U.S. Fish & Wildlife Service

California-Nevada Fish Health Center
FY 2017 Investigational Report:

Myxosporean Parasite (*Ceratonova shasta* and *Parvicapsula minibicornis*) Prevalence of Infection in Klamath River Basin Juvenile Chinook Salmon, March – August 2017

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Summary

Juvenile Klamath River Chinook salmon (*Oncorhynchus tshawytscha*) were assayed from March to August 2017 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 were assayed in real-time, with fish collected early in the week and processed for necropsy, DNA extraction and parasite QPCR assays, in order to provide timely data to fishery managers regarding flow management. *Ceratonova shasta* prevalence of infection (POI) reached 20% in the Shasta to Scott (K4) reach on May 15th, the 8th week of the monitoring program.

The seasonal *C. shasta* prevalence of infection by QPCR in Chinook salmon collected above the Trinity River confluence during the peak out-migration period (May-July) was 26%, considerably lower than 48% observed in 2016, and 91% in 2015. *Parvicapsula minibicornis* in Chinook salmon above the Trinity River confluence for the same period was 82%, compared to 89% and 99% in 2016 and 2015, respectively.

Among the fish groups tested, naturally produced Chinook salmon had a 5% prevalence of *C. shasta* infection by QPCR, considerably lower than 27% observed in 2016 and 75% in 2015. The onset of infection (first detection) in 2017 occurred on May 2 when mean daily river temperature below Iron Gate Dam was 12.5°C and at Seiad Valley was 13.1°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 (3-8%). *Ceratonova shasta* was not detected in five of the eight sample sets from the two reaches. Additionally, pathology scores were zero for seven of eight sample dates, indicating infection levels were well below clinical disease levels in natural Chinook juvenile salmon in the two upper reaches through late spring.

Due to low numbers of returning adults, Iron Gate Hatchery (IGH) was unable to collect the number of eggs required for the typical release of 4-5 million Chinook salmon in late Spring. IGH released 410,686 juvenile Chinook salmon on a single release date of May 26, 2017. Of the coded-wire tagged (CWT) Chinook release (approximately 100, 000), only 35 IGH CWT Chinook salmon were recovered during out-migration. *Ceratonova shasta* was detected in 37% of the IGH CWT fish tested by QPCR. The highest *C. shasta* prevalence of infection in marked Chinook juvenile salmon (71%) occurred in fish captured in the Klamath Estuary, however sample number was small (N=14).

In summary, 2017 presented a unique fish health monitoring year because of low adult returns, geomorphic flow events ("flushing flows"), and low numbers of juvenile Chinook released from Iron Gate Hatchery in late May. These conditions (low numbers of parasite fish hosts and high flushing flows) likely disrupted the efficiency of the *C. shasta* parasite life cycle. This resulted in low *C. shasta* infection levels and no clinical disease observed in any of the fish groups sampled in the Klamath basin during the out-migration period of March to August. Additionally, 2017 was markedly different in terms decadal disease trends (for both *C. shasta* prevalence of infection and parasite load in juvenile Chinook salmon) compared to drought years (2013-2015) and more typical monitoring years such as 2016.

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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.

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 (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 speciosa*, which (if infected) releases the actinospore stage into the water column which can subsequently infect the vertebrate salmon host. The actinospore develops within the vertebrate host, salmon or trout species, into a myxospore (Hallett et al., 2012). Once shed from an infected fish, the myxospore can infect the polychaete host to complete the life cycle (Bartholomew et al., 1997).

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 fish 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 (Nichols et al., 2009; Bolick et al., 2013; True et al., 2013).

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: 1) examine parasite prevalence in Klamath River juvenile Chinook salmon during the spring out-migration period; and 2) compare parasite prevalence in 2017 to previous years.

Methods

Pre-Release Examination

Prior to the Iron Gate Hatchery release (May 26, 2017), a fish health examination was conducted (May 9, 2017) to determine infection levels of *C. shasta* and *P. minibicornis*.

Sample Sites, Fish Groups and Number Sampled

Fish were collected in the main stem Klamath River between the Shasta River confluence and the Klamath River estuary. 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 2017, juvenile Chinook captured from the upper reach (K4) were assayed in real-time for the first 8 weeks of the study (March 26 - May 15). Juvenile Chinook were collected early in the week and processed for necropsy, DNA extraction and parasite QPCR assays, and data was reported to partners by week's end. Real-time monitoring provided timely data to fishery managers regarding flow management and when *C. shasta* POI reached 20% in out-migrating Chinook salmon.

Normally, existing salmonid downstream migrant traps are used for fish collections, but in 2017 the Kinsman rotary screw trap could not be deployed due to high flows, and fish were primarily captured by beach seining. Field crews collected weekly samples within a 1-2 day period to preclude 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 given for hatchery releases, and first pathogen detections, and histology collection dates.

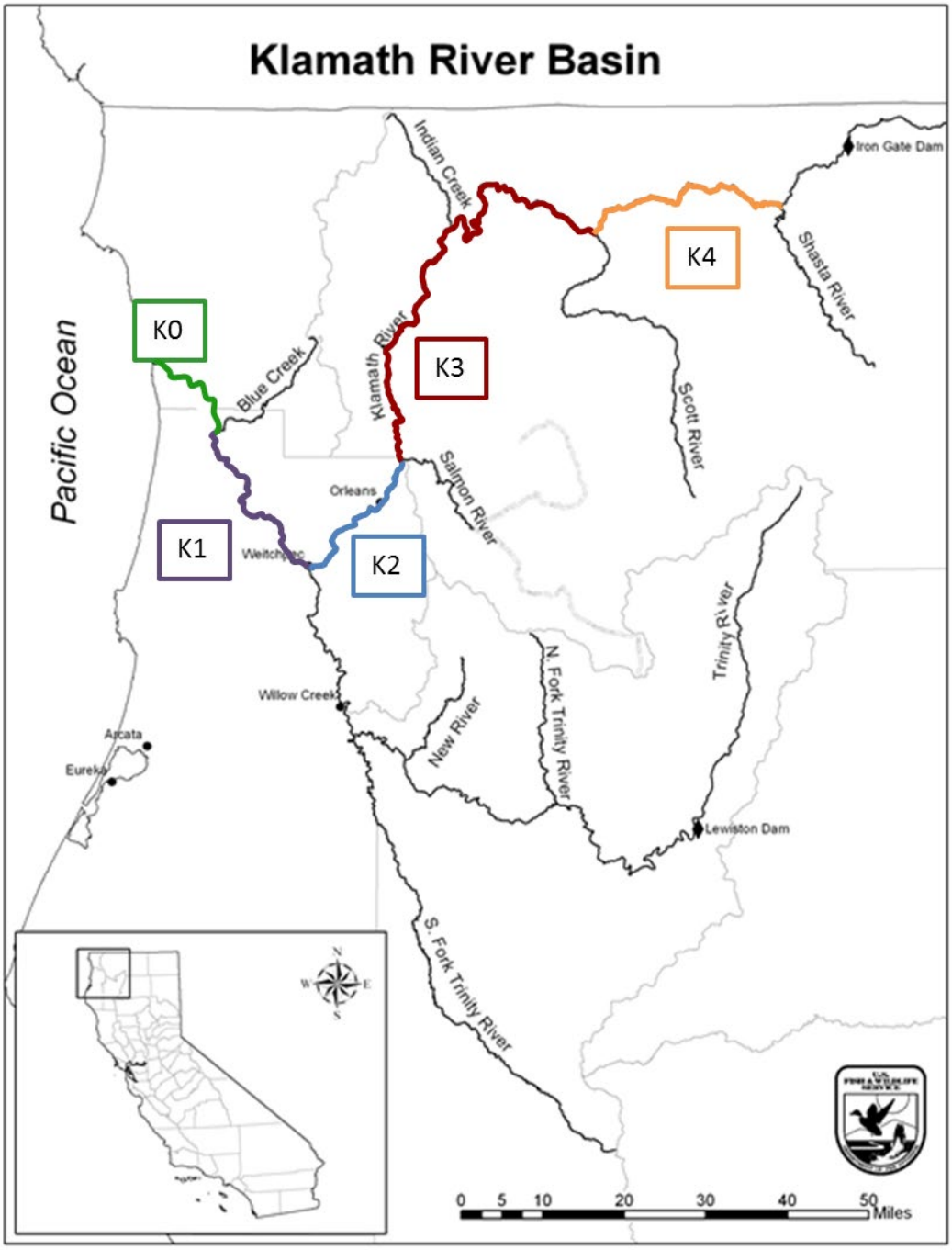


Figure 1. Klamath River watershed, major tributaries, and sample reaches: Shasta River to Scott River (K4), Scott River to Salmon River (K3), Salmon River to Trinity River confluence (K2), Trinity River to upper Estuary (K1), and Klamath River Estuary (K0). Map provided by the Arcata Fish and Wildlife 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 9). Lower reaches were sampled later in the season (June 4- August 6) as fish were migrating downstream (Appendix A – Table 1).

All fish sampled were categorized into three group types based on their origin: natural (before hatchery release), unknown (adipose fin present after hatchery release), and CWT (coded-wire tagged and adipose fin clipped). Fish numbers 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.

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 (Gene Block, IDT, Coralville Iowa) containing the 18S ribosomal DNA target sequence. Specifically, 1 ng of DNA, corresponding to 6.83×10^9copies of *C. shasta* DNA was serially diluted over 8 orders of magnitude in molecular grade water. Using QPCR analysis software, the cycle threshold (C_T) values for each standard concentration were calculated (SDS software 7300 SDS v 1.3.1, Applied Biosystems). The standard curve was used to evaluate PCR amplification efficiency (slope of the standard curve, efficiency was 93%), fit to the curve (R^2 value = 0.997) and the y-intercept (C_T value for a single copy of parasite DNA). Positive test results are tissue samples with C_T values below the statistically valid detection limit of the QPCR assay (C_T value of 39.5, 40 cycles).

