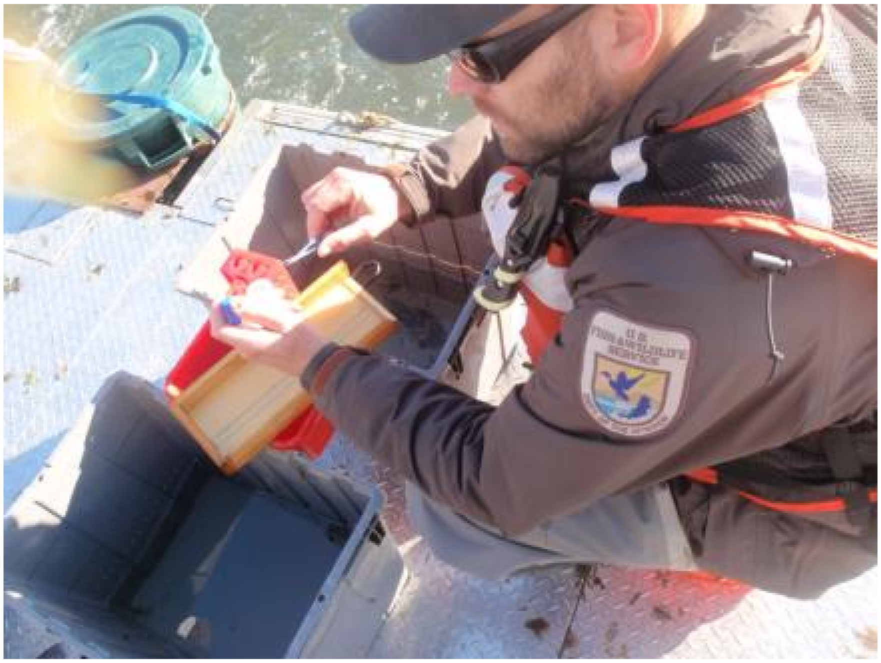
U.S. Fish & Wildlife Service

California Nevada Fish Health Center

FY2016 Technical Report:

Ceratonova shasta and *Parvicapsula minibicornis* (Phylum Cnidaria: Myxosporea) infectivity for juvenile Chinook salmon (*Oncorhynchus tshawytscha*) in the Upper Sacramento River: July – November 2016.



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Summary: Infectivity for *Ceratonova shasta* in the Upper Sacramento River (Anderson Cottonwood Irrigation District fish ladder to Red Bluff Diversion Dam) was monitored from July through November 2016. 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. The *C.shasta* spore (actinospore)/L concentration of the river was assayed by molecular methods. All three sample types (fry, sentinel salmon, eDNA) demonstrated low *C.shasta* infectivity in the upper Sacramento River during the study period. Neither *C.shasta* nor *P. minibicornis* infection was judged to have a significant disease impact on juvenile Winter-run Chinook in the upper Sacramento during 2016. Infectivity was greater at the downriver sites and during the summer months.

The correct citation for this report:

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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. 2016, Foott 2016 memorandum). *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). In the Klamath River, *M. speciosa* tolerates mean column velocities ranging between 0-0.31 meters per second and is found in both fine (silt/sand) and coarse (boulder/bedrock) substrates (Stocking and Bartholomew 2007, Malakuskas and Wilzbach 2013). High densities of *M. speciosa* are observed on Cladophora-covered boulder substrates in depositional areas of the Klamath River.

Infected polychaetes release actinospores into the water where they 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 muscularis (primarily) 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.

The life cycle of *C.shasta* offers several potential management approaches to reduce actinospore (infectious to fish) concentrations. While research in this area has been conducted in the Klamath River, there has not been a conclusive study on specific flows to disrupt either myxospore transmission (adult carcasses to polychaete worms) or polychaete densities (habitat disruption). Some observations suggest that water temperatures below 12°C reduce actinospore release and cool spring conditions have delayed the timing of lethal actinospore concentrations past the peak outmigration of natural Chinook juveniles (True et al. 2011).

The objectives of this study were:

a. Examine naturally-produced Winter-run fry tissues by histology to determine the prevalence of diseased individuals (both *C.shasta* and *P. minibicornis)*.

b. Deploy sentinel juvenile salmon (hatchery Late-fall run Chinook) and monitor development of disease state in these fish.

2. Determine whether there is a specific zone of infectivity and use this data to focus any future polychaete habitat surveys.
   a. Assay river water for eDNA (*C. shasta* spore concentration)
   b. Verify eDNA data with sentinel fish exposure

Methods:

Winter-run fry - Between September 9 and November 3, 2016, five to 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 (78 of 80 fish identified as Winter-run with 2 rated as unassigned). Gill tissue was placed into RNA later solution for Dr. Hasenbein (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. All tissues for a given fish were placed on one slide and identified by a unique sample code. Each slide was 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 / 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 was performed to identify disease status (parasite and lesion) and is less sensitive than molecular methods in detecting asymptomatic infections.

Sentinel exposures- Groups of 20 juvenile Late-fall run Chinook salmon (*Oncorhynchus tshawytscha*) from Coleman NFH were exposed for 5 d to the Sacramento River at four locations : 1) Posse Grounds (approximately 1 river mile downstream of the Anderson Cottonwood Irrigation District fish ladder water sample site), 2) Anderson River Park, 3) Bend Bridge, and 4) Red Bluff Diversion Dam (Figure

1). Exposures were conducted every 4 weeks between July 25 and October 6, 2016. At each site, two cages (0.34 ft^3) containing 10 fish each were deployed with an Onset temperature probe that recorded hourly river temperature. Rearing temperature for sentinel salmon was 16 – 17°C in July through September 26. Due to heating cost limitations, it was lowered to 13 - 14°C from September 26 – October 31. Sentinels were fed 1% BW/d salmon diet during the weekdays and mortalities frozen. Survivors were examined for clinical signs of infection (pale gill, hemorrhage or catarrhal exudate within the intestine, and swollen kidney) and sampled for histology after 24 d post exposure.

C.shasta eDNA analysis of river water– River water was collected from eight sites (Fig. 1) between July 13 and November 16, 2016. The Jelly's Ferry bridge site was sampled starting September 21 after the bridge was repaired and became operational. Four 1-Liter samples were collected approximately 30 cm below the surface after wading into 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 spores/Liter (we assume the majority are actinospores) from the triplicate samples. Due to high variability in some samples, censor rules were applied to the data:

1. If two of three samples were $\geq$ 1.0 and the third was $\geq$ 1.0 = site was 0. (e.g. 0, 0, ~~4.5)~~
2. If two of three samples were $\geq$ 1.0, and third was 0 it was censored (e.g. 13.2, 6.6, ~~0)~~
3. If a single sample was $\geq$ 10 spore/L than the other two non-zero samples were averaged for the site mean and the high replicate censored (e.g. 0.5, 0.6, ~~10.8)~~

