

California-Nevada Fish Health Center

FY 2015 Investigational Report:

**Myxosporean Parasite (*Ceratonova shasta* and *Parvicapsula minibicornis*)
Prevalence of Infection in Klamath River Basin Juvenile Chinook Salmon,
April - July 2015**

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Summary

Juvenile Klamath River Chinook salmon (*Oncorhynchus tshawytscha*) were assayed from April to July 2015 by quantitative polymerase chain reaction (QPCR) and histology for myxosporean parasite infections, *Ceratonova shasta* and *Parvicapsula minibicornis*. The seasonal prevalence of infection of *C. shasta* by QPCR in Chinook salmon collected above the Trinity River confluence during the peak out-migration period (May-July) was 91%, and higher than 81% observed in 2014. *Parvicapsula minibicornis* in Chinook salmon above the Trinity River confluence for the same period was 99% compared to 92% in 2014.

Among the various fish groups tested, naturally produced Chinook salmon had a 75% prevalence of *C. shasta* infection by QPCR and juvenile fish were infected three weeks earlier than normally observed. First detection of *C. shasta* in previous years (2009-2013) has occurred April 21 to May 10. In both 2014 and 2015, *C. shasta* was detected in early April. Based on both QPCR testing and histology, natural fish had relatively high infection levels (DNA copy number) and disease severity (pathology scores) by early to mid-May.

Among coded-wire tagged (CWT) juvenile Chinook salmon released from Iron Gate Hatchery from May 21 – June 2, *C. shasta* was detected in 90% of fish screened by QPCR. The highest *C. shasta* prevalence of infection observed was 92-93% in IGH CWT Chinook salmon residing 1-2 Weeks At Large (WAL) at time of recapture, indicating fish became infected rapidly upon entering the main stem Klamath River.

In the fourth year of drought in California, 2015 represented another year of minimal flows and elevated water temperatures for out-migrating juvenile Chinook salmon in the Klamath Basin. *Ceratonova shasta* prevalence of infection by QPCR, histology, and field observations of clinical disease was high for both natural fish and coded-wire tagged juvenile Chinook salmon. In 2015, *C. shasta* prevalence of infection in juvenile Chinook salmon during peak emigration was the highest to date (2006-2015) by QPCR and by histology.

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Introduction

The Klamath River drainage is approximately 30,000 km² 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. Once shed from an infected fish, the myxospore can infect the polychaete host to complete the life cycle (Bartholomew et al., 1997).

The 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 actinospore stage in the water column 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 (17-24°C), 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).

In 2015, California was in the fourth year of drought resulting from low snowpack in the Cascade and Sierra Nevada mountain ranges, combined with lower than normal annual precipitation from 2011-2015 and warmer than normal air temperatures each consecutive summer. Drought conditions resulted in limited water supplies in the upper basin and reservoirs. Decreased river flows in the Klamath River can result in higher spore concentrations per volume of water if polychaete actinospore production remained similar to previous years. Low flows and higher than average river temperatures can create conditions that are more favorable to *C. shasta* transmission including earlier release and concentration of the infectious actinospore stage in the water column, which results in higher infectious doses for fish hosts. The prevalence of disease (enteronecrosis) is typically higher in years characterized by these conditions. In contrast, we have observed lower prevalence of disease when annual peak flows are high and spring temperatures are lower, likely due to decreased spore concentration and slower development of parasite spores in both hosts (and disease progression in fish).

The primary objectives of this study were: 1) examine parasite prevalence in Klamath River juvenile Chinook salmon during the spring outmigration period; and 2) compare parasite prevalence in 2015 to previous years.

Methods

Pre-Release Examination

Prior to the Iron Gate Hatchery releases (May 21 through June 2, 2015) of approximately 5 million fall Chinook salmon, a fish health examination of 39 hatchery fish was conducted at the hatchery on May 6 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). When possible, existing salmonid downstream migrant traps were used for collection, but beach seining was also performed to collect fish in some weeks/reaches. 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, first pathogen detections, and diagnostic casework.

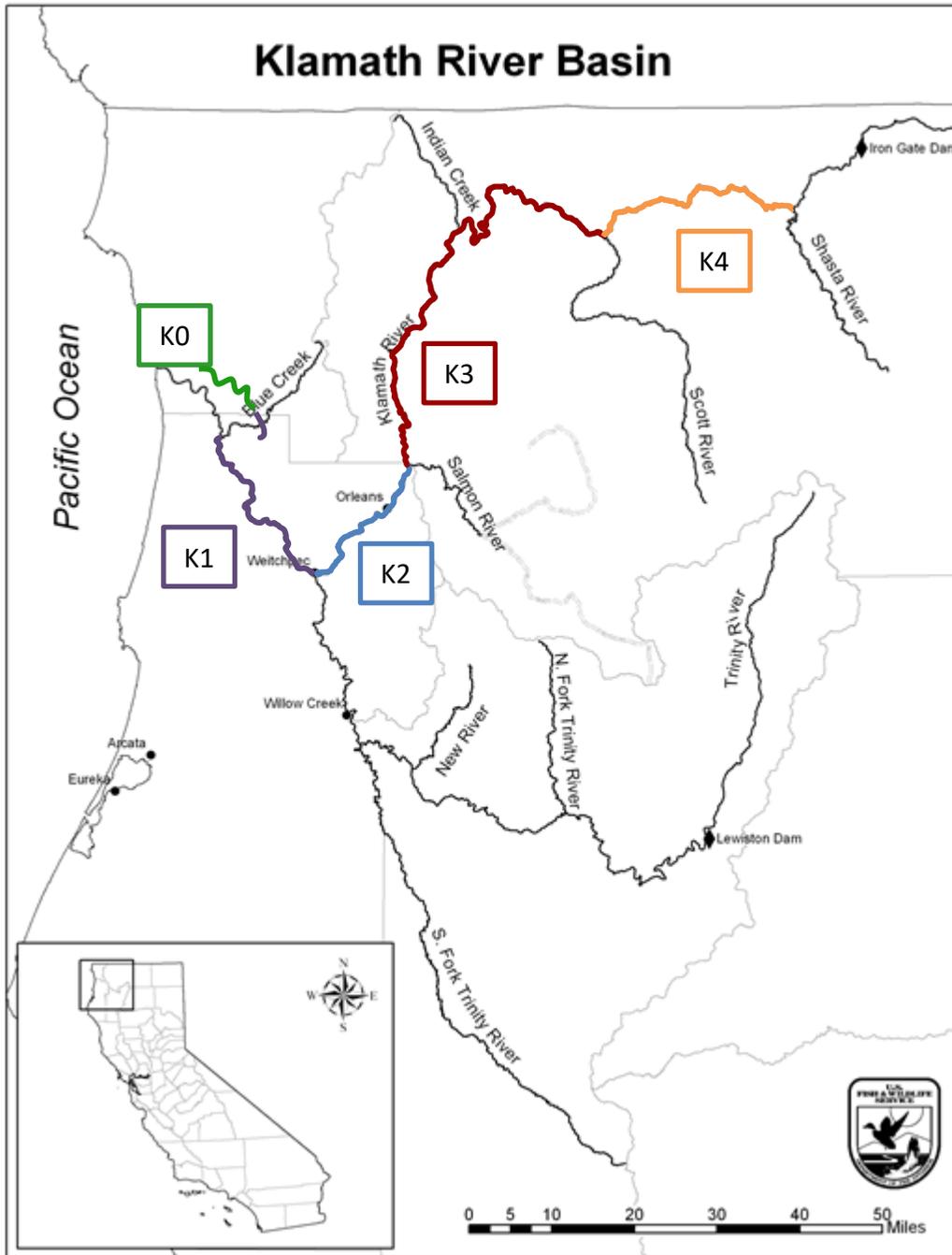


Figure 1. Klamath River watershed, major tributaries, and sample reaches: Shasta River to Scott River (K4), Scott River to Salmon River (K3), Salmon River to Trinity River confluence (K2), Trinity River to upper Estuary (K1), and Klamath River Estuary (K0). Map provided by the Arcata Fish and Wildlife 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 (the week of March 29-July19). Lower reaches were sampled later in the season (June 7- July 26) as fish were migrating downstream (Appendix A – Table 1).

All fish sampled were categorized into three group types based on their origin: natural, unknown, 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 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). All samples collected were tested for *C. shasta* (Appendix A – Table 2).