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 3% decrease in assay efficiency were retested and reevaluated. A total of two plates were re-run over the 2017 field season.

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 1.3.1, Applied Biosystems). The standard curve was used to evaluate PCR amplification efficiency (slope of the standard curve, efficiency was 90%), fit to the curve (R^2 value = 0.998) and the y-intercept (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 2017 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 2017, histology samples were collected in the Shasta to Scott reach (K4) and the Scott to Salmon reach (K3) between the week of April 16 and May 28 (Appendix A - Table 3). 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 IGH Chinook Salmon

Juvenile Chinook salmon reared at Iron Gate Hatchery were screened for infections of *C. shasta* and *P. minibicornis* by QPCR on May 9, prior to release of 410, 686 juveniles on May 26. *Ceratonova shasta* and *Parvicapsula minibicornis* were not detected in the 30 juvenile Chinook salmon tested. Klamath River temperature below Iron Gate Dam on the single release date of May 26 was 16.0ºC (60.8ºF).

Number of All Fish Collected by Origin

In 2017 we tested 1215 juvenile Chinook salmon collected from the main stem Klamath River. The sample consisted of 462 natural fish, and 753 fish collected after hatchery release which included 106 CWTs. In typical years such as 2016 when IGH releases approximately 4-5 million juvenile Chinook, CWT account for a largest proportion of fish sampled (Figure 2). However in 2017, CWT Chinook salmon accounted for only a small proportion at 8.7% (106/1215) of all fish sampled.

In 2017, natural fish accounted for a larger proportion of fish sampled at 38% (462/1215), compared to 30% in 2016. Due to the difficulty in recapturing the much smaller group of CWT juvenile Chinook released from IGH, fish of unknown origin (unmarked hatchery fish or natural fish collected after hatchery release) comprise the largest proportion of juvenile Chinook sampled in 2017 at 53% (647/1215). This is much higher proportion than in a typical monitoring year, such as 2016, when CWT juvenile Chinook comprised the largest proportion and unknown fish comprised the smallest (Figure 2).

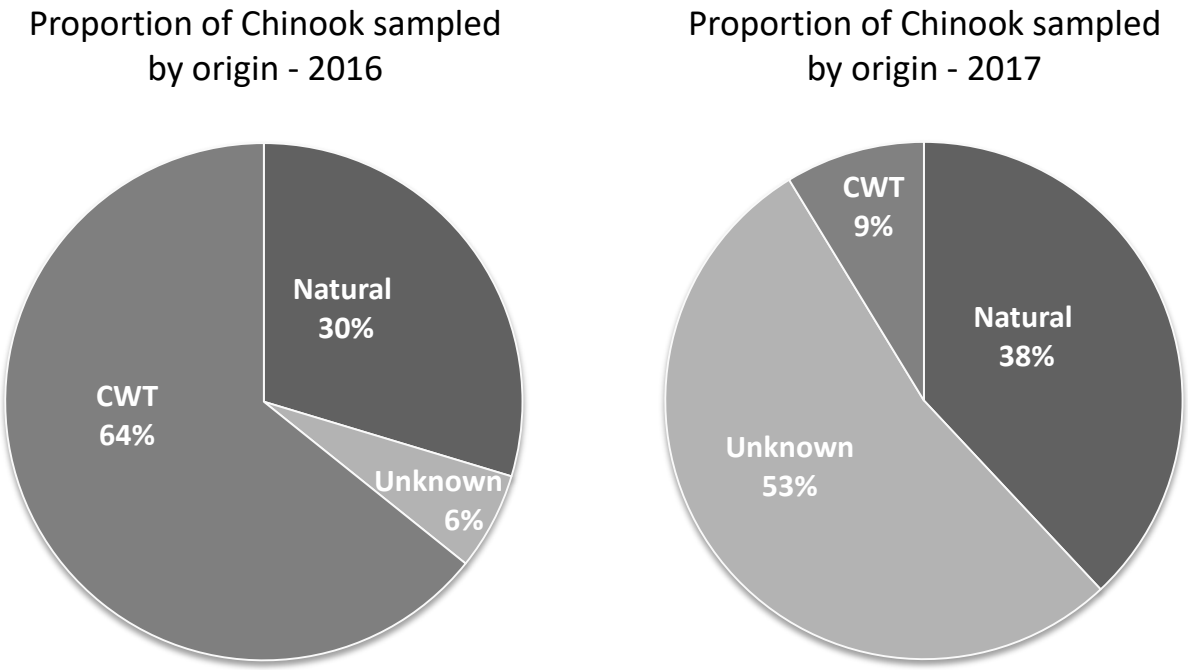


Figure 2. Proportion and origin of Chinook salmon collected (N=1215) in 2017, compared to (N = 934) in 2016.

After removing unreadable and/or Trinity River Hatchery (TRH) coded-wire tags, the total number of juvenile Chinook salmon analyzed for prevalence of infection in this report was 1144 : this consisted of 462 (40%) natural fish, 647 (57%) unknown fish, and 35 (3%) IGH CWT (Figure 3).

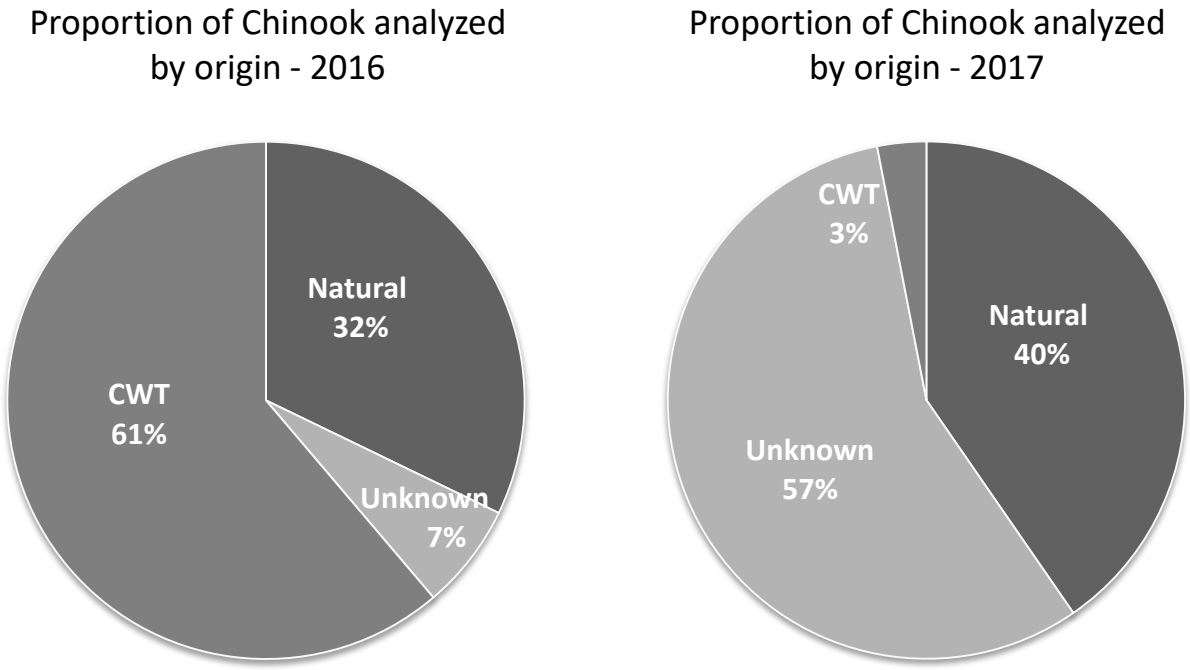


Figure 3. Proportion and origin of Chinook salmon used for prevalence of infection analysis (N=1144) in 2017, compared to (N = 861) in 2016. Unreadable tag codes or lost tags, and TRH tags have been removed from total number of fish collected, shown in Figure 1.

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

In 2017, juvenile Chinook salmon captured from the upper reach (K4) were assayed in real-time for the first 8 weeks of the study (March 26 - May 15). Juvenile Chinook were collected early in the week and processed for necropsy, DNA extraction and parasite QPCR assays, and data was reported to partners by week's end. Real-time monitoring provided timely data to fishery managers regarding flow management of when *C. shasta* POI reached 20% in out-migrating Chinook salmon. An analysis of prior monitoring years, when *C. shasta* POI first reached ≥20%, was conducted as a comparison to 2017 real-time monitoring
(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 Cs POI reached or exceeded 20%.

