	date sampled
FL (mm)	Fork length (mm)
ichgillskin histo	Presence (1) or absence (0) of Ich trophont in section
Pm#	0=no parasite seen in kidney, 1= parasite without inflammation, 2= parasite and inflammation
GMN	glomerulonephritis
IntHP	Interstitial hyperplasia of kidney
Prot	Protein casts in tubules
CS	Cshasta trophozoites (0=none,1= present without inflammation, 2= present with inflammation/necrosis)
EpNec	intestinal epithelium necrosis
Helm	Helminth
ICRVfat	inflammation of visceral fat
gill&liver(0)	examined with no abnormalities noted
na	tissue not available for examination
	eye in each section = no abnormalities

RBFWO ID 18-#	block RNAlater Number	block #	Collect date	FL(mm)	ichgill skin histo	Pm#	GMN	IntHP	Prot	CS	EpNe	helm	ICRVI	gill0	liver0
77901	245	10963	9/19/2018	37	0	0	0	0	0	0	0	0	0	0	0
77902	246	10964	9/19/2018	36	0	0	0	0	0	0	0	0	0	0	0
77903	247	10965	9/19/2018	37	0	0	0	0	0	0	0	0	0	0	0
77904	248	10966	9/19/2018	37	0	0	0	0	0	0	0	0	0	0	0
77905	249	10967	9/19/2018	37	0	0	0	0	0	0	0	0	0	0	0
77906	250	10968	9/19/2018	43	0	0	0	0	0	0	0	0	0	0	0
77907	251	10969	9/19/2018	37	0	0	0	0	0	0	0	0	0	0	0
77908	252	10970	9/19/2018	39	0	0	0	0	0	0	0	0	0	0	0
77909	253	10971	9/19/2018	33	0	0	0	0	0	0	0	0	0	0	0
77910	254	10972	9/19/2018	36	0	0	0	0	0	0	0	0	0	0	0
77911	255	10986	9/26/2018	36	0	0	0	0	0	0	0	0	0	0	0
77912	256	10987	9/26/2018	34	0	0	0	0	0	0	0	0	0	0	0
77913	257	10988	9/26/2018	36	0	0	0	0	0	0	0	0	0	0	0
77914	258	10989	9/26/2018	34	0	na	na	na	na	0	0	0	0	0	0
77915	259	10990	9/26/2018	38	0	na	na	na	na	0	0	0	0	0	0
77916	260	10991	9/26/2018	38	0	0	0	0	0	0	0	0	0	0	0
77917	261	10992	9/26/2018	36	0	0	0	0	0	0	0	0	0	0	0
77918	262	10993	9/26/2018	35	0	0	0	0	0	0	0	0	0	0	0
77919	263	10994	9/26/2018	38	0	0	0	0	0	0	0	0	0	0	0
77920	264	10995	9/26/2018	35	0	0	0	0	0	0	0	0	0	0	0
77921	265	11060	10/3/2018	36	0	0	0	0	0	0	0	0	0	0	0
77922	266	11061	10/3/2018	37	0	0	0	0	0	0	0	0	0	0	0
77923	267	11062	10/3/2018	36	0	0	0	0	0	0	0	0	0	0	0
77924	268	11063	10/3/2018	36	0	na	na	na	na	0	0	0	0	0	0
77925	269	11064	10/3/2018	38	0	0	0	0	0	0	0	0	0	0	0
77926	270	11065	10/3/2018	38	0	0	0	0	0	0	0	0	0	0	0
77927	271	11066	10/3/2018	35	0	0	0	0	0	0	0	0	0	0	0
77928	272	11067	10/3/2018	36	0	0	0	0	0	0	0	0	0	0	na
77929	273	11068	10/3/2018	35	0	0	0	0	0	0	0	0	0	na	0
77930	274	11069	10/3/2018	36	0	0	0	0	0	0	0	0	0	na	na
77931	275	11084	10/10/2018	53	0	0	0	0	0	0	0	0	0	0	0
77932	276	11085	10/10/2018	55	0	0	0	0	0	0	0	0	0	0	0
77933	277	11086	10/10/2018	54	0	na	0	0	0	0	0	0	0	0	0
77934	278	11087	10/10/2018	37	0	0	0	0	0	0	0	0	0	0	0
77935	279	11088	10/10/2018	35	0	0	0	0	0	0	0	0	0	0	0
77936	280	11089	10/10/2018	48	0	0	0	0	0	0	0	0	0	0	0
77937	281	11090	10/10/2018	36	0	0	0	0	0	0	0	0	0	0	0
77938	282	11091	10/10/2018	35	0	0	0	0	0	0	0	0	0	0	0
77939	283	11092	10/10/2018	57	0	**1**	0	0	0	0	0	0	0	0	0
77940	284	11093	10/10/2018	55	0	**1**	0	0	0	**1**	0	0	0	0	0