Fish collected early in the season (40 samples from week of March 29-April 5) were too small to assay kidney tissue for *P. minibicornis* (Appendix A – Table 3). Therefore, the number of fish tested for *C. shasta* and *P. minibicornis* were not equal.

Diagnostic Casework

Diagnostic examinations for *C. shasta*, columnaris disease, or other fish health abnormalities were conducted when requested by Tribal partners or USFWS field crews who observed clinical disease signs or fish mortality during normal field surveys. Two diagnostic examinations were conducted in 2015: Ti Creek on June 18 (N=36 fish) and Bluff Creek on June 24 (N=19 fish), using QPCR and histology to assess myxozoan parasite infection level and screen for other pathogens of interest.

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 6.83×10^9 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 1.3.1, Applied Biosystems). The standard curve was used to evaluate PCR amplification efficiency (slope of the standard curve, efficiency was 95%), fit to the curve (R^2 value = 0.999) and the y-intercept (C_T value for a single copy of parasite DNA).

Quantification of fish tissue (*C. shasta* DNA copy number) was determined using 5 μ L of DNA template in a 30 μ L 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 2015 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 2.41×10^8 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 92%), 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 μ L. 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. No plates required retesting over the 2015 field season.

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

Parasite Infection Levels by Histology

Histological assays were done according to True et al. (2013). In 2015, histology samples were collected in the Shasta to Scott reach (K4) and the Scott to Salmon reach (K3) between the week of April 19 and May 31 (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 did not affect the overall prevalence of infection reported for histological assessments. Supplemental histology was done for Ti Creek diagnostic examinations (N=5) conducted June 18, 2015 and is reported in that section of the report.

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 prior to release (May 21 through June 2, 2015) on May 6th. *Ceratonova shasta* was detected in 18% (7/39, ci = 8-34%) and *Parvicapsula minibicornis* was detected in 23% (9/39, ci = 11-39%) of hatchery juveniles sampled on May 6 at IGH. River temperatures on initial release of May 21 were 15.7°C (60.3°F), and 18.0°C (64.4°F) on final release date of June 2 (data from Arcata FWO, temperature logger below Iron Gate Dam).

Number of All Fish Collected by Origin

In 2015 we examined 925 juvenile Chinook salmon collected from the main stem Klamath River. The sample consisted of 307 natural fish, and 618 fish collected after hatchery release which included 594 CWTs. Coded-wire tagged Chinook salmon accounted for 64% (594/925) of all fish sampled in 2015 (Figure 2). Natural fish accounted for 33% (307/925) and 3% (24/925) of the fish are of unknown origin (unmarked hatchery fish or natural).

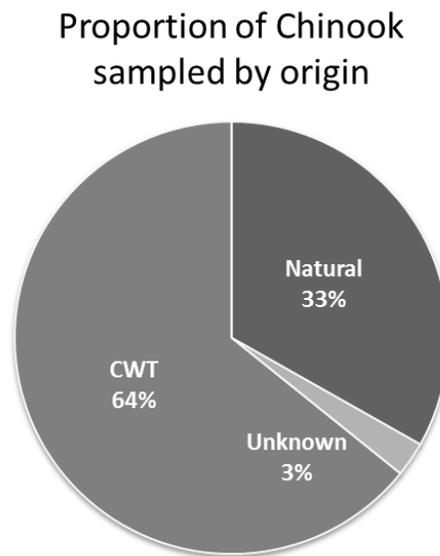


Figure 2. Proportion and origin of Chinook salmon collected (N = 925) in 2015.

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 820: this consisted of 307 natural fish (37%), 24 unknown fish (3%), and 489 IGH CWT (60%, Figure 3).

Proportion of Chinook used for report analysis

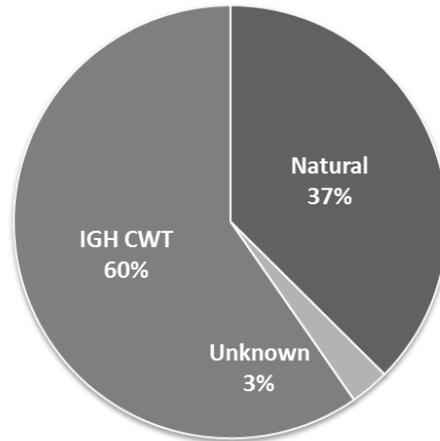


Figure 3. Proportion and origin of Chinook salmon used for prevalence of infection analysis (N = 820). Unreadable tag codes or lost tags, and TRH tags have been removed from total number of fish collected.

Annual Prevalence of Infection by Klamath River Reach

The annual prevalence of *C. shasta* infection in all Chinook salmon collected in 2015 by QPCR was 84% (690/820, ci = 81-87%). *Ceratonova shasta* was first detected on April 9 in the Shasta to Scott reach (K4). *Ceratonova shasta* POI was highest in the Salmon to Trinity (K2) at 97%, followed by 96% in the Trinity to Estuary (K1) reach. The lowest prevalence of 72% was observed in the Shasta to Scott reach (K4, Figure 4).

The annual *P. minibicornis* POI by QPCR was 97% (754/780, ci = 95-98%). *Parvicapsula minibicornis* was detected after April 16 in the Shasta to Scott reach (K4). Prevalence was highest in the Salmon to Trinity reach (K2) at 100%, followed closely by the Estuary (K0) at 99% (Figure 4). The lowest prevalence of 94% was observed in the Scott to Salmon reach (K3).

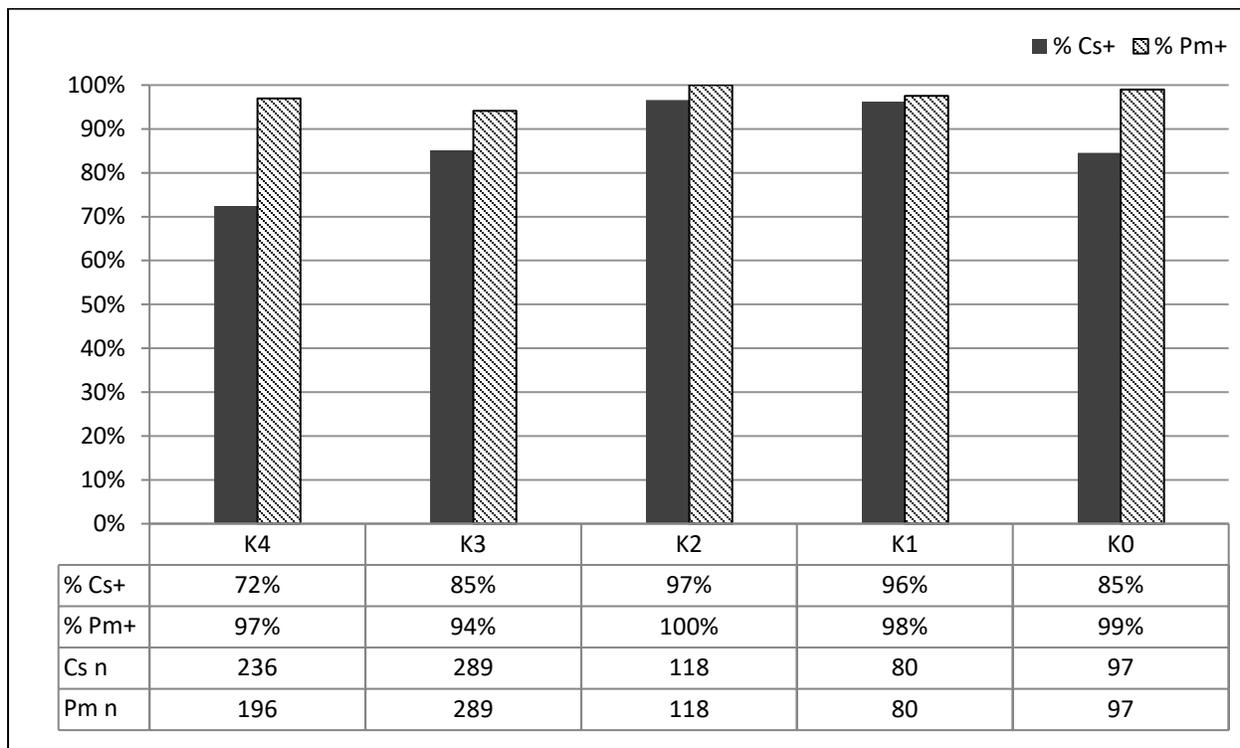


Figure 4. Prevalence of *Ceratonova shasta* (Cs+) and *Parvicapsula minibicornis* (Pm+) infection in juvenile Klamath River Chinook salmon by collection reach in 2015. Shasta River to Scott River (K4), Scott River to Salmon River (K3), Salmon River to Trinity River confluence (K2), Trinity River to upper Estuary (K1), and Klamath River Estuary (K0). Sample numbers collected (N) are displayed in the table below each column for both pathogens.