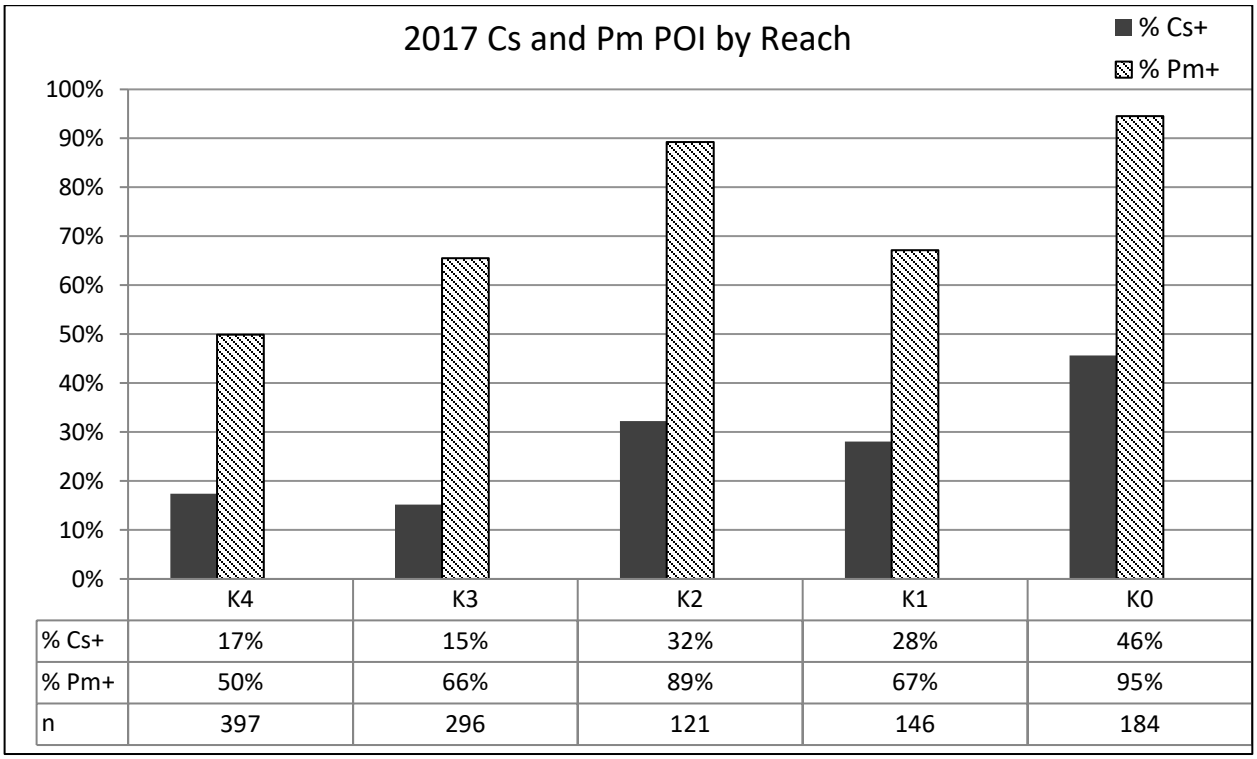
Year	Reach	Sample Week	Capture Date	Cs+	N Sampled	Cs POI
2009	K4	1	4/21/2009	3	30	10%
		3	5/5-6/2009	29	30	97%
		5	5/19/2009	22	30	73%
2010	K4	1	4/8/2010	0	20	0%
		3	4/21/2010	0	20	0%
		5	5/6/2010	0	20	0%
		7	5/19/2010	0	20	0%
		8	5/28/2010	8	31	26%
		9	6/2/2010	8	20	40%
2011	K4	2	4/15/2011	0	10	0%
		3	4/21/2011	0	10	0%
		4	4/27/2011	0	10	0%
		5	5/5/2011	0	10	0%
		6	5/12/2011	0	10	0%
		7	5/18/2011	4	10	40%
		8	5/25/2011	0	10	0%
		9	6/2/2011	2	10	20%
2012	K4	2	4/11-12/2012	0	15	0%
		3	4/18/2012	0	15	0%
		4	4/25/2012	0	15	0%
		5	5/3/2012	0	15	0%
		6	5/10/2012	2	15	13%
		7	5/17/2012	2	15	13%
		8	5/24/2012	1	15	7%
		9	5/31/2012	2	15	13%
2013	K4	1	3/28/2013	0	20	0%
		2	4/4/2013	0	20	0%

		3	4/11/2013	0	20	0%
		4	4/18/2013	0	20	0%
		5	4/25/2013	5	20	25%
		6	5/2/2013	0	20	0%
		7	5/9/2013	9	20	45%
		8	5/16/2013	13	19	68%
		9	5/22-23/2013	6	20	30%
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	K4	1	4/2/2015	0	20	0%
		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%

Annual Prevalence of Infection by Klamath River Reach

The annual prevalence of *C. shasta* infection in all Chinook salmon analyzed in 2017 by QPCR was 24% (278/1144, ci = 22-27%). *Ceratonova shasta* was first detected on May 2 in the Shasta to Scott reach (K4). *Ceratonova shasta* POI was highest in the Estuary (K0) reach at 46%, followed by 32% in the Salmon to Trinity (K2) reach. The lowest prevalence of 15% was observed in the Scott to Salmon reach (K3) (Figure 4).

The annual *P. minibicornis* POI in all Chinook salmon by QPCR was 68% (772/1144, ci = 65-70%). *Parvicapsula minibicornis* was first detected on April 24 in the Scott to Salmon reach (K3). Prevalence was highest in the Estuary (K0) at 95%, followed by the Salmon to Trinity reach (K2) at 89% (K0) at 85% (Figure 4). The lowest prevalence of 50% was observed in the Shasta to Scott reach (K4).



	K4	K3	K2	K1	K0
% Cs+	17%	15%	32%	28%	46%
% Pm+	50%	66%	89%	67%	95%
n	397	296	121	146	184

Figure 4. Prevalence of *Ceratonova shasta* (Cs+) and *Parvicapsula minibicornis* (Pm+) infection in juvenile Klamath River Chinook salmon by collection reach in 2017. 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.

As described in methods, histology sampling occurred during the week of April 16 to May 28 in the K4 reach and K3 reaches (Appendix B, Table 2 and Table 3). The annual *C. shasta* POI by histology for all fish tested in 2017 was 5% (4/80, ci = 1-12%) and for *P. minibicornis* was 22% (17/79, ci = 13-32%).

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 462 natural fish were collected in the Klamath River above the Trinity River confluence (K4 and K3) for testing by QPCR. Natural fish were collected from March 28 through June 1 in Shasta to Scott (K4) reach and from April 10 through May 31 in the Scott to Salmon (K3) reach. Mean daily river temperature was 9.9°C (data from Karuk Tribe, below IGD) at first detection of *C. shasta* (April 17) in natural fish collected in K4 reach.

Ceratonova shasta was detected by QPCR in 5% (25/462, ci = 4-8%) of natural fish in 2017, compared to 27% in 2016. *Ceratonova shasta* POI was highest at 6% (17/293, ci = 3-9%) in the Shasta to Scott (K4) reach compared to 5% (8/169, ci = 2-9%) in the Scott to Salmon (K3) reach below. The Fish Health Center did not observe any clinical disease signs in natural fish during necropsy in 2017. Comparatively, *P. minibicornis* was detected in 38% (175/462, ci = 33-42%) of naturally produced Chinook salmon by QPCR. The highest *P. minibicornis* prevalence of 43% (72/169, ci = 35-50%) was detected in Scott to Salmon reach (K3) and the lowest prevalence 35% (103/293, ci = 30-41%) was observed in the upper Shasta to Scott reach (K4).

Natural fish were collected for histology prior to first IGH releases on May 26. The prevalence of *C. shasta* infection by histology was low in both collection reaches, at 8% (3/40, ci = 2-20%) in the Shasta to Scott (K4) reach and 3% (1/40, ci = 0-13%) in the Scott to Salmon (K3) reach. In the Shasta to Scott (K4) reach, *C. shasta* prevalence of infection by histology was negative for the first and third sample of four collection dates from April 16 to May 28. The *C. shasta* pathology score was zero for all but one sample date (Appendix B, Table 2). 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 22% (17/79, ci = 13-32%). Prevalence was highest in the upper Shasta to Scott (K4) reach at 28% (11/40, ci = 15-44%) compared to 15% (6/39, ci = 6-31%) in the Scott to Salmon (K3) reach. The kidney pathology scores were zero for three of four collection dates in the Shasta to Scott (K4) reach, and zero for all four collection dates for the Scott to Salmon (K3) reach (Appendix B, Table 3). For reference, pathology scores of 6-8 have been observed in clinical disease, in previous monitoring years (True et al., 2010).

In an analysis of *C. shasta* disease threshold in natural fish, DNA copy number by QPCR is assessed in juvenile fish from the upper K4 and K3 reaches. For these analyses, QPCR data from previous studies (True et al. 2012) compare daily parasite DNA levels with histological assessments (rankings of clinical disease by tissue damage that would likely lead to mortality). In this analysis, we determined an infection threshold of 2-4 logs *C. shasta* DNA represents advanced clinical disease that is highly likely to result in mortality as the infection progresses at temperatures (15-18ºC) that are common during natural juvenile Chinook migration period.

The 5-year period (2013-2017) of these analyses represent a moderate *C. shasta* disease year (2013), two severe years associated with drought (2014-2015), a relatively low infection year (2016) and the most recent data for 2017 (Figure 5). The QPCR disease profile of *C. shasta* POI and parasite infection level for 2017 is markedly lower than the previous 4 years and well below the disease threshold of 2-4 logs of *C. shasta* DNA.