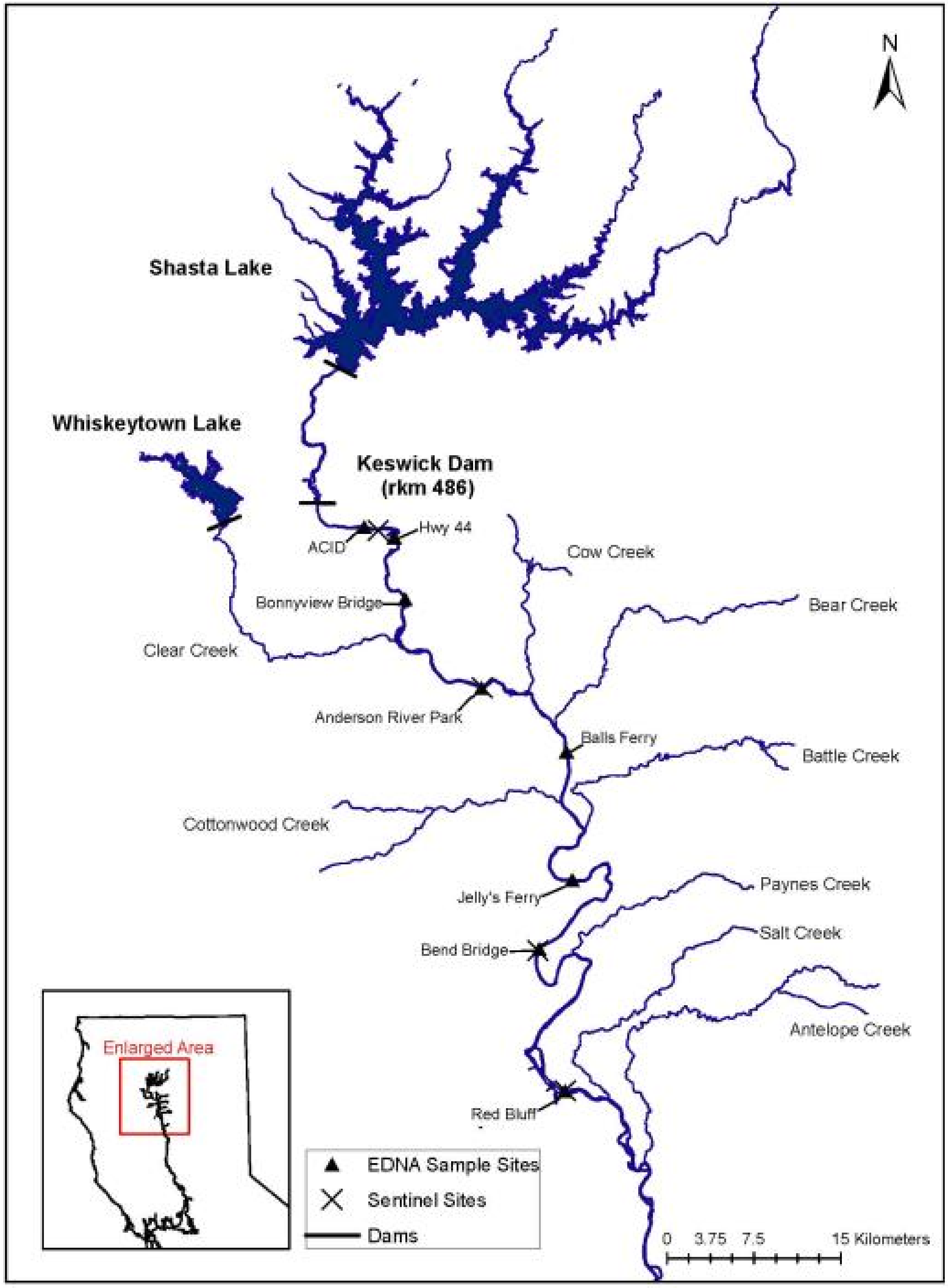

Figure 1. Map of water sample and sentinel cage sites.

Results:

Wild Winter-run fry – A total of 80 fry were collected at the Red Bluff Diversion Dam rotary screw traps for histological examination between September 9 and November 3, 2016. Fork length of the collection group ranged from 32 to 72mm (Fig. 2). The increase in length in October suggests fry were rearing within the study area. 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 7.6% (6 / 79) and 9.7% (6 / 62), respectively (Table 1). Only one fry (September 20) showed enteronecrosis associated with *C.shasta* trophozoites and was considered diseased. The remaining infected fry had fewer than 4 trophozoites observed in their intestinal tract with little to no inflammation. These fish were considered to have asymptomatic infections. Similarly, low numbers of *Parvicapsula minibicornis* trophozoites were observed in kidney glomeruli and were not associated with inflammation. The detection of these two myxozoan parasites increased after late October through the November 3 final sample. No gill parasites or abnormalities were observed in the sample set (see Appendix 1).

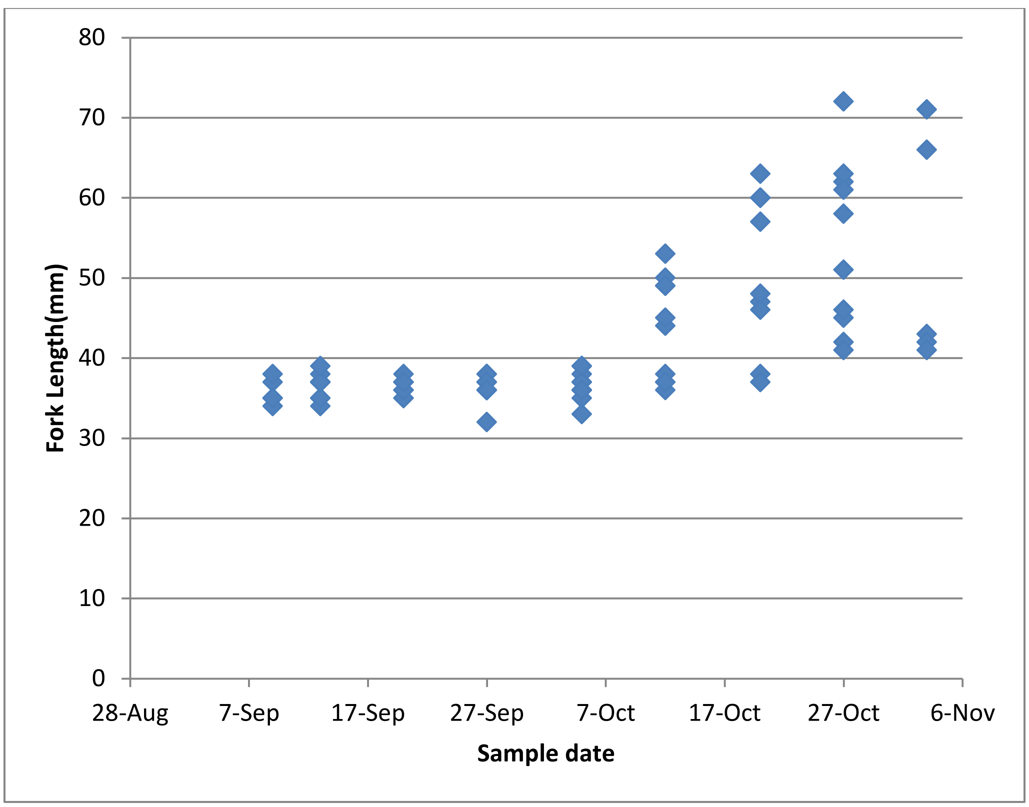

Figure 2. Fork length (mm) of naturally-produced Winter-run fry sampled at Red Bluff Diversion Dam rotary screw traps between September 9 and November 3, 2016.

Table 1. 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 and total sample number (N). Season prevalence is summation of individual sample dates.