As described in methods, histology sampling occurred during the week of April 19 to May 31 in the K4 reach and K3 reaches (Appendix B, Table 2 and Table 3). The annual *C. shasta* POI in 2015 by histology for all fish tested was 68% (54/80, ci = 56-78%) and for *P. minibicornis* was 88% (70/80, ci = 78-94%).

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 8-10°C cooler in the collection months of April and May compared to hatchery fish sampled during the peak salmon migration period of May-July. A total of 307 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 29 through May 17 in Shasta to Scott (K4) reach and from April 12 through May 24 in the Scott to Salmon (K3) reach. Mean daily river temperature was 10.6°C (data from Arcata FWO, below IGD) at first detection of *C. shasta* in natural fish collected in K4 on April 9.

Karuk Tribe field crews reported increased mortality and clinical disease signs early in the field season. On April 23, crews reported gross external disease signs of distended abdomen (17%) and pale gills/anemia (83%) at the Kinsman Rotary Screw Trap (KMN RST, Figure 5). River temperature at

the trap site was 11.3°C (52.3°F). By May 7, crews reported increased clinical signs at the KMN RST in the daily catch: distended abdomens (88%) and anemia (52%). River temperature at the RST was 15.4°C (59.7°F, Ca-Nv FHC temp logger).

Figure 5. Juvenile Chinook with external clinical disease signs, collected from Kinsman RST on April 23, 2015. (Photo courtesy of Michael Sundman, USFWS, Arcata FWO)



Ceratonova shasta was detected by QPCR in 75% (229/307, ci = 69-79%) of natural fish. *Ceratonova shasta* POI was highest (84%, ci = 77-89%) in the Scott to Salmon (K3) reach compared to 66% (ci = 58-74%) in the K4 reach above. Comparatively, *P. minibicornis* was detected in 92% (245/267 ci = 88-95%) of naturally produced Chinook salmon by QPCR. The highest *P. minibicornis* prevalence of 95% (ci = 89-98%) was detected in Shasta to Scott (K4) compared to 89% (ci = 83-94%) in the Scott to Salmon reach (K3) below.

Natural fish were collected for histology prior to May 21. One additional histological sample set (N=10) was collected post hatchery release on May 31, and these unmarked fish were considered to be of unknown origin. The prevalence of infection by histology, by collection location was highest at 77% (23/30, ci = 58-90%) in the Shasta to Scott (K4) reach compared to 67% (20/30, ci = 47-83%) in the Scott to Salmon (K3) reach. In the Shasta to Scott (K4) reach, *C. shasta* prevalence of infection by histology was similar for all three sample dates (70-80%). The peak pathology score (5.2) occurred on the earliest sample date of April 19, followed by scores of 3.5 for May 3 and 3.9 for May 17 (Appendix B, Table 2). In the Scott to Salmon (K3) reach below, the highest prevalence (80-90%) in natural fish occurred the week of April 19 and May 3, and the highest pathology score (4.4) occurred on May 3. For comparison, clinically infected salmon 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 95% (57/60, ci = 86-99%). Prevalence was highest in the Scott to Salmon (K3) reach at 97% (ci = 83-100%), compared to 93% (ci = 78-99%) in the Shasta to Scott (K4) reach above. The kidney pathology score in fish collected in K4 increased from 3.3 on April 19 to 6.7 and 5.9 on samples collected May 3 and May 17 respectively. Pathology scores of 6-8 have been observed in clinical disease, in previous monitoring years (True et al., 2010). In the Scott to Salmon (K3) reach, kidney pathology for scores *P. minibicornis* were low (1.2) in mid-April, and increased to 7.4 and 6.7 in early to mid-May (Appendix B, Table 3).

Unknown Chinook salmon

Unknown origin Chinook salmon were fish collected after hatchery release that could not be differentiated from either natural fish or unmarked hatchery fish. A total of 24 fish of unknown origin were collected from May 24 to July 26 primarily from the Kinsman RST in the Shasta to Scott reach (K4). *Ceratonova shasta* was detected by QPCR in 96% (23/24, ci = 79-100%) of unknown origin Chinook salmon. *Parvicapsula minibicornis* POI in was 100% (24/24).

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 six past years of the monitoring program (Buttars et al., 2009). A total of 594 CWT Chinook salmon were collected this season from the Klamath River. Iron Gate Hatchery CWT fish accounted for 82% (489/594) and TRH CWT fish accounted for 5% (28/594) of CWT fish tested. Additionally, 77 fish (13%) had lost or unreadable tags, which meant their release date is unknown. For fish collected below the confluence of the Trinity River, hatchery origin is not known for unreadable tags, and 24 of the 77 unreadable tags collected over the sampling season were recovered from fish captured in the Trinity to Estuary reach (K1) or the Estuary (K0).

Coded-wire tagged salmon originating from IGH were collected in the Klamath River from May 24 to July 26. *Ceratonova shasta* was detected in 90% (438/489, ci = 87-92%) of all IGH CWT screened by QPCR. The largest proportion of IGH CWT (N=141 fish), were collected in the Scott to Salmon reach (K3). Prevalence of infection for *C. shasta* was highest in the Salmon to Trinity (K2) reach at 97% (ci = 91-99%) followed closely by the Trinity to Estuary (K1) reach at 96% (ci = 89-99%). The lowest prevalence was in the Shasta to Scott reach (K4) at 83% (ci = 70-91%). A turbidity event in the lower basin tributaries and subsequently the main stem Klamath River in mid-July made fish collections difficult for field crews in the lower reaches (Figure 6). By August 6 CWTs were not found in the Trinity to Estuary (K1) reach.

Parvicapsula minibicornis was detected by QPCR in 99% (485/489, ci = 98-100%) of IGH CWT. Prevalence of infection for *P. minibicornis* was 100% in two reaches: Shasta to Scott (K4), and Salmon to Trinity (K2). The lowest prevalence was in the Trinity to Estuary (K1) reach at 98% (ci = 91-100%).



Figure 6. Heavy sedimentation and turbidity in North Fork Salmon River on July 6, 2015. (Photo courtesy of Tom Hotaling, Salmon River Restoration Council).

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 periods (hatchery release to in-river recapture dates). The period of how long fish reside in the Klamath River post hatchery release is Weeks At Large (WAL). *Ceratonova shasta* POI in IGH CWT Chinook salmon by WAL analysis shows a high prevalence of infection (85-92%) across all groups of fish residing 1-8 Weeks At Large (Figure 7).

The highest *C. shasta* prevalence of infection occurred in groups residing 1-3 WAL (92, 93 and 89% respectively). In 2015 the lowest *C. shasta* prevalence of infection occurs in fish residing at 0 (less than 1 WAL, 77%), and 4 and 6 WAL (85% for both groups).

As stated in the methods, the QPCR assay can quantify parasite DNA copies within fish tissue and therefore describe infection level at specific exposure periods. In IGH CWT Chinook salmon, the highest mean DNA copy number was observed in groups residing 1-3 WAL (79,000, 118,000 and 92,000 copies respectively (Figure 7)). Mean DNA copy was similar across the remaining weeks (less than 10,000 copies) except for WAL 6 (45,000 copies). Sample size was small (12-13 fish) for <1 and 8 WAL groups (sample size shown at base of each column in Figure 7).