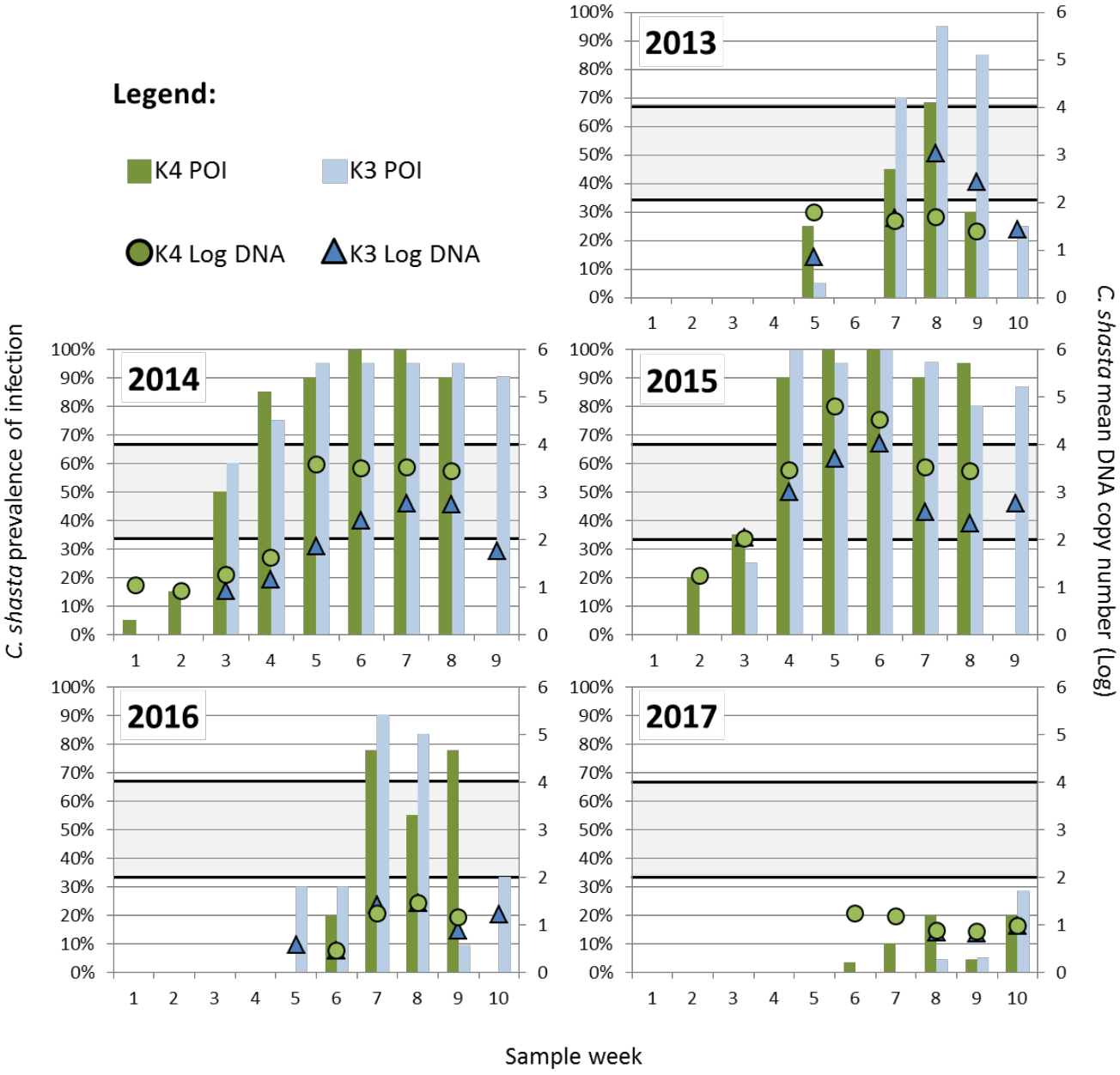


Figure 5. *Ceratonova shasta* prevalence of infection in natural Chinook juveniles, captured in Shasta to Scott (K4) reach and Scott to Salmon (K3). Prevalence of infection shown in columns (Y axis) and *C. shasta* mean DNA copy number (log) shown in circles and triangles (secondary Y axis). *Ceratonova shasta* mean DNA range of 2-4 logs determined to correlate with unrecoverable infection levels by histology, likely resulting in mortality as parasite proliferation continues. Sample Week shown on X axis, beginning with natural fish collected the end of March-first week of April (Week 1), through end of May.

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 647 fish of unknown origin were collected from June 6 to August 8. *Ceratonova shasta* was detected by QPCR in 37% (240/647, ci = 33-41%) of unknown origin Chinook salmon. *Parvicapsula minibicornis* POI in fish of unknown origin was 87% (566/647, ci = 85-90%).

Iron Gate Hatchery (CWT) Chinook salmon

The 25% constant fractional mark rate at IGH since 2009 has facilitated the capture of a large proportion of IGH CWT Chinook salmon in the past seven years of the monitoring program (Buttars et al., 2009). Coded-wire tagged salmon originating from IGH were collected in the Klamath River from June 7 to July 20; however as noted in the methods section sample size for the entire season was very small due to difficulty recapturing the small number of marked juvenile Chinook released from IGH. A total of 106 CWT Chinook salmon were collected of which Iron Gate Hatchery CWT fish accounted for 33% (35/106) and TRH CWT fish accounted for 57% (60/106). Additionally, 11 fish (10%) had lost or unreadable tags, which meant their release date is unknown.

Ceratonova shasta was detected in 37% (13/35, ci = 21-55%) of IGH CWT screened by QPCR. Prevalence of infection for *C. shasta* was highest at 71% (10/14, ci = 77-100%) in the Estuary reach (K0) and not detected in the Scott to Salmon (K3) reach where only 3 CWT fish were recovered.

Parvicapsula minibicornis was detected by QPCR in 89% (31/35, ci = 73-97%) of IGH CWT. Prevalence of infection for *P. minibicornis* was highest at 100% (14/14) in the Estuary reach (K0) and lowest at 75% (12/16, ci = 48-93%) in the Shasta to Scott reach (K4).

IGH CWT Weeks At Large

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).

Due to the very small numbers of IGH CWT juvenile Chinook salmon collected in 2017, Weeks at Large (WAL) analysis was not informative. The largest sample size (N=13) recovered for IGH CWT occurred for fish residing 2 WAL however *C. shasta* was not detected in this group. Only 3 fish residing 3 WAL were recovered. The highest *C. shasta* POI (50%) and DNA copy number (1.9 logs) occurred in fish residing 4 WAL, but again the small sample size of 6 fish does not provide the temporal data needed to draw meaningful conclusions about infection levels associated with exposure period. The fact that *C. shasta* was not detected in the thirteen fish residing at 2 WAL does reinforce that infectivity was low in early June in 2017.

Historical Comparison

Prevalence of infection by QPCR is the metric that 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 26% (153/600, ci = 22-29%) in 2017, and lower than the average of 43% for the past decade (2006-2017, Table 3). *Parvicapsula minibicornis* prevalence of infection by QPCR in Chinook salmon above the Trinity River confluence for the same period was 82% (493/600, ci = 79-85%) compared to 89% in 2016 and 99% in 2015.

Prevalence of *C. shasta* infection by histology was very low in 2017, compared to the previous years, and was similar to the lowest disease years such as 2011 and 2012.

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, 2006-2017.

Year	Histology (% Positive)	QPCR (% Positive)
2006	21	34
2007	21	31
2008	37	49
2009	54	47
2010	15	17
2011	2[1]	17
2012	9[1]	30
2013	16[1]	46
2014	42[1]	81
2015	62[1]	91
2016	14[1]	48
2017	**8[1]**	**26**
Mean	**25**	**43**

[1] Histology limited to two reaches in 2011 (K4 and K1); and two reaches in 2012-2017 (K4 and K3).

Environmental Conditions

Water temperatures in 2017 were 1-2ºC lower for most of the spring and summer, when compared to 2016. In 2017, water temperatures started out low in early March (approximately 5ºC) below Iron Gate dam and quickly rose to 9ºC by the end of the month. Water temperatures gradually increased through the end of May with a small spike in temperatures at the beginning of June. Klamath River water temperatures below Iron Gate dam peaked at 21ºC on July 9, 2017 (Figure 6).

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 2017 as the mean daily water temperature during peak juvenile migration was 17.6ºC.

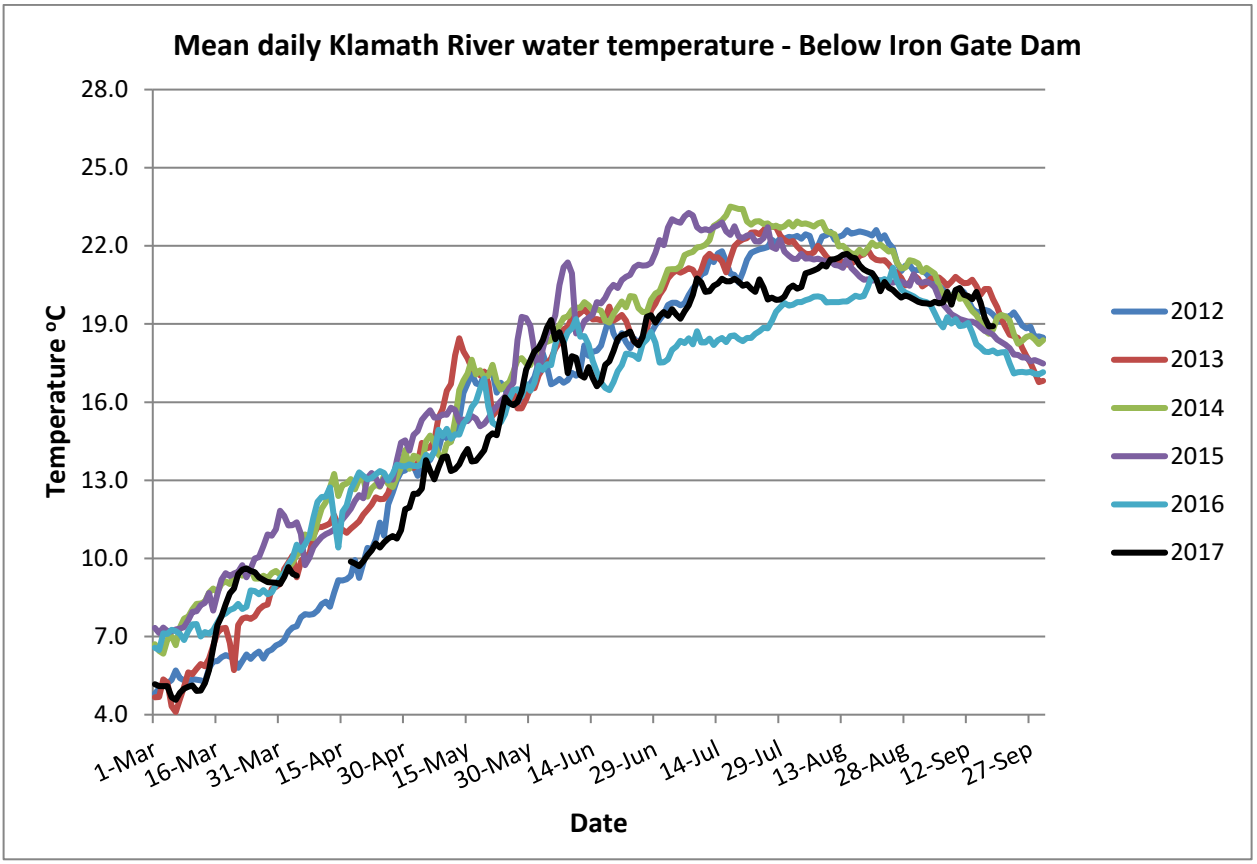


Figure 6. Mean daily Klamath River water temperature below Iron Gate Dam for 2012-2017. Temperature data for 2013-2015 was acquired from Arcata Fish and Wildlife Field Office. Temperature data for 2012 acquired from Iron Gate Hatchery and taken from the main stem Klamath River, not the hatchery facility. Temperature data for 2016-2017 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 substantial to modest (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 2017, the operation of the barrier curtain had little influence on water temperatures in the Klamath River below Iron Gate dam. The curtain was deployed at a shallow depth for most of the summer due to large algae bloom and low dissolved oxygen (personal communication with Demian Ebert, PacifiCorp). The deepest the curtain was operated in 2017 was at 25ft, compared to the maximum depth of 35ft used in 2016.