77941	285	11101	10/17/2018	37	0	0	0	0	0	0	0	0	0	0	na
77942	286	11102	10/17/2018	37	0	0	0	0	0	0	0	0	0	na	na
77943	287	11103	10/17/2018	57	0	1	0	0	0	0	0	0	0	0	na
77944	288	11104	10/17/2018	56	0	0	0	0	0	0	0	0	0	0	0
77945	289	11105	10/17/2018	56	0	0	0	0	0	0	0	0	0	0	0
77946	290	11106	10/17/2018	35	0	0	0	0	0	0	0	0	0	0	0
77947	291	11107	10/17/2018	35	0	na	na	na	na	0	0	0	0	0	0
77948	292	11108	10/17/2018	35	0	na	na	na	na	0	0	0	0	0	na
77949	293	11109	10/17/2018	34	0	0	0	0	0	0	0	0	0	0	0
77950	294	11110	10/17/2018	35	0	0	0	0	0	0	0	0	0	0	na
77951	295	11132	10/24/2018	51	0	1	0	0	0	1	0	0	0	0	0
77952	296	11133	10/24/2018	54	0	1	0	0	0	0	0	0	0	0	0
77953	297	11134	10/24/2018	36	0	0	0	0	0	0	0	0	0	0	0
77954	298	11135	10/24/2018	36	0	0	0	0	0	0	0	0	0	0	0
77955	299	11136	10/24/2018	35	0	0	0	0	0	0	0	0	0	0	0
77956	300	11137	10/24/2018	39	0	na	0	0	0	0	0	0	0	0	0
77957	301	11138	10/24/2018	37	0	na	0	0	0	0	0	0	0	0	0
77958	302	11139	10/24/2018	36	0	na	0	0	0	0	0	0	0	0	0
77959	303	11140	10/24/2018	36	0	0	0	0	0	0	0	0	0	0	na
77960	304	11141	10/24/2018	61	0	1	0	0	0	0	0	0	0	0	0
77961	305	11234	10/31/2018	53	0	1	0	0	0	0	0	0	0	0	0
77962	306	11235	10/31/2018	51	0	1	0	0	0	0	0	0	0	0	0
77963	307	11236	10/31/2018	49	0	1	0	0	0	0	0	0	0	0	0
77964	308	11237	10/31/2018	49	0	0	0	0	0	0	0	0	0	0	0
77965	309	11238	10/31/2018	48	0	0	0	0	0	0	0	0	0	0	0
77966	310	11239	10/31/2018	65	0	1	0	0	0	1	0	0	0	0	0
77967	311	11240	10/31/2018	54	0	0	0	0	0	0	0	0	0	0	0
77968	312	11241	10/31/2018	52	0	1	0	0	0	0	0	0	0	0	0
77969	313	11242	10/31/2018	56	0	1	0	0	0	1	0	0	0	0	0
77970	314	11243	10/31/2018	50	0	na	na	na	na	0	0	0	0	0	0
77971	315	11244	11/7/2018	59	0	1	0	0	0	0	0	0	2	na	na
77972	316	11245	11/7/2018	59	0	0	0	0	0	0	0	0	2	na	na
77973	317	11246	11/7/2018	57	0	1	0	0	0	1	0	0	2	na	na
77974	318	11247	11/7/2018	64	0	1	0	0	0	1	0	0	2	na	na
77975	319	11248	11/7/2018	54	0	0	0	0	0	0	0	0	na	na	na
77976	320	11249	11/7/2018	53	0	0	0	0	0	1	0	0	na	na	na
77977	321	11250	11/7/2018	69	0	1	0	0	0	1	0	0	0	na	na
77978	322	11251	11/7/2018	57	0	1	0	0	0	0	0	0	0	na	na
77979	323	11252	11/7/2018	42	0	1	0	0	0	0	0	0	0	0	0
77980	324	11253	11/7/2018	47	0	0	0	0	0	0	0	0	0	0	0

