

California-Nevada Fish Health Center

FY 2014 Investigational Report:

**Myxosporean Parasite (*Ceratonova shasta* and *Parvicapsula minibicornis*)
Prevalence of Infection in Klamath River Basin Juvenile Chinook Salmon,
April-August 2014**

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Summary

Juvenile Klamath River Chinook salmon (*Oncorhynchus tshawytscha*) were assayed from April to August 2014 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 outmigration period (May-July) was 81%, an increase from 46% in 2013. *Parvicapsula minibicornis* in Chinook salmon above the Trinity River confluence for the same period was 92% compared to 88% in 2013. Historically, the *C. shasta* prevalence of infection for juvenile Chinook salmon during outmigration in 2014 was the highest to date (2006-2014) by QPCR and second highest by histology.

Among the various fish groups tested, naturally produced Chinook salmon had a 76% prevalence of *C. shasta* infection by QPCR and were infected three weeks earlier in the season, compared to previous monitoring years. Based on both QPCR testing and histology, natural fish had relatively high infection levels (DNA copy number) and disease severity (pathology scores) in early to mid-May compared to natural fish tested in 2013.

Among coded-wire tagged (CWT) juvenile Chinook salmon released from Iron Gate Hatchery (IGH), *C. shasta* was detected in 79% of fish screened by QPCR. The highest *C. shasta* prevalence of infection observed was 92-96% in IGH CWT Chinook salmon residing 1-2 Weeks At Large (WAL) at time of recapture. Iron Gate Hatchery Chinook salmon had a much higher parasite infectious load, measured as quantity of *C. shasta* DNA present in intestinal tissue, in 2014 compared to 2013. By diagnostic examination of moribund fish, approximately 80% of IGH CWT juvenile Chinook salmon examined at the mouth of Ti Creek experienced end stage clinical disease, including myxospore production and mortality, in the Scott to Salmon reach in mid-June just 16 days (2 WAL) post release. The majority of juvenile CWT Chinook salmon examined in routine sampling in late June through August in the lower reaches appeared relatively healthy despite high *C. shasta* prevalence of infection. Of all fish examined by necropsy for this period, 6% were scored as severe for clinical disease and an additional 9% were scored moderate for clinical disease.

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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 the lower basin extends 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; Bartholomew, et al., 2007; Jones, et al., 2004). The parasites' life cycles include the invertebrate polychaete host, *Manayunkia speciosa*, which (if infected) releases an infectious actinospore stage into the water column that can infect the vertebrate salmon host. The actinospore infects and develops within the vertebrate host, salmon or trout species, into a myxospore. Once shed from a 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 ceratomyxosis and is a significant contributor to mortality in juvenile fish that migrate through the region (Hoffmaster, et al., 1988; Stocking, et al., 2006; Bartholomew, et al., 1997). Infectivity patterns of ceratomyxosis 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 (Bartholomew, et al., 2010; Bjork, et al., 2009; Stone, et al., 2008; True, et al., 2013) and annual monitoring of coded-wire tagged (CWT) Chinook salmon with known exposure periods in the main stem Klamath (True et al., 2013, Bolick et al., 2013, Nichols et al., 2009).

Klamath River juvenile Chinook salmon can experience high prevalence and severity of infection with these two myxosporean parasites, particularly when river temperatures promote early proliferation and maturation of polychaete populations (Bartholomew, et al., 2010; True, et al., 2011). Mortality from ceratomyxosis 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 affected 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 2014, California experienced severe drought following an extremely dry year in 2013 (February through October; NOAA, 2013). The drought conditions primarily resulted from low snowpack in the Cascade and Sierra Nevada mountain ranges, combined with low annual precipitation throughout the winter of 2013-2014 and warmer than normal air temperatures in the summer of 2014. 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 habitat conditions that are more favorable to *C. shasta* transmission: early release and concentration of the parasite infectious spore stage in the water column result in higher infectious dose to the fish host. Ceratomyxosis prevalence of disease can be higher under such conditions, compared to environmental conditions that promote higher river flows (decreased spore concentration) and cooler river water temperatures (slowed spore development and disease progression).

The primary objectives of this study were to: 1) examine parasite prevalence in Klamath River juvenile Chinook salmon during the spring out-migration period; and 2) compare parasite prevalence in 2014 to previous years.

Methods

Pre-Release Examination

Prior to the Iron Gate Hatchery releases (May 20 through June 13, 2014) of approximately 5 million fall Chinook salmon, a fish health examination of 30 hatchery fish was conducted at the hatchery on May 15 to determine infection levels of *C. shasta* and *P. minibicornis*. A fish health examination of 30 fall Chinook salmon was also conducted at Trinity River Hatchery (TRH) on May 14, prior to approximately 1.5 million fall Chinook salmon and approximately 1.3 million spring Chinook salmon being released from June 1 through June 4, 2014.

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.