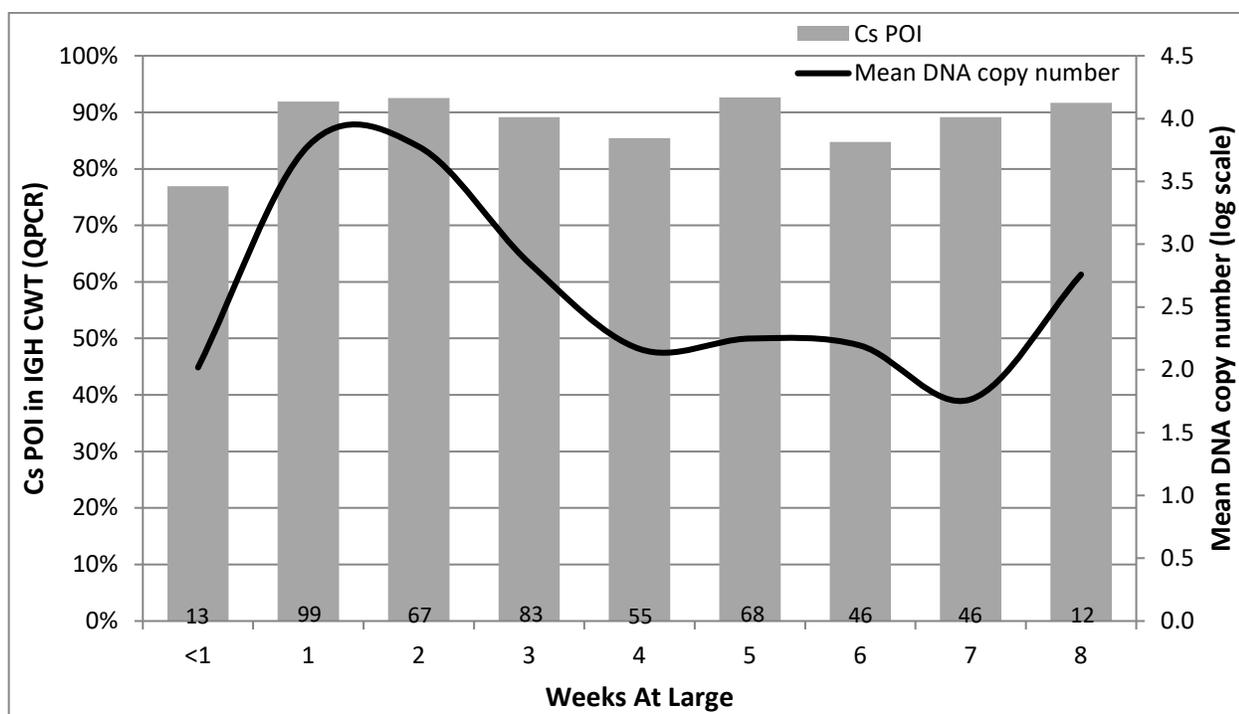


Figure 7. *Ceratonova shasta* prevalence of infection in IGH CWT by Weeks At Large (WAL) post hatchery release. The bar graph is prevalence of infection (%) on the primary y-axis and the line graph is the mean *C. shasta* DNA copy number (log scale) on the secondary y-axis for Chinook salmon tested by QPCR. The number of fish collected is listed inside the base of each bar.

Diagnostic Casework

Two diagnostic examinations were conducted in 2015 on June 18 at the mouth of Ti Creek (Scott to Salmon, K3 reach) and on June 24 at mouth of Bluff Creek (Salmon to Trinity, K2 reach). At Ti Creek, 36 CWT juvenile Chinook were examined for clinical disease signs of myxozoan parasites (abdominal swelling and/or hemorrhaged vent) and necropsied for QPCR testing. Clinical infection with *Parvicapsula minibicornis* was apparent in the Ti Creek CWT group in mid-June: *Parvicapsula minibicornis* POI was 100% (36/36 fish) and by coded-wire tag codes, all but one fish had been released either June 1st or 2nd and had therefore been residing 2 WAL upon recapture. The DNA copy number in the kidney of fish from the highest infection level group ranged from 119,000 to 3.6 million (median copy number 172,000) and these fish comprised 64% of the fish sampled at this site. By histological examination, the most severely infected fish were in end-stage renal failure and would not be expected to survive. Eleven percent of the 36 CWT fish examined had relatively low levels of *P. minibicornis* (less than 20,000 DNA copies) and 25% had moderate infection levels in the 20,000-100,000 copy range.

On June 24th, 19 CWT fish were examined at Bluff Creek thermal refugium, in the same manner as the Ti Creek fish sampled approximately one week earlier. The Bluff Creek group also showed *P. minibicornis* clinical disease signs in 74% of the 19 CWT Chinook sampled. While the range of DNA copy number was not as high as the previous sample at Ti Creek (210,000 to 2.0 million) the median DNA copy number for this group of fish was higher at 405,000. The majority of fish collected from Bluff Creek had been residing 3 WAL (1 fish at 4 WAL) and therefore had similar release dates (May 28-29 and June 2) as the fish sampled at Ti Creek.

In addition to QPCR testing, five CWT Chinook juveniles with moderate to severe (N=1) clinical disease signs were fixed for histology. Microscopic examination showed severe edema of kidney interstitium and necrosis of intestinal epithelium, indicating dual infection with both *C. shasta* and *P. minibicornis* resulting in complete loss of osmoregulation. One *C. shasta* myxospore was observed in one fish. The fish with severe clinical signs on field exam had a high *P. minibicornis* kidney pathology score, and overall this group of fish were scored 4.7 for *P. minibicornis* and 6.7 for *C. shasta*. Generally, *P. minibicornis* kidney pathology scores for severe clinical disease (glomerular nephritis) have ranged from 4-5. Intestinal pathology scores for clinical *C. shasta* (enteronecrosis) typically have ranged from 4-8.

One hand-caught juvenile CWT Chinook salmon exhibiting severe clinical disease signs and lethargy (eminent mortality) was captured at Blue Hole on June 18 in the Trinity to Estuary (K1) reach by tribal biologists. QPCR testing found high levels of parasite DNA both for *C. shasta* (840, 000 copies) and *P. minibicornis* (460,000 copies). This fish was released June 2 and resided two weeks (16 days) upon recapture, demonstrating that dual infections of this severity disrupt osmoregulatory functions of both the kidney and the intestine and lead to rapid mortality.

Historical Comparison

Prevalence of infection by QPCR is the metric that been used for historical comparisons of disease prevalence 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.

Prevalence of *C. shasta* infection by QPCR during the peak outmigration period was very high at 91% (437/482, ci = 88-93%) in 2015, compared to previous years (Table 2). *Parvicapsula minibicornis* in Chinook salmon above the Trinity River confluence for the same period was 99% (479/482, ci = 98-100%) compared to 92% in 2014.

Table 2. 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, 2006-2015.

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
Mean	28	44

¹ Histology limited to two reaches in 2011 (K4 and K1); and two reaches in 2012-2015 (K4 and K3).

Environmental Conditions

In previous study years, we typically observed mean daily water temperatures of approximately 18°C, and often as high as 22°C, during the peak juvenile migration period of May through July. The exception was 2010 and 2011 in which mean daily water temperatures were cooler for an extended period in May and June. These cooler temperatures coincided with the lowest *C. shasta* POI observed to date for the juvenile fish health monitoring program (17% by QPCR for both years).

Klamath River water temperatures in the spring of 2012 were similar to 2011; however spring temperatures in 2013 were 2-3°C higher. Spring temperatures in 2014 were also warmer than the previous year (~ 2°C warmer); however all these years (2012-2014) had water temperatures similar to historic temperatures from May to September.

In 2015, mean daily water temperature in March peaked at 11.8°C and at 14.5°C in April below Iron Gate Dam. Temperatures were approximately 2°C higher than 2014 in late March and early April. Temperatures rose throughout the month of May with the monthly peak on May 28 at 19.3°C. A temperature spike (21.4°C) was observed on June 8, however the highest mean daily temperature observed this season below Iron Gate dam occurred on July 7 at 23.3°C (Figure 8).

