Ceratonova shasta infection in lower Feather River Chinook juveniles and trends in water-borne spore stages

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FULL RESEARCH ARTICLE

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Abstract

We performed a five-year (2015–2020) survey of juvenile natural Chinook Salmon (*Oncorhynchus tshawytscha*), adult salmon carcasses, and river water from the lower Feather River to determine infection prevalence, distribution, and spore quantity of the myxozoan parasite *Ceratonova shasta*. Average prevalence of infection in juvenile salmon collected from the high flow channel ranged from 45–58% depending on assay method. Initial infection of fry and detection of actinospore stage in river water began in late January or early February. Overt disease occurred in March and was lethal. Infection of the gill was detected weeks ahead of intestinal infection. Water-borne spore measurement and fish infection demonstrate an infectious zone beginning at the outlet of the Thermalito Afterbay. This zone is expanding downriver past the confluence of the Yuba River. Adult carcasses produce billions of myxospores annually that move downriver over the winter. *C. shasta* infection is one of several factors (predation, limited rearing habitat, elevated water temperature, water withdrawal, etc.) limiting natural Chinook Salmon recruitment in the Feather River.

Key words: *Ceratonova shasta,* Chinook Salmon, Feather River, infectious zone, myxospore

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Introduction

The lower Feather River is a tributary of the Sacramento River in Northern California and divided by flow regime into two distinct reaches (Fig. 1). The low flow channel (LFC) is a partially bypassed reach of the natural channel which extends from Oroville dam (river kilometer (rkm) 117.5) to the Thermalito Afterbay Outlet (TAO, rkm 94.9), a LFC spawning reach (rkm 96.5 – 107.8), and a high flow channel (HFC) beginning below the outlet to the confluence with the Sacramento River. Most of Lake Oroville's water is diverted through the Thermalito Forebay complex with approximately 25% or less of annual flow released down the LFC. The Thermalito complex warms cold reservoir water for agriculture and additional storage (Seesholtz et al. 2004). Water not diverted for agriculture is returned to the lower Feather River via the TAO. The HFC receives planktonic organisms flushed from the mesotrophic Thermalito complex (Seesholtz et al. 2004).

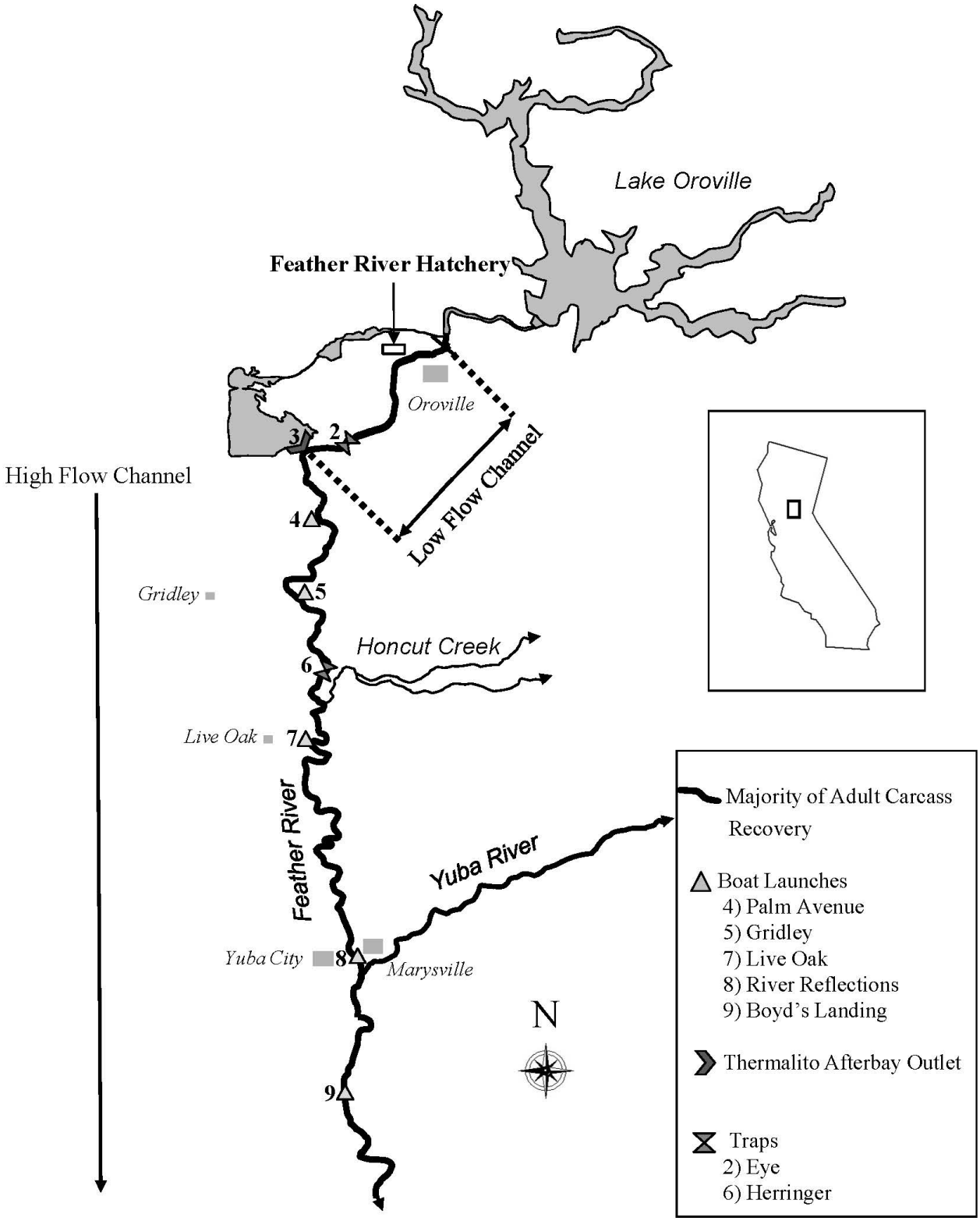


Figure 1. Map of study site on the lower Feather River (below Oroville Dam) and associated tributaries in

northern California, USA.
Both fall and spring-run Chinook salmon (*Oncorhynchus tshawytscha*) are endemic to the lower Feather River with ≥ 95% of adult spawning occurring in the LFC (Sommer et al. 2001). Since 2008, Feather River Fish Hatchery returns dominate the adult spawning population (Willmes et al. 2018). Emigration of juveniles occurs primarily as fry (< 50 mm) beginning in December and peaking in the January – February period (Sommer et al. 2001; Seeshotz et al. 2004). Despite natural spawning populations averaging 60,000 adults, natural recruitment is deemed quite low. Mercer and Kurth (2014) report a range of < 1% to 10% natural-spawner contribution to the hatchery between 2009–2011.