Appendix 2. CS spore/L data for Anderson R. Park and Reading Island.

tube n	sample date	sampl	filter	volume (ml)	extracted? archive	C.shasta sp/L	C. shasta sp/L average 3 sample
17	8/13/2018	ARP	A	1000	extracted	< 1	< 1
18	8/13/2018	ARP	B	1000	extracted	2	
19	8/13/2018	ARP	C	1000	extracted	< 1	
20	8/13/2018	ARP	D	1000	archived	#N/A	
37	8/17/2018	ARP	A	1000	extracted	0	0
38	8/17/2018	ARP	B	1000	extracted	0	
39	8/17/2018	ARP	C	1000	extracted	0	
40	8/17/2018	ARP	D	1000	archived	#N/A	
57	8/27/2018	Reading I	A	1000	extracted	0	< 1
58	8/27/2018	Reading I	B	1000	extracted	< 1	
59	8/27/2018	Reading I	C	1000	extracted	0	
60	8/27/2018	Reading I	D	1000	archived	#N/A	
77	9/6/2018	Reading I	A	1000	extracted	< 1	< 1
78	9/6/2018	Reading I	B	1000	extracted	0	
79	9/6/2018	Reading I	C	1000	extracted	0	
80	9/6/2018	Reading I	D	1000	archived	#N/A	
97	9/10/2018	Reading I	A	1000	extracted	0	< 1
98	9/10/2018	Reading I	B	1000	extracted	0	
99	9/10/2018	Reading I	C	1000	extracted	1	
100	9/10/2018	Reading I	D	1000	archived	#N/A	
117	9/17/2018	Reading I	a	1000	extracted	3	1
118	9/17/2018	Reading I	b	1000	extracted	0	
119	9/17/2018	Reading I	c	1000	extracted	0	
120	9/17/2018	Reading I	d	1000	archived	#N/A	
138	10/5/2018	Reading I	a	1000	extracted	2	2
139	10/5/2018	Reading I	b	1000	extracted	2	
140	10/5/2018	Reading I	c	1000	extracted	2	
141	10/5/2018	Reading I	d	1000	archived	#N/A	
158	10/9/2018	Reading I	a	1000	extracted	4	3
159	10/9/2018	Reading I	b	1000	extracted	3	
160	10/9/2018	Reading I	c	1000	extracted	1	
161	10/9/2018	Reading I	d	1000	archived	#N/A	
179	10/23/2018	Reading I	a	1000	extracted	< 1	1
180	10/23/2018	Reading I	b	1000	extracted	2	
181	10/23/2018	Reading I	c	1000	extracted	< 1	
182	10/23/2018	Reading I	d	1000	archived	#N/A	
199	11/1/2018	Reading I	a	1000	extracted	< 1	3
200	11/1/2018	Reading I	b	1000	extracted	2	
201	11/1/2018	Reading I	c	1000	extracted	4	
202	11/1/2018	Reading I	d	1000	archived	#N/A	
219	11/5/2018	Reading I	a	1000	extracted	1	3
220	11/5/2018	Reading I	b	1000	archived	#N/A	
221	11/5/2018	Reading I	c	1000	extracted	2	
222	11/5/2018	Reading I	d	1000	extracted	5	
239	11/19/2018	Reading I	a	1000	extracted	4	3
240	11/19/2018	Reading I	b	1000	extracted	3	
241	11/19/2018	Reading I	c	1000	extracted	< 1	
242	11/19/2018	Reading I	d	1000	archived	#N/A	

Appendix 3. CS spore/L data for Red Bluff Diversion Dam.