At the Seiad Valley temperature gauge, water temperatures were cooler in the spring then in recent years. The decrease in temperature observed below Iron Gate Dam in mid-June can also be observed at Seiad Valley where mean daily temperature decreased from 17ºC on June 6 to 14ºC on June 11 (Figure 7). Temperature rose back to 20ºC by June 20. Klamath River water temperatures at Seiad Valley peaked at 25ºC on August 2, 2017.

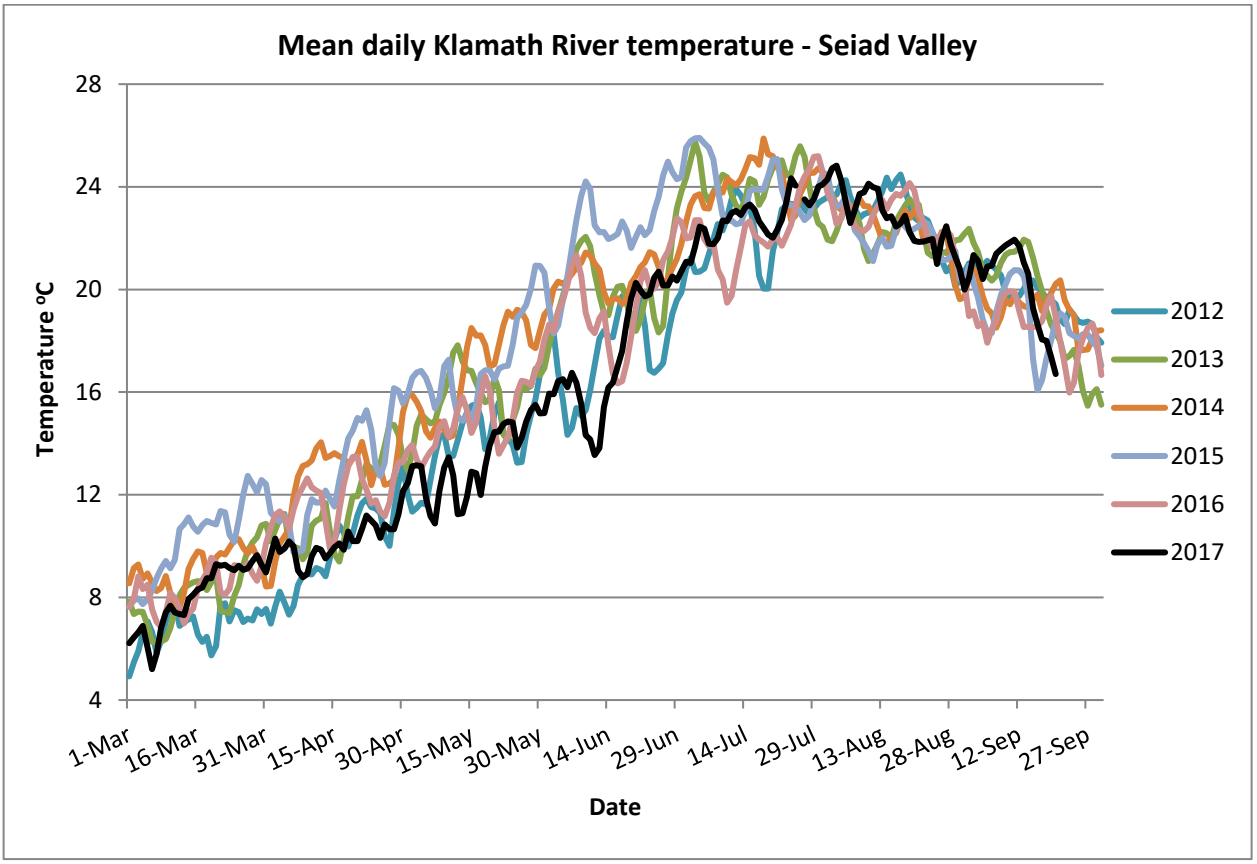


Figure 7. Mean daily Klamath River temperature from March through September 2012-2017 at Seiad Valley. Data from 2012 to 2015 were provided by the Arcata Fish and Wildlife Field Office, with the exception of 2014 temperature data that were provided by Arcata FWO and Karuk Tribe. Temperature data for 2016-2017 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 2017 as the mean daily water temperature during peak juvenile migration was 17.9ºC.

River Flows

High amounts of fall and winter precipitation occurred in California during the 2017 water year (Figure 8). Based on California Precipitation Rankings, 2017 was the wettest year since 1998 (NOAA, 2017). Much of the precipitation fell during an extremely wet January and February as numerous atmospheric river events drenched the state (Di Liberto, 2017). The 2017 water year also came in second place for statewide runoff, behind the wettest year of 1983 (CA Department of Water Resources, 2017).

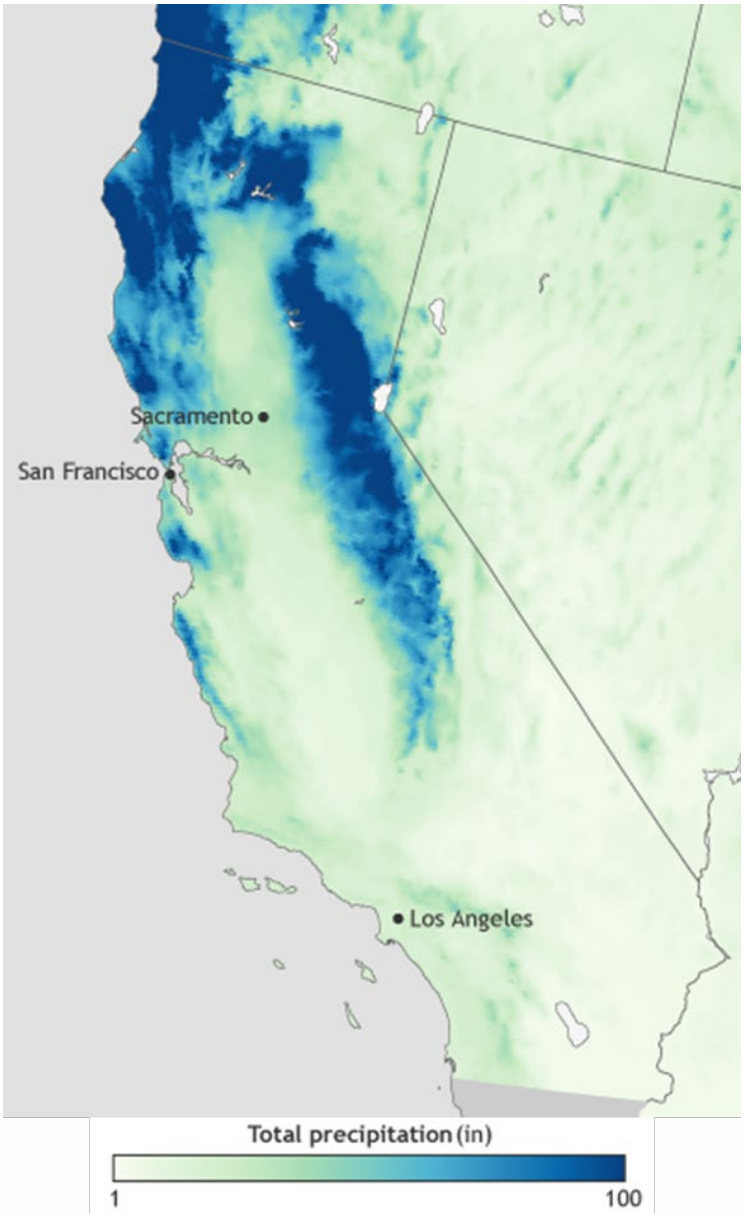


Figure 8. Precipitation totals in California from October 2016 to September 2017. NOAA Climate.gov

The wet year was reflected in Klamath River discharge, especially early in the year. Klamath River flow below Iron Gate dam increased sharply in early February to 8,280 cfs, followed by a second peak at 8,600 cfs near the end of the month (Figure 9). River discharge increased for most of the month of March. The peak river discharge below Iron Gate Dam was 10,100 cfs on March 23. Collection of juvenile Chinook began on March 28th when flow was 9,380 cfs and this was the maximum discharge recorded during the sampling season. River discharge dropped in mid-April and gradually decreased for the remainder of the sampling season. The minimum discharge observed during the sampling season was 857 cfs on July 29 (Figure 9).

