U.S. Fish & Wildlife Service

California-Nevada Fish Health Center
FY 2018 Investigational Report:

Myxosporean Parasite (*Ceratonova shasta* and *Parvicapsula minibicornis*) Prevalence of Infection in Klamath River Basin Juvenile Chinook Salmon, March – August 2018

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Summary

Juvenile Klamath River Chinook salmon (*Oncorhynchus tshawytscha*) were assayed from late March to August 2018 by quantitative polymerase chain reaction (QPCR) and histology for myxosporean parasite infection of *Ceratonova shasta* and *Parvicapsula minibicornis*. During the first 8 weeks of the season, juvenile Chinook salmon were assayed in real-time for *C. shasta*. Fish were collected early in the week and processed for necropsy, DNA extraction, and QPCR, in order to provide timely data to fishery managers regarding flow management. *Ceratonova shasta* prevalence of infection (POI) exceeded the emergency dilution flow criteria of 20% in the Shasta to Scott (K4) reach on April 30th, the 6th week of the monitoring program.

Ceratonova shasta prevalence of infection by QPCR in Chinook salmon collected above the Trinity River confluence during the peak out-migration period (May-July) was 20%, lower than 26% observed in 2017, and 48% in 2016. *Parvicapsula minibicornis* prevalence of infection in Chinook salmon above the Trinity River confluence for the same time period was 92%, compared to 82% and 89% in 2017 and 2016, respectively.

Among the fish groups tested, naturally produced Chinook salmon had a 11% prevalence of *C. shasta* infection by QPCR, which was higher than the 5% observed in 2017 but lower than the 27% in 2016. The onset of infection (first detection) in 2018 occurred on April 23 when mean daily river temperature below Iron Gate Dam was 12.5°C. By histology, natural fish sampled from the Shasta to Scott (K4) and Scott to Salmon (K3) reaches from mid-April through the end of May had very low *C. shasta* POI (0-3%). *Ceratonova shasta* was not detected in six of the seven sample sets from the two reaches. Additionally, pathology scores were zero for six of seven sample dates, indicating infection levels were well below clinical disease levels in natural Chinook juvenile salmon in the two upper reaches through late spring.

In coded-wire tagged (CWT) juvenile Chinook salmon released from Iron Gate Hatchery from June 8-27, *C. shasta* was detected in 35% of fish screened by QPCR. The highest *C. shasta* prevalence of infection in marked juvenile Chinook salmon ranged from 42-51% in fish residing 2-4 Weeks At Large (WAL) at time of recapture. The infection pattern (infection within 2-3 weeks post exposure) is commonly observed for juvenile Chinook salmon entering the main stem Klamath River, under typical temperature regimes (15-18°C) at time of release (mid-May to early June).

In 2018, the annual *C. shasta* prevalence of infection, historical comparison, and POI in Iron Gate Hatchery CWTs were all lower in both QPCR and histology samples, compared to 2017. The only increase in *C. shasta* POI was observed in natural fish by QPCR. *Parvicapsula minibicornis* infection increased in almost every sample type by both QPCR and histology testing. In 2018, 61% of all fish sampled were infected with *P. minibicornis* only. Clinical pathology scores were zero in natural fish by histology, however clinical signs of *P. minibicornis* were observed during necropsy later in the sampling season, especially in fish collected in mid-July in both the Salmon to Trinity (K2) and the Trinity to Estuary (K1) reaches.

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Introduction

The Klamath River drainage is approximately 30,000 km^2 located in southern Oregon and northern California. It consists of an upper basin which extends northeast from Iron Gate Dam (IGD) on the main stem Klamath River, and a lower basin extending southwest to the Pacific Ocean (Figure 1).

The lower Klamath River supports 19 species of native fishes including Chinook salmon (*Oncorhynchus tshawytscha)*, which continues to be the most abundant anadromous fish in the river (National Research Council, 2004). Also present in the Klamath River are two myxozoan parasites, *Ceratonova shasta* (*syn. Ceratomyxa shasta*, Atkinson et al., 2014) and *Parvicapsula minibicornis*. The parasites share both vertebrate and invertebrate hosts (Bartholomew et al., 1997; Jones et al., 2004; Bartholomew et al., 2007). The parasites' life cycles include the invertebrate polychaete host, *Manayunkia* sp., which (if infected) releases the actinospore stage into the water column and can subsequently infect the vertebrate salmonid host. The actinospore develops within the vertebrate host, salmon or trout species, into a myxospore. Once shed from an infected fish, the myxospore can infect the polychaete host to complete the life cycle (Bartholomew et al., 1997). Since *C. shasta* and *P. minibicornis* share the same invertebrate host (Bartholomew et al., 2006) there is the potential for a fish to encounter actinospores from both parasites, if polychaetes are infected. Coinfection (concurrent multiple species infections) of *C. shasta* and *P. minibicornis* have been well documented in juvenile Chinook salmon monitoring and sentinel studies from the Klamath River.

The two myxozoan parasites have overlapping distributions throughout the Pacific Northwest, where they are present in many of the larger river systems (Ching et al., 1984; Hoffmaster et al., 1988; Hendrickson et al., 1989; Bartholomew et al., 1997; Jones et al., 2004; Bartholomew et al., 2006; Stocking et al., 2006). *Ceratonova shasta* and *P. minibicornis* are distributed throughout the main stem Klamath River system including the lower reaches of the Williamson and Sprague Rivers, Agency Lake, Klamath Lake, Copco Reservoir, and the Klamath River from Iron Gate Dam to the estuary (Hendrickson et al., 1989; Stocking et al., 2006; Bartholomew et al., 2007). A 2006 study monitoring the waterborne stages showed that *C. shasta* abundance was low at the outflow of Iron Gate Reservoir (RM 190), but increased in the main stem Klamath River between the interstate five bridge crossing (RM 177) and the confluence of the Scott River (RM 144; Hallett et al., 2006). This section of the Klamath River has been termed the "infectious zone" and this general pattern of parasite abundance remains

steady, but the size of the infectious zone and the magnitude of parasite densities change seasonally and annually (Bartholomew et al., 2010).

Ceratonova shasta causes enteronecrosis and is a significant contributor to mortality in juvenile salmonids that migrate through the region (Hoffmaster et al., 1988; Bartholomew et al., 1997; Stocking et al., 2006). Infectivity patterns of enteronecrosis are well defined for native Klamath basin salmonid species. At river temperatures commonly observed in the Klamath River during peak juvenile Chinook salmon migration of April to August (17-24ºC), clinical disease occurs within three weeks of initial exposure resulting in moderate to high levels of mortality. This infectivity pattern has been established through sentinel susceptibility studies (Stone et al., 2008; Bjork et al., 2009; Bartholomew et al., 2010; True et al., 2012) and annual monitoring of coded-wire tagged (CWT) Chinook salmon with known exposure periods in the main stem Klamath River (Nichols et al., 2009; Bolick et al., 2013; True et al., 2013). *Parvicapsula minibicornis* accumulates in the kidney, as seen histologically, and pathology can vary from minor inflammation in lightly infected fish to congestive necrosis of kidney tubules in heavily infected fish (True et al., 2009).

Klamath River juvenile Chinook salmon can experience high prevalence and severity of infection with these two myxosporean parasites, particularly when river temperatures promote earlier reproduction and expansion of the polychaete host population (Bartholomew et al., 2010) which can lead to earlier infection and proliferation of the parasite within the fish host (True et al., 2011). For salmonids, mortality from enteronecrosis is temperature dependent as demonstrated by Udey et al. (1975), but water discharge can also play an important role. Bjork et al., (2009) found prevalence of *C. shasta* infection was higher in a smaller volume of water when fish were exposed to the same number of parasites. Therefore, parasite concentration affects infection prevalence. Higher flows may not only dilute the infectious spore stages, but transmission efficiency may also be decreased (Hallett et al., 2012; Ray et al., 2013).

The primary objectives of this study were to: 1) examine parasite prevalence in Klamath River juvenile Chinook salmon during the spring out-migration period; and 2) compare parasite prevalence in 2018 to previous years.

Methods

Pre-Release Examination

Prior to the first Iron Gate Hatchery release (June 8, 2018), a fish health examination of 30 fall-run Chinook salmon was conducted (May 15, 2018) to determine infection levels of *C. shasta* and *P. minibicornis*.

Sample Sites, and Fish Groups

Fish were collected in the main stem Klamath River between the Shasta River confluence and the Klamath River estuary (a distance of ~177 river miles). The middle and lower Klamath River is divided into five sample reaches at major tributaries, with study cooperators collecting fish in each reach (Figure 1, Table 1).

In 2018, juvenile Chinook salmon captured from the upper reach (K4) were assayed in real-time for the first 8 weeks of the study (March 25 - May 13). Juvenile Chinook salmon were collected early in the week and processed for necropsy, DNA extraction, and QPCR. Results were reported to partners by the end of the week. Real-time monitoring provided timely data to fishery managers regarding flow management and when *C. shasta* POI exceeded 20% in out-migrating Chinook salmon. A 2017 court order from the U.S. District Court Northern District of California requires the Bureau of Reclamation to release emergency dilution flows when POI at the Kinsman site exceeds 20% or 5 *C. shasta* spores/L in water samples (case no. 16-cv-04294-WHO, judge William Orrick, February 8, 2017).

When possible, existing salmonid downstream migrant traps are used for fish collections. Beach seining was also performed to collect fish in some weeks/reaches, especially when fish were hard to locate. Field crews collected weekly samples within a 1-2 day period to prevent protraction of the sampling period within the sample week. The date reported for fish collection is the start date (Sunday) of the sampling week. Specific dates are provided for hatchery releases, first pathogen detections, and histology collection dates.