Ceratonova (syn. *Ceratomyxa*) *shasta* Noble 1950 (Atkinson et al. 2014) infects freshwater salmonid fishes and is enzootic to anadromous fish tributaries of the Pacific Northwest of the USA, including the Feather River (Hendrickson et al. 1989). Infection by *C. shasta* often results in enteronecrosis and anemia in juvenile salmon. This disease is a significant mortality factor for juvenile Chinook salmon in the Klamath River (Foott et al. 2004; Stocking et al. 2006; Fujiwara et al. 2011; Hallett et al. 2012). *C. shasta* has a complex life cycle, involving an invertebrate polychaete host (*Manayunkia occidentalis*)as well as the vertebrate salmon host (Atkinson et al. 2020; Bartholomew et al. 1997). 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 genotype and density, innate host resistance, and water temperature, infected fish can develop varying degrees of enteritis and associated anemia (Udey et al. 1975; Foott et al. 2004; Bjork and Bartholomew 2009; Ray et al. 2010; Hallett et al. 2012).

After approximately two weeks post-infection, the parasite can form the myxospore stage. Myxospore release typically occurs after the fish dies. If ingested by the filter-feeding polychaete it completes the life cycle after invading the worm's gut epithelium (Meaders and Hendrickson 2009). *M. occidentalis* inhabits tubes constructed of fine sediment and mucus in a variety of lotic conditions with highest densities found in low velocity areas (Stocking and Bartholomew 2007). It likely has an annual generation time (Willson et al. 2010). The myxosporean, *Parvicapsula minibicornis*, shares the same hosts and range as *C. shasta* (Bartholomew et al. 2006). It has a tropism for the kidney nephron and can induce glomerulonephritis in the salmon host. It is often a co-infection with *C. shasta* with 30–52% prevalence of infection in juvenile Feather River salmon during the 2015–2020 study period (Foott et al. 2004). While glomerulonephritis is an additive stressor, hemorrhagic enteronecrosis associated with *C. shasta* is the primary mortality factor for juvenile salmon. This report describes trends of *C. shasta* infection in lower Feather River natural Chinook juveniles and the water borne spore stages of the parasite.

Methods

Natural Chinook Salmon juveniles collected by California Department of Water Resource (CDWR) biologists from either rotary screw traps or beach seine (under California Department of Fish and Wildlife Scientific Collection Permit 9611 and 13341). Collections occurred from 2015–2020; however, no data are presented for 2017. Extreme high flows in 2017 reduced sample numbers to approximately 30% of our target (LFC = 84 and HFC = 315 over the 15-week period), limited actual collection period by half, and changed collection methods from rotary screw traps to limited beach seine collections. Staff transported natural fry (prognosis study) or hatchery "cage exposure" fish (sentinel) to the California–Nevada Fish Health Center wet lab and reared in 370 L circular tanks with aeration and flow-through, ambient river temperature water with daily feeding. Fish rearing and euthanization followed published guidelines (Jenkins et al. 2014). Dates reported as Julian Week (JW; **Table 1**). In 2018, staff transported eight groups

of “prognosis” fry from the Herringer trap to the wet laboratory and reared at ambient river temperatures for 21 days. This action occurred on a bi-weekly basis beginning JW5 through JW19 (due to high *C. shasta* mortality, the last group sampled after only 6 days). We used juvenile fall-run Chinook Salmon from Coleman National Fish Hatchery (Anderson, CA, USA) and Feather River Fish Hatchery (Oroville, CA, USA) in sentinel fish trials in 2015, 2016, and 2019. These populations are historically negative for *C. shasta* infection and tested negative prior to their exposures. Sentinel trials provide data on infection response of salmon to known exposure durations at specific river locations and dates. We exposed sentinels in the river within 0.01 m^3 live cages, transported and reared in the wet lab for 17 to 21 days post-exposure, and sampled for histology. Intestines from mortalities assayed for *C. shasta* DNA by Quantitative Polymerase Chain Reaction (QPCR). Water temperature for sentinel exposures and the Gridley site measured by Onset probes (Onset, Bourne, MA, USA, **https://www.onsetcomp.com**).

Table 1. Julian week and corresponding calendar dates.

Julian Week	Inclusive Dates
1	01 Jan–07 Jan
2	08 Jan–14 Jan
3	15 Jan–21 Jan
4	22 Jan–28 Jan
5	29 Jan– 04 Feb
6	05 Feb–11 Feb
7	12 Feb–18 Feb
8	19 Feb–25 Feb
9	26 Feb–04 Mar
10	05 Mar–11 Mar
11	12 Mar–18 Mar
12	19 Mar–25 Mar
13	26 Mar–01 Apr
14	02 Apr–08 Apr
15	09 Apr–15 Apr
16	16 Apr–22 Apr
17	23 Apr–29 Apr
18	30 Apr–06 May

Histology

We fixed euthanized fish in Davidson's fixative for histological examination or froze for QPCR analysis. Kidney, gill, and gastrointestinal tract were processed for 5µm paraffin sections and stained with hematoxylin and eosin (Humason 1979). Fry, smaller than 50mm, were de-calcified and processed as sagittal sections. We placed all tissues for a given fish on one slide and identified by a unique number code. Histological rankings of *C. shasta*-2 (CS2) 'clinical disease' were based on the presence of multifocal lesions of lamina propria hyperplasia or necrosis associated with parasite infection. The CS1 rating required the presence of the parasite but with minimal inflammatory changes.

Quantitative Polymerase Chain Reaction (QPCR)

We collected gill tissue (all arches) and intestine (rectum to the junction of small intestine with the pyloric ceca) separately and extracted DNA with Applied Biosystems MagMax express-96 Magnetic Particle Processer. A *C. shasta* QPCR assay targeting the 18S ribosomal DNA sequence was used to assay DNA extracted from fish tissues: forward primer (Cs-1034F 5' CCA GCT TGA GAT TAG CTC GGT AA), reverse primer (Cs-1104R CCC CGG AAC CCG AAA G), and probe (CsProbe-1058T 6FAMCGA GCC AAG TTG GTC TCT CCG TGA AAA C TAMRA) (Hallett and Bartholomew 2006). We performed all reactions (30µL) in a 96-well optical reaction plate using 0.9 µM of both primers, 0.25 µM of probe, 15 µL of TaqMan Universal PCR Master Mix, and 5uL of DNA template. Samples were tested in single well, and reactions were assayed using a 7300 Sequence Detection System (Applied Biosystems) with the following cycling conditions: 2 min at 50°C and 10 min at 95°C, followed by 40 cycles of 95°C for 15 sec and 60°C for 1 min. Each assay plate included a standard curve with three concentrations of reference standards (two replicates each) at known DNA copy number and two negative control wells. We obtained the *C. shasta* reference standard curve using synthesized DNA (gBlock Gene Fragments, Integrated DNA Technology, Coralville Iowa) containing the 18S ribosomal DNA target sequence. Specifically, 1 ng of DNA, corresponding to 6.83×10^9 copies of *C. shasta* DNA was serially diluted over ten orders of magnitude in Tris-ethylenediaminetetraacetic acid buffer. Using QPCR analysis software, we calculated the cycle threshold (C_T) values for each standard concentration (SDS software 7300 SDS v 1.4, Applied Biosystems). Criteria for a positive test result required samples to produce a change in normalized fluorescent signal (Δ Rn) greater than or equal to 100,000 florescent units and ≥ 5 copies. Low copy numbers are not reliable given the limitations of the Poisson distribution (Applied Biosystems 2016). A "disease" threshold of > 3.0 logs of *C. shasta* DNA were used to segregate data based on the relationship between this value and histological lesion of the intestine (Voss et al. 2020).