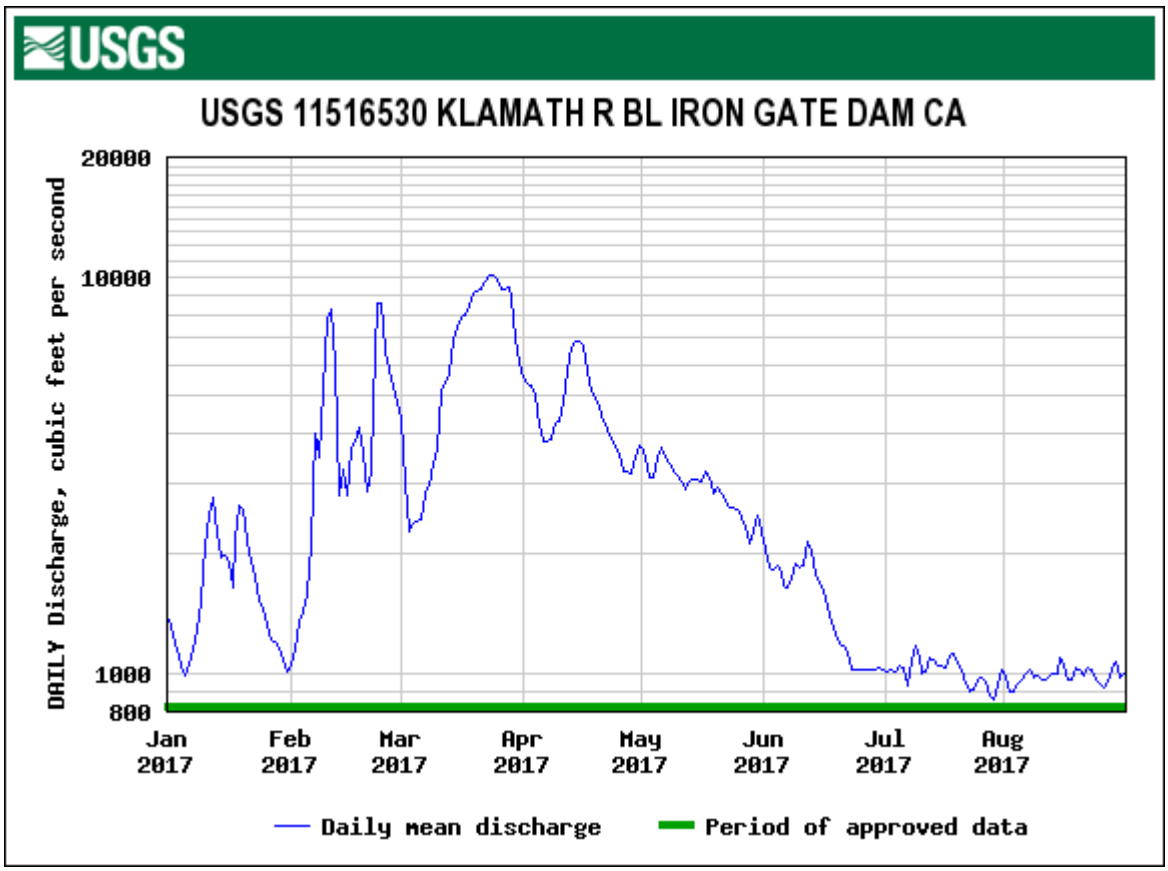


Figure 9. Daily discharge (cfs) below Iron Gate Dam from January 2017 through August 2017. Data collected from USGS gaging station 11516530 at waterdata.usgs.gov.

A similar hydrograph was observed downstream at Seiad Valley, with high flows in February and March. The peak river discharge for the year at Seiad Valley occurred in February (37,700 cfs) before juvenile Chinook collection began (Figure 10). Flows remained above 6,000 cfs for most of the summer, before dropping below 2,000 cfs in July.

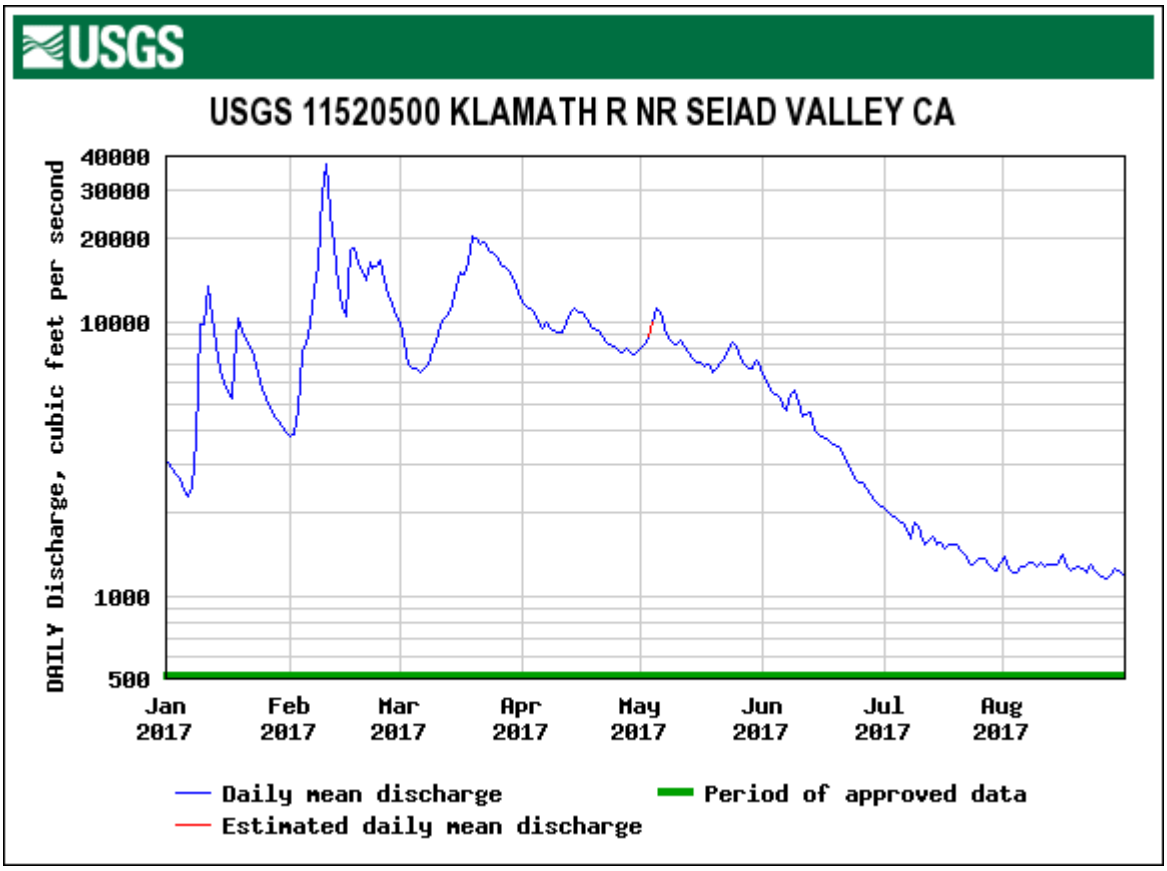


Figure 10. Daily discharge (cfs) near Seiad Valley from January 2017 through August 2017. Data collected from USGS gaging station 11520500 at waterdata.usgs.gov.

Klamath River flows in 2017 were high compared to previous years (Figure 11). 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. However, in 2017 flows remained above 2,000 cfs for most of the spring and summer, with multiple peaks between 8,000 and 10,000 cfs.

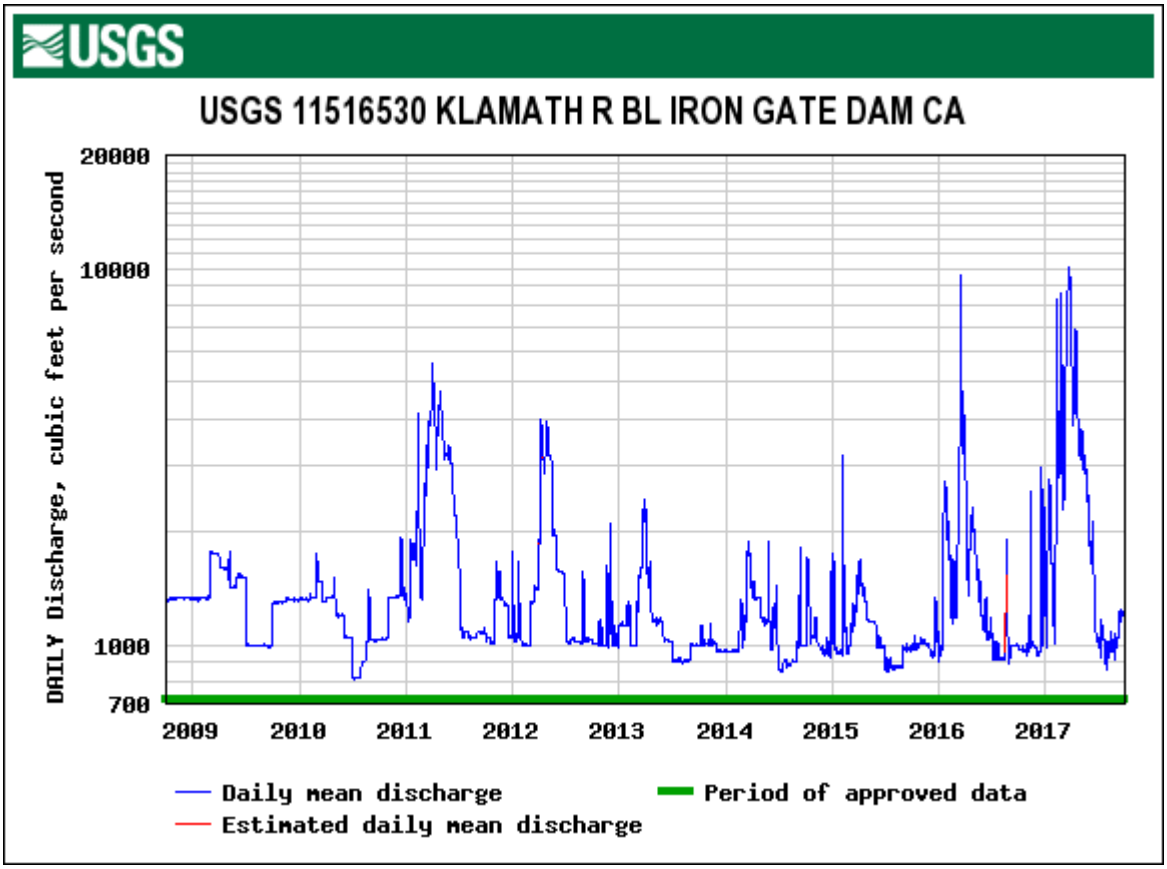


Figure 11. Daily discharge (cfs) below Iron Gate Dam from October 2009 through September 2017. Data acquired from USGS waterdata.usgs.gov