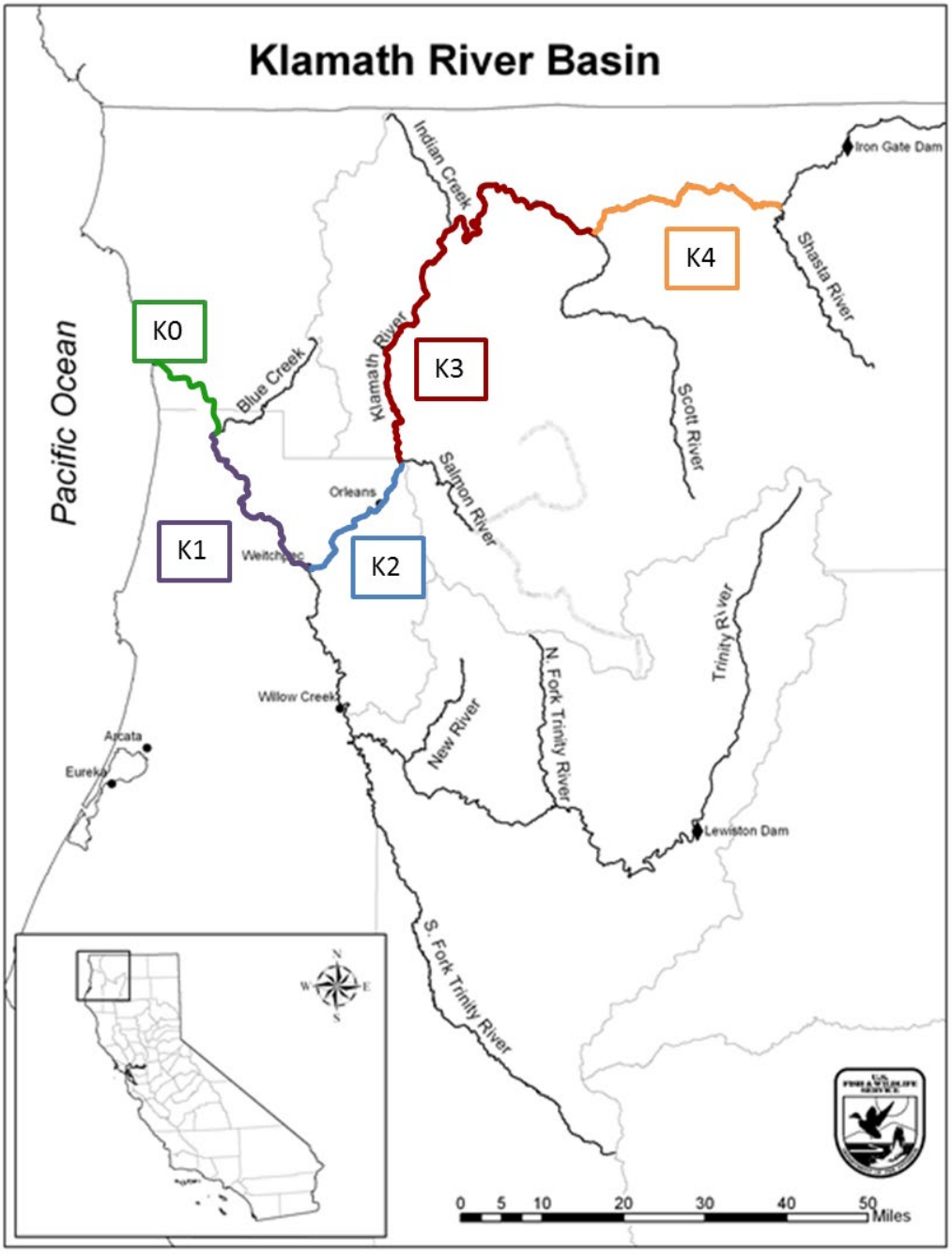


Figure 1. Klamath River watershed, major tributaries, and sample reaches: Shasta River to Scott River (K4), Scott River to Salmon River (K3), Salmon River to Trinity River confluence (K2), Trinity River to upper Estuary (K1), and Klamath River Estuary (K0). Map provided by the Arcata Fish and Wildlife Office.

Table 1. Sample reach locations, distances, and cooperating agencies performing fish collection on the main stem Klamath River.

Sample Reach	Reach Code	River miles (Upstream – Downstream)	Primary Collector
Klamath River main stem			
Shasta R. to Scott R.	K4	177-144	USFWS
Scott R. to Salmon R.	K3	144-66	Karuk Tribe
Salmon R. to Trinity R.	K2	66-44	Karuk Tribe
Trinity R. to Estuary R.	K1	44-4	Yurok Tribe
Estuary	K0	4-0	Yurok Tribe

Fish were sampled, according to True et al., 2013 from the Shasta River confluence to the Klamath River estuary. Fish were collected in the upper reaches, K4 and K3, early in the sampling season (week of March 26-July 7). Lower reaches were sampled later in the season (June 4- August 16) as fish were migrating downstream (Appendix A – Table 1).

All fish sampled were categorized into three group types based on their origin: natural (collected before release of hatchery fish), unknown (adipose fin present after hatchery release), and CWT (coded-wire tagged and adipose fin clipped). Fish tested in the Klamath River varied by reach, with emphasis on natural fish in the reaches below IGD initially, then available hatchery CWT fish for the remainder of the spring/summer migration.

The 25% constant fractional mark rate (percent of the fall-run Chinook production that is marked/tagged) at IGH since 2009 has facilitated the capture of a large proportion of IGH CWT Chinook salmon in the past decade (Buttars et al., 2009). In the monitoring program, temporal data is derived from IGH CWT codes obtained from juvenile Chinook salmon with known exposure period (hatchery release to in-river recapture date). The period of how long fish reside in the Klamath River post hatchery release is Weeks At Large (WAL).

Historical comparison between monitoring years restricts data to the peak migration period (May to end of July) and to reaches above the Trinity confluence.

Both quantitative polymerase chain reaction (QPCR) and histological assays were used to identify and quantify infectivity patterns for both *C. shasta* and *P. minibicornis* in juvenile Chinook salmon tissues (Hallett et al., 2006; True et al., 2009).

Parasite Infection Levels by Quantitative PCR Assays

Fish collection, necropsy, and DNA extraction were done according to True et al., 2013. The *C. shasta* reference standard curve was obtained 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 8 orders of magnitude in molecular grade water. Using QPCR analysis software, the cycle threshold (C_T) values for each standard concentration were calculated (SDS software 7300 SDS v 1.4, Applied Biosystems). The standard curve was used to evaluate PCR amplification efficiency (slope of the

standard curve), fit to the curve (R^2 value), and the y-intercept (theoretical C_T value for a single copy of parasite DNA when assays are 100% efficient; Applied Biosystems, 2014).

Two standard curves were used for *C. shasta* in 2018. The initial standard set of *C. shasta* gBlock was prepared in April, diluted in molecular grade water, and used for standard curve analysis on plates 1-10. This standard curve had an amplification efficiency of 90%, R^2 value = 0.997, and y-intercept was 41.7. Additional standards were prepared in June by diluting *C. shasta* gBlock in Tris-EDTA (ethylenediamine tetraacetic acid) buffer, and used for standard curve analysis on the remaining assay plates. This second standard set had an amplification efficiency of 95.2%, R^2 value = 0.998, and y-intercept was 40.5.

Quantification of fish tissue (*C. shasta* DNA copy number) was determined using 5 µL of DNA template in a 30 µL reaction. Each assay plate included a standard curve with three concentrations of reference standards (two replicates each) at known DNA copy number, and two negative control wells. Each assay was evaluated for expected C_T values of the reference standards, and assay efficiency. Any plates with more than a 5% decrease in assay efficiency were retested and reevaluated. Positive test results are tissue samples with C_T values below the statistically valid detection limit of the QPCR assay standards (Applied Biosystems, 2016).

One *C. shasta* assay plate did exceed a 5% decrease in efficiency, and this was due to the reference standards being off by ~1 C_T and some inhibition on this plate. Samples with abnormal amplification curves were diluted 1:10 and retested.

The *P. minibicornis* reference standard curve was obtained in a similar manner by using plasmid DNA containing the 18S ribosomal DNA target sequence. Specifically, 1 ng of DNA, corresponding to 2.41 $x10^8$ copies of *P. minibicornis* DNA was serially diluted over 8 orders of magnitude in molecular grade water. Using QPCR analysis software, the cycle threshold (C_T) values for each standard concentration were calculated (SDS software 7300 SDS v 1.4, Applied Biosystems). The standard curve was used to evaluate PCR amplification efficiency (slope of the standard curve, efficiency was 94%), fit to the curve (R^2 value = 0.994) and the y-intercept (42.4, C_T value for a single copy of parasite DNA).

Quantification of fish tissue (*P. minibicornis* DNA copy number) was determined using the same reaction volume of 5 µL. Each assay plate included a standard curve with three concentrations of reference standards (two replicates each) at known DNA copy number, and two negative control wells. Each assay was evaluated for expected C_T values of the reference standards, and assay efficiency. One plate required retesting over the 2018 field season.

In the results section, QPCR data are presented first for each group of fish or type of analysis, followed by histology data in a separate paragraph.

Parasite Infection Levels by Histology

Histological assays were done to assess clinical disease (disease severity that results in tissue damage) according to True et al., 2013. In 2018, histology samples were collected in the Shasta to Scott reach (K4) between the week of April 15 and May 13. Histology samples in the Scott to Salmon reach (K3) were collected between the week of April 15 and May 27. Histology samples are designated with

an “H” in parentheses in Appendix A - Table 1. Histology results are presented in a separate paragraph in appropriate sections.

Histological assays were assigned a pathology score: a numeric index of disease severity for kidney and intestine. The pathology was based on the degree of specific tissue abnormalities and parasite distribution (Appendix B -Table 1), but do not affect the overall prevalence of infection reported for histological assessments. Pathology scores are reported for fish grouped by collection date, not as pathology scores for individual fish.

Statistical Analysis

Point prevalence of infection and annual prevalence (defined by Durfee, 1978; USFWS, 2004) for *C. shasta and P. minibicornis* were reported with 95% confidence intervals (denoted ci) for each sample reach. Prevalence of infection (POI) was used to describe the proportion of infected Chinook salmon (numerator) in the sample (number of animals examined) for a particular calendar week. Annual prevalence was used to describe the overall prevalence of infection in the sampled population during the entire sampling period that year. Annual prevalence estimate is not an estimate of the annual proportion of the population that is infected, because weekly estimates are not weighted by abundance values.

Results

Pre-Release Examination of Iron Gate Hatchery Chinook Salmon

Juvenile Chinook salmon reared at Iron Gate Hatchery were screened for infections of *C. shasta* and *P. minibicornis* by QPCR on May 15, prior to release of ~4.2 million juveniles starting on June 8. *Ceratonova shasta* was not detected in the 30 juvenile Chinook salmon tested. However, *Parvicapsula minibicornis* was detected in 7% (2/30, ci = 1-22%) of fish tested in very low DNA copy numbers (19 and 23 copies), indicative of presence of the parasite rather than active disease. River temperature on the initial release of June 8 was 18.3°C (65.0°F), and 20.5°C (68.9°F) on final release date of June 27 (data from Karuk Tribe, below Iron Gate Dam).

Number of Fish Collected by Origin

In 2018 we tested 1063 juvenile Chinook salmon collected from the main stem Klamath River (unreadable and/or Trinity River Hatchery (TRH) coded-wire tags were removed). The sample consisted of 537 natural fish, and 526 fish collected after hatchery release which included 460 CWTs.

The proportion of fish sampled was typical this year in that the majority of fish were natural and CWTs (Figure 2). Natural fish accounted for 51% (537/1063) of fish sampled, and fish of unknown origin accounted for 6% (66/1063). Coded-wire tagged fish from Iron Gate Hatchery accounted for 43% (460/1063), compared to 9% in 2017. Last year the proportion of CWTs was unusually low due to a much smaller group of CWT juvenile Chinook salmon released from the hatchery (True et al., 2017).

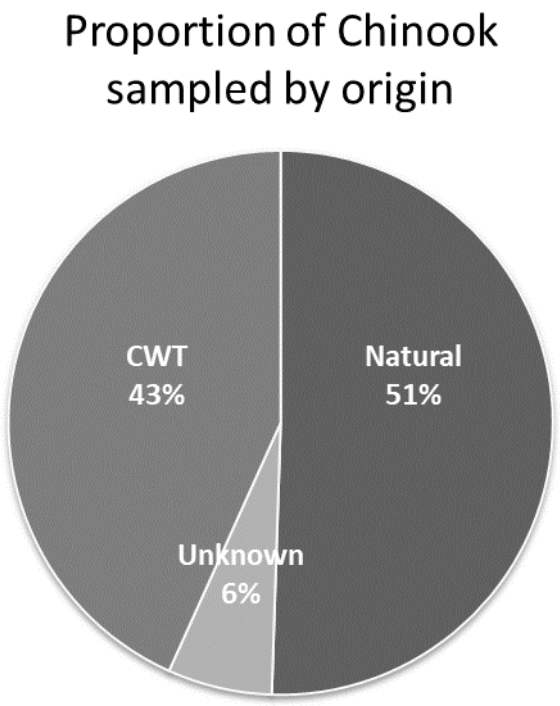


Figure 2. Proportion and origin of Chinook salmon used for prevalence of infection analysis (N=1063) in 2018. Unreadable tag codes or lost tags, and TRH tags have been removed from total number of fish collected.

Real-time Monitoring of *C. shasta* POI in Shasta to Scott (K4) reach

In 2018, juvenile Chinook salmon captured from the upper reach (K4) were assayed in real-time for the first 8 weeks of the study (March 25 - May 13). *Ceratonova shasta* prevalence of infection (POI) exceeded the emergency dilution flow criteria of 20% in the Shasta to Scott (K4) reach on April 30th, the 6th week of the monitoring program. A comparison of when *C. shasta* POI first reached ≥ 20% in previous monitoring years (last five years) is presented in Table 2.