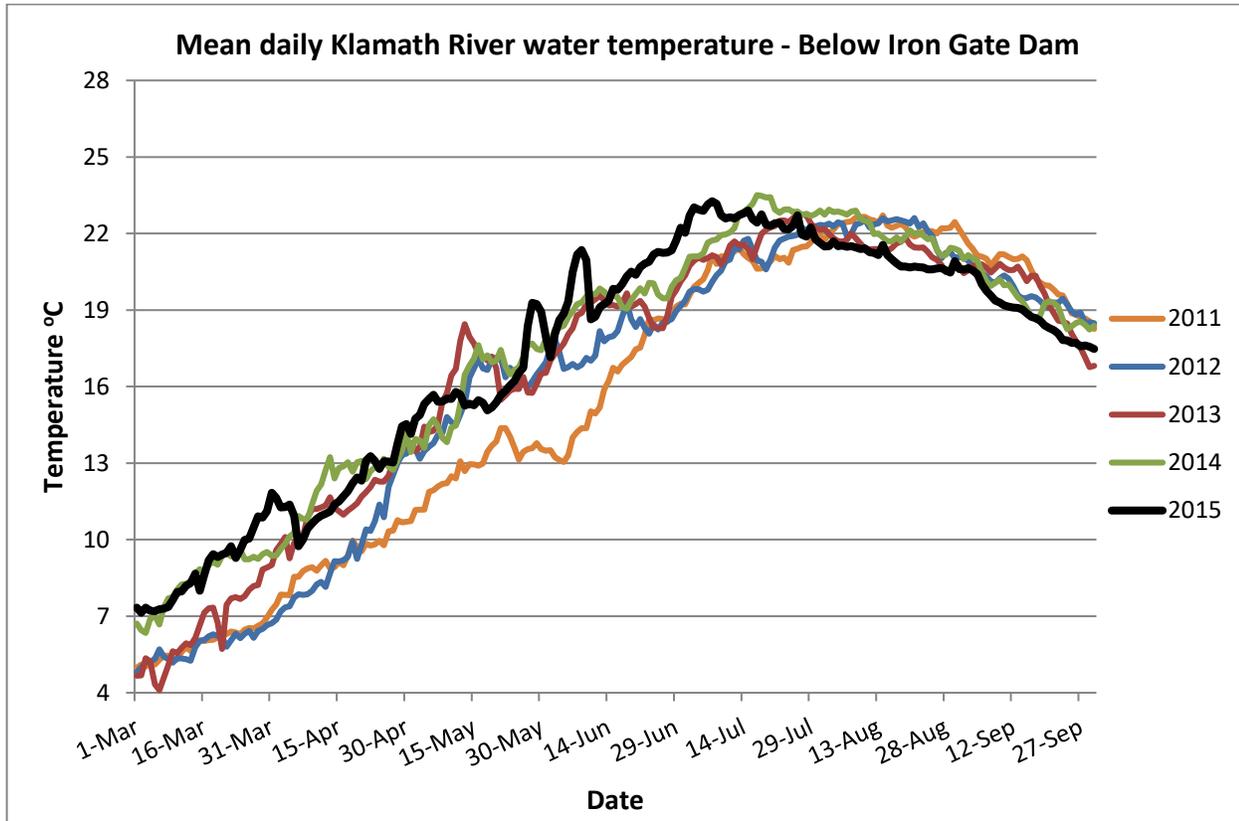


Figure 8. Mean daily Klamath River water temperature below Iron Gate Dam for 2011-2015. Temperature data for 2011 and 2013-2015 acquired from Arcata Fish and Wildlife Field Office. Temperature data for 2012 acquired from Iron Gate Hatchery and taken from the main stem Klamath River, not the hatchery facility.

At the Seiad Valley temperature gauge, mean daily Klamath River temperatures were more variable than below Iron Gate Dam and comparable to 2014 for most of the season. There were a few time periods where this part of the river was approximately 3-5°C warmer than observed during the same period in 2014. This was observed in late March and early June through early July (Figure 9).

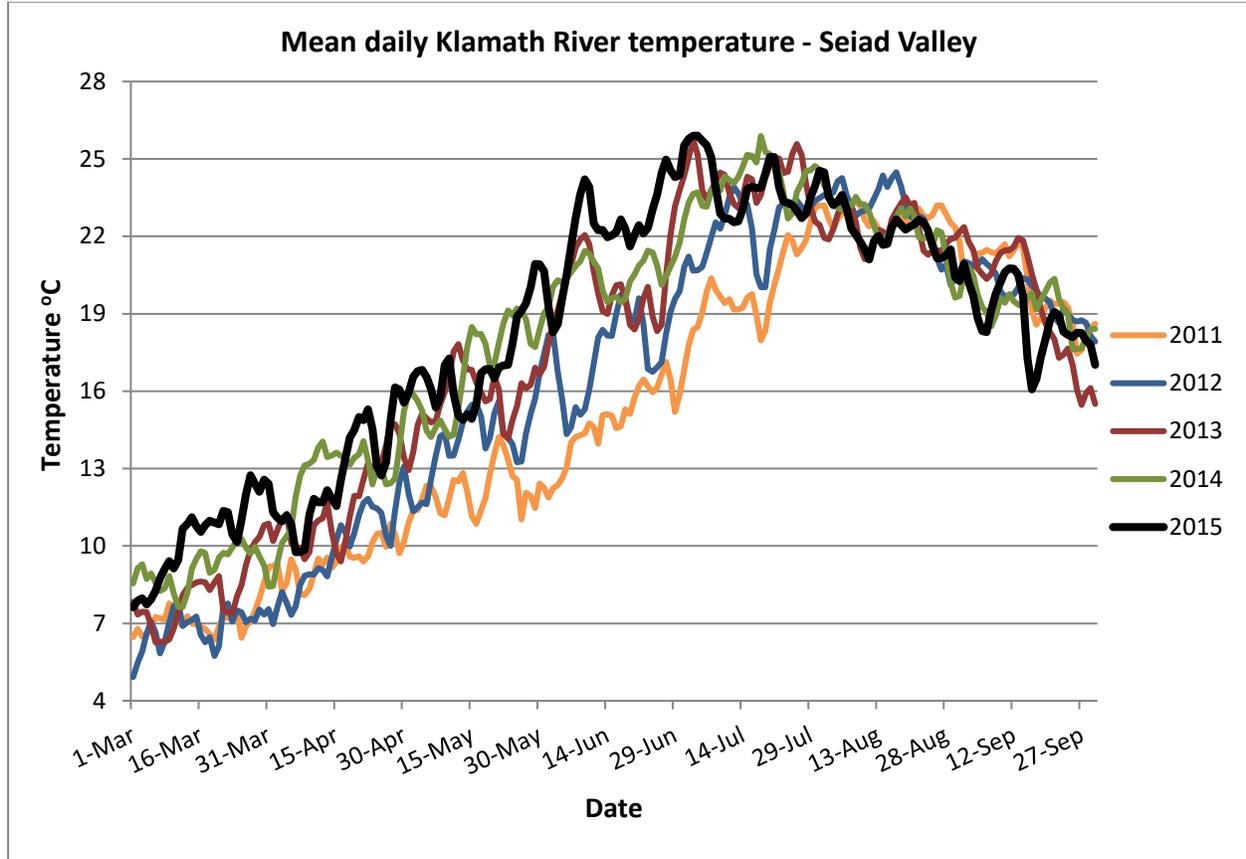


Figure 9. Mean daily Klamath River temperature from March through September 2011-2015 at Seiad Valley. Data from 2011 to 2015 were provided by the Arcata Fish and Wildlife Field Office, with the exception of 2014 temperature data that were provided by Arcata FWO and Karuk Tribe.

River Flows

Early in 2015 there was a precipitation event that occurred and the Klamath River below Iron Gate dam peaked at 3580 cfs on February 7. Discharge dropped below 1000 cfs by February 19. This peak did not occur during sampling of juvenile Chinook in the Klamath River.

River discharge fluctuated in March and April, but increased overall to 1690 cfs on April 6. This was the maximum discharge recorded during the sampling season. Flow then decreased gradually in May and June with the minimum discharge observed during the sampling season at 845 cfs on July 10 (Figure 10). Flow remained relatively steady in July and August at approximately 885 cfs.