Discussion

In the Klamath Basin, 2017 represented a unique "natural experiment" in the efficiency, and perhaps disruption, of the *C. shasta* parasite life cycle. *Ceratonova shasta* requires both the vertebrate fish host (primarily adult salmon that carry the parasite to the upper watershed), and the invertebrate polychaete worm to complete the transformation to the waterborne infectious actinospore stage for fish (adults and juvenile salmon). Adult returns in fall of 2016 and release of juveniles in 2017 did not provide the number of fish hosts normally present in the system (adult returns to IGH in the fall of 2016 were low at 2586 fish, KRTT Report, 2017). Low numbers of adults, coupled with geomorphic flow events (high river and/or flushing flows) that were sufficient to flush polychaetes from their habitats, left short supplies of both of the parasite's obligate hosts. The subsequent release of smaller numbers of fall Chinook salmon from IGH (410,686 compared to 4-5 million in most years) further limited the number of vertebrate fish hosts that could be infected during spring and summer, and potentially releasing myxospores in reaches below IGH and the infectious zone.

Record precipitation in the basin beginning in September 2016 (Figure 8) coupled with Klamath River flows that were higher in 2017 than the previous 8 years (Figure 11) likely did not provide environmental conditions that were favorable to polychaete worm (either for overwintering populations, or for early or high abundance levels in spring). Klamath river flows had multiple peaks between 8,000 and 10,000 cfs in February, March and April and flows also remained above 2,000 cfs for most of the late-spring and early summer.

Klamath River mean temperatures below IGD and at Seiad Valley were not out of step with previous temperature profiles, and were not likely a significant disease factor in 2017. Water temperatures were 1-2ºC lower for most of the spring and early summer (Figure 6 and 7). Mean daily temperatures at Seiad Valley decreased from 17ºC on June 4 to 14ºC on June 11 and slowly returned to 20ºC by June 20. This period may have provided some thermal refuge for the 80% of natural fish that emigrated past the Kinsman trap site (Shasta to Scott reach) by May 20 (Arcata FWO Technical Memorandum, May 15, 2017). Iron Gate Hatchery fish were also out-migrating through the upper and middle Klamath reaches during this period, which was approximately two weeks post-hatchery release (May 26, 2017). High river flows and low fish numbers (adult and juveniles) were more likely to have a played a larger role in disease dynamics in 2017.

Natural Chinook Salmon

Ceratonova shasta prevalence of infection and parasite infection levels were low in all fish groups examined, but particularly low in natural fall Chinook juveniles at 5%, compared to 27% in 2016, 75% in 2015 and 76% in 2014. The mean *C. shasta* POI for natural fish for the past 9 years (2009-2017) is 34%, and has ranged from a low of 5% in 2012 (and 2017) to 75-76% during the drought years of 2014-2015. Not only was prevalence of infection low for natural fish, but parasite infection levels (DNA copy number) were also the lowest observed in the disease threshold analyses (Figure 5) for all years examined (2013-2017). *Ceratonova shasta* POI did not reach or exceed 20% in natural fish during the real-time monitoring conducted in 2017 until May 15, the eighth week of the monitoring program.

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 low at 8% compared to 27% in 2016 and 75% in 2015. *Ceratonova shasta* was not detected by histology on two of four sample dates in the upper K4 reach, and three of four dates in the lower K3 reach. Pathology scores indicating tissue damage were zero for all sample dates with the exception of May 10 in the K3 reach, with a low pathology score of 0.10. Natural fish had an overall *P. minibicornis* POI by histology of 22%, compared to 39% in 2016 and 92% in 2015.

Unknown Origin Chinook Salmon

As described in the results section (Figure 3), juvenile Chinook salmon of unknown origin (unmarked fish collected after IGH releases) made up the largest proportion (57%) of fish tested and analyzed by QPCR in 2017. Normally when IGH CWT Chinook are abundant, unknown origin fish are not targeted for sampling because without a known origin or release date, they provide little value in assessing myxozoan infection levels in terms of exposure period. *Ceratonova shasta* POI in the large unknown origin group was 37% (240/647, ci = 33-41%) and *P. minibicornis* was 87% (566/647, CI = 85-90%).

Iron Gate Hatchery Coded-wire Tagged Chinook Salmon

Among the small number of CWT juvenile Chinook salmon released from Iron Gate Hatchery, *C. shasta* was detected in 37% (13/35, ci = 21-55%) by QPCR. It's difficult to evaluate *C. shasta* POI in IGH CWT in 2017, compared to prior monitoring years (45% in 2016 and 90% in 2015) because the sample size was so small. Normally, IGH CWT juvenile Chinook comprise the largest proportion of fish tested and sample sizes are large (647 in 2016 and 489 in 2015). *Parvicapsula minibicornis* was 89% (31/35, ci = 73-97%) in IGH CWT Chinook in 2017.

Likewise, Weeks at Large (WAL) analysis of the small sample numbers of IGH CWT juvenile Chinook salmon was not informative in 2017. The largest sample size (N=13) obtained for recaptured IGH CWT Chinook occurred for fish residing 2 WAL and *C. shasta* was not detected in this group. The highest *C. shasta* POI of 50% occurred in a sample set of 4 CWT Chinook residing 4 WAL.

Clearly, *C. shasta POI* was extremely low in natural Chinook (5%), and low in unknown origin Chinook (37%) and similar (37%) in the few CWT Chinook recaptured in 2017. But temporal analysis regarding exposure period and infection level was not possible without a larger data set of CWT Chinook.

Therefore the *C. shasta* POI of 46% in all fish collected from the Estuary, regardless of fish origin, is likely the best overall measure of infection in juvenile Chinook salmon in 2017. The parasite infection level in this mixed origin group was relatively low (mean DNA copy number of 568 and 1.2 logs) compared to prior years. Therefore, while the prevalence of infection may seem proportionally high for the group, the parasite loads are light and therefore *C. shasta* infections are not likely impacting the group in terms of expected survival.

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 26% (153/600, ci = 22-29%) in 2017, and lower than the average of 43% for the past decade (2006-2017, Table 3). More striking than the lower *C. shasta* POI during the peak migration period of 2017 is the extremely low mean DNA copy number of 58. This very low level of parasite load in emigrating juvenile Chinook is far below what has been observed in the monitoring program previously, as illustrated in Figure 5. And while *C. shasta* POI of 26% in this group appears significant in terms of the proportion of fish that are infected, the extremely low parasite DNA levels (mean of 58 copies) are more indicative of background infections in a system, i.e., where *C. shasta* is endemic but not causing disease. Levels this low are not likely to cause tissue damage, impaired function of the intestine, or expected mortality as indicated by the 2017 histology findings. Histology results for 2017 were similar to the lowest disease years (2011 and 2012) of the monitoring program (Table 3).

Parvicapsula minibicornis prevalence of infection by QPCR in Chinook salmon above the Trinity River confluence for the same period was 82% (493/600, ci = 79-85%) compared to 89% in 2016 and 99% in 2015. Mean parasite copy number was 38,000 (log 3.1) but pathology scores were zero for seven of eight sample weeks, and well below the clinical thresholds (path scores of 6-8) by histology.

In summary, sampled fish groups benefited from high spring flows and to a lesser degree from slightly lower river temperatures in 2017. The lack of clinical disease (enteronecrosis) by *C. shasta* by both QPCR and histology in natural and hatchery fish was unique in 2017. And while *C. shasta* POI was 26% for the peak migration period, the extremely low parasite levels indicate infectivity was very low in the Klamath River in 2017. The infectious waterborne actinospores either were not present, or in low abundance in the infectious zone when juvenile Chinook were migrated through the upper reaches. The most likely explanation is decreased efficiency, or disruption, of the parasite life cycle in both the vertebrate fish host and the invertebrate worm host. This was likely due to low numbers of returning adults and high winter and spring flushing flows. Flows appear to have been the primary environmental factor affecting parasite density (infectious actinospore stage) as river temperatures were only slightly (1-2ºC) lower than previous years. Environmental conditions were advantageous for out-migrating juvenile Chinook salmon, and in the absence of high infectivity by *C. shasta*, their over health prognosis is positive. The annual trends in *C. shasta* prevalence of infection in juvenile Chinook salmon demonstrate that while actinospore density (exposure dose) is the key infectivity factor, flushing river flows (as well as temperature) are also important environmental factors that influence disease development and severity in juvenile salmonids.

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Julie Alexander, Oregon State University
Don Reck, NOAA

Photo contributions:
Cover Photo: Klamath River Estuary and mouth, 2017 by Kimberly True.

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 annual report.
- Anne Voss – Data management, QPCR necropsy extraction and assays, histology processing, environmental data and figures, assistance with pivot tables and written report and reviewer's comments.
- Scott Foott – Histological sample processing and assessments.

REVISION
April 2018 – This report was revised due to an error found post publication, as follows:

1) Revision to 2009 report resulted in corrections to Table 3 in this report, page 16, for the 2009 QPCR value (see 2009 revision note #1 for details).