Table 2. *Ceratonova shasta* POI in natural juvenile Chinook salmon, collected from the Shasta to Scott reach (K4). Highlighted rows indicate Sample Week (and Capture Date) when *C. shasta* POI reached or exceeded 20%**.**

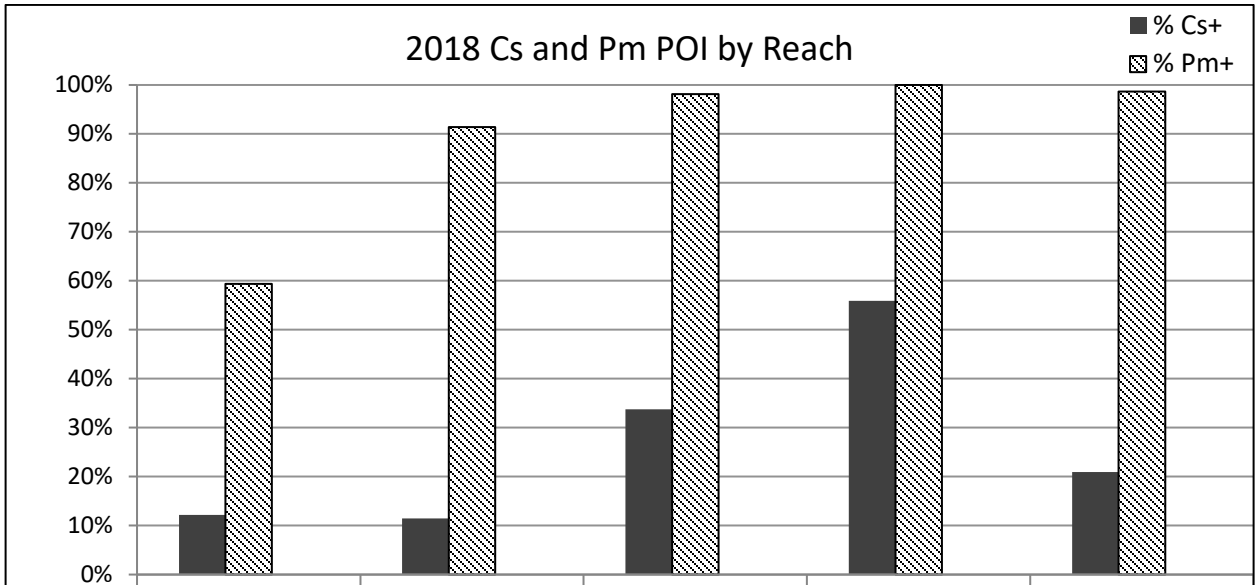
Year	Reach	Sample Week	Capture Date	Cs+	N Sampled	Cs POI
2014	K4	1	4/3/2014	1	20	5%
		2	4/10-11/2014	3	20	15%
		3	4/17/2014	10	20	50%
		4	4/24/2014	17	20	85%
		5	5/1/2014	18	20	90%
		6	5/8-9/2014	20	20	100%
		7	5/15/2014	20	20	100%
		8	5/20/2014	18	20	90%

2015		1	4/2/2015	0	20	0%
	K4	2	4/9/2015	4	20	20%
		3	4/16/2015	7	20	35%
		4	4/22/2015	18	20	90%
		5	4/30/2015	20	20	100%
		6	5/7/2015	20	20	100%
		7	5/14/2015	18	20	90%
		8	5/21/2015	19	20	95%
2016	K4	1	3/31/2016	0	20	0%
		2	4/7/2016	0	20	0%
		3	4/14/2016	0	19	0%
		4	4/21/2016	0	21	0%
		5	4/28/2016	0	20	0%
		6	5/5/2016	4	20	20%
		7	5/12/2016	14	18	78%
		8	5/19/2016	11	20	55%
2017	K4	1	3/28/2017	0	30	0%
		2	4/3/2017	0	30	0%
		3	4/10/2017	0	30	0%
		4	4/17/2017	0	30	0%
		5	4/21/2017	0	30	0%
		6	5/2/2017	0	30	0%
		7	5/8/2017	3	30	10%
		8	5/15/2017	6	30	20%
		9	5/22/2017	1	23	4%
		10	5/30/2017	6	30	20%
2018	K4	1	3/26/2018	0	30	0%
		2	4/2/2018	0	30	0%
		3	4/9/2018	0	30	0%
		4	4/16/2018	0	30	0%
		5	4/23/2018	3	30	10%
		6	4/30/2018	14	30	47%
		7	5/7/2018	8	31	26%
		8	5/14/2018	11	30	37%
		9	5/21/2018	3	30	10%
		10	5/29-6/1/2018	2	9	22%
		11	6/4-6/2018	1	4	25%

Annual Prevalence of Infection by Klamath River Reach

The annual prevalence of *C. shasta* infection in all Chinook salmon analyzed in 2018 by QPCR was 21% (220/1063, ci = 18-23%). *Ceratonova shasta* was first detected on April 23 in both the Shasta to Scott reach (K4) and Scott to Salmon River reach (K3). *Ceratonova shasta* POI was highest in the Trinity to Estuary reach (K1) at 56%, followed by 34% in the Salmon to Trinity (K2) reach. The lowest prevalence of 11% was observed in the Scott to Salmon reach (K3, Figure 3).

The annual *P. minibicornis* POI in all Chinook salmon by QPCR was 83% (867/1042, ci = 81-85%). *Parvicapsula minibicornis* was first detected on April 2 in the Shasta to Scott reach (K4). Prevalence was highest in the Trinity to Estuary reach (K1) at 100%, closely followed by the Estuary (K0) at 99% (Figure 3). The lowest prevalence of 59% was observed in the Shasta to Scott reach (K4).



	K4	K3	K2	K1	K0
% Cs+	12%	11%	34%	56%	21%
% Pm+	59%	91%	98%	100%	99%
n	362	288	163	102	148

Figure 3. Prevalence of *Ceratonova shasta* (Cs+) and *Parvicapsula minibicornis* (Pm+) infection in juvenile Klamath River Chinook salmon by collection reach in 2018. Shasta River to Scott River (K4), Scott River to Salmon River (K3), Salmon River to Trinity River confluence (K2), Trinity River to upper Estuary (K1), and Klamath River Estuary (K0). Sample numbers collected (N) are displayed in the table below and were the same for both pathogens.

The annual *C. shasta* POI by histology for all fish tested in 2018 was 1% (1/68, ci = 0-8%) and for *P. minibicornis* was 51% (35/68, ci = 39-64%). Histology results are listed in Appendix B, Table 2 and Table 3.

Coinfection

In 2018, 0.19% (2/1063) of all fish sampled were infected with *C. shasta* only, 61% (649/1063) were infected with *P. minibicornis* only, 21% (218/1063) were coinfected, and 16% (173/1063) were uninfected. Twenty-one fish were not included in coinfection analysis, as they were only tested for one parasite.

Prevalence of Infection by Fish Origin

Naturally produced Chinook salmon

Naturally produced Chinook salmon represent early infection status by these two myxozoan parasites in the Klamath River, as river temperatures are generally 8-10ºC cooler in the collection months of late March to late May compared to hatchery fish sampled during the peak salmon migration period of late May to end of July. A total of 537 natural fish were collected in the Klamath River above the Trinity River confluence for testing by QPCR. Natural fish were collected from March 25 through June 3 in Shasta to Scott (K4) reach, from April 8 through June 10 in the Scott to Salmon (K3) reach, and June 3 to June 10 in the Salmon to Trinity (K2) reach. Mean daily river temperature was 12.5°C (data from Karuk Tribe, below IGD) at first detection of *C. shasta* (April 23) in natural fish collected in K4 reach.

Ceratonova shasta was detected by QPCR in 11% (57/537, ci = 8-14%) of natural fish in 2018, compared to 5% in 2017. *Ceratonova shasta* POI was highest at 15% (42/284, ci = 11-19%) in the Shasta to Scott (K4) reach compared to 6% (13/211, ci = 3-10%) in the Scott to Salmon (K3) reach and 5% (2/42, ci = 1-16%) in the Salmon to Trinity (K2) reach. The Fish Health Center did not observe any clinical disease signs in natural fish during necropsy in 2018.

Parvicapsula minibicornis was detected in 73% (379/516, ci = 69-77%) of naturally produced Chinook salmon by QPCR, compared to 38% in 2017. The highest *P. minibicornis* prevalence of 98% (41/42, ci = 87-100%) was detected in Salmon to Trinity (K2) reach and the lowest prevalence of 60% (171/284, ci = 54-66%) was observed in the upper Shasta to Scott (K4) reach.

All histology samples in 2018 were from natural fish and were collected prior to the first IGH release. The prevalence of *C. shasta* infection by histology was very low; 3% (1/30, ci = 0-17%) in the Shasta to Scott (K4) reach and 0% (0/38) in the Scott to Salmon (K3) reach. In the Shasta to Scott (K4) reach, *C. shasta* was detected by histology only in one sample collected on May 13 (Appendix B, Table 2). The pathology score of 0.1 suggests an early stage infection with very little tissue damage. For comparison, clinically infected salmon (those showing disease signs and symptoms), generally have *C. shasta* intestine pathology scores between 3 and 4 (True et al., 2010).

Natural fish had an overall *P. minibicornis* POI by histology of 51% (35/68, ci = 39-64%). Prevalence was highest in the Scott to Salmon (K3) reach at 53% (20/38, ci = 36-69%) compared to 50% (15/30, ci = 31-69%) in the Shasta to Scott (K4) reach. The highest pathology score was 1.9 in the May 13 collection date from the Shasta to Scott (K4) reach. These fish had inflammation of the kidney due the presence of the parasite, but did not have severe tissue damage. For reference, pathology scores of 6-8 have been observed in clinical disease, in previous monitoring years (True et al., 2010).

In order to assess *C. shasta* disease impacts to out-migrating juvenile fish, it is important to look at both prevalence of infection, as well as the quantity of parasite DNA within fish tissue (DNA copy number). For natural fish, early *C. shasta* infections in the range of 2-4 logs (mean DNA copy number) correlated with clinical disease and mortality in a sentinel exposure study conducted in 2008 (True et al. 2012). In that study, daily DNA levels were compared with histological assessments where tissue damage was irreversible (i.e., fish unlikely to recover) and lead to significant mortality (~80%) within three weeks at 18°C. The 2-4 log mean DNA range correlates with clinical disease that we believe has a high probability of resulting in mortality of juvenile Chinook salmon. However, this "disease threshold" is based on the caveat that early *C. shasta* infections detected in the upper reaches continue to progress (parasite proliferation) as juvenile fish migrate through the lower reaches at water temperatures that are common (exceeding 18°C) during the out-migration period. For example, in 2018, river temperatures surpassed 18°C by early to mid-June (Figures 6 and 7).

Similar analysis of natural fish, sampled in the upper reaches, was conducted in recent years (Figure 4). The past four years represent a severe *C. shasta* disease year (2015), an intermediate year (2016), a relatively low year (2017), and the most recent data for 2018. The parasite infection level for 2018 does include some samples in the range of 2-4 logs of *C. shasta* DNA, which was not observed in 2016 or 2017.