Water-borne Spore Measurement

We collected water samples with two methods: 1) four 1-liter simultaneous grab samples (majority of "single day longitudinal" water samples), or 2) four 1-liter samples obtained from a 24 h composite sample at the Herringer trap (Global Water Sampler WS700, ~600 mL each hour). Sample sites for both fish and water collection identified in **Figure 1**. Filtered (47mm, 5µm nitrocellulose, Millipore) water samples were analyzed for *C. shasta* DNA by Oregon State University (Hallett et al. 2012) and reported as mean spore/L for a one-day site collection.

Myxospores in Carcass and Water

In 2014, 2015, and 2020, we collected intestines from both fall and spring-run Chinook adult carcasses in the spawning reach (rkm 96.5–107.8). A scraping of the intestinal epithelium and lamina propria layers were examined microscopically and myxospores enumerated per mass of scraping material (Foott et al. 2016). Water sampling from the spawning reach and immediately above TAO (Eye trap rkm 96) conducted by three different methods to estimate myxospore concentration post-spawning. All filter extraction and QPCR analysis were conducted by Dr. Stephen Atkinson's laboratory at Oregon State University. In 2015, triplicate 1-L grab samples analyzed for *C. shasta* DNA as described above (Hallett et al. 2012). In 2016, 15-L samples were collected at the same locations, suspended particles allowed to settle for 24 hours, slow siphoning of the water column to the 1-L mark, and filtering of the lower 1-L suspension for *C. shasta* DNA analysis as described above. Spore/L estimates are divided by 15 to obtain concentration data. In 2020, 15-L samples passed through a 142 mm, 5.0 µm nitrocellulose filter (Merck Millipore Ltd) held in an in-line filter holder (Geotech Environmental Equipment, Inc.) under vacuum and the filter was processed in similar fashion as done for water samples.

Data Analysis

We performed linear regression and t-tests with SigmaPlot 12 software (Systat software Inc., San Jose, CA, USA) and calculated 95% confidence limit as standard error of the mean (SEM = standard deviation/squared root of sample number) multiplied by 1.96.

Results

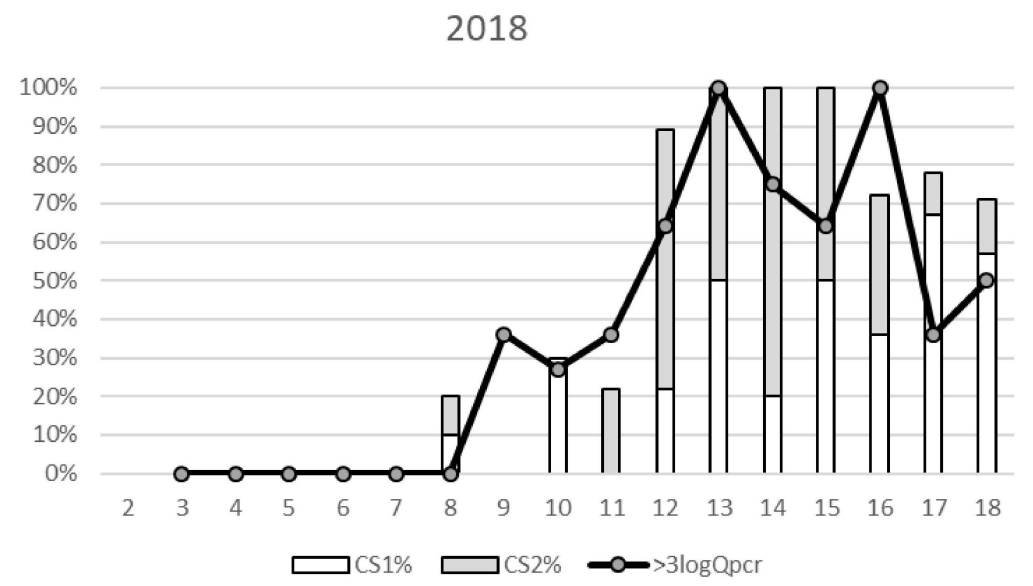
Juvenile Salmon Infection

Prevalence of infection and severity.—Limited histological examination of Chinook juveniles in April and May 2012–2014 demonstrated a marked difference in prevalence of *C. shasta* infection between LFC (0–5%) and HFC (≥ 68%) (USFWS National Wild Fish Health Survey, **www.fws.gov/project/national-wild-fish-health-survey-mapper**, June 2023). This disparity continued in the 2015–2020 surveys (**Table 2**). The more sensitive QPCR assay detected a range of 45–72% prevalence of infection in HFC salmon compared to 9–38% from LFC. Weekly prevalence data shows a marked increase in both infection and severity between Julian weeks (JW) 9–12 (**Fig. 2**). Prevalence of histological rating of CS2 (clinical disease) was similar to QPCR samples with ≥ 3 logs of parasite DNA in 2018, 2019, and 2020 (**Fig. 2**). Despite high flows in 2017 and relatively low spore/L concentrations (≤ 25) in 2018, most fish sampled in 2018 were rated as diseased (**Table 2**).

Table 2. Prevalence of *Ceratonova shasta* infection (± 95% CI) in juvenile Chinook Salmon intestine samples collected in the low or high flow reaches of the lower Feather River. Collections occurred from 1 Jan–30 Apr from 2015–2020. Data from fish assayed by histology or quantitative polymerase chain reaction (QPCR).

Year	High Flow Histology	High Flow QPCR	Low Flow Histology	Low Flow QPCR
2015	58% ± 12% (n = 64)	72% ± 10% (n = 71)	0% (n = 60)	9% ± 6% (n = 70)
2016	46% ± 9%, (n = 106)	61% ± 11% n = 64)	0% (n = 15)	28% ± 15% (n = 36)

Year	High Flow Histology	High Flow QPCR	Low Flow Histology	Low Flow QPCR
2018	40% ± 8% (n = 149)	58% ± 8% (n = 154)	0% (n = 32)	38% ± 16% (n = 37)
2019	34% ± 8% (n = 128)	45% ± 8% (n = 164)	18% ± 12% (n = 38)	32% ± 14% (n = 44)
2020	48% ± 8% (n = 159)	56% ± 8% (n = 164)	2% ± 4% (n = 46)	19% ± 11% (n = 53)