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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	26-Mar	30				
2	2-Apr	30				
3	9-Apr	30	21			
4	16-Apr	30 (H10)	22 (H10)			
5	23-Apr	30	21			
6	30-Apr	30 (H10)	22 (H10)			
7	7-May	30	19			
8	14-May	30 (H10)	23 (H10)			
9	21-May	23	20			
10	28-May	30 (H10)	21 (H10)			
11	4-Jun	34	20	20		
12	11-Jun	51	20	20	20	
13	18-Jun	19	21	21	20	30
14	25-Jun		21	21	18	25
15	2-Jul		23	20	21	19
16	9-Jul		22	19	17	28
17	16-Jul				16	16
18	23-Jul				16	18
19	30-Jul					24
20	6-Aug				18	24

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	26-Mar	0% (0/30)				
2	2-Apr	0% (0/30)				
3	9-Apr	0% (0/30)	0% (0/21)			
4	16-Apr	0% (0/30)	0% (0/22)			
5	23-Apr	0% (0/30)	0% (0/21)			
6	30-Apr	3% (1/30)	0% (0/22)			
7	7-May	10% (3/30)	0% (0/19)			
8	14-May	20% (6/30)	4% (1/23)			
9	21-May	4% (1/23)	5% (1/20)			
10	28-May	20% (6/30)	29% (6/21)			
11	4-Jun	65% (22/34)	20% (4/20)	55% (11/20)		
12	11-Jun	43% (22/51)	35% (7/20)	25% (5/20)	40% (8/20)	
13	18-Jun	42% (8/19)	29% (6/21)	43% (9/21)	25% (5/20)	67% (20/30)
14	25-Jun		52% (11/21)	38% (8/21)	6% (1/18)	32% (8/25)
15	2-Jul		4% (1/23)	0% (0/20)	24% (5/21)	32% (6/19)
16	9-Jul		36% (8/22)	32% (6/19)	12% (2/17)	64% (18/28)
17	16-Jul				13% (2/16)	50% (8/16)
18	23-Jul				25% (4/16)	39% (7/18)
19	30-Jul					33% (8/24)
20	6-Aug				78% (14/18)	38% (9/24)
		K4 Total **17% (69/397)**	K3 Total **15% (45/296)**	K2 Total **32% (39/121)**	K1 Total **28% (41/146)**	K0 Total **46% (84/184)**

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	26-Mar	0% (0/30)				
2	2-Apr	0% (0/30)				
3	9-Apr	0% (0/30)	0% (0/21)			
4	16-Apr	3% (1/30)	0% (0/22)			
5	23-Apr	10% (3/30)	14% (3/21)			
6	30-Apr	10% (3/30)	32% (7/22)			
7	7-May	70% (21/30)	42% (8/19)			
8	14-May	87% (26/30)	70% (16/23)			
9	21-May	96% (22/23)	90% (18/20)			
10	28-May	90% (27/30)	95% (20/21)			
11	4-Jun	94% (32/34)	95% (19/20)	85% (17/20)		
12	11-Jun	92% (47/51)	100% (20/20)	95% (19/20)	90% (18/20)	
13	18-Jun	84% (16/19)	100% (21/21)	100% (21/21)	95% (19/20)	93% (28/30)
14	25-Jun		100% (21/21)	90% (19/21)	17% (3/18)	88% (22/25)
15	2-Jul		91% (21/23)	70% (14/20)	62% (13/21)	89% (17/19)
16	9-Jul		91% (20/22)	95% (18/19)	29% (5/17)	100% (28/28)
17	16-Jul				63% (10/16)	100% (16/16)
18	23-Jul				88% (14/16)	100% (18/18)
19	30-Jul					96% (23/24)
20	6-Aug				89% (16/18)	92% (22/24)
		K4 Total **50% (198/397)**	K3 Total **66% (194/296)**	K2 Total **89% (108/121)**	K1 Total **67% (98/146)**	K0 Total **95% (174/184)**

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 16	April 30	May 14	May 28	POI
Kidney					
Pm Troph.	0 /10 (0)	0 /10 (0)	3 /10 (30)	8 / 10 (80)	11 / 40 (28)
Pm Myxosp.	0 /10 (0)	0 /10 (0)	0 /10 (0)	0 /10 (0)	0 / 40 (0)
Metacercaria	0 /10 (0)	1 /10 (10)	1 / 10 (10)	0 /10 (0)	2 / 40 (5)
C. shasta troph.	0 /10 (0)	0 /10 (0)	0 /10 (0)	0 /10 (0)	0/ 40 (0)
Chloromyxum sp	0 /10 (0)	0 /10 (0)	0 /10 (0)	0 /10 (0)	0 / 40 (0)
Pathology Score	0.0	0.0	0.0	0.50	
Intestinal tract					
C. shasta troph.	0 /10 (0)	1 /10 (10)	0 /10 (0)	2 /10 (20)	3 / 40 (8)
C. shasta myxosp.	0 /10 (0)	0 /10 (0)	0 /10 (0)	0 /10 (0)	0 / 40 (0)
Helminth	0 /10 (0)	0 /10 (0)	0 /10 (0)	0 /10 (0)	0 / 40 (0)
Pathology Score	0.0	0.0	0.0	0.0	
Adipose steatitis	0 /10 (0)	3 /4 (75)	5 /8 (63)	6 /6 (100)	14 / 28 (50)
Adipose lipofuscin	0 /10 (0)	0 /4 (0)	0 /8 (0)	0 /5 (0)	0 / 27 (0)
Gill					
Metacercaria	0 /10 (0)	0 /10 (0)	9 /10 (90)	9 /10 (90)	18 /40 (45)
Multif. Hyperplasia	0 /10 (0)	1 /10 (10)	4 /10 (40)	7 /10 (70)	12 /40 (30)

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 16	April 30	May 14	May 28	POI
Kidney					
Pm Troph.	0 /10 (0)	0 /9 (0)	4 /10 (40)	2 /10 (20)	6 / 39 (15)
Pm Myxosp.	0 /10 (0)	0 /9 (0)	0 /10 (0)	0 /10 (0)	0 / 39 (0)
Metacercaria	0 /10 (0)	0 /9 (0)	0 /10 (0)	0 /10 (0)	0 / 39 (0)
C. shasta troph.	0 /10 (0)	0 /9 (0)	0 /10 (0)	0 /10 (0)	0 / 39 (0)
Chloromyxum sp	0 /10 (0)	0 /9 (0)	0 /10 (0)	0 /10 (0)	0 / 39 (0)
Pathology Score	0.0	0.0	0.0	0.0	
Intestinal tract					
C. shasta troph.	0 /10 (0)	0 /10 (0)	1 /10 (10)	0 /10 (0)	1 / 40 (3)
C. shasta myxosp.	0 /10 (0)	0 /10 (0)	0 /10 (0)	0 /10 (0)	0 / 40 (0)
Helminth	0 /10 (0)	0 /10 (0)	0 /10 (0)	0 /10 (0)	0 / 40 (0)
Pathology Score	0.0	0.0	0.10	0.0	
Adipose steatitis	2 /3 (67)	2 /6 (33)	3 /6 (50)	2 /6 (33)	9 / 21 (43)
Adipose lipofuscin	0 /3 (0)	0 /6 (0)	0 /6 (0)	0 /6 (0)	0 / 21 (0)
Gill					
Metacercaria	0 /9 (0)	0 /10 (0)	8 /9 (89)	3 /10 (30)	11 / 38 (29)
Multif. Hyperplasia	0 /9 (0)	0 /10 (0)	6 /9 (67)	1 /10 (10)	7 /38 (18)

Appendix C - Reviewer comments

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

General comments: Reviewer had general comment that the terms (1) "clinical disease" (2) "geomorphic flow" and (3) "flow threshold of 2000 cfs" should be better defined for the reader in the summary.

Response:

1) Language is included in the methods section (QPCR and histology) that defines "clinical disease." The term is used for the pathology score by histology (fish host tissue damage) and the "disease threshold" assessment by QPCR which links tissue damage and *C. shasta* mean DNA copy number (parasite load).

2) "Geomorphic flow" is a broad term used by water managers to describe flows that are sufficient to mobilize the river sediment and maintain natural hydrological functions. In terms of *C. shasta* disease considerations, this term is generally used in the context of flows adequate to remove polychaete worms. Commonly called "flushing flows" when discussing sediment movement (USFWS Technical Memo: Response to Request for Technical Assistance – Sediment Mobilization and Flow History in Klamath River below Iron Gate Dam, September 2016) and described in Summary Guidelines:

> "Environmental flows are developed by river managers to mitigate the detrimental impacts of dams and water diversions on river form and ecological functions. Environmental flow regimes designed to induce geomorphic changes are broadly divided into two categories, sediment maintenance or "flushing flows" used to modify substrate composition and channel maintenance flows intended to maintain channel form and floodplains."

3) The mention of 2000 cfs flows are simply reporting flow conditions, and are not describing any "flow threshold".

Pg. 8 - Results: In regards to the pre-release examination, the reader wanted to know the detection threshold for the QPCR assay.

Response: Language about the detection threshold was added in the methods section.

Pg. 13 – Results (Prevalence of Infection by Fish Origin): In regards to natural fish, the reader wanted to know why results for lower reaches are not reported.

Response: Due to the sampling method, natural fish are not collected in the lower reaches of the river, and therefore no data exists. The sampling method focuses on collecting natural fish in the upper reaches early in the season and then hatchery fish during out-migration (Appendix A, Table 1).

Pg. 13 – Results: In regards to natural fish, the reader wanted more explanation about the infection threshold and the term "advanced clinical disease"

Response: We added language to make it clearer regarding how the analysis was done. We also think the figure caption (Figure 5) explains that the end point of the analysis is fish mortality and the "advanced clinical disease" is unrecoverable infection levels by histology.