	Sample (N)	CS1	CS2	PM1	Gill Para
9-Sep	5	0 / 5	0 / 5	0 / 4	ND
13-Sep	10	0 / 10	0 / 10	0 / 5	ND
20-Sep	10	0 / 10	**1** / 10	0 / 9	ND
27-Sep	10	0 / 10	0 / 10	0 / 6	0 / 10
5-Oct	10	0 / 10	0 / 10	0 / 6	0 / 10
12-Oct	10	0 / 10	0 / 10	0 / 9	0 / 10
20-Oct	10	**1** / 10	0 / 10	0 / 10	0 / 10
27-Oct	10	**1** / 10	0 / 10	4 / 9	0 / 9
3-Nov	5	**3** / 5	0 / 5	2 / 4	0 / 3
	80				
Prevalence		5 / 79 (6.3%)	1 / 79 (1.3%)	6 / 62 (9.7%)	0 / 52

Cshasta eDNA - River samples had a wide range of *C.shasta* spore/L concentrations (0 – 25.8 spores/L) with the majority of samples below the 10 spore/L concentration reported as a mortality threshold for Klamath R. juvenile Chinook (Hallett et al. 2012). Spore concentration tended to increase in a downstream fashion with low spore concentrations in the ACID to Anderson River Park reach (Fig. 3). The reach between Balls Ferry and Red Bluff Diversion Dam had higher spore/L concentrations throughout the July to November sample period. Outlier sub-samples were censored from the spore/L data at 15 of 75 sites (underlined in Table 2). Appendix 2 contains all replicate spore/L data. Water temperature ranged from 11° to 15°C in the study areas with Red Bluff Diversion Dam having 2 - 4°C greater temperature than up-river (Keswick dam gauge)(Fig. 4). Sentinel salmon *C.shasta* infection occurred over this range of temperatures.

Table 2. Mean *C. shasta* spore/L in water samples collected from the Sacramento River at ACID, Highway 44 bridge (Hwy44), Bonnyview bridge (BON), Anderson River Park (ARP), Balls Ferry (BALLF), Jelly's Ferry bridge (JELLY), Bend bridge (BEND), and Red Bluff Diversion Dam (RBDD) between July 13 and November 16, 2016. Values where one of the subsamples was censored are underlined; the complete data set is listed in Appendix 2.

	ACID	HWY44	BON	ARP	BALLF	JELLY	BEND	RBDD
7/13/2016	0	2.1	1.1	3.6	3.1	ND	8.1	9.2
7/27/2016	0	0.3	0	9.9	3.3	ND	9.8	25.8
8/10/2016	0	1.7	0	2.6	5.9	ND	12.5	12
8/24/2016	0.3	0	0	0	5.3	ND	11.5	9.4
9/7/2016	1.1	2.1	2.6	2.5	6	ND	2.1	3.3
9/21/2016	3.2	0.7	1.4	2.8	1.2	5.4	6.9	7.9
10/5/2016	0.5	0.6	0.9	3.6	0	0.8	5.7	5.8
10/19/2016	0.6	0.1	2	5.9	0.5	2.4	6.1	4.9
11/2/2016	0	0.1	1.3	3.4	1.8	0.5	0.8	0.2
11/16/2016	1.3	0	0.5	1	4.3	4.2	12.3	4.6

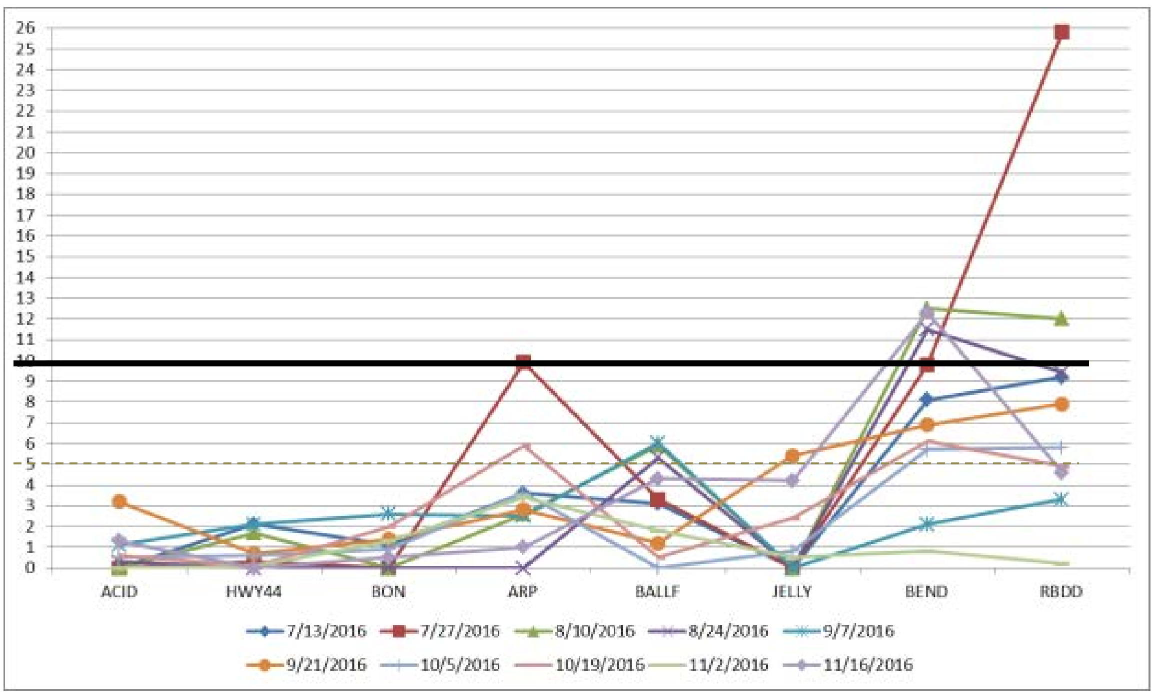

Figure 3. Mean *C. shasta* spore/L data for river samples collected from July 13 to November 16, 2016 at ACID, Highway 44 bridge (HWY44), Bonnyview bridge (BON), Anderson River Park (ARP), Balls Ferry bridge (BALLF), Jelly Ferry bridge (JELLY), Bend bridge (BEND) and Red Bluff Diversion Dam (RBDD). Solid line is the 10 spore/L threshold reported for mortality and dotted line is 5 spore/L.