2018
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
CS1%
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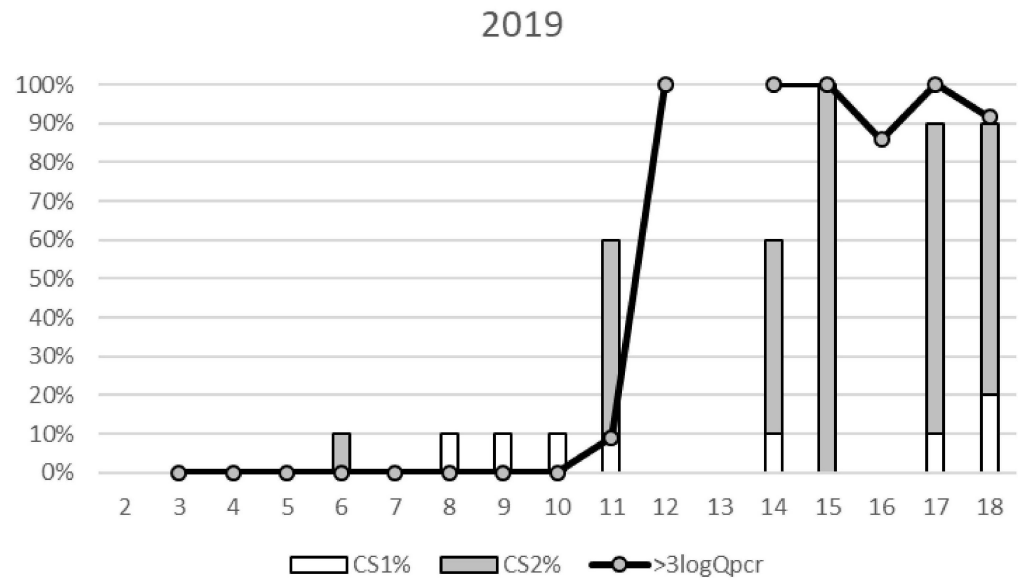
>3logQpcr


2019
100%
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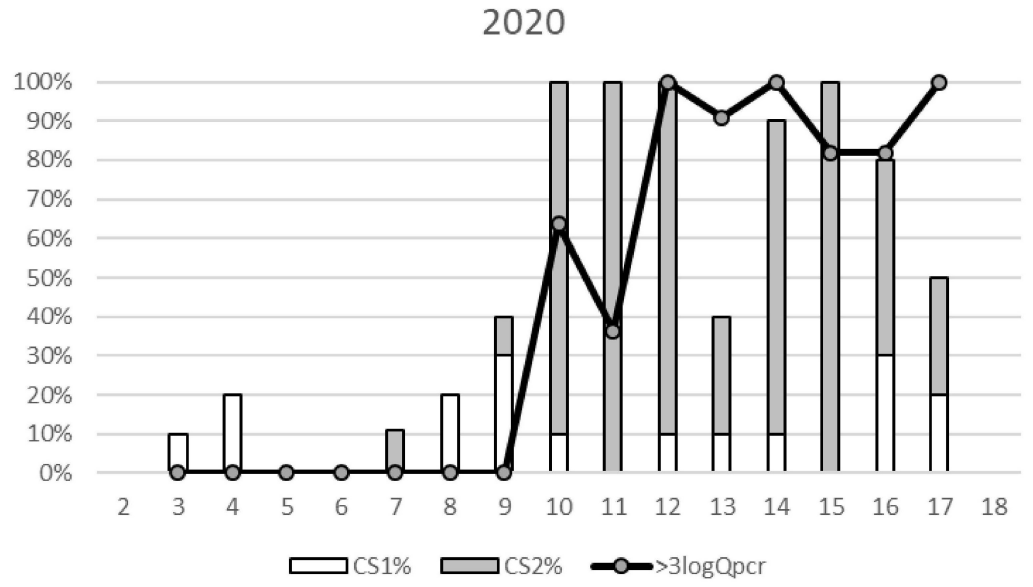
2020
100%
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40%
30%
20%
10%
0%
2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
CS1%
CS2%
>3logQpcr

Figure 2. Prevalence of *Ceratonova shasta* (CS) infection in Chinook juveniles sampled from Herringer trap in 2018 (n = 414), 2019 (n = 292), and 2020 (n = 324). Samples histologically rated as not clinical (CS1%, open bar) or clinical disease state (CS2%, gray bar). Separate QPCR samples rated as clinical disease when ≥ 3 logs of *C. shasta* DNA (> 3logQpcr, black line with gray circles). Data for Julian weeks 3–18.

Ceratonova shasta-associated mortality in the 2018 prognosis fry ranged from 0 to 85% with a peak in JW12. Disease prevalence in 21-day survivors mirrored the mortality with the peak (100%) in JW 12. Fry collected at Herringer trap were larger compared to Eye trap cohorts captured during JW10-13 (Fig. 3). This period corresponded with marked increase in the number of disease- rated fry at the HFC trap (Fig. 3). For instance, between JW10-13 of 2019 there was good correlation (R^2 of 0.506) between fork length and parasite copy number in Herringer trap fry (Fork length = 56.972 – (0.512 *Copy number), df = 52, P < 0.001). JW10 is also when more than 85% of natural fry have migrated past the Herringer trap (five-year average, Fig 3). 2015 was an outlier to this pattern as the increase in diseased fry (55%) occurred in JW8. In 2020, both gill and intestine were assayed by QPCR. We detected infection much earlier in gill tissue (JW 3 compared to JW6 from intestine) and at greater prevalence for weeks prior to any increase in intestinal infections (Fig. 4).