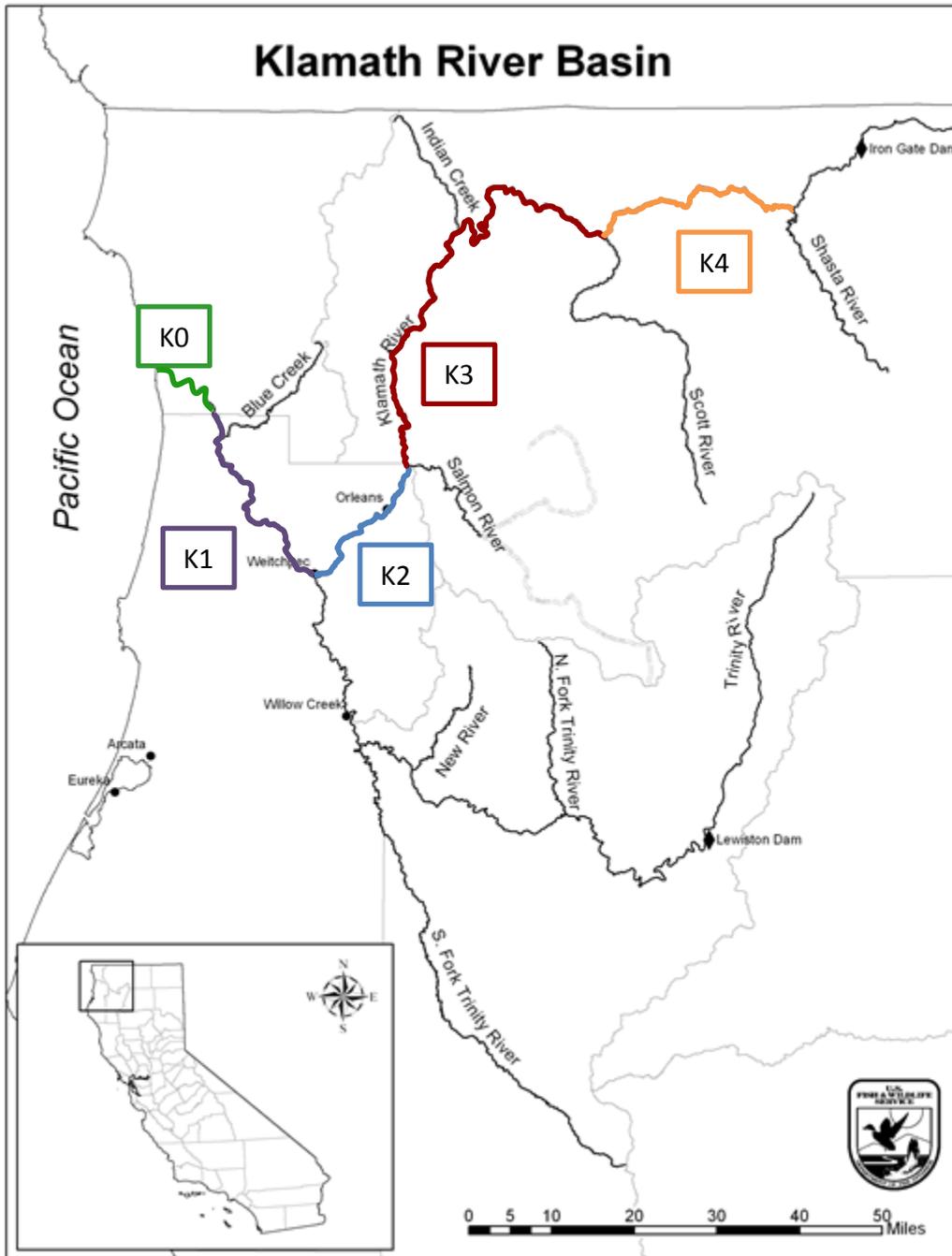


Figure 1. Klamath River watershed, major tributaries, and sample reaches: Shasta River to Scott River (K4), Scott River to Salmon River (K3), Salmon River to Trinity River confluence (K2), Trinity River to upper Estuary (K1), and Klamath River Estuary (K0). Map provided by the Arcata Fish and Wildlife 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 (March 30-July 20) while the lower reaches were sampled later in the season (June 8-August 17) 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 (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 (37 samples) 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 exams 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 or fish mortality during normal field surveys. The Yurok Tribe field crews observed clinically diseased fish and mortality in juvenile Chinook salmon during routine field surveys in the Scott to Salmon reach (K3). Clinical and moribund juvenile Chinook salmon were sampled by QPCR, including 17 CWT juvenile Chinook with known exposure periods. A subset (N=3) of fish were tested by histology.

A second diagnostic examination was conducted on July 8 by Fish Health Center staff at the thermal refugia located at the mouth of Pecwan Creek, in the Trinity to Estuary (K1) reach. Juvenile Chinook salmon were beach seined and examined for clinical disease signs and 30 CWT fish were collected for QPCR testing. Additionally, laboratory testing was conducted on a subset (N=28) of juvenile coho salmon (*Oncorhynchus kisutch*) rescued from the Scott River in early July prior to their transfer to Iron Gate Hatchery.

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, the cycle threshold (C_T) values were calculated (SDS software 7300 SDS v 1.3.1, Applied Biosystems) and a standard curve was constructed to calculate PCR efficiency (94%), fit to the curve (R^2 value = 0.997) and estimated DNA copy number:

$$y = -3.48x + 40.0 + \varepsilon$$

Quantification of fish tissue (*C. shasta* DNA copy number) was determined using 5 μ L of DNA template in a 30 μ L reaction. Individual plate assays were evaluated against the standard curve efficiency. When assay efficiency for an individual plate varied by more than 3%, the C_T value and DNA copy number were adjusted proportionally to bring data within 3% of the standard curve range. Over the field season, two plates required adjustment to the standard curve.

The *P. minibicornis* reference standard curve was obtained 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, the C_T values were calculated (SDS software 7300 SDS v 1.3.1, Applied Biosystems) and a standard curve was constructed to calculate PCR efficiency (92%), fit to the curve (R^2 value = 0.995) and estimated DNA copy number:

$$y = -3.54x + 43.3 + \varepsilon$$

Quantification of fish tissue (*P. minibicornis* DNA copy number) was determined using the same reaction volume. *Parvicapsula minibicornis* data was not fit to a single reference standard curve, but to standard curves produced for each individual plate. Assay efficiencies were monitored over the field season, and no plates required adjustment of C_T or DNA copy number. In the Results section, QPCR data are presented first for each group of fish or type of analysis, followed by histology in a separate paragraph.