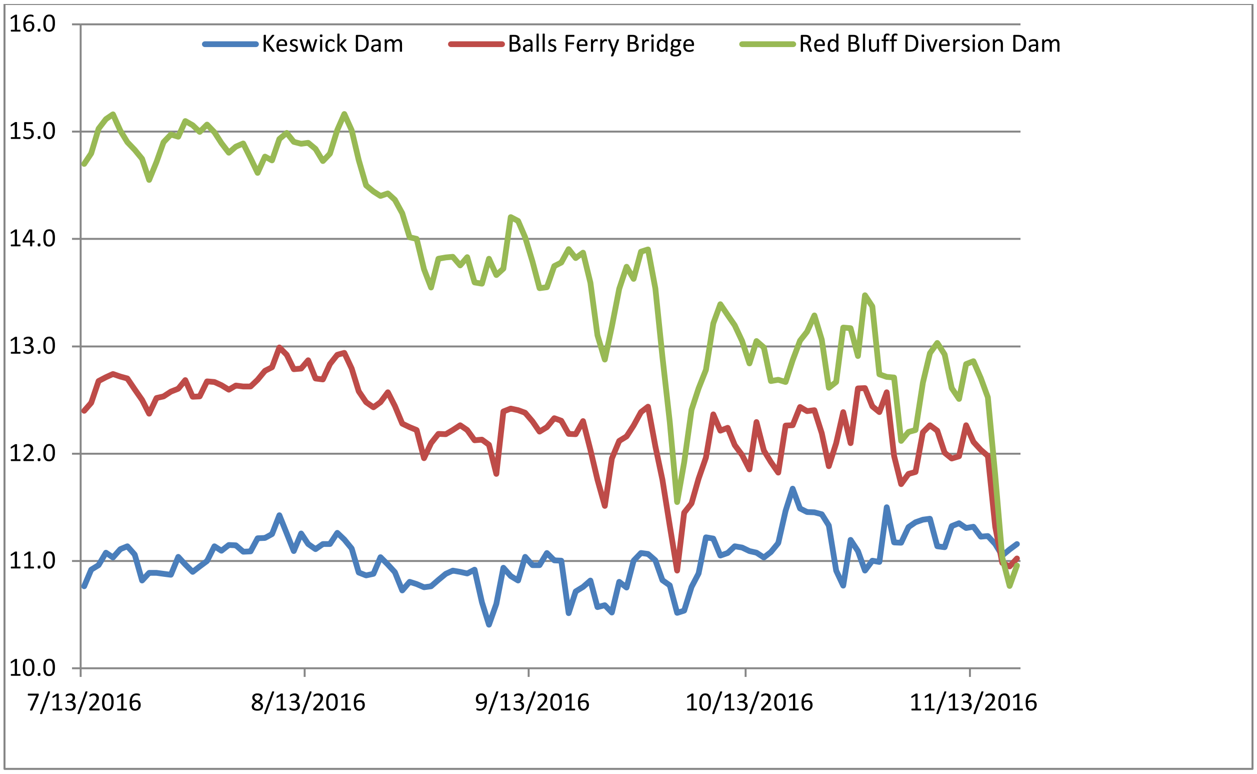

Figure 4. Mean daily temperature profile at Keswick Dam, Balls Ferry, and Red Bluff Diversion Dam during the study period (July – November 2016)

Sentinel salmon- 16 separate sentinel groups were exposed between July 25 and October 11, 2016 (Table 3). Three cages and their onset probes (Anderson River Park August 15-19 and one cage replicate at Posse Grounds October 6-11) were missing at the end of their respective exposure. Additionally all September 26-30 exposure groups, except the Red Bluff group, were killed soon after return to the wetlab due to a chlorine contamination of a partially open recirculation circuit. River temperatures ranged from 11° to 15°C and mean fork length of the sentinels ranged from 74 to 100 mm (Table 3).

C. shasta trophozoites were observed in the intestinal tracts of 20% of the sentinel survivors (58 / 290) while *P.minibicornis* trophozoites were observed in the kidney glomeruli of 69% (199 / 290;Table 4). None of the infected fish had histological ratings indicating a diseased state (#2). There was a general trend of increasing infection with distance downriver (Fig. 5 and 6). The upstream Posse Grounds site had little (1 fish in July) to no infectivity (3 of 4 exposure had 0 prevalence) for either *C. shasta* or *P.minibicornis* (Table 4). In contrast, sentinel salmon exposed at both Bend Bridge and Red Bluff Diversion dam showed similar prevalence of infection per exposure for *C. shasta* (6 – 55%) and *P. minibicornis* (≥ 72%). This prevalence pattern was higher than cohorts held at the upstream sites (Table 4; Figure 5 and 6). Based on both the eDNA and sentinel data, it appears there is an "infectious zone" in the study area between Anderson River Park and Red Bluff Diversion Dam (approximately 40 rm). Spore concentration (sampled during week of exposure) and sentinel *C. shasta* prevalence of infection was moderately correlated (Linear Regression, R^2=0.55, P< 0.01).

Limited mortality (5 – 15%) during the rearing period occurred in 6 of 16 exposure groups. Nine sentinel mortalities were collected and frozen over the study. Molecular analysis of their tissues was not performed as they died $\leq$ 11 d post exposure. At the 13 - 17°C rearing temperature, it is unlikely that clinical enteronecrosis would occur in this short of time unless actinospore concentration during the exposure was quite high. The lack of clinical enteronecrosis in the survivors indicates that exposure actinospore challenge was not severe. In general, low actinospore exposure produced asymptomatic infections in the sentinel groups.

Table 3. Sentinel exposure mean river temperature (°C) and fork length (FL, mm) for juvenile Chinook held for 5 d (Exposure dates) at Posse Grounds , Anderson River Park, Bend Bridge, and Red Bluff Diversion Dam between July and October 2016 and reared for 23- 25 d post-exposure in the wetlab prior to tissue sample collection.

Exp. Dates	Group	River Temp	FL
July25-29	POSSE GR.	11.2	79
	ANDERSON RP	12.5	74
	BEND BRDG	14.3	80
	RBDD	15.1	81
Aug15-19	POSSE GR.	11.5	78
	ANDERSON RP	N/A	N/A
	BEND BRDG	14.0	92
	RBDD	15.0	89
Sep6-10	POSSE GR.	11	97
	ANDERSON RP	11.8	94
	BEND BRDG	13.4	82
	RBDD	13.8	96
Sep26-30	POSSE GR.	N/A	N/A
	ANDERSON RP	N/A	N/A
	BEND BRDG	N/A	N/A
	RBDD	13.9	ND
Oct6-11	POSSE GR.	N/A	100
	ANDERSON RP	11.9	100
	BEND BRDG	12.9	96
	RBDD	13.3	100

N/A not available as group or temperature probe was lost prior to sampling

Table 4. Prevalence of *C. shasta* and *P.minibicornis* infection (POI) in sentinel salmon (Posse Grounds, Anderson River Park, Bend Bridge, and Red Bluff Diversion Dam sites) intestine and kidneys by histological disease rating (CS and PM #1 = parasite present with little to no tissue inflammation, #2 = parasite present with significant inflammation, "disease state"). Percent mortality data is during the 21 d rearing period post-exposure.

		Percent Mortality	CS1	CS2	POI-CS	PM1	PM2	POI-PM
July25-29	POSSE GR.	0%	1 /20	0/20	1/20 (5%)	1 /20	0/20	1/20 (5%)
	ANDERSON RP	0%	3/20	0/20	3/20 (15%)	19/20	0/20	18/20 (95%)
	BEND BRDG	0%	11/20	0/20	11/20 (55%)	16/20	0/20	16/20 (80%)
	RBDD	0%	9 /20	0/20	9/20 (45%)	19/19	0/19	19/19 (100%)
Aug15-19	POSSE GR.	0%	0/20	0/20	0/20 (0%)	0/20	0/20	0/20 (0%)
	ANDERSON RP	N/A *	N/A *	N/A *	N/A *	N/A *	N/A *	N/A *
	BEND BRDG	0%	6/20	0/20	6/20 (30%)	17/20	0/20	17/20 (85%)
	RBDD	5%	6/19	0/19	6/19 (32%)	18/ 19	0/19	18/19 (95%)
Sep6-10	POSSE GR.	0%	0/20	0/20	0/20 (0%)	0/20	0/20	0/20 (0%)
	ANDERSON RP	10%	2/19	0/19	2/19 (11%)	11/19	0/19	11/19 (58%)
	BEND BRDG	10%	1/18	0/18	1/18 (6%)	13/18	0/18	13/18 (72%)
	RBDD	15%	2/18	0/18	2/18 (11%)	15/18	0/18	15/18 (83%)
Sep26-30	POSSE GR.	N/A **	N/A **	N/A **	N/A **	N/A **	N/A **	N/A **
	ANDERSON RP	N/A **	N/A **	N/A **	N/A **	N/A **	N/A **	N/A **
	BEND BRDG	N/A **	N/A **	N/A **	N/A **	N/A **	N/A **	N/A **
	RBDD	0%	7/20	0/20	7/20 (35%)	19/20	0/20	19/20 (95%)
Oct6-11	POSSE GR.	0%	0/10	0/10	0/10 (0)%)	0/10	0/10	0/10 (0)%)
	ANDERSON RP	10%	5/18	0/18	5/18 (28)%)	13/20	0/20	13/20 (65)%)
	BEND BRDG	10%	3/18	0/18	3/18 (11)%)	18/18	0/18	18/18 (100)%)
	RBDD	0%	2/20	0/20	2/20 (10)%)	20/20	0/20	20/20 (100)%)

N/A* sample not available as cage missing at pickup
N/A** sample not available as entire exposure group killed by chlorine exposure in wetlab.