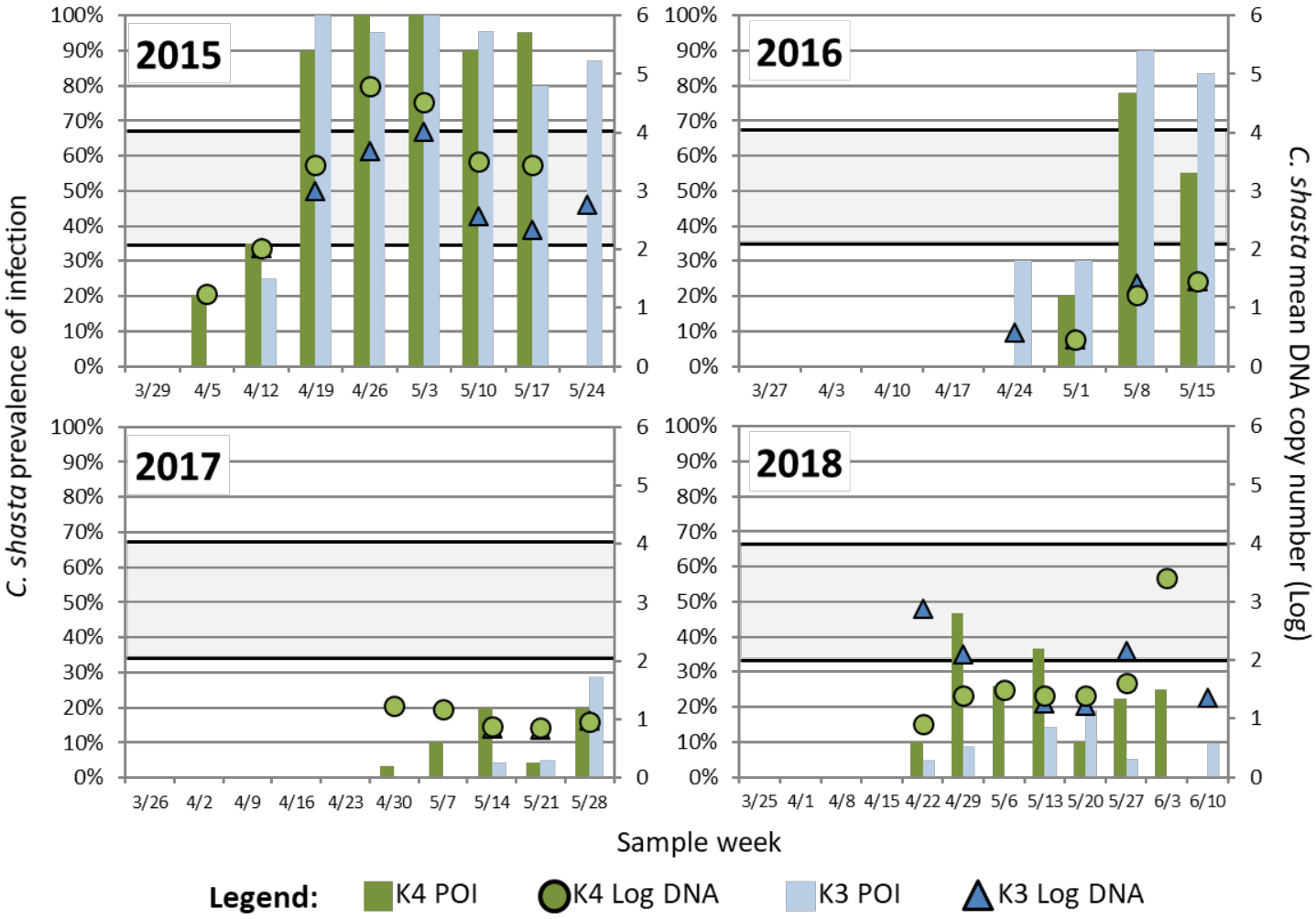


Figure 4. *Ceratonova shasta* prevalence of infection and mean DNA copy number (log) in natural juvenile Chinook salmon, captured in upper reaches: Shasta to Scott (K4) reach and Scott to Salmon (K3). Prevalence of infection shown in columns (Y axis) and *C. shasta* mean DNA copy number (log) shown in circles and triangles (secondary Y axis). Sample week date is shown on the X axis. *Ceratonova shasta* mean DNA range of 2-4 logs correlates with clinical infection levels by histology, considered irreversible and likely to result in mortality.

Unknown Chinook salmon

Unknown origin Chinook salmon are unmarked fish (adipose fin present) collected after hatchery release that could not be differentiated from either natural fish or unmarked hatchery fish. A total of 66 fish of unknown origin were collected from June 13 to June 21. *Ceratonova shasta* was detected by QPCR in 5% (3/66, ci = 1-13%) of unknown origin Chinook salmon. *Parvicapsula minibicornis* POI in fish of unknown origin was 71% (47/66, ci = 59-82%).

Iron Gate Hatchery (CWT) Chinook salmon

Coded-wire tagged salmon originating from IGH were collected in the Klamath River from June 13 to August 16. *Ceratonova shasta* was detected in 35% (160/460, ci = 30-39%) of IGH CWT screened by QPCR. Prevalence of infection for *C. shasta* was highest at 56% (57/102, ci = 46-66%) in the Trinity to Estuary reach (K1), followed by 51% (51/100, ci = 41-61%) in the Salmon to Trinity (K2) reach. Prevalence of infection was lowest in the Shasta to Scott reach (K4) at 3% (1/33, ci = 0-16%).

Parvicapsula minibicornis was detected by QPCR in 96% (441/460, ci = 94-97%) of IGH CWT. Prevalence of infection for *P. minibicornis* was highest at 100% in both the Scott to Salmon (K3) and Trinity to Estuary (K1) reaches. Prevalence of infection was lowest at 52% (17/33, ci = 34-69%) in the Shasta to Scott reach (K4).

IGH CWT Weeks At Large

The highest *C. shasta* prevalence of infection occurred in groups residing 2-4 WAL (51-42%, Figure 5). Intermediate POI ranges occurred for WAL 1 and WAL 6 at 39% and 27%, respectively. In 2018, the lowest *C. shasta* prevalence of infection occurred in fish residing at 1 WAL (5%) and 9 WAL (0%). Sample size was small (2 fish) for 9 WAL group (sample size shown at base of each column in Figure 5).

As stated in the methods, the QPCR assay can quantify parasite DNA copies within fish tissue and therefore describe infection level for a group of fish at specific exposure periods. In IGH CWT Chinook salmon, the mean DNA copy number had two peaks; one at 2 WAL (14,000 copies, 2.3 logs) and a second peak at 5 WAL (9,000 copies, 2.4 logs). Log transformation can reduce data variability and the log values for these two peaks are similar, while the mean DNA copy numbers are different. This is due to three samples at 2 WAL with high DNA copy numbers that are affecting the mean. The log value is calculated by transforming the DNA copy number for an individual fish to log scale first, and then taking the mean of the log values for that group of fish. Mean DNA copy number for all IGH CWTs in 2018 was 7,853 copies (2.0 logs). Fish residing 8 WAL were collected in K1 and K0; the two fish at 9 WAL were collected in K0.

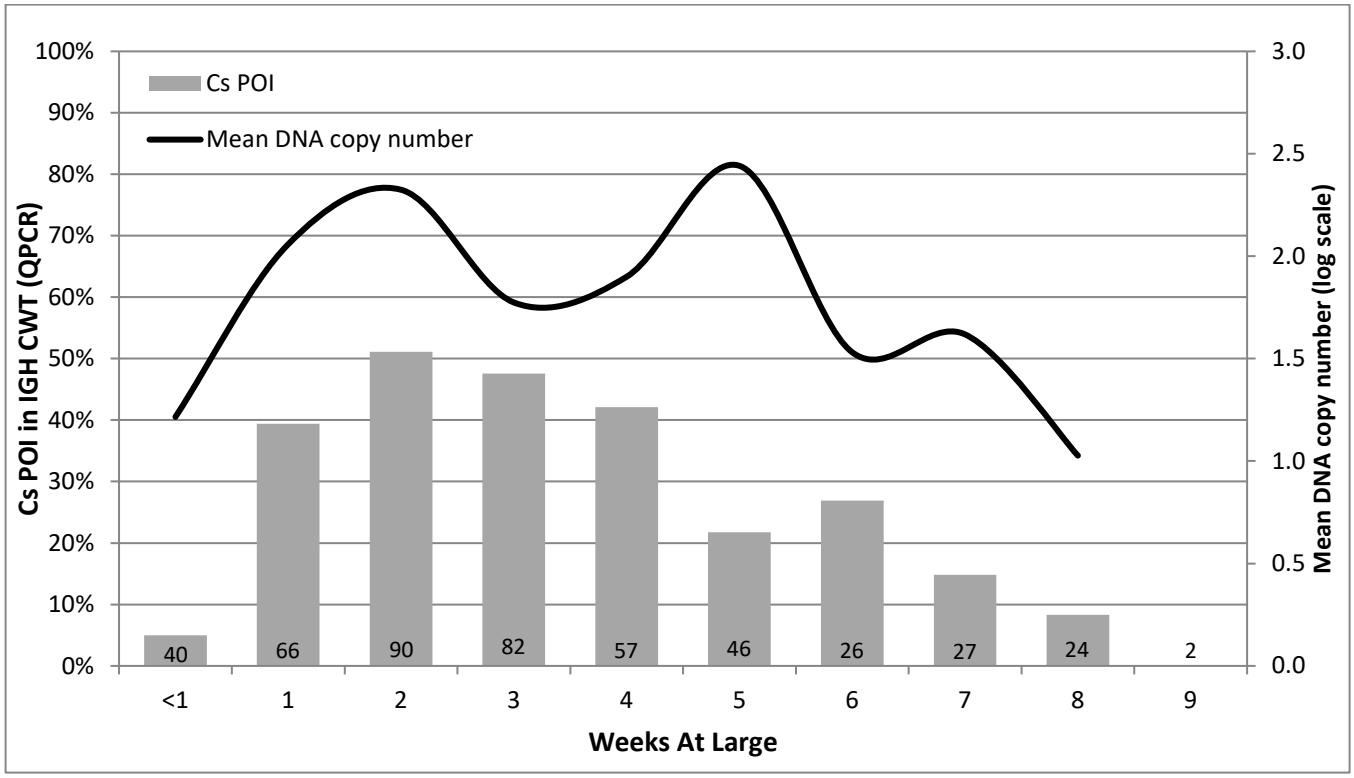


Figure 5. *Ceratonova shasta* prevalence of infection in IGH CWT by Weeks At Large (WAL) post hatchery release. The bar graph is prevalence of infection (%) on the primary y-axis and the line graph is the mean *C. shasta* DNA copy number (log scale) on the secondary y-axis for Chinook salmon tested by QPCR. The number of fish collected is listed inside the base of each bar.

Historical Comparison

Prevalence of infection by QPCR is the metric that has been used for historical comparisons of disease prevalence in the monitoring program since 2009. Data is confined to the peak migration period of May 1 to July 31 and fish collected above the Trinity confluence. Supplemental histology continues to be performed annually for select reaches to assess tissue damage associated with clinical disease and to detect other pathogens that may be present in out-migrating juvenile Chinook salmon.

Prevalence of *C. shasta* infection by QPCR during the peak out-migration period was low at 20% (114/570, ci = 17-24%) in 2018, and lower than the average of 41% for the last decade (2009-2018, Table 3). *Parvicapsula minibicornis* prevalence of infection by QPCR in Chinook salmon above the Trinity River confluence for the same period was 92% (523/570, ci = 89-94%) compared to 82% in 2017. The average POI for *P. minibicornis* over the last decade is also 82%.

Prevalence of *C. shasta* infection by histology was low in 2018; similar to the lowest disease year of 2011. It should be noted, however, that 2018 had the smallest sample size for histology over the past decade.

Table 3. Historic annual prevalence of *Ceratonova shasta* infection (% positive by assay) in all juvenile Chinook salmon collected from the main stem Klamath River between Iron Gate Dam and Trinity River confluence during May through July, 2006-2018.