Parasite Infection Levels by Histology

Histological assays were done according to True, et al. (2013). In 2014, histology samples were collected in the Shasta River to Scott River reach (K4) and the Scott River to Salmon River reach (K3) between April 20 and June 1 (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.

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 for the sampled population during the entire sampling period that year.

Results

Pre-Release Examination of IGH and TRH Chinook Salmon

Juvenile Chinook salmon reared at Iron Gate and Trinity River hatcheries were screened for infections of *C. shasta* and *P. minibicornis* by QPCR prior to release. *Ceratonova shasta* was detected in 2/30 (7%) of IGH juveniles sampled on May 15th. *Parvicapsula minibicornis* was not detected at IGH. Trinity River Hatchery juvenile Chinook salmon were sampled on May 14 and *C. shasta* was not detected in this examination, however 2/30 (7%) of fish sampled had low levels of *P. minibicornis* present.

Number of Fish Collected by Origin

In 2014 we examined 1129 juvenile Chinook salmon collected from the main stem Klamath River. The sample consisted of 301 natural fish, and 828 fish collected after hatchery release which included 740 CWTs. Coded-wire tagged Chinook salmon (marked with an adipose fin clip) accounted for 66% (740/1129) of all fish sampled in 2014 (Figure 2). Natural fish accounted for 27% (301/1129) and 8% (88/1129) of the fish are of unknown origin (unmarked hatchery fish or natural).

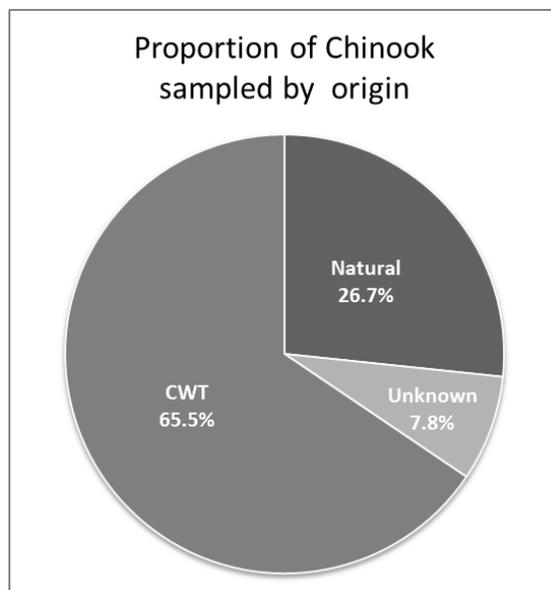


Figure 2. Proportion and origin of Chinook salmon collected (N = 1129) in 2014.

Annual Prevalence of Infection by Klamath River Reach

The annual prevalence of *C. shasta* infection in all Chinook salmon collected in in 2014 by QPCR was 78% (875/1129, ci = 75-80%). *Ceratonova shasta* was first detected on April 3 in the Shasta River to Scott River reach (K4). *Ceratonova shasta* POI was highest in the Trinity River to Estuary (K1) at 89%, followed by 87% in the Scott River to Salmon River reach (K3). The lowest prevalence of 59% was observed in the Shasta River to Scott River reach (K4) (Figure 3).

The annual *P. minibicornis* POI by QPCR was 91% (992/1092, ci = 89-92%). *Parvicapsula minibicornis* was first detected on April 3 in the Shasta River to Scott River reach (K4). Prevalence was highest in the Salmon River to Trinity River reach (K2) at 100%, followed closely by the Trinity River to Estuary (K1) at 98% (Figure 3). The lowest prevalence of 70% was observed in the Shasta River to Scott River reach (K4).