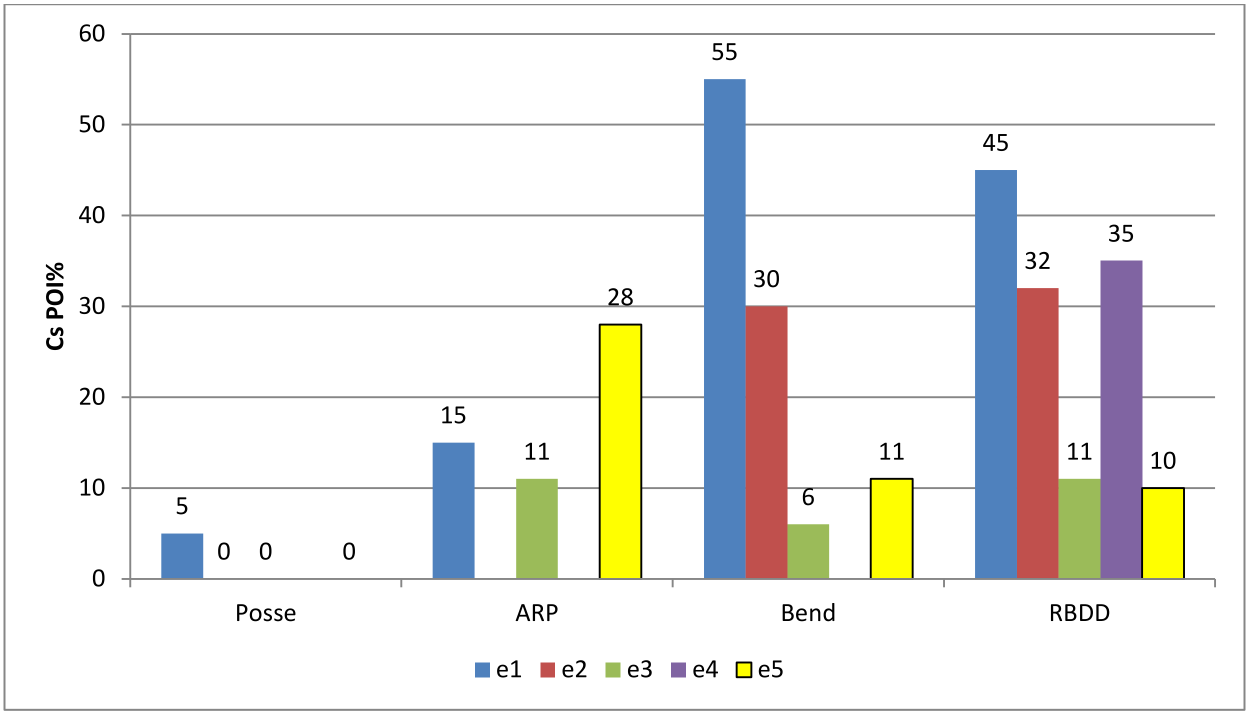

Figure 5. Prevalence of ***C.shasta*** infection in sentinel salmon from 5 exposures (e1 = 7/25-29, e2= 8/15-19, e3 = 9/6-10, e4=9/26-30, and e5=10/6-11) at Posse ground, Anderson River Park (ARP), Bend Bridge, and Red Bluff Diversion Dam . In exposure 4, only the Red Bluff Diversion Dam group was tested due to mishap.

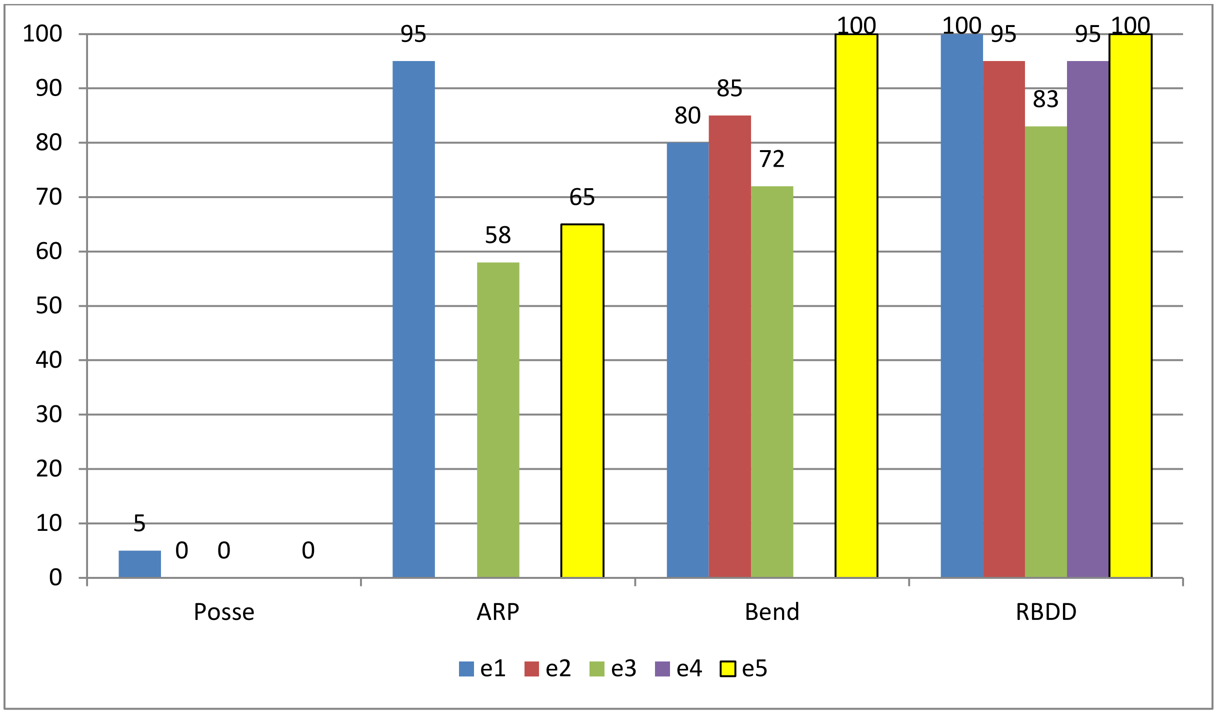

Figure 6. Prevalence of ***P.minibicornis*** infection in sentinel salmon from 5 exposures (e1 = 7/25-29, e2= 8/15-19, e3 = 9/6-10, e4=9/26-30, and e5=10/6-11) at Posse Ground, Anderson River Park (ARP), Bend Bridge, and Red Bluff Diversion Dam. In exposure 4, only the Red Bluff Diversion Dam group was tested due to mishap.

Discussion:

As in 2015, the prevalence and severity of *C. shasta* infection observed in naturally-produced Winter-run Chinook fry, captured at Red Bluff Diversion Dam, did not reflect these same data in sentinel salmon (Foott 2016). The prevalence of asymptomatic *C. shasta* infection was 7.6% in naturally-produced fry while specific sentinel groups had up to 55% prevalence. This difference in response could be influenced by their respective exposure history (specific micro environment and duration of actinospore challenge), cage stress impaired immune function in sentinels, or potential host (Winter-run vs Late fall) genetic resistance. Given the spatial pattern of infectivity observed in this study, we hypothesize that rate of movement through the approximate 40 rm infectious zone may limit fry exposure and subsequent infection response to these myxozoan parasites.