Year	Histology (% Positive)		QPCR (% Positive)	
2009	54%	(50/93)	47%	(264/561)
2010	15%	(22/146)	17%	(128/774)
2011	3%[1]	(3/118)	17%	(62/374)
2012	9% [1]	(9/98)	30%	(160/526)
2013	16% [1]	(6/37)	46%	(234/508)
2014	42% [1]	(20/48)	81%	(467/576)
2015	62% [1]	(37/60)	91%	(437/482)
2016	14% [1]	(8/58)	48%	(243/504)
2017	8% [1]	(3/40)	26%	(153/600)
2018	4% [1]	(1/27)	20%	(114/570)
Mean	**22%**	**(159/725)**	**41%**	**(2262/5475)**

[1] Histology limited to two reaches in 2011 (K4 and K1); and two reaches in 2012-2018 (K4 and K3).

Environmental Conditions

Water temperatures in 2018 were 1-3ºC higher for most of the spring and summer, when compared to 2017. In 2018, water temperatures started out low in early March (approximately 6ºC) below Iron Gate Dam and climbed steadily through the end of June to 20ºC. Water temperature dropped slightly in early July before peaking at 21.2ºC on July 17 (Figure 6). Water temperature started to gradually decrease in late August.

In previous study years, we typically observed mean daily water temperatures of approximately 18ºC, and often as high as 22ºC below Iron Gate Dam, during the peak juvenile migration period of May through July. That trend held true in 2018 as the mean daily water temperature during peak juvenile migration was 18.4ºC.

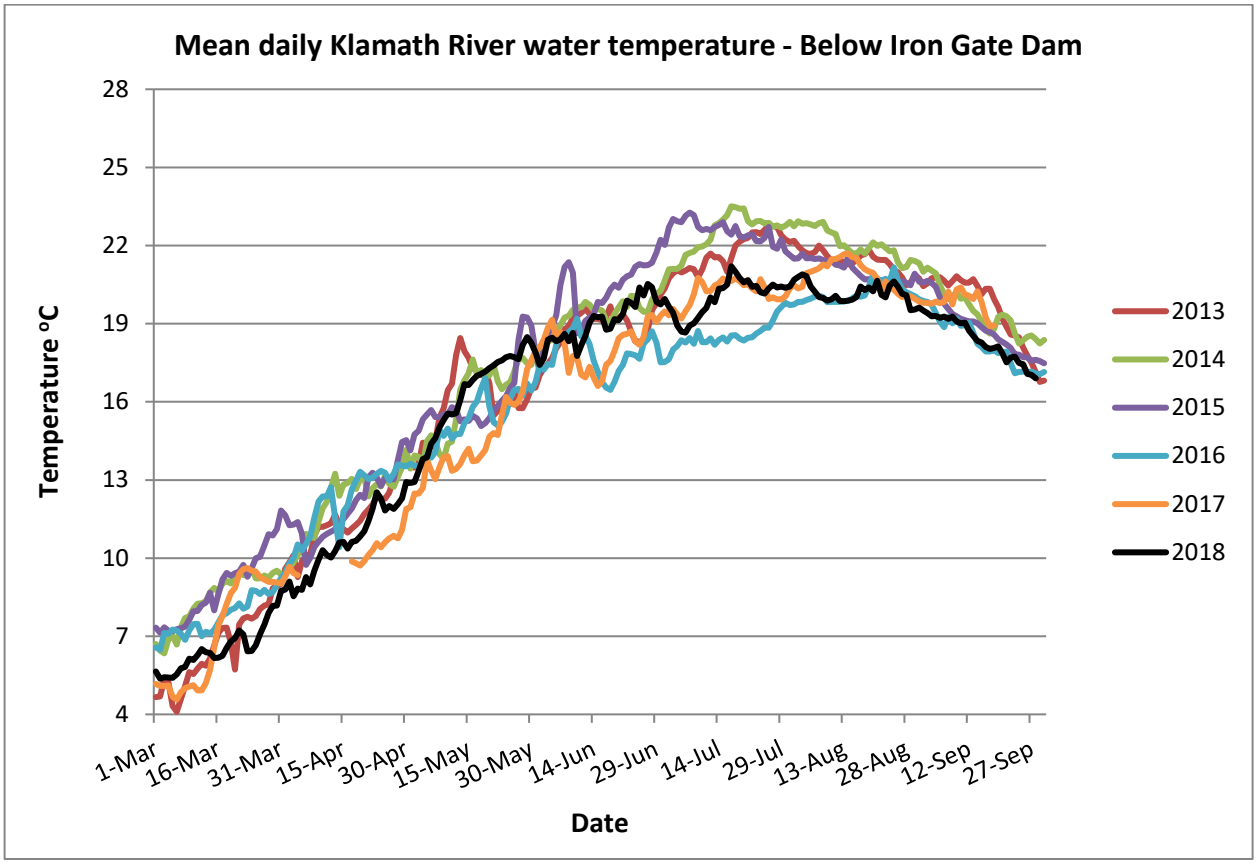


Figure 6. Mean daily Klamath River water temperature below Iron Gate Dam for 2013-2018. Temperature data for 2013-2015 was acquired from Arcata Fish and Wildlife Field Office. Temperature data for 2016-2018 was acquired from the Karuk Tribe.

In 2015 PacifiCorp installed a barrier curtain in Iron Gate reservoir upstream of the powerhouse intake structure. The purpose of the curtain is to minimize cyanobacteria and associated toxins from being released into the Klamath River; however, the curtain also has a secondary effect of reducing water temperatures downstream of the dam. The curtain can be raised or lowered vertically in the reservoir. When the curtain is deployed, water is released from deeper in the reservoir and therefore is cooler. PacifiCorp monitored water temperature in 2016 and found that cooling can be modest to substantial (2-4°C) depending on the curtain depth, the distance downstream from the curtain, and the time of year the curtain is being used (PacifiCorp, 2017).

In 2018, the operation of the barrier curtain had little influence on water temperatures in the Klamath River below Iron Gate dam. This is due to shallow curtain operation, unlike previous years where the curtain was deployed earlier in the summer and at deeper depths. The curtain, in 2018, was initially deployed at a shallow depth of 15ft in late July, lowered to a depth of 20ft in early August, and set back up to 15ft in mid-August. While the reservoir water temperature (downstream side of the curtain) dropped ~5°C, the river water temperature downstream of IGD only dropped ~1.8°C (personal communication with Demian Ebert, PacifiCorp). The deepest the curtain was operated in 2018 was 20ft, compared to the maximum depth of 35ft used in 2016.

Another temperature gauge is located in the Scott River to Salmon River (K3) reach, near Seiad Valley. This gauge has less influence from Iron Gate Dam, in contrast to the water temperatures in Figure 6, therefore this data shows the variability of water temperatures that can occur. At the Seiad Valley gauge, water temperature data were only available starting in mid-May. Temperatures increased from May through July (Figure 7) and peaked at Seiad Valley at 25.5ºC on July 24, 2018.

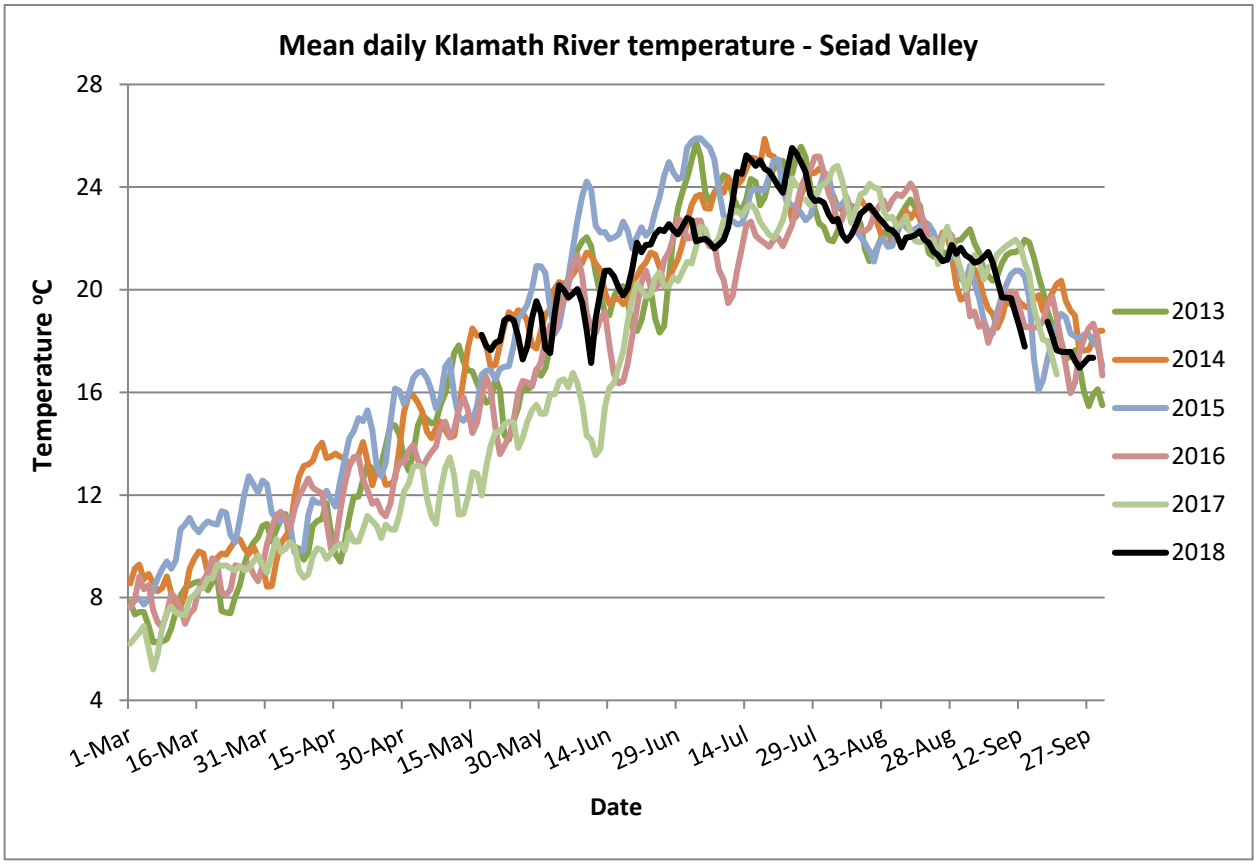


Figure 7. Mean daily Klamath River temperature from March through September 2013-2018 at Seiad Valley. Data from 2013 to 2015 were provided by the Arcata Fish and Wildlife Field Office, with the exception of 2014 temperature data that were provided by both Arcata FWO and Karuk Tribe. Temperature data for 2016-2018 was acquired from the Karuk Tribe.

In previous study years, we typically observed mean daily water temperatures of approximately 18ºC, and often as high as 24ºC at Seiad Valley, during the peak juvenile migration period of May through July. That trend held true in 2018 as the mean daily water temperature during peak juvenile migration was 21.4ºC.

River Flows

Dry conditions returned to California during the 2018 water year, with nearly all the state experiencing below average precipitation (CA Department of Water Resources, 2018). Based on California Precipitation Rankings, the 2018 water year was the driest year since 2014 (NOAA, 2018). This was also true for the north coast drainage division of the state, which includes the Klamath River.

Klamath River flow below Iron Gate Dam remained steady around 1,000 cfs early in the year. In April 2018, U.S. Bureau of Reclamation (BOR) implemented surface flushing flows paired with anticipated hydrologic conditions (high tributary flows) to maximize the potential benefit of the event. Flow peaked at 6,170 cfs on April 7, and then gradually decreased through the rest of April (Figure 8).

In May, BOR increased flows out of Iron Gate Dam again (flows reaching ~3,000 cfs) to address *C. shasta* disease concerns as criteria for implementing emergency dilution flows were exceeded on May 3 (BOR 2018). Dilution flows were implemented on May 7 and higher flows continued through May 21. The minimum discharge observed during the sampling season was 855 cfs on August 13.