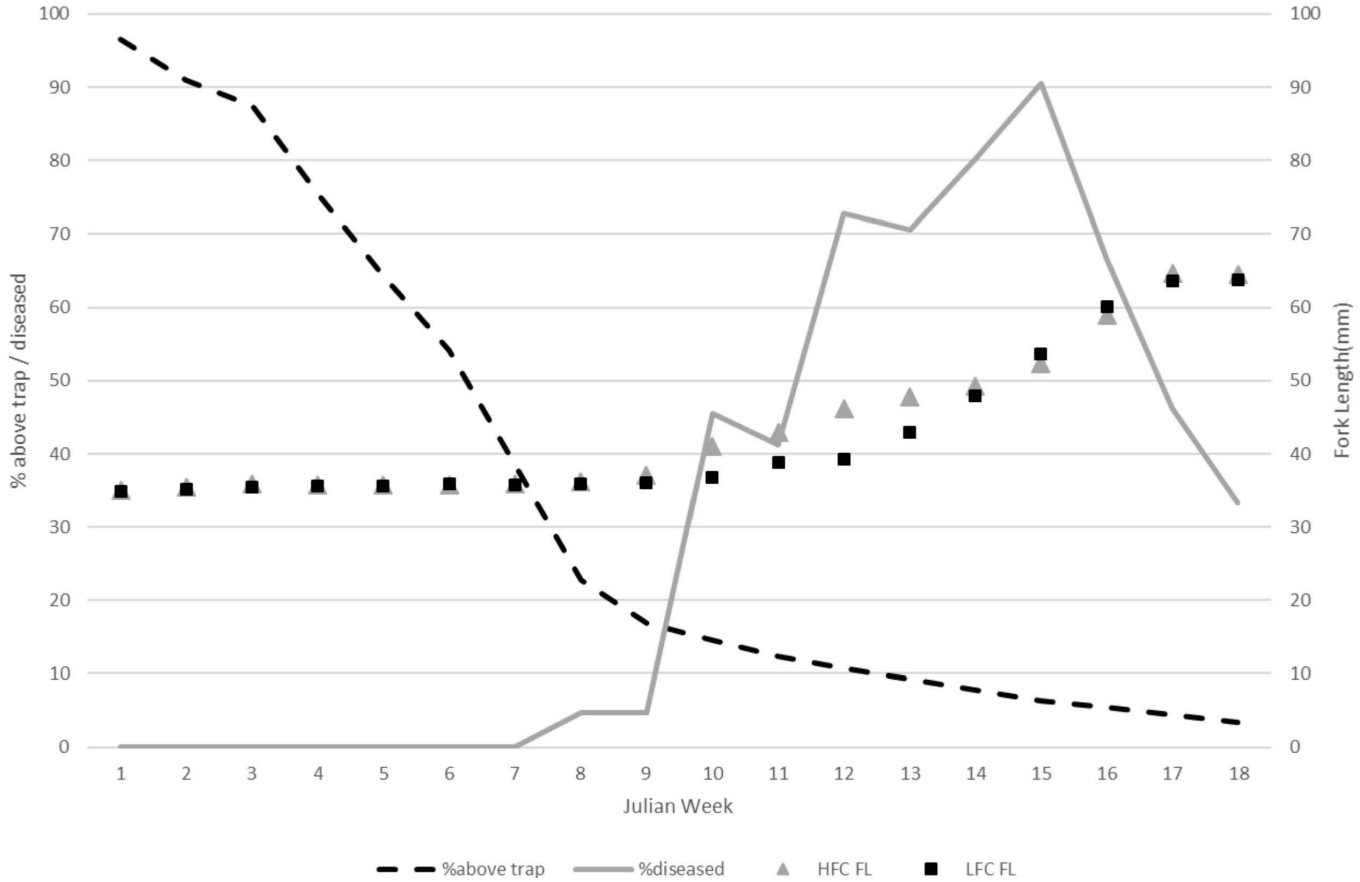

Figure 3. Relationship between 5-year average fry population above the Herringer trap (% above trap, dashed black line, n > 100,000 per year), 5-year average fork length (mm) at both Herringer (HFC FL, gray triangle) and Eye (LFC FL, black square) trap, and 5-year median percent disease-rated fry sampled per Julian week (gray line) at the Herringer trap (2015, n = 136; 2016, n = 178; 2018, n = 414; 2019, n = 292; 2020, n = 324).