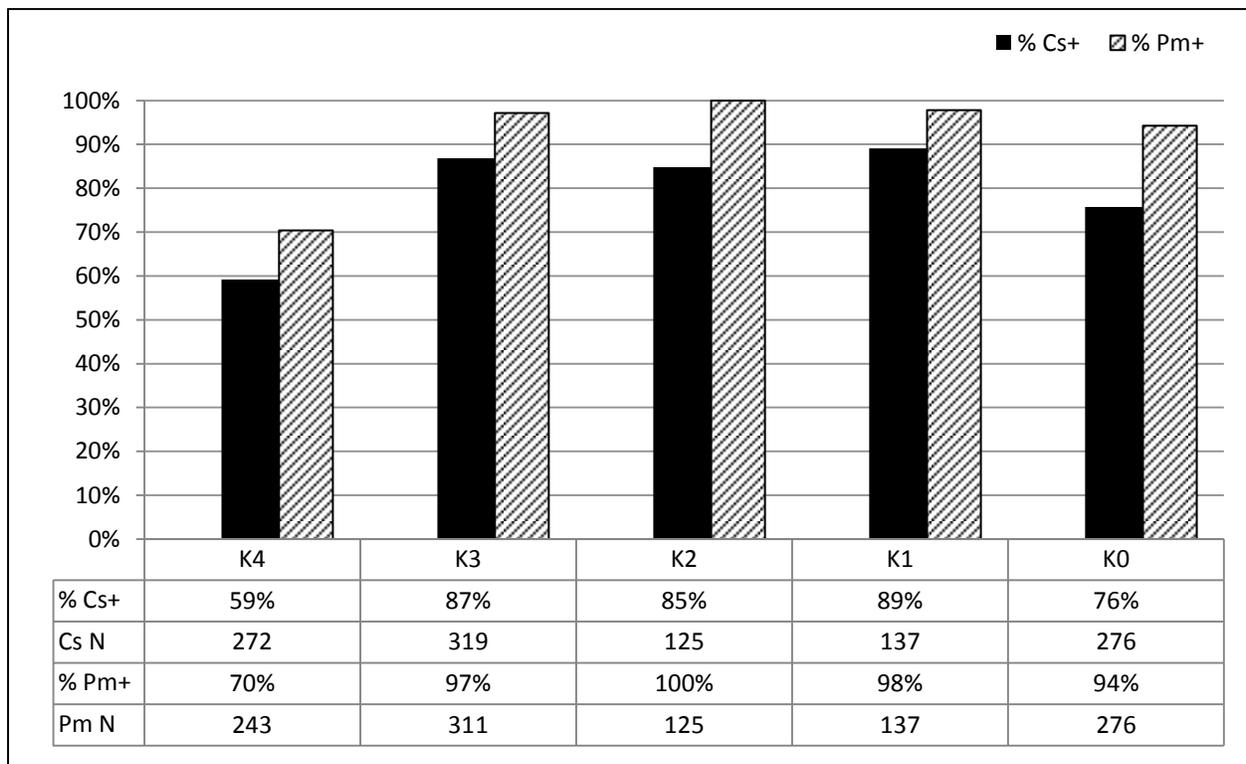


Figure 3. Prevalence of *Ceratonova shasta* (Cs+) and *Parvicapsula minibicornis* (Pm+) infection in juvenile Klamath River Chinook salmon by collection reach in 2014. K4= Shasta River to Scott River; K3= Scott River to Salmon River; K2= Salmon River to Trinity River; K1= Trinity River confluence to Estuary; K0= Klamath River Estuary. Sample numbers collected (N) are displayed in the table below each column for both pathogens.

As described in methods, histology sampling occurred from April 20 to May 18 in the K4 reach and April 20 to June 1 in the K3 reach. The annual *C. shasta* POI in 2014 by histology for all fish tested was 33% (22/66, ci = 22-46%) and for *P. minibicornis* was 68% (42/62, ci = 55-79%).

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 301 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 April 3 through May 27 in Shasta to Scott (K4) reach and from April 3 through May 25 in the Scott to Salmon River (K3) reach. River mean daily temperature was 9.8 °C at first detection of *C. shasta* in natural fish collected in K4.

Ceratonova shasta was detected by QPCR in 76% (229/301, ci = 71-81%) of natural fish. *Ceratonova shasta* POI was highest (87%) in the Scott River to Salmon River (K3) reach compared to 67% in the K4 reach above.

Comparatively, *P. minibicornis* was detected in 83% (218/264, ci = 77-87%) of naturally produced Chinook salmon by QPCR. The highest *P. minibicornis* prevalence of 95% was detected in Scott River to Salmon River reach (K3) compared to 70% in the (K4) reach above.

As stated earlier, by histological examination, all fish collected had an overall *C. shasta* POI of 33% (22/66, ci = 22-46%). Natural fish were collected for histology prior to June 1, and one histological sample set (N=10) collected on June 1 was of unknown origin. The prevalence of infection by reach was higher at 58% (N=26) in the Shasta to Scott (K4) reach compared to 18% (N=40) in the Scott to Salmon River (K3) reach. In the Shasta to Scott (K4) reach, both the highest *C. shasta* prevalence of infection by histology (88%) and the peak pathology score (5.1) occurred simultaneously during the sampling week of May 4. However in the Scott to Salmon (K3) reach, the highest prevalence (50%) also occurred on May 4, but the highest pathology score was lower (1.5) and occurred later in the season on June 1. For comparison, clinically infected salmon in 2009 had intestine pathology scores between 3 and 4 (True, et al., 2010).

Natural fish had an overall *P. minibicornis* POI by histology of 68% (42/62, ci = 55-79%). Prevalence was highest in the Scott to Salmon (K3) reach at 71%, compared to 63% in the Shasta to Scott (K4) reach above. The pathology score in K4 increased steadily over a 3 week period (May 4 – June 1) from 2.0 to 4. 1; which is below the clinical kidney pathology score of 6-8 (True, et al., 2010).

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 88 fish of unknown origin were collected from the Shasta to Scott reach (K3) through the estuary reach (K0) from May 29 through June 9. *Ceratonova shasta* was detected by QPCR in 71% (62/88, ci = 60-80%) of unknown origin Chinook salmon. *Parvicapsula minibicornis* POI in was 92% (81/88, ci = 84-97%).

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 (adipose fin clipped) in the five past years of the monitoring program (Buttars, et al., 2009). A total of 740 CWT Chinook salmon were collected this season from the Klamath River. Iron Gate Hatchery CWT fish accounted for 71% (527/740) and TRH CWT fish accounted for 24% (176/740) of CWT fish tested. Additionally, 37 fish (5%) 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: 24 of the 37 unreadable tags 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 June 8 to August 20. The largest proportion of IGH CWT (N=108 fish), were collected in the Scott River to Salmon River reach (K3). *Ceratonova shasta* was detected in 79% (418/527, ci = 76-83%) of all IGH CWT screened by QPCR. Prevalence of infection for *C. shasta* was highest in the Trinity River to Estuary (K1) reach at 91% followed closely by the Scott to Salmon (K3) reach at 88%. The lowest prevalence was in the Shasta River to Scott River reach (K4) at 45%.

Parvicapsula minibicornis was detected by QPCR in 94% (95/527, ci = 92-96%) of IGH CWT. Prevalence of infection for *P. minibicornis* was 100% in three reaches: Scott to Salmon (K3), Salmon to Trinity (K2) and Trinity to Estuary (K1). The lowest prevalence was in the Shasta River to Scott River (K4) reach at 59%.

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 and in-river recapture dates). The time period used to describe how long fish are residing in the Klamath River post hatchery release is referred to as Weeks At Large (WAL).