Spore concentrations were higher at downstream sites (Anderson River Park and downstream) and decreased after August. One exception to this trend was a 12.3 spore/L mean value at Bend Bridge on November 26, 2016. Only 6 samples were above the 10 spore/L threshold associated with 40% mortality in sentinel salmon exposed to the Klamath River for 3 d (Hallett et al. 2012). This disease response was not observed in our sentinel fish exposed at sites having $\geq$ 10 spore/L samples collected within a week of their exposure. Our decision to censor 20% of the *C.shasta* eDNA data was based on the high variability among some site replicates but did not change the overall trend of low spore concentration. The complete data set is listed in Appendix 2. The high variability in *C.shasta* DNA content within some river sample sets is likely an artifact of the collection method (single liter samples collected by wading) particularly when spore concentrations are low (Ellison et al. 2006, S. Atkinson Oregon State University pers. comm.). In addition to natural heterogeneity of actinospores within the river, grab samples collected by wading could be affected by actinospores associated with disturbed sediment. Future water sampling will employ a single larger volume collection, pointed upstream of the collector, from which 1-Liter subsamples are removed for filtration and analysis.

We hypothesize that the reduced *C.shasta* infectivity and disease response of sentinels exposed in 2016 compared to 2015 was influenced by higher 2016 Sacramento River flows. Increased river flow can affect infectivity by decreasing polychaete (alternate host) density, reduce transmission of both myxospores (to polychaete) and actinospores (to salmon) via both dilution and velocity effects, and through temperature reduction during the summer (Alexander et al. 2014, Ray and Bartholomew 2013, Ray et al. 2012).

All three sample types (naturally-produced fry, sentinel salmon, eDNA) demonstrated low *C.shasta* infectivity in the upper Sacramento River between July and November 2016. As in 2015, the prevalence and severity of *C.shasta* infection in wild Winter-run Chinook fry captured at Red Bluff Diversion Dam did not reflect these data in sentinel salmon exposed for 5 d (Foott 2016). There was no indication of disease impact due to either *C.shasta* or *P. minibicornis* infection on juvenile Winter-run Chinook in the upper Sacramento during the study period.

Acknowledgements: Anne Voss for transporting sample supplies between the FHC and Red Bluff FWO as well as figure 4, Samantha Adams (Red Bluff FWO) for figure 1, and Scott Freund for assistance with sentinel fish. Oregon State University Bartholomew laboratory for eDNA qPCR analysis (Stephen Atkinson, Sascha Hallett and Jamie Graen), and, both Jim Smith 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 (preparation and microscopy), and report

RS – sentinel field deployment, sampling, blocking and staining of histological specimens,

KN – filtration, coordination of sample shipments for eDNA,

S Voss – water collection, RBDD feral fry collection, and temperature data.

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Appendix 1 Histological data on RBDD winter-run fry.

CS1 = few C.shasta trophozoites with little to no inflammation
CS2 = Cshasta trophozoites associated with significant inflammation/necrosis ("diseased")
PM1 = Pminibicornis trophzoites in glomerulus and/or tubules with little to no inflammation ("glomerulonephritis")
PM2 = Pminibicornis trophzoites in glomerulus and/or tubules associated with glomerulonephritis (diseased")
gill - observation of parasite, 0 = none
abn note = observation of abnormality, rankings [HP= hyperplasia, ICR= inflammation
none = none or insufficient tissue to evaluate

block ID	sample date	2016RBDD fish ID	CS1	CS2	abn note	gill	PM#	abn note	note
9500	9-Sep	R1	0	0	0	ND	0	0	yolk present=swimup fry
9501	9-Sep	R2	0	0	0	ND	none	0	yolk present=swimup fry
9502	9-Sep	R3	0	0	0	ND	0	0	yolk present=swimup fry
9503	9-Sep	R4	0	0	0	ND	0	0	yolk present=swimup fry
9504	9-Sep	R5	0	0	0	ND	0	0	yolk present=swimup fry
9505	13-Sep	R6	0	0	0	ND	0	0	
9506	13-Sep	R7	0	0	0	ND	none	0	
9507	13-Sep	R8	0	0	0	ND	none	0	
9508	13-Sep	R9	0	0	0	ND	none	0	yolk present=swimup fry
9509	13-Sep	R10	0	0	0	ND	none	0	yolk present=swimup fry
9510	13-Sep	R11	0	0	0	ND	0	0	
9511	13-Sep	R12	0	0	0	ND	0	0	
9512	13-Sep	R13	0	0	0	ND	none	0	
9513	13-Sep	R14	0	0	0	ND	0	0	yolk present=swimup fry
9514	13-Sep	R15	0	0	0	ND	0	0	yolk present=swimup fry
9515	20-Sep	R16	0	0	0	ND	0	0	
9516	20-Sep	R17	none	none	0	ND	0	0	
9517	20-Sep	R18	0	0	0	ND	0	0	yolk present=swimup fry
9518	20-Sep	R19	0	0	0	ND	0	0	
9519	20-Sep	R20	0	0	0	ND	0	0	yolk present=swimup fry
9520	20-Sep	R21	0	2	Mutifocal HP-necrosis	ND	0	edema interstitum - likely due to gut necrosis	yolk present=swimup fry
9521	20-Sep	R22	0	0	0	ND	0	0	
9522	20-Sep	R23	0	0	0	ND	0	0	
9523	20-Sep	R24	0	0	0	ND	none	0	yolk
9524	20-Sep	R25	0	0	0	ND	0	0	yolk

9538	27-Sep	R26	0	0	0	0	none	0	
9539	27-Sep	R27	0	0	0	0	0	0	
9540	27-Sep	R28	0	0	0	0	none	0	
9541	27-Sep	R29	0	0	0	0	0	0	
9542	27-Sep	R30	0	0	0	0	0	0	
9543	27-Sep	R31	0	0	0	0	0	0	
9544	27-Sep	R32	0	0	0	0	none	0	
9545	27-Sep	R33	0	0	0	0	0	0	
9546	27-Sep	R34	0	0	0	0	none	0	
9547	27-Sep	R35	0	0	0	0	none	0	
9626	5-Oct	R36	0	0	0	0	none	0	
9627	5-Oct	R37	0	0	0	0	none	0	
9628	5-Oct	R38	0	0	0	0	0	0	
9629	5-Oct	R39	0	0	0	0	0	0	
9630	5-Oct	R40	0	0	0	0	none	0	
9631	5-Oct	R41	0	0	0	0	0	0	
9632	5-Oct	R42	0	0	0	0	0	0	
9633	5-Oct	R43	0	0	0	0	0	0	
9634	5-Oct	R44	0	0	0	0	0	0	
9635	5-Oct	R45	0	0	0	0	none	0	
9636	12-Oct	R46	0	0	0	0	0	0	
9637	12-Oct	R47	0	0	0	0	0	0	
9638	12-Oct	R48	0	0	0	0	0	0	
9639	12-Oct	R49	0	0	0	0	0	0	
9640	12-Oct	R50	0	0	0	0	0	0	
9641	12-Oct	R51	0	0	hp1	0	none	0	
9642	12-Oct	R52	0	0	0	0	0	0	
9643	12-Oct	R53	0	0	0	0	0	0	
9644	12-Oct	R54	0	0	0	0	0	0	
9645	12-Oct	R55	0	0	0	0	0	0	