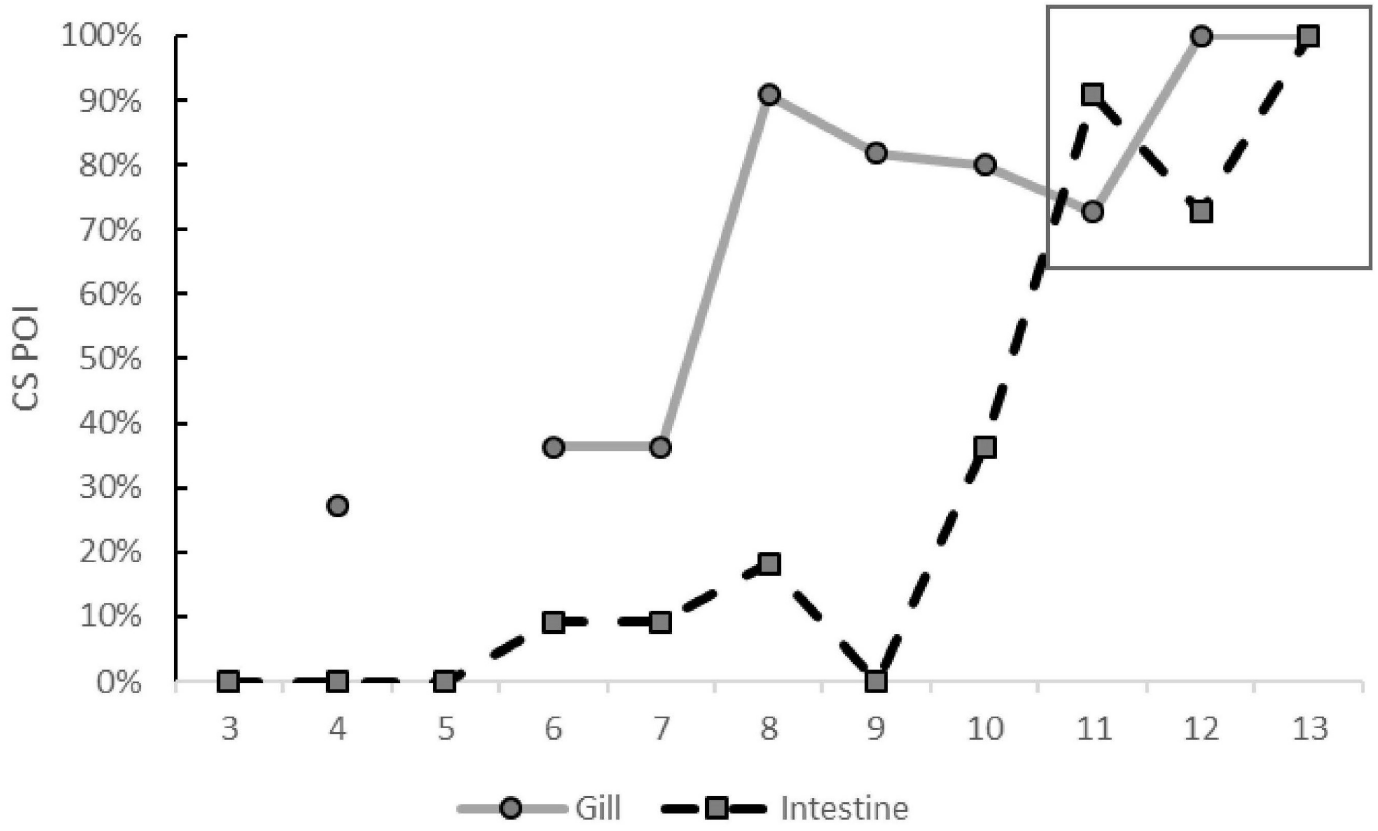

Figure 4. Prevalence of *Ceratonova shasta* (CS POI %) infection of the gill (circles and gray line, n = 108) and intestine (squares and black line, n = 110) of Chinook juveniles sampled at Herringer trap between Julian week 3 and 13 of 2020. Box area in weeks 11–13 highlights similar detection in the two tissues. *Sentinel salmon infection.*—In March and April of 2015 2016, and 2019, uninfected hatchery salmon were exposed to the lower Feather River for durations of 3–11 days (Table 3). These exposures showed the effect of exposure site, date, and duration on both infection and progression to a diseased state. In 2015, a 3-day exposure at two sites within the presumptive infectious zone showed both prevalence of infection and disease severity was greater at the downriver site (rkm 83.7) compared to rkm 93 (Table 3). A 6-day exposure at rkm 80.5 resulted in a similar prevalence of infection and disease as the 3-day exposure at rkm 83.7 however, it was much higher (70% prevalence of infection) than a similar 6-day exposure at rkm 35.9 (17%). The 11-day exposure during JW 15 showed a similar pattern with all fish from the rkm 80.5 site experiencing clinical disease compared to only 7% diseased at the lower river (rkm 29) site (Table 3). In 2016, three- or four-day exposures at rkm 80.5 had similar prevalence of infection (78 and 82%) and moderate levels of disease (7 and 13%) when conducted in February (JW 7) and late March (JW 13). Actinospore concentrations were deemed high (140 spores/L) for both exposures. In 2019, two- and four-day exposures at rkm 93, during February and March, were conducted to simulate typical fry transient times between LFC to the HFC trap site. Water samples at the exposure site measured 1–20 spores/L. The 4-day exposure groups had 2.3–6.4 greater POI (32–80%) than the 2-day groups (5–15%). None of the sentinels showed signs of disease.

Table 3. Prevalence (and %) of *Ceratonova shasta* infection (CS-POI) and fish rated as diseased from sentinel salmon exposed to the lower Feather River in Julian week (JW) 7–15 during 2015, 2016, and 2019. For each exposure, sites recorded in river kilometer (rkm), duration as days of exposure (DE), and mean daily temperature (MDT, °C).

Year	Rkm	JW	DE	MDT	CS-POI	Disease
2015	93.0	12	3	14	14/32 (44%)	2/32 (6%)
2015	93.0	15	3	16	14/32 (44%)	2/32 (6%)
2015	83.7	12	3	14	20/30 (67%)	2/30 (7%)
2015	83.7	15	3	15	24/30 (80%)	8/30 (27%)
2015	80.5	12	6	17	14/20 (70%)	4/20 (20%)
2015	80.5	15	11	16	15/15 (100%)	15/15 (100%)
2015	35.9	12	6	17	3/18 (17%)	0/18 (0%)
2015	29.0	15	11	18	15/28 (18%)	2/18(7%)
2016	80.5	7	3	12	35/45 (78%)	6/45 (13%)
2016	80.5	13	4	15	24/28 (82%)	2/28 (7%)
2019	93.0	7	2	9	1/20 (5%)	0/20 (0%)
2019	93.0	7	4	9	6/20 (32%)	0/20 (0%)
2019	93.0	9	2	10	3/21 (14%)	0/21 (0%)
2019	93.0	9	4	10	16/20 (80%)	0/20 (0%)
2019	93.0	11	2	10	3/20 (15%)	0/20 (0%)
2019	93.0	11	4	10	7/20 (35%)	0/20 (0%)

Spore Stages

Myxospore monitoring.—Prevalence of myxospores from adult carcasses was 53% (32/60), 80% (48/60), and 70% (14/20) in 2014, 2015, and 2020, respectively. The estimated quantity of myxospores observed in the intestine samples ranged from 49–875 million. We consider samples with 500,000 myxospores/scraping or more to be the major spore contributors to the river. These high spore contributors ranged from 21% (2020 samples) to 65% (2015). In 2015, two to three spores/L were measured in spawning reach (rkm 104.7) water samples between October and December (**Table 4**). In contrast, *C. shasta* DNA (2-5 spores/L) was only detected at Eye trap (directly above HFC) in October and early November (**Table 4**). A positive signal (> 0.03 spore/L) detected from both the spawning reach and Eye trap sites from late October 2016 through early January 2017. Heavy rains in the winter of 2017 increased turbidity and PCR inhibition occurred in 60% of the water samples. In 2020, positive signal was detected at Eye trap from early December through the last sample in early February. The spawning reach sample was only positive in early December. In contrast to the low spore concentrations discussed above, water samples within the HFC are >10 spores/L by mid-January. Fry infection currently indicates the presence of fish-infective actinospores. Spore concentration was markedly higher at these two sites in 2015 compared to 2016–2017 and 2020–2021. QPCR assay of filtered water cannot distinguish between fish-infective actinospores and worm-infective myxospores.

Table 4. Presumptive myxospore concentration (spore/L) in water samples collected from the spawning reach (rkm 104.7) and directly above the high flow channel (Eye trap, rkm 96.5). Sample collection occurred during and after 2015, 2016, and 2020 Chinook spawning (Oct–Feb).

Date	rkm 104.7	rkm 96.5
15 Oct 2015	1.72	1.26
21 Oct 2015	2.63	4.15
4 Nov 2015	2.77	2.13
20 Nov 2015	1.73	0
10 Dec 2015	2.93	0
27 Oct 2016	0.19	0.05
3 Nov 2016	0.39	0
9 Nov 2016	0.51	0.14
16 Nov 2016	0.26	0.03
1 Dec 2016	0.03	0.04
7 Dec 2016	0.12	0.09
15 Dec 2016	0.05	0.03
21 Dec 2016	0	0
28 Dec 2016	0.08	0.03
5 Jan 2017	0	0
2 Dec 2020	1.33	2.29
16 Dec 2020	0	0.48
31 Dec 2020	0.13	0.86
13 Jan 2021	0.28	0.67
28 Jan 2021	0.1	0.76
10 Feb 2021	0	0.57

HFC spore concentration.—Herringer trap spore/L data provides insight into conditions within the infectious zone (**Fig. 5**). Except for 2018, peak spore concentrations (≥ 120 spores/L) tend to occur between JW 7–10 (**Table 5**). Initial detection of *C. shasta* infection in Chinook fry occurred 1–7 weeks prior to the peak spore concentration and 0–4 weeks after spore concentrations reached ≥ 10 spores/L (**Table 5**). Mean daily water temperature was 9.5–13.2°C during these annual peaks. A series of samples, referred to as longitudinal sampling (collected on one day in an upstream manner at multiple locations), demonstrate spatial and temporal differences in actinospore concentration within and between years

(Table 6, Fig. 6). Timing of peak concentration biased by irregular sample collection schedule but tended to be in JW 11. Peak spore concentration was earlier (JW6) in 2019 and later (JW18) in 2018 (Table 6). 2018 was also unique in the markedly lower spore concentrations measured (peak was 4.8 times lower than 2019 peak). Longitudinal profiles demonstrate the markedly higher spore concentrations within an "infectious zone" downstream of TAO (rkm 94.9, Fig. 6). The location of peak spore concentration shifted downriver starting in 2019 from rkm 91.7–80.5 to rkm 73.7–49.1. Two lines of evidence suggest that the afterbay is not the primary source of actinospore production but rather the high flow reach itself. In May 2015, water samples collected directly above the TAO in the LFC (4.2 spores/L), within the afterbay channel (0.3 spores/L, outlet – river mixing zone (1.7 spores/L), and one rkm downriver of outlet (12.9 spores/L) indicate the minimal contribution of the afterbay. Similarly, no parasite DNA was detected in samples from the channel in January and February 2019.