Ceratonova shasta POI in IGH CWT Chinook salmon by WAL analysis shows a skewed curve with highest prevalence of infection (96% decreasing to 85%) in fish residing 1-5 Weeks At Large (Figure 4) compared to a more typical bell shaped curve observed in previous years. In 2014 the lowest *C. shasta* prevalence of infection occurs in fish residing at 0 (less than 1 WAL), 6 and 9 WAL (32%, 48% and 42% respectively). Intermediate POI ranges occur for WAL 7, 8, and 10 at 70%, 64% and 50% respectively (Figure 4). Groups residing 11-12 WAL were combined because sample numbers consisted of only 3 fish collected in the estuary reach.

As stated in the methods, the QPCR assay can quantify parasite DNA copies within fish tissue and therefore describe infection levels. In IGH CWT Chinook salmon, the highest mean DNA copy number (67,000-74,000; 3.8-3.9 logs) was observed in groups residing 1-2 WAL (Figure 4). Mean DNA copy was similar across the remaining weeks (less than 10,000 copies) except for WAL 3 (15,000 copies) and WAL 6 (11,000 copies). Seasonal mean DNA copy number for all IGH CWTs in 2014 was 32,000 copies (3.2 logs) compared to 4,500 (2.2 logs) in 2013.

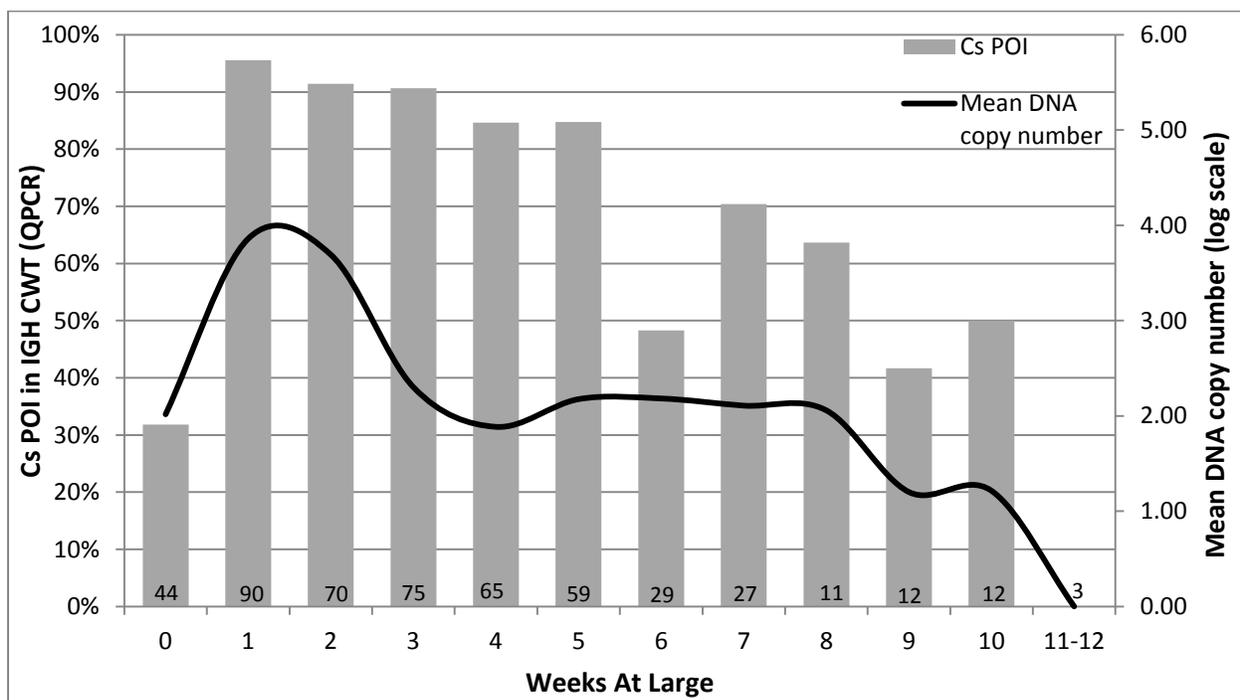


Figure 4. *Ceratonova shasta* prevalence of infection in IGH CWT by Weeks At Large (WAL) post hatchery release. The bar graph is prevalence of infection (%) on the primary y-axis and the line graph is the mean *C. shasta* DNA copy number (log scale) on the secondary y-axis for Chinook salmon tested by QPCR. The number of fish collected is listed inside the base of each bar.

Diagnostic Casework

Two diagnostic examinations were conducted in 2014 at the request of tribal partners observing clinical disease and fish mortality in juvenile Chinook salmon during routine field surveys (described below). Additionally, laboratory testing was conducted on a subset (N=28) of juvenile coho salmon (*Oncorhynchus kisutch*) rescued from the Scott River in early July prior to their transfer to Iron Gate Hatchery. These coho juveniles had modest prevalence of infection level of 32% for *C. shasta* but infection load was low (12-1200 DNA copy number). Likewise, prevalence of infection for *P. minibicornis* was 39% and DNA copy numbers (14-2500) were also low for this parasite.

On June 12, the Yurok Tribe conducted reconnaissance float surveys, and noted approximately 80 dead juvenile Chinook salmon between Big Bar (~RM 50) and Weitchpec (~RM 44). The majority of dead juvenile fish were observed above Aikens Creek (~RM 50). On June 16, USFWS Fish Health Center staff conducted a diagnostic examination of 78 juvenile Chinook salmon collected from the Klamath River at the confluence of Ti Creek (thermal refugium; RM 80) to determine the cause of the fish mortality in this reach (K3) of the Klamath River. The majority of fish collected appeared in poor condition and had clinical disease signs of ceratomyxosis: lethargy, severe distension of abdomen and bloody ascites, and hemorrhaged vent. Several fish were moribund.