tube n	sample date	sampl	filter	volume (ml)	extracted? archive	C.shasta sp/L	C. shasta sp/L average 3 sample
13	8/13/2018	RBDD	A	1000	extracted	< 1	< 1
14	8/13/2018	RBDD	B	1000	extracted	0	
15	8/13/2018	RBDD	C	1000	extracted	2	
16	8/13/2018	RBDD	D	1000	archived	#N/A	
33	8/17/2018	RBDD	A	1000	extracted	1	2
34	8/17/2018	RBDD	B	1000	extracted	4	
35	8/17/2018	RBDD	C	1000	extracted	1	
36	8/17/2018	RBDD	D	1000	archived	#N/A	
53	8/27/2018	RBDD	A	1000	extracted	2	4
54	8/27/2018	RBDD	B	1000	extracted	2	
55	8/27/2018	RBDD	C	1000	extracted	8	
56	8/27/2018	RBDD	D	1000	archived	#N/A	
73	9/6/2018	RBDD	A	1000	extracted	1	2
74	9/6/2018	RBDD	B	1000	extracted	< 1	
75	9/6/2018	RBDD	C	1000	extracted	5	
76	9/6/2018	RBDD	D	1000	archived	#N/A	
93	9/10/2018	RBDD	A	1000	extracted	5	3
94	9/10/2018	RBDD	B	1000	extracted	3	
95	9/10/2018	RBDD	C	1000	extracted	2	
96	9/10/2018	RBDD	D	1000	archived	#N/A	
113	9/17/2018	RBDD	a	1000	extracted	3	7
114	9/17/2018	RBDD	b	1000	extracted	5	
115	9/17/2018	RBDD	c	1000	extracted	13	
116	9/17/2018	RBDD	d	1000	archived	#N/A	
133	10/5/2018	RBDD	a	350+350=	extracted	< 1	< 1
134	10/5/2018	RBDD	a	300	extracted	#N/A	
135	10/5/2018	RBDD	b	500	extracted	0	
136	10/5/2018	RBDD	c	500	extracted	0	
137	10/5/2018	RBDD	d	500	archived	#N/A	
154	10/9/2018	RBDD	a	1000	extracted	12	12
155	10/9/2018	RBDD	b	1000	extracted	15	
156	10/9/2018	RBDD	c	1000	extracted	9	
157	10/9/2018	RBDD	d	1000	archived	#N/A	
174	10/23/2018	RBDD	a	1000	extracted	17	11
175	LOST	RBDD					
176	10/23/2018	RBDD	b	1000	extracted	9	
177	10/23/2018	RBDD	c	1000	extracted	6	
178	10/23/2018	RBDD	d	1000	archived	#N/A	
195	11/1/2018	RBDD	a	1000	extracted	2	1
196	11/1/2018	RBDD	b	1000	extracted	< 1	
197	11/1/2018	RBDD	c	1000	extracted	2	
198	11/1/2018	RBDD	d	1000	archived	#N/A	
215	11/5/2018	RBDD	a	1000	extracted	11	9
216	11/5/2018	RBDD	b	1000	extracted	4	
217	11/5/2018	RBDD	c	1000	extracted	11	
218	11/5/2018	RBDD	d	1000	archived	#N/A	
235	11/19/2018	RBDD	a	1000	extracted	10	11
236	11/19/2018	RBDD	b	1000	extracted	7	
237	11/19/2018	RBDD	c	1000	extracted	#N/A	
238	11/19/2018	RBDD	d	1000	extracted	15	

Appendix 4. CS spore/L data for River Ranch Road.