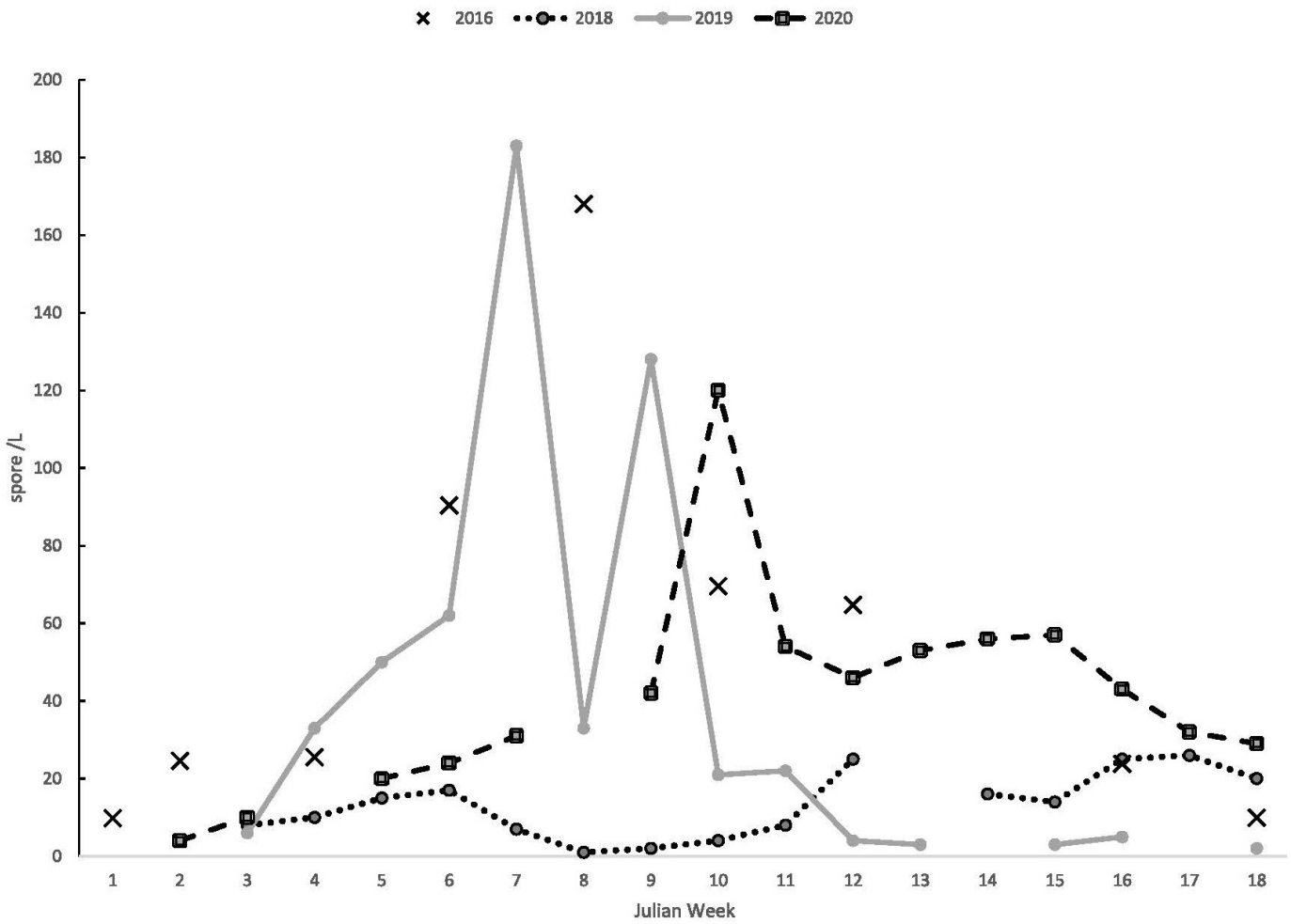

Figure 5. Mean *Ceratonova shasta* spore/L concentrations in triplicate water samples collected at Herringer trap between Julian weeks 1-18 in 2016 (black x), 2018 (circles and black dotted line), 2019 (circles and gray line), and 2020 (squares and black dashed line).

Table 5. Herringer trap *Ceratonova shasta* occurrence (spore/L) with temperature (°C) and Julian week (JW) for peak spore concentration and Julian week of initial detection of infection and greater than 10 sp/L in Chinook Salmon fry.

Year	JW (peak sp/L)	°C	sp/L	JW (≥ 10 sp/L)	JW (first infection)
2016	8	12.1	168	1	4
2018	12	10.9	25	4	8
2019	7	9.5	183	4	5
2020	10	13.2	120	3	3

Table 6. *Ceratonova shasta* spore/L longitudinal data collected from rkm 96.7 to 35.9 during Julian weeks (JW) 5–18 of 2015, 2016, 2018, 2019, and 2020. Peak spore/L for a given year is in bold type. (NT = not taken)

Year	JW	rkm 96.7	rkm 94.9	rkm 91.7	rkm 80.5	rkm 73.7	rkm 66.8	rkm 49.1	rkm 35.9
2015	11	NT	14	**294**	175	NT	NT	11	1
2016	6	NT	18	183	141	NT	62	57	6
2016	11	NT	14	**252**	141	NT	25	15	2
2016	14	NT	5	41	53	NT	9	12	2
2016	18	NT	2	22	38	NT	6	7	1
2018	5	NT	3	4	11	NT	18	13	5
2018	10	0	0	3	8	NT	2	1	2
2018	18	0	5	27	**38**	NT	19	12	6
2019	5	0	NT	37	NT	50	NT	27	15
2019	6	1	0	115	NT	**183**	103	103	4
2019	8	0	NT	NT	107	128	NT	82	2
2019	10	NT	NT	NT	NT	21	8	16	7
2019	11	NT	NT	3	NT	22	3	NT	11
2019	16	0	NT	5	NT	3	5	6	5
2020	6	1	11	8	10	NT	24	28	16
2020	11	3	33	44	76	NT	81	**138**	29
2020	14	1	34	38	43	NT	44	59	12
2020	18	3	27	26	14	NT	18	19	8