Testing by QPCR found unmarked Chinook salmon were heavily infected with *C. shasta* with C_T values of (19-22) and high DNA copy numbers (200,000- 1.4 million) indicative of severe clinical disease and lethal parasite infection levels. Histology confirmed end stage ceratomyxosis including myxospore production in 3 clinical fish examined by this method. The QPCR sample set included 17 IGH CWT Chinook salmon with known exposure periods of 0-3 weeks (3-26 days post hatchery release). The majority of fish in this group with high parasite numbers had been residing 2-3 WAL upon recapture: they were released from IGH on either May 20, 23 or 30 (Table 2). Interestingly, fish collected at 3 WAL had lower infection levels than the 2 WAL group, and their release date of May 30 occurred after a pulse flow event on May 26th.

Table 2. Summary for two diagnostic cases conducted at Ti and Pecwan Creek. Coded-wire tagged fall Chinook salmon were tested by QPCR and data summarized for Weeks at Large (WAL), Cs POI for entire group and each WAL group, and mean *C. shasta* Cycle Threshold (C_T) and DNA copy number in combined and individual WAL groups.

Collection Site	Collection Date	Number CWT	Total Cs POI	WAL Group	Cs POI for Group	Release Date	Days Post Release	Mean C_T	Mean DNA Copy Number
TI Crk.	16-Jun-14	17 CWT	+14/17 (82%)					33.0	14805
								(All WAL Groups Combined)	
Thermal Refugia				0 WAL	+1/1 (100%)	6/13/2014	3	39.0	6
				1 WAL	+1/2 (50%)	6/10/2014	6	38.4	9
				2 WAL	+5/6 (83%)	5/30/2014	16	28.3	40951
				3 WAL	+7/8 (88%)	5/23/2014	23	34.6	358
						5/20/14 (1 fish)	26		
PECWAN Crk.	8-Jul-14	29 CWT	+20/29 (69%)					38.4	4127
								(All WAL Groups Combined)	
Thermal Refugia				3 WAL	+6/6 (100%)	6/13/2014	25	32.4	13134
				4 WAL	+3/4 (75%)	6/10/2014	28	35.8	114
				5 WAL	+10/17 (59%)	6/2/2014	36	35.4	192
				6 WAL	1/2 (50%)	5/23/2014	45	39.6	4

A second diagnostic examination was conducted on July 8 by Fish Health Center staff at the thermal refugia located at the mouth of Pecwan Creek (RM 25), in the Trinity to Estuary (K1) reach. Juvenile Chinook salmon were beach seined and examined for clinical disease signs and 30 CWT fish were tested by QPCR. The clinical picture was quite improved from juvenile Chinook observed on June 16 at Ti Creek in the K3 reach. At Pecwan Creek thermal refugium, juvenile fish appeared healthy overall. Only a few fish exhibited mild to moderate clinical disease signs: 2/60 (3%) showing hemorrhaged fins and vents and 1/60 (2%) with gill lesions indicative of *Flavobacterium columnare* bacterial infection. QPCR testing and CWT codes showed individual fish with the highest infection levels (C_T values of 24-28 and DNA copy number ranging from 7000-71,000) would have been released from IGH on June 13 and resided for 3 WAL prior to recapture. The mean C_T value was 32 and mean DNA copy number was 131,000 for the entire 3 WAL group (N=6; Table 2). The next highest ranking infection group (N=21) had very low DNA copy numbers (mean less than 200 copies), and were residing 4-5 WAL (released from June 2 to June 10).

Historical Comparison

Prevalence of infection by histology had been utilized as the metric for historical comparisons of disease prevalence (data confined to peak migration period of May 1 to July 31 and above the Trinity confluence) from 2006-2008. The metric transitioned to QPCR data in 2009, due to the higher sensitivity and ability to quantify parasite DNA copy number within fish tissue. 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 out-migration period was very high at 81% (467/576, ci = 78-84%) in 2014, compared to previous years (Table 3). *Parvicapsula minibicornis* in Chinook salmon above the Trinity River confluence for the same period was 92% compared to 88% in 2013.

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, 2006-2014.

Year	Histology (% Positive)	QPCR (% Positive)
2006	21	34
2007	21	31
2008	37	49
2009	54	45
2010	15	17
2011	2 ¹	17
2012	9 ¹	30
2013	16 ¹	46
2014	42 ¹	81
Mean (SE)	24 (6)	39 (7)

¹ Histology limited to two reaches in 2011 (K4 and K1); and two reaches in 2012-2014 (K4 and K3).

Environmental Conditions

In previous study years (2006-2009) 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. However in 2010 and 2011, average spring river 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). In 2013, mean daily Klamath River temperatures were warmer in the spring, compared to the 2010-2011, but similar to historic temperatures in May through September.

In 2014, temperatures in March were approximately 2°C higher than 2013 and notably 2-4°C higher than 2012 from March through April. Mean daily temperatures rose steadily throughout the season with small peaks in mid-April and mid-May, and the seasonal mean daily temperature peak of 23.2°C occurred on July 16 (Figure 5).