tube n	sample date	sampl	filter	volume (ml)	extracted? archive	C.shasta sp/L	C. shasta sp/L average 3 sample
9	8/13/2018	River Roa	A	1000	extracted	0	< 1
10	8/13/2018	River Roa	B	1000	extracted	2	
11	8/13/2018	River Roa	C	1000	extracted	1	
12	8/13/2018	River Roa	D	1000	archived	#N/A	
29	8/17/2018	River Roa	A	1000	extracted	3	2
30	8/17/2018	River Roa	B	1000	extracted	2	
31	8/17/2018	River Roa	C	1000	extracted	< 1	
32	8/17/2018	River Roa	D	1000	archived	#N/A	
49	8/27/2018	River Roa	A	1000	extracted	< 1	< 1
50	8/27/2018	River Roa	B	1000	extracted	2	
51	8/27/2018	River Roa	C	1000	extracted	< 1	
52	8/27/2018	River Roa	D	1000	archived	#N/A	
69	9/6/2018	River Roa	A	1000	extracted	< 1	< 1
70	9/6/2018	River Roa	B	1000	extracted	< 1	
71	9/6/2018	River Roa	C	1000	extracted	1	
72	9/6/2018	River Roa	D	1000	archived	#N/A	
89	9/10/2018	River Roa	A	1000	extracted	3	3
90	9/10/2018	River Roa	B	1000	extracted	< 1	
91	9/10/2018	River Roa	C	1000	extracted	5	
92	9/10/2018	River Roa	D	1000	archived	#N/A	
109	9/17/2018	River Roa	a	1000	extracted	3	3
110	9/17/2018	River Roa	b	1000	extracted	3	
111	9/17/2018	River Roa	c	1000	extracted	1	
112	9/17/2018	River Roa	d	1000	archived	#N/A	
129	10/5/2018	River Roa	a	1000	extracted	1	1
130	10/5/2018	River Roa	b	1000	extracted	2	
131	10/5/2018	River Roa	c	1000	extracted	< 1	
132	10/5/2018	River Roa	d	1000	archived	#N/A	
150	10/9/2018	River Roa	a	1000	extracted	4	3
151	10/9/2018	River Roa	b	1000	extracted	2	
152	10/9/2018	River Roa	c	1000	extracted	2	
153	10/9/2018	River Roa	d	1000	archived	#N/A	
170	10/23/2018	River Roa	a	1000	extracted	2	4
171	10/23/2018	River Roa	b	1000	extracted	7	
172	10/23/2018	River Roa	c	1000	extracted	3	
173	10/23/2018	River Roa	d	1000	archived	#N/A	
191	11/1/2018	River Roa	a	1000	extracted	< 1	1
192	11/1/2018	River Roa	b	1000	extracted	2	
193	11/1/2018	River Roa	c	1000	extracted	< 1	
194	11/1/2018	River Roa	d	1000	archived	#N/A	
211	11/5/2018	River Roa	a	1000	extracted	10	7
212	11/5/2018	River Roa	b	1000	extracted	5	
213	11/5/2018	River Roa	c	1000	extracted	7	
214	11/5/2018	River Roa	d	1000	archived	#N/A	
231	11/19/2018	River Roa	a	1000	extracted	5	4
232	11/19/2018	River Roa	b	1000	extracted	< 1	
233	11/19/2018	River Roa	c	1000	extracted	7	
234	11/19/2018	River Roa	d	1000	archived	#N/A	

Appendix 5. CS spore/L data for Highway 162 Bridge.

tube n	sample date	sampl	filter	volume (ml)	extracted? archive	C.shasta sp/L	C. shasta sp/L average 3 sample
5	8/13/2018	SR162	A	1000	extracted	0	< 1
6	8/13/2018	SR162	B	1000	extracted	< 1	
7	8/13/2018	SR162	C	1000	extracted	< 1	
8	8/13/2018	SR162	D	1000	archived	#N/A	
25	8/17/2018	SR162	A	1000	extracted	< 1	< 1
26	8/17/2018	SR162	B	1000	extracted	< 1	
27	8/17/2018	SR162	C	1000	extracted	0	
28	8/17/2018	SR162	D	1000	archived	#N/A	
45	8/27/2018	SR162	A	1000	extracted	0	< 1
46	8/27/2018	SR162	B	1000	extracted	0	
47	8/27/2018	SR162	C	1000	extracted	< 1	
48	8/27/2018	SR162	D	1000	archived	#N/A	
65	9/6/2018	SR162	A	1000	extracted	< 1	2
66	9/6/2018	SR162	B	1000	extracted	< 1	
67	9/6/2018	SR162	C	1000	extracted	3	
68	9/6/2018	SR162	D	1000	archived	#N/A	
85	9/10/2018	SR162	A	1000	extracted	2	< 1
86	9/10/2018	SR162	B	1000	extracted	< 1	
87	9/10/2018	SR162	C	1000	extracted	< 1	
88	9/10/2018	SR162	D	1000	archived	#N/A	
105	9/17/2018	SR162	a	1000	extracted	3	2
106	9/17/2018	SR162	b	1000	extracted	< 1	
107	9/17/2018	SR162	c	1000	extracted	< 1	
108	9/17/2018	SR162	d	1000	archived	#N/A	
125	10/5/2018	SR162	a	1000	extracted	< 1	2
126	10/5/2018	SR162	b	1000	extracted	4	
127	10/5/2018	SR162	c	1000	extracted	2	
128	10/5/2018	SR162	d	1000	archived	#N/A	
146	10/9/2018	SR162	a	1000	extracted	2	2
147	10/9/2018	SR162	b	1000	extracted	< 1	
148	10/9/2018	SR162	c	1000	extracted	3	
149	10/9/2018	SR162	d	1000	archived	#N/A	
166	10/23/2018	SR162	a	1000	extracted	0	1
167	10/23/2018	SR162	b	1000	extracted	3	
168	10/23/2018	SR162	c	1000	extracted	< 1	
169	10/23/2018	SR162	d	1000	archived	#N/A	
187	11/1/2018	SR162	a	1000	extracted	2	2
188	11/1/2018	SR162	b	1000	extracted	1	
189	11/1/2018	SR162	c	1000	extracted	2	
190	11/1/2018	SR162	d	1000	archived	#N/A	
207	11/5/2018	SR162	a	1000	extracted	< 1	1
208	11/5/2018	SR162	b	1000	extracted	3	
209	11/5/2018	SR162	c	1000	extracted	< 1	
210	11/5/2018	SR162	d	1000	archived	#N/A	
227	11/19/2018	SR162	a	1000	extracted	2	2
228	11/19/2018	SR162	b	1000	extracted	1	
229	11/19/2018	SR162	c	1000	extracted	2	
230	11/19/2018	SR162	d	1000	archived	#N/A	