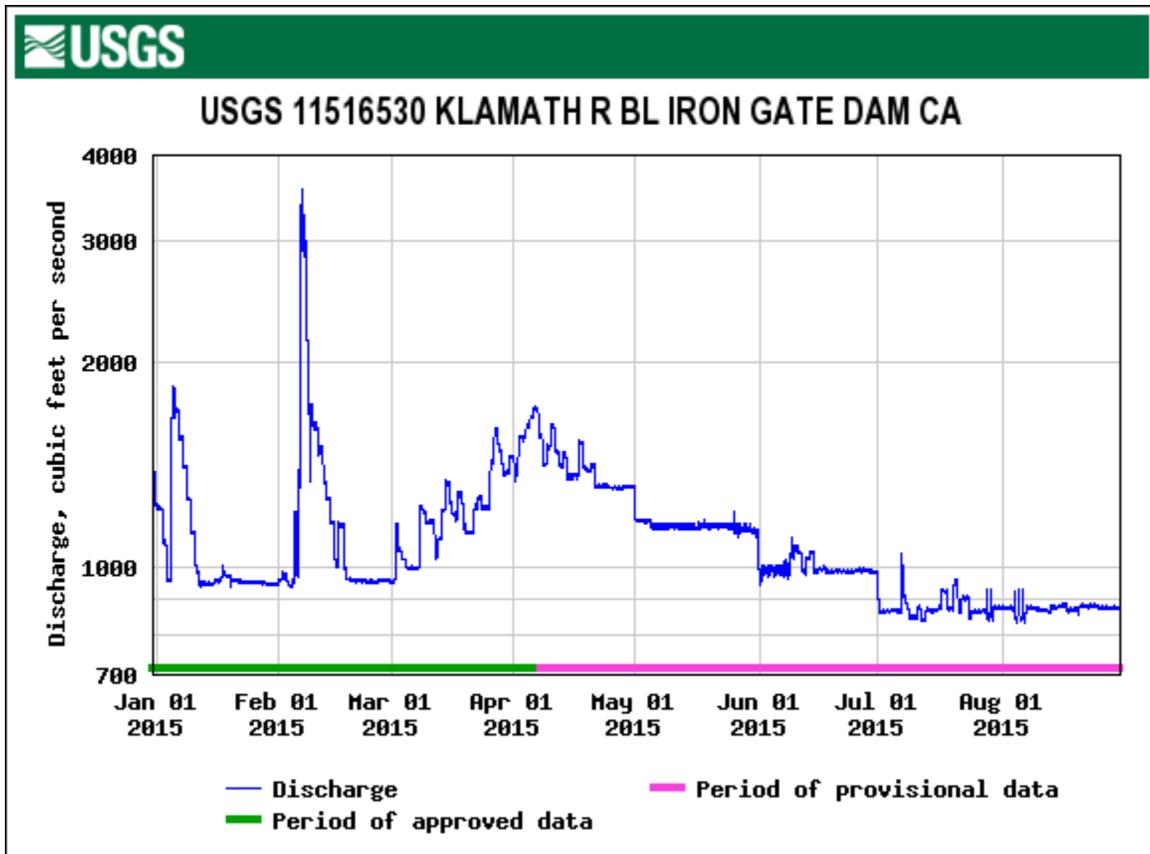


Figure 10. Daily discharge (cfs) below Iron Gate Dam from January 2015 through August 2015. Data collected after April 7, 2015 is provisional data. Data collected from USGS gaging station 11516530 at waterdata.usgs.gov.

Klamath River flows in 2015 were intermediate between low flow years (2009-2010) and high flow years (2011-2012, Figure 11).

In 2009 and 2010, flows did not reach above 2000 cfs. In 2011, two peak spring flows exceeded 5000 cfs, the first of which was a manipulated pulse flow released from IGD in February where flow was ramped up to 5000 cfs for approximately 6 hours (Moore, 2011). In 2012, spring flows were close to 4000 cfs. Flows in 2013 and 2014 were also intermediate, even with a pulse flow close to 1900 cfs in late May 2014.

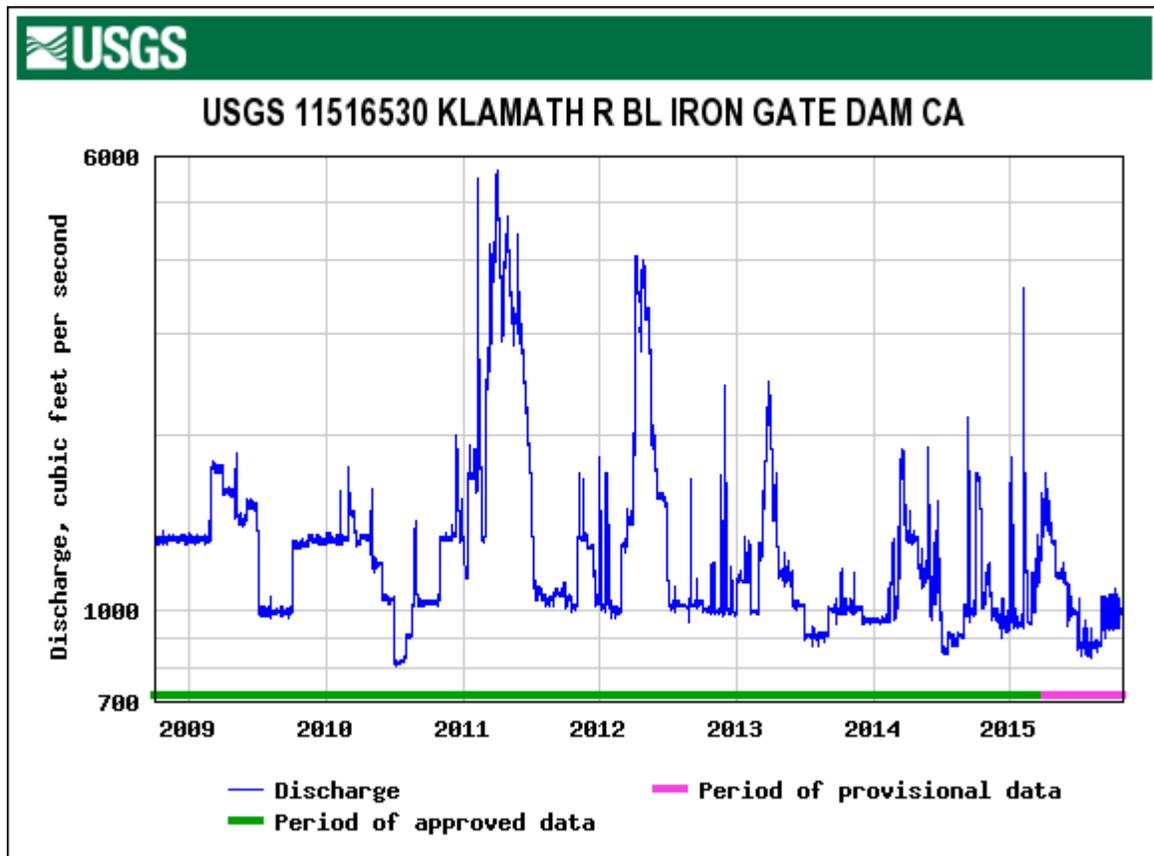


Figure 11. Daily discharge (cfs) below Iron Gate Dam from October 2008 through October 2015. Data collected after April 7, 2015 is provisional data. Data acquired from USGS waterdata.usgs.gov

Discussion

Natural Chinook salmon

In the fourth year of drought in California, 2015 represented another year of minimal flows and elevated water temperatures for out-migrating juvenile Chinook salmon in the Klamath Basin.

Ceratonova shasta prevalence of infection by QPCR, histology, and field observations of clinical disease was high for both natural fish and coded-wire tagged juvenile Chinook salmon.

Ceratonova shasta prevalence of infection by QPCR in natural fish was 75% in 2015, similar to 76% in 2014, and higher than 25% observed in 2013. Natural origin fish were collected from the upper reaches below Iron Gate Dam where *C. shasta* was first detected in fish on April 9 at the Kinsman trap, approximately 3 weeks earlier than normally observed in the monitoring program. First detections in previous years occurred in late April to early May: April 21-22 (2009, 2011 and 2013), May 4 (2010) and May 10 (2012). An exception to this trend occurred in 2014 when the first detection of April 3 was similarly early as in 2015. The prevalence of infection at first detection in 2015 was low (20%) and occurred at river temperatures of 10.6°C (USGS gauge below Iron Gate Dam). Infection prevalence rose steadily to 90% by April 19 in the Shasta to Scott (K4) reach, and remained high (90-100%) through the last sample date of May 17. In the Scott to Salmon (K3) reach, *C. shasta* was similarly detected early (April 12) at relatively low prevalence (25%) but rapidly rose to 100% by the following week and remained high (87-100%) through May 24. Clinical disease signs in natural juvenile Chinook salmon were reported by field crews at the Kinsman trap on April 23 and included swollen abdomens (17%) and pale gills/anemia (85%). Ninety percent of the twenty fish tested by QPCR on this date were positive for *C. shasta* and two fish had high *C. shasta* DNA copy numbers (145,000 and 330,000) indicative of clinical disease. *Ceratonova shasta* mean DNA copy number (88,000) for all natural fish sampled in 2015 was higher than 2014 (18,000 copies). And 2015 DNA copy number was also higher than 2013 (6,000 copies) which was a year with low infection prevalence (25%) in natural fish (Bolick et al., 2013).

Parvicapsula minibicornis prevalence of infection by QPCR (100%) in the April 19 group indicated nearly all fish examined were dual infected with both myxosporean parasites. *Parvicapsula minibicornis* prevalence of infection (92%) in all natural fish tested by QPCR was higher in 2015 compared to 2014 (83%) and 2013 (64%).