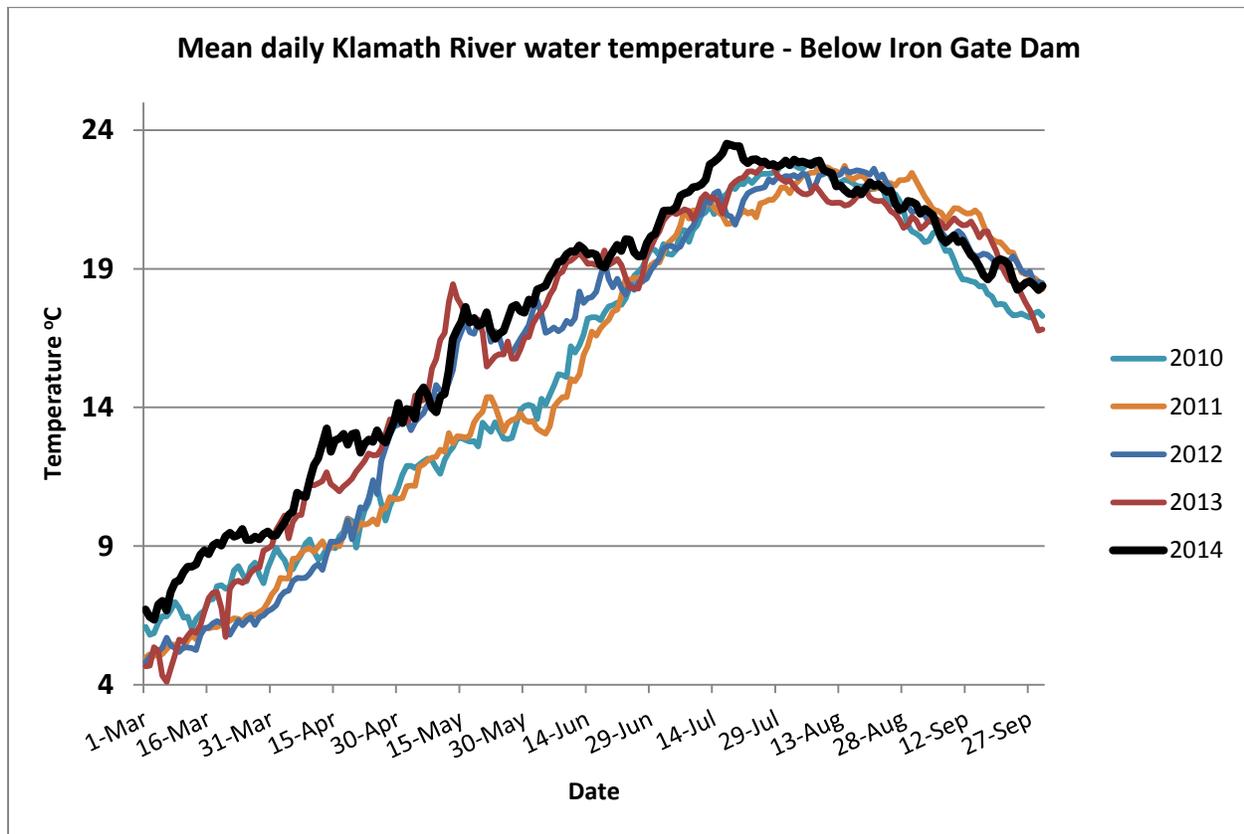


Figure 5. Mean daily Klamath River water temperature below Iron Gate Dam for 2010-2014. Temperature data for 2010-2011 and 2013-2014 acquired from Arcata Fish and Wildlife Field Office. Temperature data for 2012 acquired from Iron Gate Hatchery, and taken from the main stem Klamath River, not the hatchery facility.

At the Seiad Valley temperature gauge, mean daily Klamath River temperatures were more variable than below Iron Gate Dam and higher overall than the previous five years during the April to May period (Figure 6). Average daily temperatures reached 22°C on June 9 and peaked at 26°C on July 3. At several points in early May, early June, and early July temperatures in this part of the river were approximately 2-6°C warmer than observed during the same period in 2012, but similar to 2013. The 2012 monitoring year was an intermediate year in terms of thermal profile and disease prevalence.