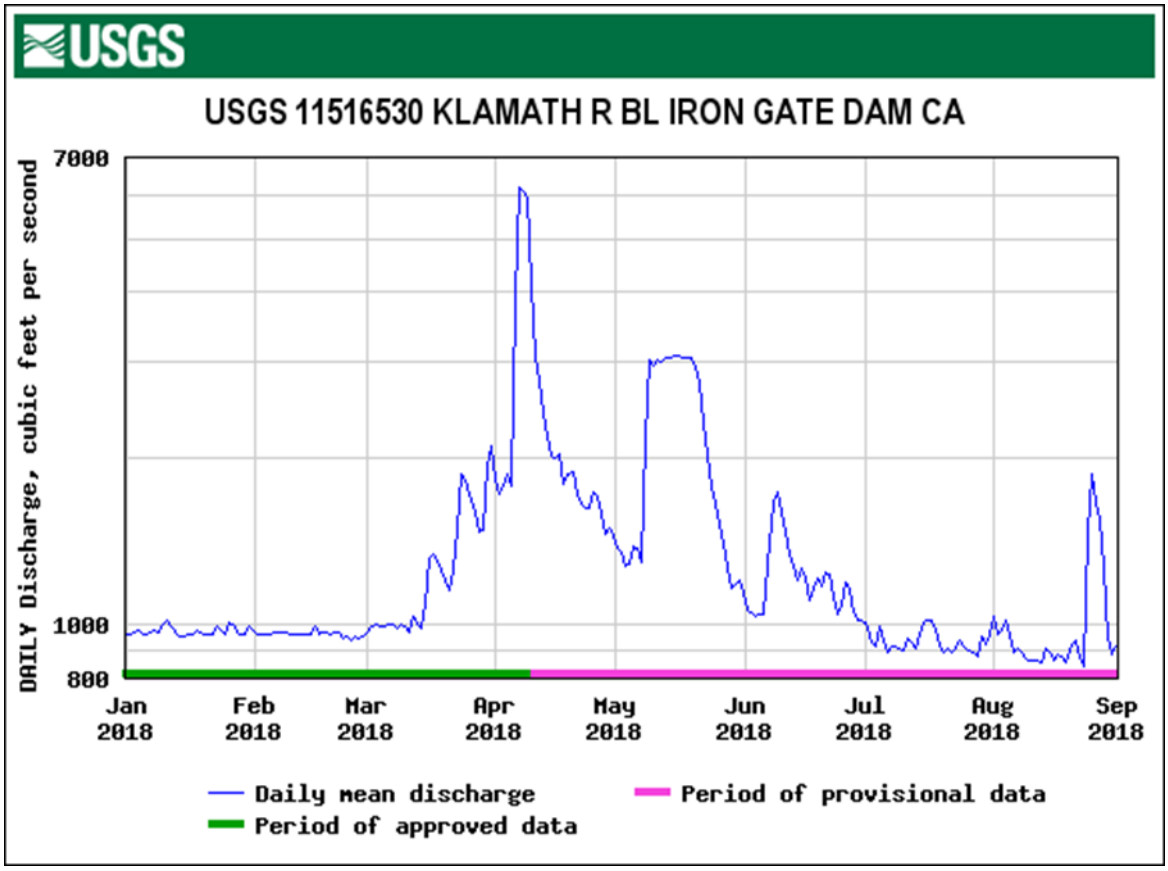


Figure 8. Daily discharge (cfs) below Iron Gate Dam from January 2018 through August 2018. Data collected from USGS gaging station 11516530 at waterdata.usgs.gov.

A similar hydrograph was observed downstream at Seiad Valley, with peaks in flow associated with BOR flow events in April and May 2018. The peak discharge at Seiad Valley occurred on April 8 at 11,400 cfs (Figure 9). Flows remained below 2,000 cfs at the end of summer, and the minimum discharge observed during the sampling season was 977 cfs on August 14.

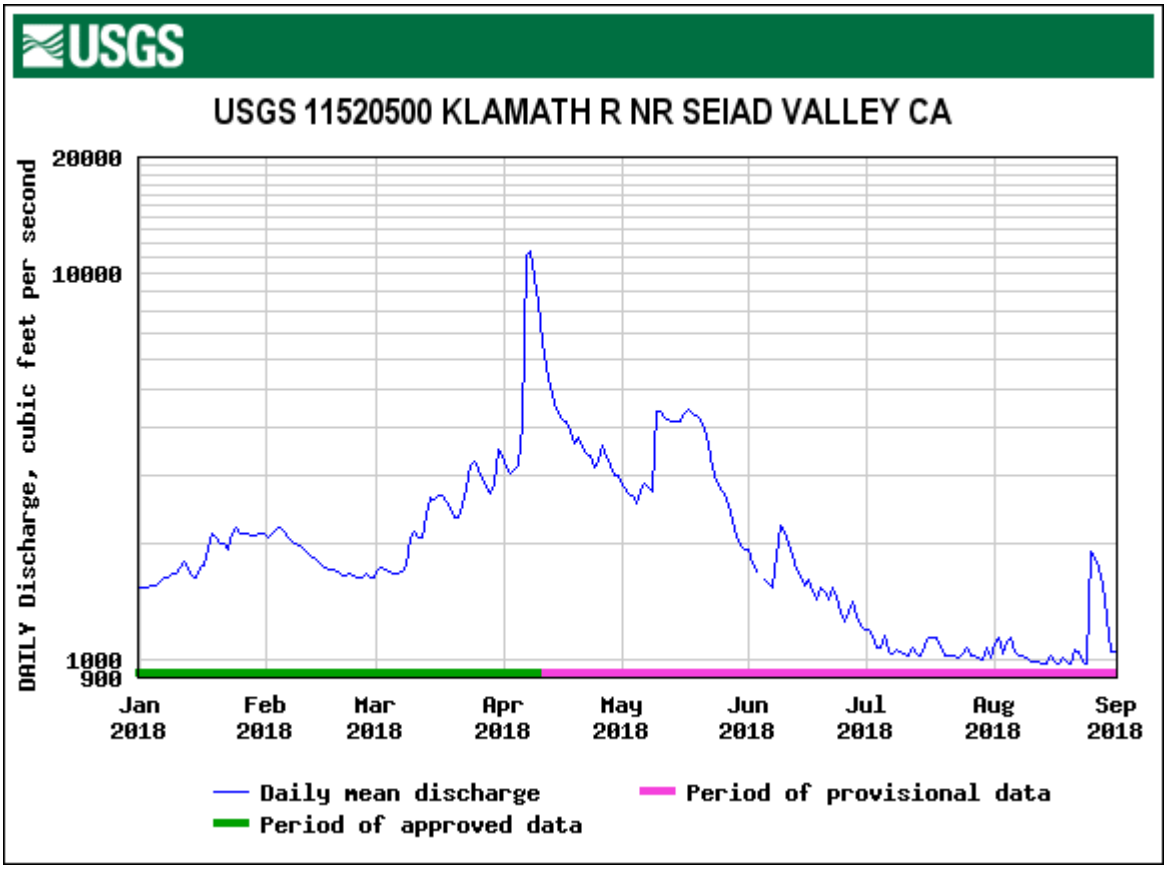


Figure 9. Daily discharge (cfs) near Seiad Valley from January 2018 through August 2018. Data collected from USGS gaging station 11520500 at waterdata.usgs.gov.

Klamath River flows in 2018 were lower compared to previous years (Figure 10). In 2009 and 2010, flows did not reach above 2,000 cfs at the USGS gage below Iron Gate Dam. In 2011, two peak spring flows exceeded 5,000 cfs, the first of which was a manipulated pulse flow released from Iron Gate Dam. In 2012, spring flows were close to 4,000 cfs. Both 2014 and 2015 were ranked as extreme drought years. In 2017 flows remained above 2,000 cfs for most of the spring and summer, as this was a wet year. Flow in 2018 was low, in comparison to 2017, as most of the year was less than 2,000 cfs except for the peaks when BOR released water.

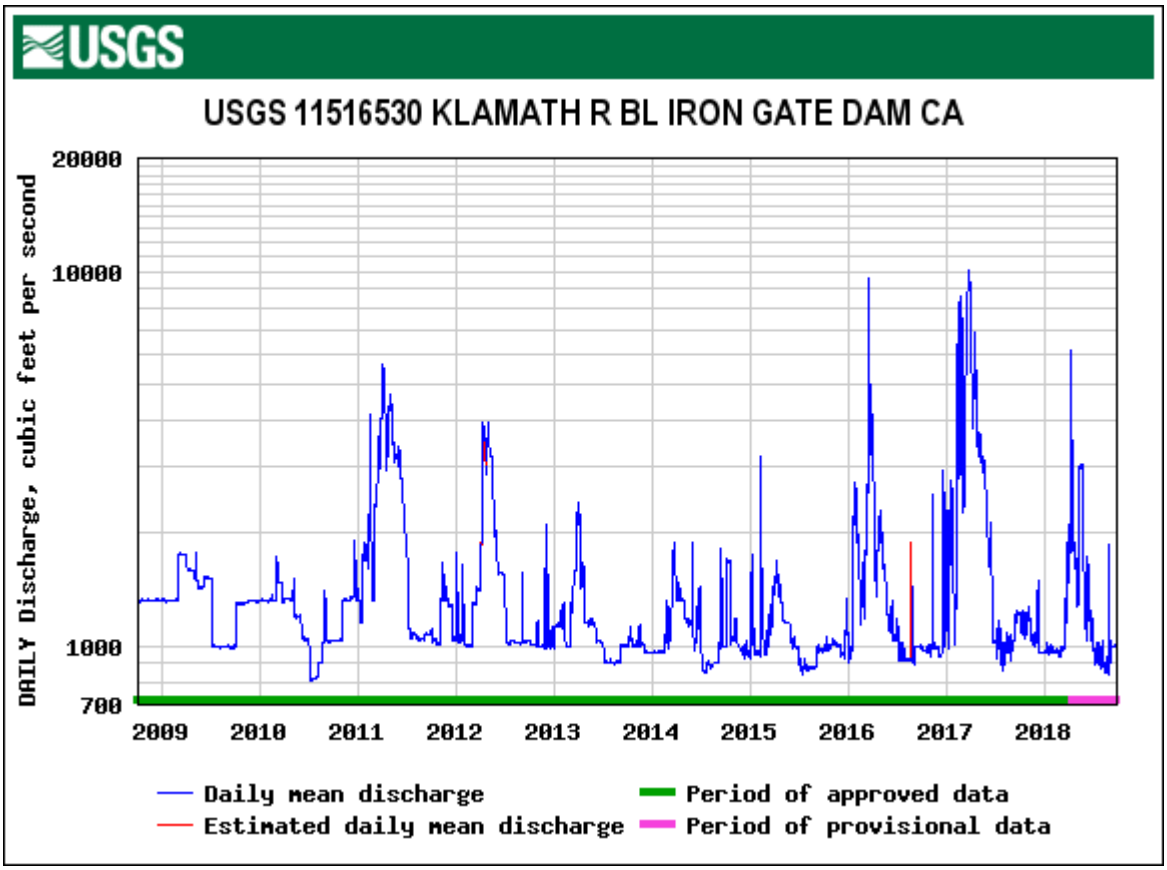


Figure 10. Daily discharge (cfs) below Iron Gate Dam from October 2009 through September 2018. Data acquired from USGS waterdata.usgs.gov

Discussion

In 2018, the annual *C. shasta* prevalence of infection, historical comparison, and POI in Iron Gate Hatchery CWTs were all lower in both QPCR and histology samples, compared to 2017. The only increase in *C. shasta* POI was observed in natural fish by QPCR. *Parvicapsula minibicornis* infection increased in almost every sample type by both QPCR and histology testing.

The year was usual in that Iron Gate Hatchery released ~4.2 million juvenile Chinook salmon, and therefore CWTs made up a large percentage of our sampling efforts (43%). This was not the case in 2017 when Iron Gate Hatchery released a tenth of normal production, making it very difficult to recover tagged hatchery fish, and resulted in only 35 CWTs being collected that year.