Appendix 6 CS spore/L data for Tisdale.

tube n	sample date	sampl	filter	volume (ml)	extracted ? archive	C.shasta sp/L	C. shasta sp/L average 3 sample
1	8/13/2018	Tisdale	A	1000	extracted	1	1
2	8/13/2018	Tisdale	B	1000	extracted	< 1	
3	8/13/2018	Tisdale	C	1000	extracted	1	
4	8/13/2018	Tisdale	D	1000	archived	#N/A	
21	8/17/2018	Tisdale	A	1000	extracted	< 1	< 1
22	8/17/2018	Tisdale	B	1000	extracted	0	
23	8/17/2018	Tisdale	C	1000	extracted	0	
24	8/17/2018	Tisdale	D	1000	archived	#N/A	
41	8/27/2018	Tisdale	A	1000	extracted	< 1	< 1
42	8/27/2018	Tisdale	B	1000	extracted	< 1	
43	8/27/2018	Tisdale	C	1000	extracted	< 1	
44	8/27/2018	Tisdale	D	1000	archived	#N/A	
61	9/6/2018	Tisdale	A	1000	extracted	0	< 1
62	9/6/2018	Tisdale	B	1000	extracted	2	
63	9/6/2018	Tisdale	C	1000	extracted	< 1	
64	9/6/2018	Tisdale	D	1000	archived	#N/A	
81	9/10/2018	Tisdale	A	1000	extracted	0	< 1
82	9/10/2018	Tisdale	B	1000	extracted	1	
83	9/10/2018	Tisdale	C	1000	extracted	0	
84	9/10/2018	Tisdale	D	1000	archived	#N/A	
101	9/17/2018	Tisdale	a	1000	extracted	0	< 1
102	9/17/2018	Tisdale	b	1000	extracted	< 1	
103	9/17/2018	Tisdale	c	1000	extracted	0	
104	9/17/2018	Tisdale	d	1000	archived	#N/A	
121	10/5/2018	Tisdale	a	1000	extracted	3	2
122	10/5/2018	Tisdale	b	1000	extracted	1	
123	10/5/2018	Tisdale	c	1000	extracted	2	
124	10/5/2018	Tisdale	d	1000	archived	#N/A	
142	10/9/2018	Tisdale	a	1000	extracted	< 1	< 1
143	10/9/2018	Tisdale	b	1000	extracted	0	
144	10/9/2018	Tisdale	c	1000	extracted	0	
145	10/9/2018	Tisdale	d	1000	archived	#N/A	
162	10/23/2018	Tisdale	a	1000	extracted	0	1
163	10/23/2018	Tisdale	b	1000	extracted	3	
164	10/23/2018	Tisdale	c	1000	extracted	< 1	
165	10/23/2018	Tisdale	d	1000	archived	#N/A	
183	11/1/2018	Tisdale	a	1000	extracted	< 1	< 1
184	11/1/2018	Tisdale	b	1000	extracted	< 1	
185	11/1/2018	Tisdale	c	1000	extracted	-	
186	11/1/2018	Tisdale	d	1000	extracted	0	
203	11/5/2018	Tisdale	a	1000	extracted	0	< 1
204	11/5/2018	Tisdale	b	1000	extracted	< 1	
205	11/5/2018	Tisdale	c	1000	extracted	0	
206	11/5/2018	Tisdale	d	1000	archived	#N/A	
223	11/19/2018	Tisdale	a	1000	extracted	< 1	< 1
224	11/19/2018	Tisdale	b	1000	extracted	< 1	
225	11/19/2018	Tisdale	c	1000	extracted	< 1	
226	11/19/2018	Tisdale	d	1000	archived	#N/A	