The majority of juvenile Chinook tested by histology in 2015 were of natural origin. *C. shasta* prevalence of infection ranged from 70-80% in early-April sampling, compared to 25% for the same sample period in 2014. *Ceratonova shasta* pathology scores were high early and higher overall in 2015, compared to 2013-2014, indicating more severe infection levels including moderate to severe intestinal necrosis (enteronecrosis).

Parvicapsula minibicornis was first detected by histology on April 16 in fish collected from the Kinsman trap. *Parvicapsula minibicornis* infection by histology (95%) was higher in fish collected from the two upper reaches in 2015, compared to 2014 (63%). Juvenile Chinook salmon from both reaches had high prevalence of infection patterns (90-100%) as well as high pathology scores (6.7-7.4), indicating clinical disease by this myxozoan by early May.

Iron Gate Hatchery Coded-wire Tagged Chinook salmon

Ceratonova shasta prevalence of infection in IGH CWT salmon by QPCR (90%) was high in 2015 compared to previous sampling years: 79% in 2014, 46% in 2013 and 42% in 2012. Hatchery release dates (May 21 through June 2) were similar to 2013-2014 dates.

We detected infection in WAL fish earlier and at higher levels than in previous years, indicating fish experienced high parasite exposure doses immediately upon release in the upper reaches. The 1-2 WAL groups had the highest prevalence of infection (92-93%) and high mean DNA copy number (79,000-118,000), but infection prevalence was high (80-93%) in all of the WAL groups (except WAL <1). This unusual pattern of elevated prevalence across all WAL groups, indicated all fish developed high prevalence of infection once exposed to the main stem, regardless of exposure period. In previous monitoring years, the highest *C. shasta* prevalence of infection generally occurs in fish residing for 3-5 WAL and relates to the typical period required for disease progression at temperatures and spore concentrations typically occurring during the juvenile outmigration period (True et al., 2012, Bolick et al., 2013 and True et al. 2013).

For *P. minibicornis*, the overall prevalence of infection in IGH CWT was 99%. Prevalence increased rapidly to 100% following hatchery releases and remained high in juvenile fish collected in the Salmon to Trinity (K2) reach. Considering the lowest *P. minibicornis* prevalence of infection was 97% in the Trinity to Estuary reach and 99% in the Estuary, the majority of hatchery fish were infected with both *C. shasta* and *P. minibicornis*. Moreover, it is highly likely that the most severely infected juvenile Chinook salmon died as a result of dual infections, and dropped out of the population prior to reaching the two lowest reaches.

When comparing infection prevalence in IGH CWT by capture reach, *C. shasta* POI in CWT Chinook was high in the upper two reaches (79%) comprising the 'infectious zone' (K4 and K3 reaches). This *C. shasta* POI was similar for these upper reaches (77%) in 2014. However an analysis of previous monitoring years (2005-2013) confirmed *C. shasta* prevalence of infection in IGH CWT in the upper reaches was higher in 2015, when compared to the most severe disease years on record for the monitoring program (2007-2008: 68% and 69%). The high *C. shasta* prevalence of infection in 2014-2015 contrasts dramatically with low prevalence of infection in IGH CWT in the upper reaches during 2010 (16%) and 2011 (13%), years characterized by cooler temperatures (2010-2011) and higher peak flows (2011).

In the Estuary reach, *C. shasta* infection (85%) was higher than in 2014 (76%) and in 2013 (49%). However sampling in the Estuary ended approximately two weeks earlier (July 26) in 2015 than in the past two years (Aug 11 and 17) of the monitoring program. A high turbidity event in July led to early cessation of sampling in the lowest reaches (Trinity to Estuary and Estuary) as field crews were unable to capture adequate numbers of IGH CWT fish. Thus *C. shasta* prevalence of infection may have been even higher in the estuary if sampling had continued another two weeks into August, as in prior years.

For inter-annual comparison of average parasite load in the juvenile Chinook salmon, mean DNA copy number for all IGH CWT Chinook salmon sampled in 2015 was 53,000 copies compared to 32,000 copies in 2014, and 4,500 copies in 2013. When considered along with the high *C. shasta* prevalence of infection observed in all reaches, the high mean DNA copy number observed in IGH

CWT indicates fish were exposed to high spore concentrations upon release into the main stem Klamath in 2015.

Historical Comparison

For historical comparisons between monitoring years, data is restricted to the peak migration period (May to end of July) and reaches above the Trinity confluence. The mean *C. shasta* prevalence of infection for the past ten years (2006-2015) of the monitoring program is 44% by QPCR and 28% by histology. However, the range of *C. shasta* POI been quite variable over the past decade (17-91% by QPCR and 2-62% by histology). The annual *C. shasta* POI in all Chinook salmon tested by QPCR (91%) in 2015 was the highest to date and nearly double *C. shasta* prevalence in two other severe disease years (49% in 2008 and 45% in 2009). During the past two years of an extended drought in California, the *C. shasta* POI by QPCR has ranged from 81-91%. Similarly, *C. shasta* prevalence of infection by histology was the highest to date (62%) and also highest on record during the past ten years of the monitoring program.

In 2015, we observed infections in natural fish in early April, high prevalence of infection and high rates of clinical disease signs by the end of April. Both *C. shasta* and *P. minibicornis* pathology scores were elevated to clinical disease ranges by mid-April in the upper reaches of the Klamath River, below Iron Gate Dam.

Hatchery juvenile Chinook salmon experienced early infections, high *C. shasta* prevalence of infection in 1-2 WAL groups (and across all exposure groups) and higher parasite DNA copy numbers compared to previous study years.

Severe drought conditions in 2014 and 2015 appear to have provided habitat conditions more favorable to myxozoan parasites such as *C. shasta* and *P. minibicornis*. Based on the clinical disease and high prevalence and intensity of myxozoan infections in both natural and hatchery-reared juvenile Chinook salmon, some elevated level of mortality (although not precisely known) occurred in out-migrating juvenile Chinook salmon the Klamath River in 2015. Over the course of the monitoring program, there have been diverse environmental conditions with regard to river temperature and seasonal flows. These conditions appear to correlate well with severe disease years in 2008-2009, intermediate infection levels in 2012 and 2013, and low prevalence of infection in the more favorable environmental conditions that occurred in 2010 and 2011. The trends in annual *C. shasta* prevalence of infection in juvenile Chinook salmon demonstrate that river temperatures and flows are connected factors that influence disease severity in salmonids.

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Sarah Borok, CDFW (cover photo, Klamath River Estuary Oct. 2015)

Michael Sundman, USFWS-Arcata FWO (clinical fish at KMN rotary screw trap – Figure 5)

Tom Hotaling, Salmon River Restoration Council (sediment NF Salmon River sediment – Figure 6)

Author Roles

The contributions of each author have been summarized below.

- Kimberly True – Project lead and coordination, data management and quality control, QPCR methodology and quality assurance, data analysis, and written report.
- Anne Voss – Data management and quality control, QPCR necropsy extraction and assays, pivot tables and environmental data figures, assistance with written report.
- Scott Foott – Project support, examination of histological specimens, diagnostic assessments, and report review.

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 2 in this report, page 15, 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	Weekly 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	29-Mar	20				
2	5-Apr	20				
3	12-Apr	20	20			
4	19-Apr	20 (H10)	20 (H10)			
5	26-Apr	20	21			
6	3-May	20 (H10)	21 (H10)			
7	10-May	20	22			
8	17-May	20 (H10)	20 (H10)			
9	24-May	20	23			
10	31-May	18 (H10)	20 (H10)			
11	7-Jun	19	17		19	
12	14-Jun	19	19		20	20
13	21-Jun		21	19	20	17
14	28-Jun		21	21	9	13
15	5-Jul		27	21	7	14
16	12-Jul		7	19	5	16
17	19-Jul		10	17		15
18	26-Jul			21		2

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	Weekly 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	29-Mar	0% (0/20)				
2	5-Apr	20% (4/20)				
3	12-Apr	35% (7/20)	25% (5/20)			
4	19-Apr	90% (18/20)	100% (20/20)			
5	26-Apr	100% (20/20)	95% (20/21)			
6	3-May	100% (20/20)	100% (21/21)			
7	10-May	90% (18/20)	95% (21/22)			
8	17-May	95% (19/20)	80% (16/20)			
9	24-May	95% (19/20)	87% (20/23)			
10	31-May	61% (11/18)	95% (19/20)			
11	7-Jun	84% (16/19)	100% (17/17)		100% (19/19)	
12	14-Jun	100% (19/19)	84% (16/19)		95% (19/20)	95% (19/20)
13	21-Jun		81% (17/21)	100% (19/19)	100% (20/20)	88% (15/17)
14	28-Jun		67% (14/21)	95% (20/21)	78% (7/9)	92% (12/13)
15	5-Jul		93% (25/27)	100% (21/21)	100% (7/7)	86% (12/14)
16	12-Jul		86% (6/7)	89% (17/19)	100% (5/5)	69% (11/16)
17	19-Jul		90% (9/10)	100% (17/17)		80% (12/15)
18	26-Jul			95% (20/21)		50% (1/2)
		K4 Total 72% (171/236)	K3 Total 85% (246/289)	K2 Total 97% (114/118)	K1 Total 96% (77/80)	K0 Total 85% (82/97)

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. A number of samples (n=40) were too small to collect kidney tissue. Therefore, the number of fish tested for *C. shasta* and *P. minibicornis* are not equal.