While the number of fish released in 2018 was comparable to most years, the hatchery did release fish later in the summer. In the last five years (2013-2017), juvenile Chinook salmon releases from Iron Gate Hatchery all started around late May, but in 2018 the releases started in early June. This later release date in 2018 allowed more time to collect natural fish in the system. This is the first time in the past decade, with the unique exception of 2017, where more natural fish were collected than CWTs. It is also the largest number of natural fish collected (537) in the past ten years.

Precipitation was below average in the state, including the Klamath River basin. Klamath River flows in 2018 were lower compared to previous years as most of the year was less than 2,000 cfs. Bureau of Reclamation implemented two water releases from Iron Gate Dam in 2018. The first one took place in April and was a surface flushing flow paired with anticipated hydrologic conditions. *Ceratonova shasta* remained undetected during this higher flow with the first detection date almost immediately after flows declined (April 23). The second release was an emergency dilution flow to address disease concerns, and went into effect on May 7. The total duration of higher flow was approximately 14 days, which corresponded with sample week 7 through the start of sample week 9. An increase in *C. shasta* POI was observed after one week of higher flow (real-time monitoring sample week 8, Table 2) in the Shasta to Scott (K4) reach, and a decrease was observed after two weeks of higher flow (sample week 9). Water sampling data appears to shows a similar pattern with waterborne spores increasing the second week of May in water collected from the Kinsman site (Oregon State University, 2018). It is difficult to determine if dilution flow had an immediate impact on *C. shasta* POI in juvenile Chinook salmon because exposure history is unknown in natural fish. Weekly *C. shasta* POI most likely reflects prior exposure for the sample groups tested each week, and POI may not be a sensitive enough metric to evaluate the immediate impacts of pulse/dilution flows.

Klamath River mean temperatures below IGD and at Seiad Valley were consistent with previous temperature profiles. Water temperatures were 1-3ºC higher for most of the spring and early summer below IGD, compared to 2017. Mean daily water temperatures at Seiad Valley were similar to 2017, though data were only available starting in mid-May.

Natural Chinook Salmon

Ceratonova shasta prevalence of infection was relatively low in natural fish at 11% by QPCR, but was an increase from 5% in 2017. The mean *C. shasta* POI for natural fish for the past ten years (2009-2018) is 27%, and has ranged from a low of 4% in 2012, to 75-76% during the drought years of 2014-2015. The increase in POI observed in 2018 could be due to lower flows and slightly higher spring water temperatures. Environmental conditions were very favorable for fish in 2017, therefore it is not surprising to see an increase in POI in the drier year of 2018. The increase seen in 2018 could also be attributed to testing a large number of natural fish.

There was also an increase in parasite infection level (mean DNA copy number) as seen in Figure 4. In the Scott to Salmon reach (K3), the first weekly *C. shasta* detection fell in the range of 2-4 logs of DNA (2.9 logs the week of April 22) and remained in that range the following week as well at 2.1 logs of *C. shasta* DNA. The Shasta to Scott reach (K4) had one sample that was in the range of 2-4 logs at 3.4 logs of DNA during the week of June 3. The previous year, 2017, had the lowest parasite infection levels in Figure 4; whereas 2015 (a year associated with drought) had many samples in the 2-4

log range. Early *C. shasta* infection levels (mean DNA copy number) in 2018 fall between 2017 (low infection) and 2015 (high infection), therefore 2018 is considered a moderate disease year.

All of the juvenile Chinook examined histologically were natural origin fish sampled from the two upper reaches (K4 and K3) prior to IGH release. Overall *C. shasta* prevalence of infection by histology was very low with one positive fish (1% POI), compared to 5% in 2017 and 13% in 2016. The positive fish had a *C. shasta* pathology score of 0.1 which suggests an early stage infection with very little tissue damage.

Iron Gate Hatchery Coded-wire Tagged Chinook Salmon

Among coded-wire tagged (CWT) juvenile Chinook salmon released from Iron Gate Hatchery from June 8-27, *C. shasta* was detected in 35% of fish screened by QPCR, compared to 37% in 2017. When comparing infection prevalence in IGH CWT by capture reach, *C. shasta* POI in CWT Chinook was highest in the Trinity to Estuary (K1) reach at 56%. In the Estuary, *C. shasta* POI was low at 21% indicating the majority of the fish sampled were not infected. The latest release groups (June 26-27) may have benefited from the slight decrease in river temperatures observed below IGD in early July; given that a six percent decrease in POI was observed between the first and last IGH release groups.

In terms of CWT Chinook infection prevalence regarding exposure period, the highest *C. shasta* prevalence of infection ranged from 42-51% in IGH CWT Chinook salmon residing 2-4 WAL. Prevalence of infection observed in IGH CWT Chinook followed a bell curve for groups residing between less than 1 to 5 WAL (Figure 5). The prevalence of infection pattern suggests that fish were probably exposed to actinospores soon after entering the river as prevalence increased quickly from ≤ 1 WAL to 1 WAL.

Two peaks in mean DNA copy number were observed at 2 WAL and 5 WAL. Both prevalence of infection and DNA copy number indicate that while a larger proportion of Chinook salmon residing at 2 WAL were infected upon recapture, the mean DNA copy number was similar to fish residing at 5 WAL. The 5 WAL group had fewer infected fish (22%), but the fish that were infected had the highest parasite levels. The onset and magnitude of *C. shasta* infections in CWT juvenile Chinook salmon was fairly typical in 2018, both in terms of overall POI and parasite loads in WAL groups.

Historical Comparison

For historical comparisons between monitoring years, data are restricted to all Chinook sampled during the peak migration period (May to end of July) in reaches above the Trinity confluence. Prevalence of *C. shasta* infection by QPCR during the peak out-migration period was low at 20% in 2018, and lower than the average of 41% for the past decade. The range of *C. shasta* POI has been quite variable (17-91% by QPCR and 3-62% by histology) but has correlated well with environmental conditions. Histology results for *C. shasta* in 2018 were the second lowest recorded since 2009 (Table 3).

Parvicapsula minibicornis

Prevalence of *Parvicapsula minibicornis* infection increased in 2018 in almost every sample type. The most notable increases were observed in the overall annual POI by QPCR which was 83% in 2018, compared to 67% in 2017 and 75% in 2016. A large increase was also seen in natural fish by QPCR where *P. minibicornis* POI was 73% in 2018, compared to 38% in 2017, and 39% in 2016.

Compared to 2017, the increase in prevalence of infection appears considerable, however the infection pattern (onset and progression) is typical for *P. minibicornis*. Many years we observe *P. minibicornis* infection early and the magnitude of POI remains high throughout the sampling season. Every year (2009-2018) has had at least three reach locations where weekly *P. minibicornis* POI values were 100%; five out of the ten years had 100% weekly values observed in every reach.

Waterborne *C. shasta* and *P. minibicornis* spores have different temporal and spatial distributions in the Klamath River; in 2006, *P. minibicornis* was present earlier in the year, in greater numbers, and at more sites when compared to *C. shasta* (Hallett et al., 2009). These findings resembled our results from 2018, in that *P. minibicornis* was first detected in the Klamath River three weeks before the first *C. shasta* detection. This early detection might also explain the reason why the parasite was identified in the pre-release examination at Iron Gate Hatchery this year, as the first *P. minibicornis* detection in the Klamath River was on April 2 and fish from the hatchery were inspected on May 15th.

Lastly, the increase in *P. minibicornis* prevalence of infection in 2018 is not surprising relative to 2017. Low adult returns, high spring flows that may have disrupted the number of infected polychaete hosts, and a smaller number of fish released from IGH in 2017 all made for a unique fish health monitoring year (True et al., 2017). Results for 2018 should not be closely compared to 2017 as conditions were uncharacteristic that year.

Coinfection

Analysis of coinfection was conducted in 2018 by looking at the proportion of fish that were concurrently infected with both *C. shasta* and *P. minibicornis*. The proportion of fish coinfected was almost identical to the annual *C. shasta* POI for the season (20.5% and 20.7% respectively). This would suggest that very few fish were infected with *C. shasta* only, and that was indeed the case. Two fish in 2018 were infected with only *C. shasta*. One fish was of natural origin and was collected on May 7 from the Kinsman trap (K4); the second fish was an IGH CWT that was collected on August 16, the last week of the sampling season, in the Estuary reach (K0). Using tag code information, this fish was in the river for seven weeks before being recaptured.

Over the years it has been anecdotally observed that most fish infected with *C. shasta* are also infected with *P. minibicornis*. In one study where the majority of fish were coinfected, fish that died of enteronecrosis and fish that survived both had similar *P. minibicornis* infection levels (True et al., 2012). This study suggests that *P. minibicornis* was not the driving factor of mortality in the study year of 2008. However, *P. minibicornis* has been detected at high levels by QPCR and caused clinical disease as observed by histology in 2009, 2010, 2012, and 2015. Questions arose early in the monitoring program about whether the two parasites have a synergistic effect or if a fish infected with *P. minibicornis* has a

greater chance of becoming infected with *C. shasta*. Additional studies are needed to answer these questions, but there is difficulty in obtaining or naturally exposing fish to a single myxozoan parasite.

In summary, 2018 was a moderately-low year for myxozoan disease impacts in natural and out-migrating juvenile Chinook salmon in the Klamath River. Over the course of the monitoring program, there have been diverse environmental conditions with regard to river temperature and seasonal flows. The environmental conditions in 2018 appear to correlate well with moderate disease levels observed in 2010-2012 (Table 3). The trends in annual *C. shasta* prevalence of infection in juvenile Chinook salmon demonstrate that river temperatures, flows, and myxozoan exposure dose are connected factors that influence disease severity in salmonids.

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Sascha Hallett and Kalyn Hubbard, Oregon State University

Photo contributions:
Cover Photo: Klamath River, CA-NV Fish Health Center.

Author Roles

The contributions of each author have been summarized below.

- Kimberly True – Project lead and real-time sampling coordination and reporting, data management and quality control, QPCR methodology and quality assurance, data analysis and pivot tables, and assistance with written annual report.
- Anne Voss – Data management, QPCR necropsy extraction and assays, histology processing, environmental data and figures, graphs, data analysis, and pivot tables. Primary author for 2018 annual report and reviewer's comments.
- Scott Foott – Histological sample processing and assessments for natural fish.

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Appendix A – Samples Collected

Table 1. Number of fish collected for QPCR testing and histology (H) by Klamath River reach (reach code) and sampling week.

Week	Sample date	Shasta R. to Scott R. (K4)	Scott R. to Salmon R. (K3)	Salmon R. to Trinity R. (K2)	Trinity R. to Estuary (K1)	Klamath R. Estuary (K0)
1	25-Mar	30				
2	1-Apr	30				
3	8-Apr	30	21			
4	15-Apr	30 (H10)	21 (H10)			
5	22-Apr	30	21			
6	29-Apr	30 (H10)	23 (H10)			
7	6-May	31	21			
8	13-May	30 (H10)	21 (H10)			
9	20-May	30	21			
10	27-May	9	20 (H10)			
11	3-Jun	4	21	21		
12	10-Jun	40	21	21		
13	17-Jun	30	19	21	5	
14	24-Jun	8	20	19	16	16
15	1-Jul		18	21	19	22
16	8-Jul		20	21	21	18
17	15-Jul			19	9	17
18	22-Jul			20	11	17
19	29-Jul				12	19
20	5-Aug				9	21
21	12-Aug					18

Table 2. *Ceratonova shasta* infection by QPCR in juvenile Chinook salmon sampled from 5 reaches within the Klamath River. The prevalence [% (number positive/number tested)] is presented for each sample reach by collection week and sample date.