9666	20-Oct	R56	0	0	0	none	0		
9667	20-Oct	r57	0	0	0	0	0		
9668	20-Oct	R58	0	0	0	0	1		
9669	20-Oct	R59	0	0	Inflam acinar/visceralfat	0	0		
9670	20-Oct	R60	0	0	0	0	1		
9671	20-Oct	R61	1	0	0	0	1		1troph in muscularis
9672	20-Oct	R62	0	0	0	0	0		monocyte cuffing in liver
9673	20-Oct	R63	0	0	0	0	0		
9674	20-Oct	R64	0	0	0	0	0		
9675	20-Oct	R65	0	0	0	0	0		
9750	42670	R66	0	0	0	0	1	0	
9751	42670	R67	0	0	0	0	0	0	
9752	42670	R68	0	0	0	0	none	0	
9753	42670	R69	0	0	0	0	0	0	
9754	42670	R70	0	0	0	none	0	0	
9755	42670	R71	1	0	0	0	0	?0	GMN1-2,thick mesangium
9756	42670	R72	0	0	0	0	1	0	
9757	42670	R73	0	0	0	0	0	0	
9758	42670	R74	0	0	0	0	1	0	
9759	42670	R75	0	0	0	0	1	0	
9745	42677	R76	1	0	0	0	1	0	high prev PM trophs withot GMN
9746	42677	R77	1	0	0	0	1	0	
9747	42677	R78	0	0	0	none	0	0	
9748	42677	R79	0	0	0	0	0	0	
9749	42677	R80	1	0	0	none	none	0	

Appendix 1. eDNA data set.

date	Locality	bottle	spore equivalents / L	3-bottle average	outlier drop average
7/13/2016	ACID	1	0.0	0.0	
7/13/2016	ACID	2	0.0		
7/13/2016	ACID	3	0.0		
7/13/2016	Anderson River Park	1	6.6	3.6	
7/13/2016	Anderson River Park	2	3.4		
7/13/2016	Anderson River Park	3	0.8		
7/13/2016	Balls Ferry Bridge	1	1.2	3.1	
7/13/2016	Balls Ferry Bridge	2	3.6		
7/13/2016	Balls Ferry Bridge	3	4.3		
7/13/2016	Bend Bridge	1	7.7	8.1	
7/13/2016	Bend Bridge	2	5.8		
7/13/2016	Bend Bridge	3	10.8		
7/13/2016	Bonnyview Bridge	1	2.7	1.1	
7/13/2016	Bonnyview Bridge	2	0.0		
7/13/2016	Bonnyview Bridge	3	0.5		
7/13/2016	Hwy44	1	5.1	2.1	
7/13/2016	Hwy44	2	0.0		
7/13/2016	Hwy44	3	1.2		
7/13/2016	Red Bluff	1	7.5	9.2	
7/13/2016	Red Bluff	2	11.3		
7/13/2016	Red Bluff	3	8.9		
7/27/2016	ACID	1	0.0	0.0	
7/27/2016	ACID	2	0.0		
7/27/2016	ACID	3	0.0		
7/27/2016	Anderson River Park	1	13.2	6.6	9.9
7/27/2016	Anderson River Park	2	6.6		
7/27/2016	Anderson River Park	3	0.0		
7/27/2016	Balls Ferry Bridge	1	3.5	3.3	
7/27/2016	Balls Ferry Bridge	2	1.2		
7/27/2016	Balls Ferry Bridge	3	5.1		
7/27/2016	Bend Bridge	1	8.3	9.8	
7/27/2016	Bend Bridge	2	9.4		
7/27/2016	Bend Bridge	3	11.5		
7/27/2016	Bonnyview Bridge	1	4.5	1.5	0
7/27/2016	Bonnyview Bridge	2	0.0		
7/27/2016	Bonnyview Bridge	3	0.0		
7/27/2016	Hwy44	1	0.0	0.3	
7/27/2016	Hwy44	2	0.6		
7/27/2016	Hwy44	3	0.2		
7/27/2016	Red Bluff	1	19.7	25.8	
7/27/2016	Red Bluff	2	27.1		
7/27/2016	Red Bluff	3	30.7		

Appendix 1. eDNA data set.

8/10/2016	ACID	1	0.0	1.8	0
8/10/2016	ACID	2	0.0		
8/10/2016	ACID	3	5.4		
8/10/2016	Anderson River Park	1	3.2	2.6	
8/10/2016	Anderson River Park	2	2.9		
8/10/2016	Anderson River Park	3	1.6		
8/10/2016	Balls Ferry Bridge	1	1.2	5.9	
8/10/2016	Balls Ferry Bridge	2	8.2		
8/10/2016	Balls Ferry Bridge	3	8.2		
8/10/2016	Bend Bridge	1	10.7	12.5	
8/10/2016	Bend Bridge	2	18.4		
8/10/2016	Bend Bridge	3	8.5		
8/10/2016	Bonnyview Bridge	1	6.1	2.0	0
8/10/2016	Bonnyview Bridge	2	0.0		
8/10/2016	Bonnyview Bridge	3	0.0		
8/10/2016	Hwy44	1	0.0	17.6	1.7
8/10/2016	Hwy44	2	4.7		
8/10/2016	Hwy44	3	34.0		
8/10/2016	Hwy44	3	49.0		
8/10/2016	Hwy44	4	0.3		
8/10/2016	Red Bluff	1	12.6	12.0	
8/10/2016	Red Bluff	2	5.4		
8/10/2016	Red Bluff	3	18.0		
8/24/2016	ACID	1	0.0	0.3	
8/24/2016	ACID	2	0.0		
8/24/2016	ACID	3	0.8		
8/24/2016	Anderson River Park	1	17.2	5.7	0
8/24/2016	Anderson River Park	2	0.0		
8/24/2016	Anderson River Park	3	0.0		
8/24/2016	Balls Ferry Bridge	1	4.7	3.5	
8/24/2016	Balls Ferry Bridge	2	0.0		
8/24/2016	Balls Ferry Bridge	3	5.8		
8/24/2016	Bend Bridge	1	9.0	7.7	11.5
8/24/2016	Bend Bridge	2	0.0		
8/24/2016	Bend Bridge	3	14.0		
8/24/2016	Bonnyview Bridge	1	0.0	0.0	
8/24/2016	Bonnyview Bridge	2	0.0		
8/24/2016	Bonnyview Bridge	3	0.0		
8/24/2016	Hwy44	1	0.0	0.0	
8/24/2016	Hwy44	2	0.0		
8/24/2016	Hwy44	3	0.0		
8/24/2016	Red Bluff	1	9.7	9.4	
8/24/2016	Red Bluff	2	14.0		
8/24/2016	Red Bluff	3	4.6		

Appendix 1. eDNA data set.