Appendix 7. OSU spore/L analysis criteria (pers. Comm. S. Atkinson Oregon State University, 2019) **2019 – OSU qPCR assessment guidelines summary:**

Notes on replicates

Two levels of replication are used: 3 samples make up a typical water sample collection (at one locality/time) = 3 x 1L.

These can either be regarded as technical replicates or biological replicates:

TECHNICAL replicate 3 x 1L samples: a volume of water >3L is sampled, pooled, mixed, and three x 1L are taken from this; typically when an automatic sampler is used.

BIOLOGICAL replicate 3 x 1L samples: 3 separate 1-L samples are taken from the waterbody, at the same sampling time; typically a manual "grab" sample. Variation between each of the 3 samples in a "grab" sample is usually higher than from three technical replicates.

qPCR assay

Each sample is then run in 3 wells on the qPCR (= technical triplicate).

We assess the well results based on several criteria. Two of the three wells must return valid readings (see below) for the sample to count, otherwise it is re-run. Also, we assess inhibition, with valid wells having an IPC value less than 1 cycle different from the average IPC value of the non-template control wells.

Best case: all three wells return values <1 cycle apart (SD <~.6) and the internal positive control (IPC) values of wells are <1 cycle different from the average of the non-template control wells run on the plate. In this case all three wells are converted to spores per liter based on the on-plate spore standard, and the three wells averaged to produce the sp/L value for the sample.

Cs-negative: We consider a sample to be *C. shasta*-negative (0 sp/L) when at least 2 of the 3 replicate wells are undetected, and there is no significant inhibition.

<1 sp/L: We assign a sample as <1sp/L (but greater than 0) if at least 2 of the 3 replicate wells give fluorescence readings, which equate to less than 1 sp/L. This category is used to flag samples that have a valid *C. shasta* detection (i.e. not 0) but are less than a single-spore. **We leave it up to the project lead** to decide how to handle these samples – can be regarded as "trace" or biologically negative. We regard it as a flag for the presence of *C. shasta* in the system, but at low levels. Biologically, a reading of <1sp/L could mean that some parasite fragment was captured on the filter, or an immature spore, or mutant/damaged spore.

>=1 sp/L: We assign a sample as 1 or >1 sp/L if at least 2 of the 3 wells give valid readings. These are averaged to produce the sp/L for the sample.
Censoring data: One of the three wells is censored if it is an outlier when standard deviation between the three wells is > ~0.6 (1 cycle).

If all wells of a single sample vary from each other (no one is an outlier), and have sp/L values **>1**, then we examine the other two water samples (i.e. 2 sets of triplicate wells) from the same set to determine if the sample itself might be valid or if the sample needs to be re-run. When in doubt, we re-run the sample.

If all wells of a single sample vary from each other (no one is an outlier), and have sp/L values **<1**, then we regard the sample as valid at <1 sp/L, because variation between replicates increases towards the detection limit (i.e. spore values <1sp/L), hence we are more likely to retain values with higher standard deviation, but low overall sp/L, as re-running them is unlikely to produce a less varied result, and would likely not change the overall value of “<1 sp/L”).