Week	Weekly 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	29-Mar					
2	5-Apr					
3	12-Apr	70% (14/20)	30% (6/20)			
4	19-Apr	100% (20/20)	100% (20/20)			
5	26-Apr	100% (20/20)	100% (21/21)			
6	3-May	100% (20/20)	100% (21/21)			
7	10-May	100% (20/20)	95% (21/22)			
8	17-May	100% (20/20)	95% (19/20)			
9	24-May	100% (20/20)	100% (23/23)			
10	31-May	100% (18/18)	100% (20/20)			
11	7-Jun	100% (19/19)	100% (17/17)		100% (19/19)	
12	14-Jun	100% (19/19)	100% (19/19)		90% (18/20)	100% (20/20)
13	21-Jun		100% (21/21)	100% (19/19)	100% (20/20)	100% (17/17)
14	28-Jun		100% (21/21)	100% (21/21)	100% (9/9)	100% (13/13)
15	5-Jul		96% (26/27)	100% (21/21)	100% (7/7)	100% (14/14)
16	12-Jul		100% (7/7)	100% (19/19)	100% (5/5)	100% (16/16)
17	19-Jul		100% (10/10)	100% (17/17)		93% (14/15)
18	26-Jul			100% (21/21)		100% (2/2)
		K4 Total 97% (190/196)	K3 Total 94% (272/289)	K2 Total 100% (118/118)	K1 Total 98% (78/80)	K0 Total 99% (96/97)

Appendix B – Histological Summary

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

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

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 Monday of given week.

Collection week	April 19	May 3	May 17	May 31	POI
<u>Kidney</u>					
<i>P. minibicornis</i> troph.	9 / 10 (90)	9 / 10 (90)	10 / 10 (100)	3 / 10 (30)	31 / 40 (78)
<i>P. minibicornis</i> myxosp.	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 40 (0)
Metacercaria	0 / 10 (0)	0 / 10 (0)	1 / 10 (10)	0 / 10 (0)	1 / 40 (3)
<i>C. shasta</i> troph.	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 40 (0)
<i>Chloromyxum</i> sp	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 40 (0)
Pathology Score	3.3	6.7	5.9	1.5	
<u>Intestinal tract</u>					
<i>C. shasta</i> troph.	8 / 10 (80)	8 / 10 (80)	7 / 10 (70)	3 / 10 (30)	26 / 40 (65)
<i>C. shasta</i> myxosp.	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	1 / 10 (10)	1 / 40 (3)
Helminth	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 40 (0)
Pathology Score	5.2	3.5	3.9	0.6	
Adipose steatitis	2 / 2 (100)	1 / 4 (25)	7 / 8 (88)	1 / 7 (14)	11 / 21 (52)
Adipose lipofuscin	0 / 2 (0)	0 / 4 (0)	0 / 8 (0)	0 / 7 (0)	0 / 21 (0)
<u>Gill</u>					
Metacercaria	1 / 10 (10)	5 / 10 (50)	8 / 10 (80)	5 / 10 (50)	19 / 40 (48)
Multif. Hyperplasia	2 / 10 (20)	4 / 10 (40)	6 / 10 (60)	5 / 10 (50)	17 / 40 (43)

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 Monday of given week.

Collection Week	April 19	May 3	May 17	May 31	POI
<u>Kidney</u>					
<i>P. minibicornis</i> troph.	9 /10 (90)	10 /10 (100)	10 /10 (100)	10 /10 (100)	39 / 40 (98)
<i>P. minibicornis</i> myxosp.	0 /10 (0)	3 /10 (30)	0 /10 (0)	0 /10 (0)	3 / 40 (8)
Metacercaria	0 /10 (0)	0 /10 (0)	2 /10 (20)	0 /10 (0)	2 / 40 (5)
<i>C. shasta</i> troph.	0 /10 (0)	0 /10 (0)	2 /10 (20)	0 /10 (0)	2 / 40 (5)
<i>Chloromyxum</i> sp	0 /10 (0)	0 /10 (0)	0 /10 (0)	0 /10 (0)	0 / 40 (0)
Pathology Score	1.2	7.4	6.7	3.8	
<u>Intestinal tract</u>					
<i>C. shasta</i> troph.	9 /10 (90)	8 /10 (80)	3 /10 (30)	8 /10 (80)	28 / 40 (70)
<i>C. shasta</i> 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)	1 /10 (10)	1 / 40 (3)
Pathology Score	2.5	4.4	0.0	5.1	
Adipose steatitis	8 /8 (100)	10/10 (100)	2 /9 (22)	4 /5 (80)	24 / 32 (75)
Adipose lipofuscin	0 /8 (0)	0 /10 (0)	0 /9 (0)	0 /5 (0)	0 / 32 (0)
<u>Gill</u>					
Metacercaria	7 /10 (70)	9 /10 (90)	8 /10 (80)	10 /10 (100)	34 / 40 (85)
Multif. Hyperplasia	6 /10 (60)	8 /10 (80)	9 /10 (90)	9 /10 (90)	32 /40 (80)

Appendix C - Reviewer comments

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

Reviewer #1

Pg. 7 - Parasite Infection Levels by Quantitative PCR Assays: Reviewer suggested changes to the standard curve equation for Cs and Pm QPCR assays, including clarification of x and y definitions.

Response: The equations for single standard curve used for each parasite assay were removed and language added to clarify the methodology now compares a standard curve included on each assay plate (and no longer refers to a single standard curve for all data generated by QPCR assays).

Pg. 7 - Statistical Analysis: Reviewer suggested clarification on implication for the annual prevalence estimate so reader did not infer the weekly estimates are weighted by abundance values. (Not weighted, represent proportion of fish tested that are infected)

Response: Language added to this section to state weekly estimates are not weighted by abundance.

Pg. 11 - Prevalence of Infection by Fish Origin: Reviewer suggested including ci (confidence interval) for all prevalence percentages be reported. In some sections they were not reported.

Response: Confidence intervals were included for the majority of prevalence data (including POI by histology and individual WAL data points). Confidence intervals were not included where ranges of similarly high WAL POI were reported (too cumbersome to display them for ranges). The reader can see the similar POIs across the WAL range and infer the confidence intervals are likely similar.

Pg 13 – IGH CWT Weeks at Large: Similar to above comment, reviewer stating confidence intervals are not shown when range of POI discussed, but acknowledged specific WAL POI don't appear any different than those around them.

Response: Language included stating confidence intervals around these percentages don't appear different (confidence intervals included for all single POI percentages given).

Pg 15 – Historical Comparison – Table 2: Reviewer suggested removing the SE stat given for mean Cs POI in Table 2 because it does not consider the variability of each estimate (annual POI for histology and QPCR).

Response: Comment is valid and SE removed.

Reviewer #2

General comments: Reviewer had general comments regarding writing style and usage.

Response: Minor edits were done throughout the report to reword some sentences and incorporate these suggestions.

Pg. 3 - Introduction: In regards to “river temperatures promote early proliferation and maturation of polychaete populations” the reviewer asked for clarification on whether this sentence means the parasite within the polychaete or the polychaete population itself.

Response: The sentence was intended to point towards the polychaete population itself and the sentence has been rephrased to add that clarification.

Pg. 20 – Discussion: Reviewer states that references to the first detection of the parasite should be clarified that these parasite detections are in fish.

Response: The method section tells the reader that fish tissues are being used for parasite identification and quantification, therefore that clarification is not needed.

Pg. 23 – Discussion – Historical Comparison: Reviewer suggests including range of Cs POI for historic period, in addition to mean given for past decade.

Response: Added Cs POI ranges for QPCR and histology results.