9/7/2016	ACID	1	0.2	1.1	
9/7/2016	ACID	2	0.5		
9/7/2016	ACID	3	2.4		
9/7/2016	Anderson River Park	1	2.0	2.5	
9/7/2016	Anderson River Park	2	1.6		
9/7/2016	Anderson River Park	3	3.7		
9/7/2016	Balls Ferry Bridge	1	4.1	4.0	6.0
9/7/2016	Balls Ferry Bridge	2	7.9		
9/7/2016	Balls Ferry Bridge	3	0.0		
9/7/2016	Bend Bridge	1	1.0	2.1	
9/7/2016	Bend Bridge	1	1.4		
9/7/2016	Bend Bridge	2	3.2		
9/7/2016	Bend Bridge	2	2.9		
9/7/2016	Bend Bridge	3	5.5		
9/7/2016	Bonnyview Bridge	1	0.3	2.6	3.8
9/7/2016	Bonnyview Bridge	2	2.6		
9/7/2016	Bonnyview Bridge	3	5.0		
9/7/2016	Hwy44	1	1.6	2.1	
9/7/2016	Hwy44	2	3.1		
9/7/2016	Hwy44	3	1.6		
9/7/2016	Red Bluff	1	1.9	3.3	
9/7/2016	Red Bluff	2	4.3		
9/7/2016	Red Bluff	3	3.8		
9/21/2016	ACID	1	3.4	3.2	
9/21/2016	ACID	2	3.9		
9/21/2016	ACID	3	2.1		
9/21/2016	Anderson River Park	1	2.5	1.8	2.8
9/21/2016	Anderson River Park	2	0.0		
9/21/2016	Anderson River Park	3	3.0		
9/21/2016	Balls Ferry Bridge	1	0.9	1.2	
9/21/2016	Balls Ferry Bridge	1	0.8		
9/21/2016	Balls Ferry Bridge	2	0.9		
9/21/2016	Balls Ferry Bridge	2	0.7		
9/21/2016	Balls Ferry Bridge	3	1.8		
9/21/2016	Balls Ferry Bridge	3	1.9		
9/21/2016	Bend Bridge	1	3.7	6.9	
9/21/2016	Bend Bridge	2	10.4		
9/21/2016	Bend Bridge	3	6.6		
9/21/2016	Bonnyview Bridge	1	0.4	20.2	1.4
9/21/2016	Bonnyview Bridge	2	49.0		
9/21/2016	Bonnyview Bridge	2	29.0		
9/21/2016	Bonnyview Bridge	3	2.5		
9/21/2016	Hwy44	1	2.2	0.7	
9/21/2016	Hwy44	2	0.0		
9/21/2016	Hwy44	3	0.0		
9/21/2016	Jelly's Ferry Bridge	1	0.0	3.6	
9/21/2016	Jelly's Ferry Bridge	2	5.3		
9/21/2016	Jelly's Ferry Bridge	3	5.5		
9/21/2016	Red Bluff	1	4.5	7.9	
9/21/2016	Red Bluff	2	9.6		
9/21/2016	Red Bluff	3	9.6		

Appendix 1. eDNA data set.

10/5/2016	ACID	1	0.5	4.0	0.5
10/5/2016	ACID	2	10.8		
10/5/2016	ACID	3	0.6		
10/5/2016	Anderson River Park	1	1.0	3.6	
10/5/2016	Anderson River Park	2	0.3		
10/5/2016	Anderson River Park	3	9.7		
10/5/2016	Balls Ferry Bridge	1	0.0	0.0	
10/5/2016	Balls Ferry Bridge	2	0.0		
10/5/2016	Balls Ferry Bridge	3	0.0		
10/5/2016	Bend Bridge	1	2.9	5.7	
10/5/2016	Bend Bridge	2	3.8		
10/5/2016	Bend Bridge	3	10.4		
10/5/2016	Bonnyview Bridge	1	1.9	0.9	
10/5/2016	Bonnyview Bridge	2	0.4		
10/5/2016	Bonnyview Bridge	3	0.3		
10/5/2016	Hwy44	1	0.0	0.6	
10/5/2016	Hwy44	2	1.3		
10/5/2016	Hwy44	3	0.4		
10/5/2016	Jelly's Ferry Bridge	1	2.1	0.8	
10/5/2016	Jelly's Ferry Bridge	2	0.0		
10/5/2016	Jelly's Ferry Bridge	3	0.3		
10/5/2016	Red Bluff	1	11.2	5.8	
10/5/2016	Red Bluff	2	2.0		
10/5/2016	Red Bluff	3	4.1		
10/19/2016	ACID	1	1.7	0.6	
10/19/2016	ACID	2	0.0		
10/19/2016	ACID	3	0.0		
10/19/2016	Anderson River Park	1	8.7	5.9	
10/19/2016	Anderson River Park	2	2.3		
10/19/2016	Anderson River Park	3	6.8		
10/19/2016	Balls Ferry Bridge	1	0.4	0.5	
10/19/2016	Balls Ferry Bridge	2	0.7		
10/19/2016	Balls Ferry Bridge	3	0.3		
10/19/2016	Bend Bridge	1	6.2	6.1	
10/19/2016	Bend Bridge	2	4.3		
10/19/2016	Bend Bridge	3	7.8		
10/19/2016	Bonnyview Bridge	1	3.7	2.0	
10/19/2016	Bonnyview Bridge	2	1.7		
10/19/2016	Bonnyview Bridge	3	0.5		
10/19/2016	Hwy44	1	0.0	0.1	
10/19/2016	Hwy44	2	0.2		
10/19/2016	Hwy44	3	0.0		
10/19/2016	Jelly's Ferry Bridge	1	2.8	2.4	
10/19/2016	Jelly's Ferry Bridge	2	1.2		
10/19/2016	Jelly's Ferry Bridge	3	3.1		
10/19/2016	Red Bluff	1	1.5	4.9	
10/19/2016	Red Bluff	2	4.8		
10/19/2016	Red Bluff	3	8.4		

Appendix 1. eDNA data set.

11/2/2016	ACID	1	0.0	0.0	
11/2/2016	ACID	2	0.0		
11/2/2016	ACID	3	0.0		
11/2/2016	Anderson River Park	1	3.9	2.3	3.4
11/2/2016	Anderson River Park	2	0.0		
11/2/2016	Anderson River Park	3	2.9		
11/2/2016	Balls Ferry Bridge	1	0.5	1.8	
11/2/2016	Balls Ferry Bridge	2	3.0		
11/2/2016	Balls Ferry Bridge	3	1.9		
11/2/2016	Bend Bridge	1	1.5	0.8	
11/2/2016	Bend Bridge	2	0.1		
11/2/2016	Bend Bridge	3	0.7		
11/2/2016	Bonnyview Bridge	1	0.0	1.3	
11/2/2016	Bonnyview Bridge	2	0.6		
11/2/2016	Bonnyview Bridge	3	3.3		
11/2/2016	Hwy44	1	0.2	0.1	
11/2/2016	Hwy44	2	0.2		
11/2/2016	Hwy44	3	0.0		
11/2/2016	Jelly's Ferry Bridge	1	0.6	0.5	
11/2/2016	Jelly's Ferry Bridge	2	0.1		
11/2/2016	Jelly's Ferry Bridge	3	0.7		
11/2/2016	Red Bluff	1	0.3	0.2	
11/2/2016	Red Bluff	2	0.2		
11/2/2016	Red Bluff	3	0.0		
11/16/2016	ACID	1	1.9	1.3	
11/16/2016	ACID	2	0.6		
11/16/2016	ACID	3	1.4		
11/16/2016	Anderson River Park	1	2.5	1.0	
11/16/2016	Anderson River Park	2	0.3		
11/16/2016	Anderson River Park	3	0.3		
11/16/2016	Balls Ferry Bridge	1	6.2	4.3	6.4
11/16/2016	Balls Ferry Bridge	2	6.5		
11/16/2016	Balls Ferry Bridge	3	0.2		
11/16/2016	Bend Bridge	1	11.9	12.3	
11/16/2016	Bend Bridge	2	13.8		
11/16/2016	Bend Bridge	3	11.3		
11/16/2016	Bonnyview Bridge	1	0.4	0.5	
11/16/2016	Bonnyview Bridge	2	0.9		
11/16/2016	Bonnyview Bridge	3	0.0		
11/16/2016	Hwy44	1	0.0	1.1	0
11/16/2016	Hwy44	2	0.0		
11/16/2016	Hwy44	3	3.2		
11/16/2016	Jelly's Ferry Bridge	1	4.3	2.8	4.2
11/16/2016	Jelly's Ferry Bridge	2	4.0		
11/16/2016	Jelly's Ferry Bridge	3	0.0		
11/16/2016	Red Bluff	1	6.2	3.1	4.6
11/16/2016	Red Bluff	2	0.0		
11/16/2016	Red Bluff	3	3.0		