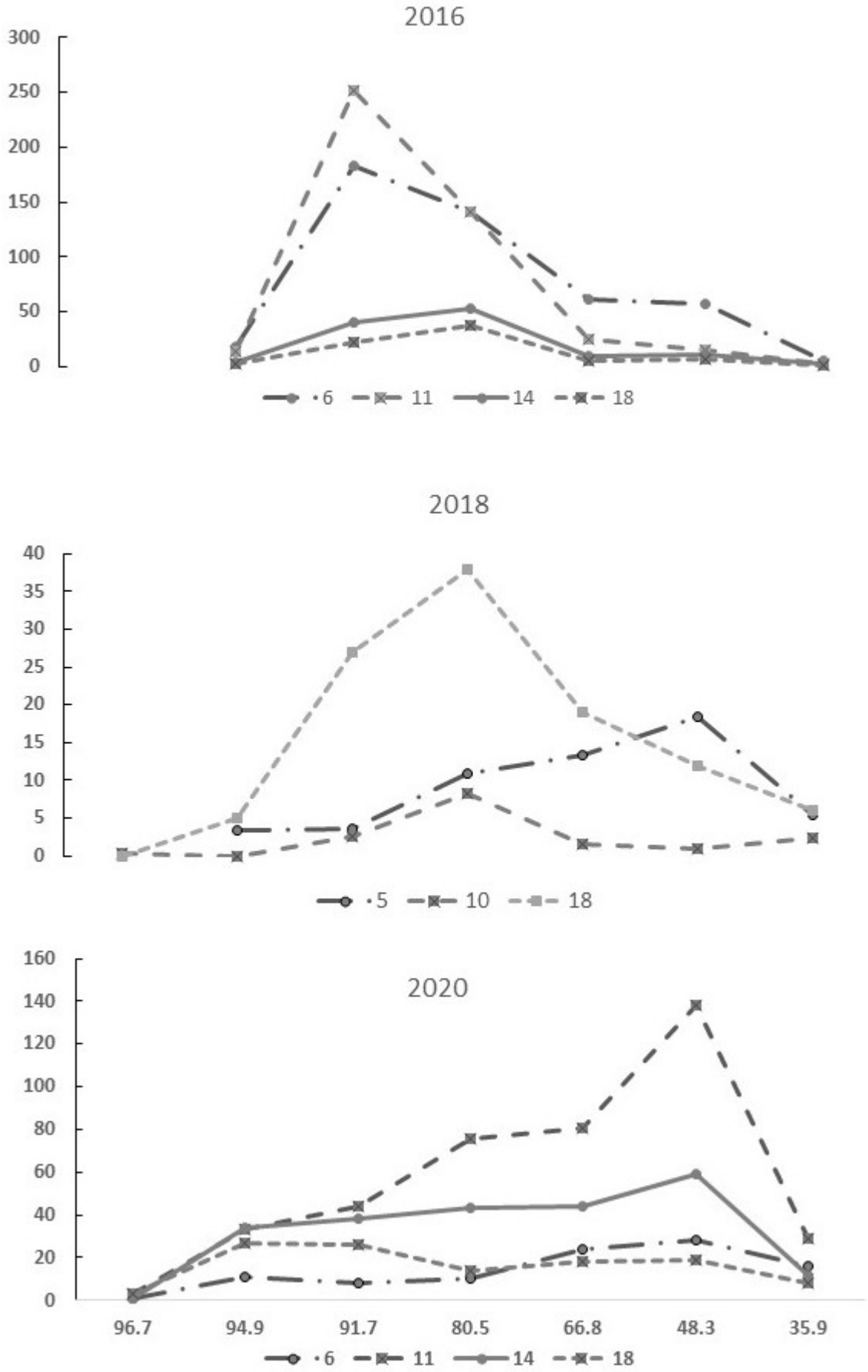

Figure 6. Longitudinal water samples collected between river kilometer 96.7–35.9 during a single day in

Julian weeks 5–18 during 2016, 2018, and 2020. Samples assayed by QPCR for mean *Ceratonova shasta* spore concentration. Note spore/L values along the y-axis vary for each year.

Discussion

Pathogen infection and disease are associated with declining Pacific salmon populations and are a concern for Central Valley Chinook stocks (Foott et al. 2007; Fujiwara et al. 2011; Furey et al. 2020; Lehman et al. 2020). The relationship between *C. shasta* infection of juveniles and the observed low recruitment of natural Feather River salmon (< 10 % of adult returns) is undefined currently. Ceratomyxosis is likely a significant factor in juvenile Feather River Chinook salmon survival. We observed prevalence of infection averaging 45% (histology) and 58% (QPCR) in natural fry within the HFC over the five-year period. Initial fry infection occur as actinospore concentration increases during late January through early February. By March over 50 % of infected fry rated as diseased with little probability of survival. Conversely, ≥ 85% of the natural fry population have migrated past the Herringer trapping site when disease severity increases in March. Determination of a fish's infectious status, from its intestine sample, is problematic as 7- 10 days post-infection is required for the parasite to move through the circulatory system and invade the intestinal tract (Bjork and Bartholomew 2010). Once fry leave the LFC, they tend to move rapidly within the HFC and pass the trap site in a week or less (Seesholtz et al. 2004, CDWR mark-recapture trap data). Our sentinel studies demonstrate that disease progression occurs if exposure within the HFC is 5 days or more. The observation of diseased fry at Herringer trap in March corresponds with the capture of larger fish (≥ 40 mm FL) suggesting they rear for several weeks within the HFC prior to capture. Disease mortality of larger fry reduces this life history "portfolio" for Feather River natural Chinook. The use of intestine samples alone will underestimate the prevalence of infection in migrant fry. Gill infection in these early out-migrants suggests some proportion will progress into a disease state. This hypothesis is also based on the wide area of infectivity and hence extended exposure fry experience in the Feather River. Survey efforts downriver of Herringer trap may elucidate the question of disease progression in fry chronically exposed to low actinospore concentrations as they migrate downriver in January and February.

An infectious zone, beginning from the TAO (rkm 94.9), has enlarged from the 2015 survey to include reaches below Yuba River confluence. It is unclear if polychaete density has also increased downriver since 2015. An understanding of the polychaete distribution, locations of protected habitats, and seasonal density would inform management decisions for this disease issue. Alexander et al. (2014) reported a modeling approach in predicting polychaete abundance and response to flow conditions. The high flows of 2017, followed by increasing levels of infection in 2018 and 2019, suggest that some portion of the polychaete population is associated with stable habitat such as lee side of boulders or riprap and can re-colonize within a year. Other workers have reported similar occurrences following disturbances (Malakauskas et al. 2013; Alexander et al. 2014). Another ecological question is why the TAO is a consistent feature of the upriver boundary for the infectious zone. Planktonic food input may be a factor, however; other environmental and habitat features could also be important (Seesholtz et al. 2004; Alexander et al. 2014). A comparison of LFC to HFC habitat characteristics is needed for this analysis.

Myxospore dispersion from adult salmon carcasses is another avenue of potential management. Adult carcasses produce billions of myxospores each year that are later ingested by the alternate worm host, *Manayunkia occidentalis,* to continue the parasite's life cycle (Bartholomew et al. 1997; Atkinson et al. 2020). Adult carcasses produce the vast majority of myxospores that feed into the annual polychaete infection cycle (Foott et al. 2016; Kent et al. 2014; Atkinson et al. 2020). Reducing myxospore

transmission to polychaetes will require an understanding of flow magnitude, duration, and seasonal timing to optimize the movement of myxospores past the current infectious zone. This study described a parasitic disease that results in high mortality for a portion of the juvenile Chinook population in the lower Feather River. This disease has a defined zone of infectivity beginning below the TAO and a seasonality that begins in late January or early February.

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