Week	Sample Date	Shasta R. to Scott R. (K4)	Scott R. to Salmon R. (K3)	Salmon R. to Trinity R. (K2)	Trinity R. to Estuary (K1)	Klamath R. Estuary (K0)
1	25-Mar	0% (0/30)				
2	1-Apr	0% (0/30)				
3	8-Apr	0% (0/30)	0% (0/21)			
4	15-Apr	0% (0/30)	0% (0/21)			
5	22-Apr	10% (3/30)	5% (1/21)			
6	29-Apr	47% (14/30)	9% (2/23)			
7	6-May	26% (8/31)	0% (0/21)			
8	13-May	37% (11/30)	14% (3/21)			
9	20-May	10% (3/30)	19% (4/21)			
10	27-May	22% (2/9)	5% (1/20)			
11	3-Jun	25% (1/4)	0% (0/21)	5% (1/21)		
12	10-Jun	3% (1/40)	10% (2/21)	5% (1/21)		
13	17-Jun	3% (1/30)	0% (0/19)	10% (2/21)	20% (1/5)	
14	24-Jun	0% (0/8)	15% (3/20)	42% (8/19)	75% (12/16)	56% (9/16)
15	1-Jul		50% (9/18)	71% (15/21)	89% (17/19)	45% (10/22)
16	8-Jul		40% (8/20)	43% (9/21)	81% (17/21)	6% (1/18)
17	15-Jul			32% (6/19)	33% (3/9)	18% (3/17)
18	22-Jul			65% (13/20)	27% (3/11)	18% (3/17)
19	29-Jul				25% (3/12)	5% (1/19)
20	5-Aug				11% (1/9)	5% (1/21)
21	12-Aug					17% (3/18)
		K4 Total **12% (44/362)**	K3 Total **11% (33/288)**	K2 Total **34% (55/163)**	K1 Total **56% (57/102)**	K0 Total **21% (31/148)**

Table 3. *Parvicapsula minibicornis* infection by QPCR in juvenile Chinook salmon sampled from 5 reaches within the Klamath River. The prevalence [% (number positive/number tested)] is presented for each sample reach by collection week and sample date.

Week	Sample Date	Shasta R. to Scott R. (K4)	Scott R. to Salmon R. (K3)	Salmon R. to Trinity R. (K2)	Trinity R. to Estuary (K1)	Klamath R. Estuary (K0)
1	25-Mar	0% (0/30)				
2	1-Apr	3% (1/30)				
3	8-Apr	10% (3/30)				
4	15-Apr	37% (11/30)	38% (8/21)			
5	22-Apr	100% (30/30)	62% (13/21)			
6	29-Apr	100% (30/30)	100% (23/23)			
7	6-May	77% (24/31)	100% (21/21)			
8	13-May	100% (30/30)	100% (21/21)			
9	20-May	100% (30/30)	100% (21/21)			
10	27-May	100% (9/9)	95% (19/20)			
11	3-Jun	75% (3/4)	95% (20/21)	100% (21/21)		
12	10-Jun	58% (23/40)	100% (21/21)	95% (20/21)		
13	17-Jun	63% (19/30)	100% (19/19)	95% (20/21)	100% (5/5)	
14	24-Jun	25% (2/8)	100% (20/20)	100% (19/19)	100% (16/16)	100% (16/16)
15	1-Jul		100% (18/18)	95% (20/21)	100% (19/19)	100% (22/22)
16	8-Jul		100% (20/20)	100% (21/21)	100% (21/21)	100% (18/18)
17	15-Jul			100% (19/19)	100% (9/9)	100% (17/17)
18	22-Jul			100% (20/20)	100% (11/11)	100% (17/17)
19	29-Jul				100% (12/12)	100% (19/19)
20	5-Aug				100% (9/9)	100% (21/21)
21	12-Aug					89% (16/18)
		K4 Total **59% (215/362)**	K3 Total **91% (244/267)**	K2 Total **98% (160/163)**	K1 Total **100% (102/102)**	K0 Total **99% (146/148)**

Appendix B – Histological Summary

Table 1. Parasite abbreviations and tissue abnormalities listed in the histological result tables.

Kidney	
P. minibicornis troph.	*Parvicapsula minibicornis* trophozoite stage
P. minibicornis myxosp.	*Parvicapsula minibicornis* myxospore stage
Metacercaria	Immature trematode stage
C. shasta troph.	*Ceratonova shasta* trophozoite stage
Chloromyxum sp.	Chloromyxum species trophozoite stage
Pathology Score	**Mean kidney pathology score for sample group**
Intestine	
C. shasta troph.	*Ceratonova shasta* trophozoite stage
C. shasta myxosp.	*Ceratonova shasta* myxospore stage
Helminth	Trematode, nematode, or cestode
Pathology Score	**Mean intestine pathology score for sample group**
Other	
Adipose steatitis	Inflammation of visceral fat tissue
Adipose lipofuscin	Oxidized lipopigments within adipose cells
Gill	
Metacercaria	Immature trematode stage
Multif. Hyperplasia	Multifocal hyperplastic regions on lamellae

Table 2. Parasite prevalence of infection [number positive / number tested (%)], pathology score for kidney and intestine, and tissue abnormalities observed in histological sections of juvenile Klamath River Chinook salmon collected from the Shasta to Scott reach (K4). Collection dates are reported as Sunday of given week.

Collection week	April 15	April 29	May 13	POI
Kidney				
Pm troph.	1/10 (10)	5/10 (50)	9/10 (90)	15/30 (50)
Pm Myxosp.	0/10 (0)	0/10 (0)	8/10 (80)	8/30 (27)
Metacercaria	0/10 (0)	0/10 (0)	0/10 (0)	0/30 (0)
C. shasta troph.	0/10 (0)	0/10 (0)	0/10 (0)	0/30 (0)
Chloromyxum sp.	0/10 (0)	0/10 (0)	0/10 (0)	0/30 (0)
Pathology Score	0.0	0.0	1.9	
Intestinal tract				
C. shasta troph.	0/10 (0)	0/11 (0)	1/9 (11)	1/30 (3)
C. shasta myxosp.	0/10 (0)	0/11 (0)	0/9 (0)	0/30 (0)
Helminth	1/10 (10)	0/11 (0)	0/9 (0)	1/30 (3)
Pathology Score	0.0	0.0	0.1	
Adipose steatitis	1/7 (14)	2/8 (25)	7/7 (100)	10/22 (45)
Adipose lipofuscin	0/7 (0)	0/8 (0)	0/7 (0)	0/22 (0)
Gill				
Metacercaria	0/10 (0)	2/11 (18)	5/10 (50)	7/31 (23)
Multif. Hyperplasia	0/10 (0)	1/11 (9)	9/10 (90)	10/31 (32)

Table 3. Parasite prevalence of infection [number positive / total (%)], pathology score for kidney and intestine, and tissue abnormalities observed in histological sections of juvenile Klamath River Chinook salmon collected from the Scott to Salmon River (K3). Collection dates are reported as Sunday of given week.

Collection Week	April 15	April 29	May 13	May 27	POI
Kidney					
Pm troph.	0/8 (0)	2/10 (20)	8/10 (80)	10/10 (100)	20/38 (53)
Pm Myxosp.	0/8 (0)	0/10 (0)	1/10 (10)	0/10 (0)	1/38 (0)
Metacercaria	0/8 (0)	1/10 (10)	0/10 (0)	0/10 (0)	1/38 (0)
C. shasta troph.	0/8 (0)	0/10 (0)	0/10 (0)	0/10 (0)	0/38 (0)
Chloromyxum sp.	0/8 (0)	0/10 (0)	0/10 (0)	0/10 (0)	0/38 (0)
Pathology Score	0.0	0.0	0.1	1.4	
Intestinal tract					
C. shasta troph.	0/10 (0)	0 /10 (0)	0/10 (0)	0/8 (0)	0/38 (0)
C. shasta myxosp.	0/10 (0)	0 /10 (0)	0/10 (0)	0/8 (0)	0/38 (0)
Helminth	1/10 (0)	0 /10 (0)	0/10 (0)	0/8 (0)	1/38 (0)
Pathology Score	0.0	0.0	0.0	0.0	
Adipose steatitis	0/8 (0)	0/5 (0)	3/4 (75)	1/7 (14)	4/24 (17)
Adipose lipofuscin	0/8 (0)	0/5 (0)	0/4 (0)	0/7 (0)	0/24 (0)
Gill					
Metacercaria	0/10 (0)	4/9 (44)	3/10 (30)	8/10 (80)	15/39 (38)
Multif. Hyperplasia	0/10 (0)	3/9 (33)	1/10 (10)	6/10 (60)	10/39 (26)

Appendix C - Reviewer comments

Significant comments provided by reviewers of this report are included below. The author's response is given below each comment.

Reviewer #1

General comments: Reviewer had a general comment that the results section includes excess language that is later repeated in the discussion.

Response: Agreed with the reviewer that some results sections are a little too verbose. Language was moved to a more appropriate section (methods or discussion) in some cases. In other cases, we decided to keep language in the results section as we feel it increases the readers understanding of the result being presented, particularly for interannual comparisons.

General comments: Reviewer had general comments on nomenclature used throughout the report, especially in regards to the acronym QPCR.

Response: Keeping the QPCR acronym as we do define its meaning in the very first sentence of the report. The term has also been used in published peer reviewed literature on assay validation (True et al., 2009) and is consistent with terminology used in previous years reports.

Pg. 4 – Sample Sites, and Fish Groups: Reviewer commented that weekly POI exceeding 20% needs to be further explained.

Response: Agree with reviewer that the reader needs additional context on POI exceeding 20%. Sentence added to reference 2017 court order and criteria for emergency dilution flows.

Pg. 15 – Results, IGH (CWT) Chinook salmon: Reviewer wanted clarification on the term "constant fractional mark" on page 16, as the term was not mentioned earlier in the report.

Response: Agreed with the reviewer that the term should be defined, and it is not defined in the 2009 report when the term was first introduced. Moved language about the mark rate to the methods section on page 7 where fish groups are categorized, and defined the term.

Pg. 19 – Environmental Conditions: Reviewer would like some context as to where the Seiad Valley site is located, relative to other sites and reaches of the Klamath.

Response: Language has been edited to clarify that Seiad Valley temperature gauge (Karuk Tribe) is located in the K3 reach.

Reviewer #2

General comments: Reviewer had a general comment that a lot of the text in the result sections is explanatory.

Response: Similar comment as Reviewer #1. Improved the language of result sections (edited or moved elsewhere), where we deemed appropriate.

Pg. 13 – Coinfection: Reviewer suggests to add language about coinfection to the introduction, since it is not mentioned before the coinfection results are given.

Response: Language about coinfection has been added to the introduction on page 3.

Pg. 16 – Results, IGH CWT Weeks At Large: Reviewer suggests placing definitions earlier in the document, such as the definition for Weeks At Large.

Response: Definition of Weeks At Large (WAL) was moved to page 7 after CWT fish are defined.

Pg. 19 – Figure 6, water temperature below Iron Gate Dam: Reviewer pointed out that the formatting on the y-axis is not the same in the temperature graphs (Figure 6 and Figure 7)

Response: The number value on the y-axis (Figure 6, page 19), was edited so that Figure 6 and Figure 7 have the same format.

Pg. 20 – Figure 7, water temperature near Seiad Valley: Reviewer suggested added more context about this temperature location and why it was chosen.

Response: Language added to page 20 to explain that we choose to look at two locations on the river for water temperature. Language added to say that the water temperature below Iron Gate Dam is influenced by the dam, therefore water temperature downstream at Seiad Valley shows more variability.

Pg. 35 – Histological Summary, Appendix B, Table 1: Reviewer questioned if results were missing on this table for the text in bold.

Response: No, results are not missing from this table. Appendix B, Table 1 is a summary showing abbreviations used for histology. Table 1 is showing the reader how the following tables will be formatted and what information is being displayed.