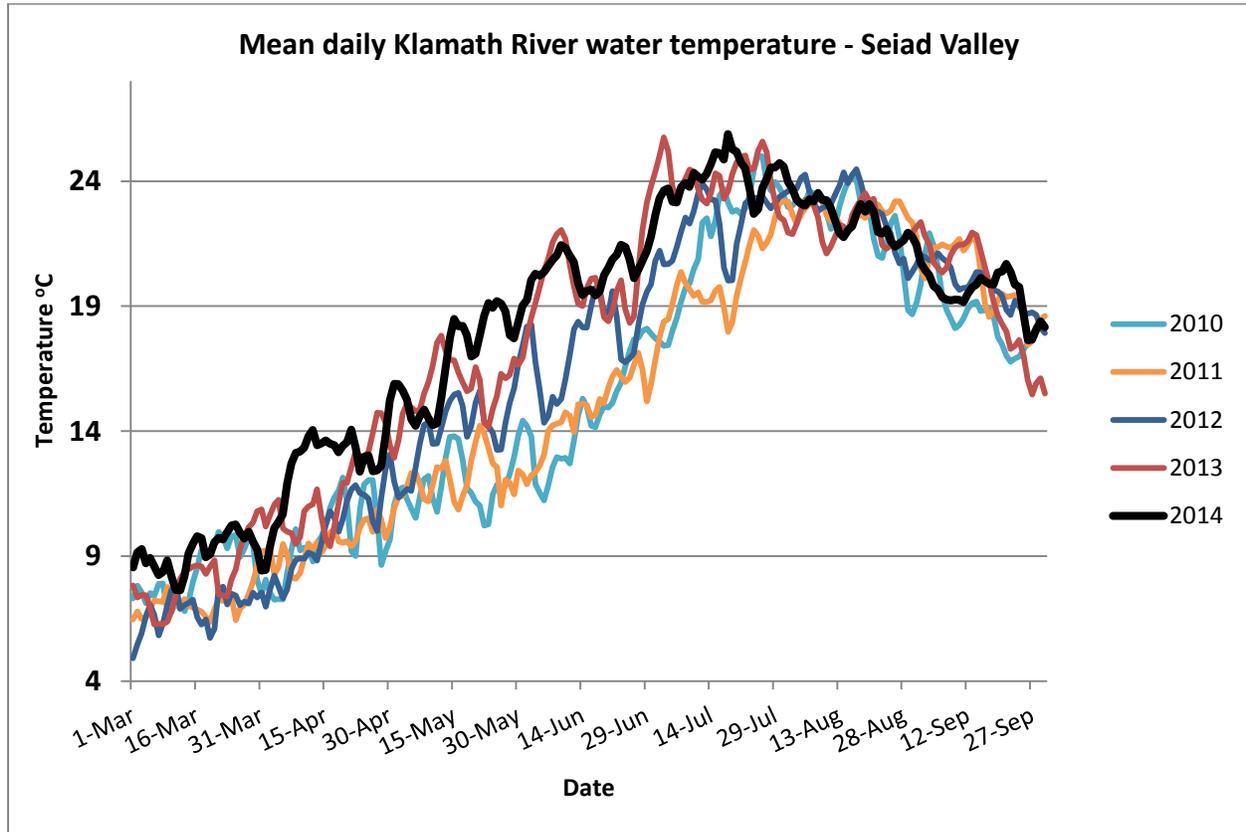


Figure 6. Mean daily Klamath River temperature from March through September 2010-2014 at Seiad Valley. Data from 2010 to 2013 were provided by the Arcata Fish and Wildlife Field Office, while the 2014 temperature data were provided by Arcata FWO and Karuk Tribe.

River Flows

In the extreme drought conditions of 2014, Klamath River flows below IGD increased starting in mid-Feb and peaked below 2000 cfs in mid-March, before declining from April through the end of May to less than 1500 cfs (Figure 7). Pulse flow events occurred on May 26th (1850 cfs to assist juvenile Chinook salmon emigration) and September 12-14 (for tribal boat dance ceremonies). In an effort to reduce *Ichthyophthirius multifiliis* parasite infections in adult Chinook and coho salmon in the Klamath River, emergency flow releases from Iron Gate Dam of approximately 1750 cfs occurred for 10 days from October 4-15th (Figure 7). The minimum flows during the sampling season were approximately 850 cfs from July 1 to July 15 followed by flows of approximately 900 cfs from July 15 to September 1. Iron Gate Hatchery fish were released May 20-June 13.

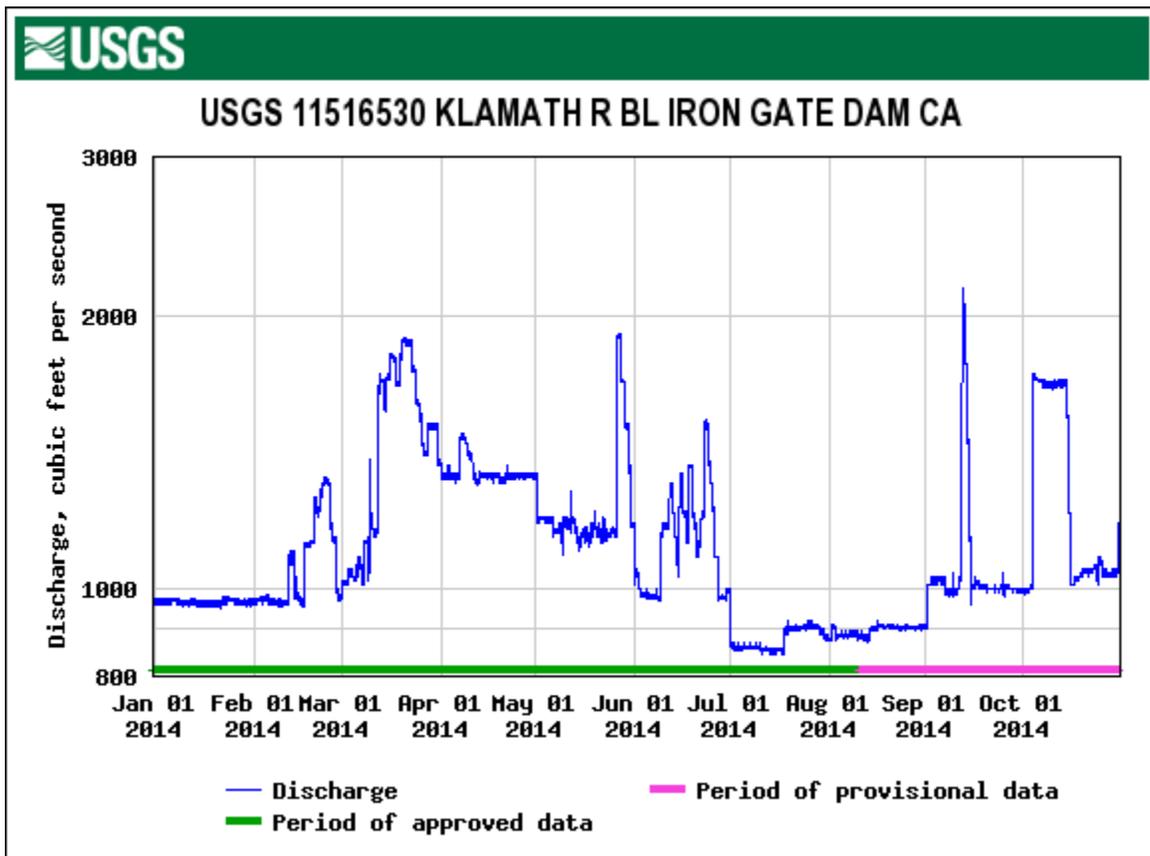


Figure 7. Daily discharge (cfs) below Iron Gate Dam from January 2014 through October 2014. Data collected after August 10, 2014 is provisional data. Data collected from USGS gaging station 11516530 at waterdata.usgs.gov.

Klamath River flows in 2014 were lower than flows seen in the previous three years (Figure 8). 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 were intermediate between low flows years (2009-2010) and high flow years (2011-2012). Comparatively, in 2014 the peak flow during the fish sampling period was 1910 cfs on May 27 and minimum flow was 845 cfs on July 14.

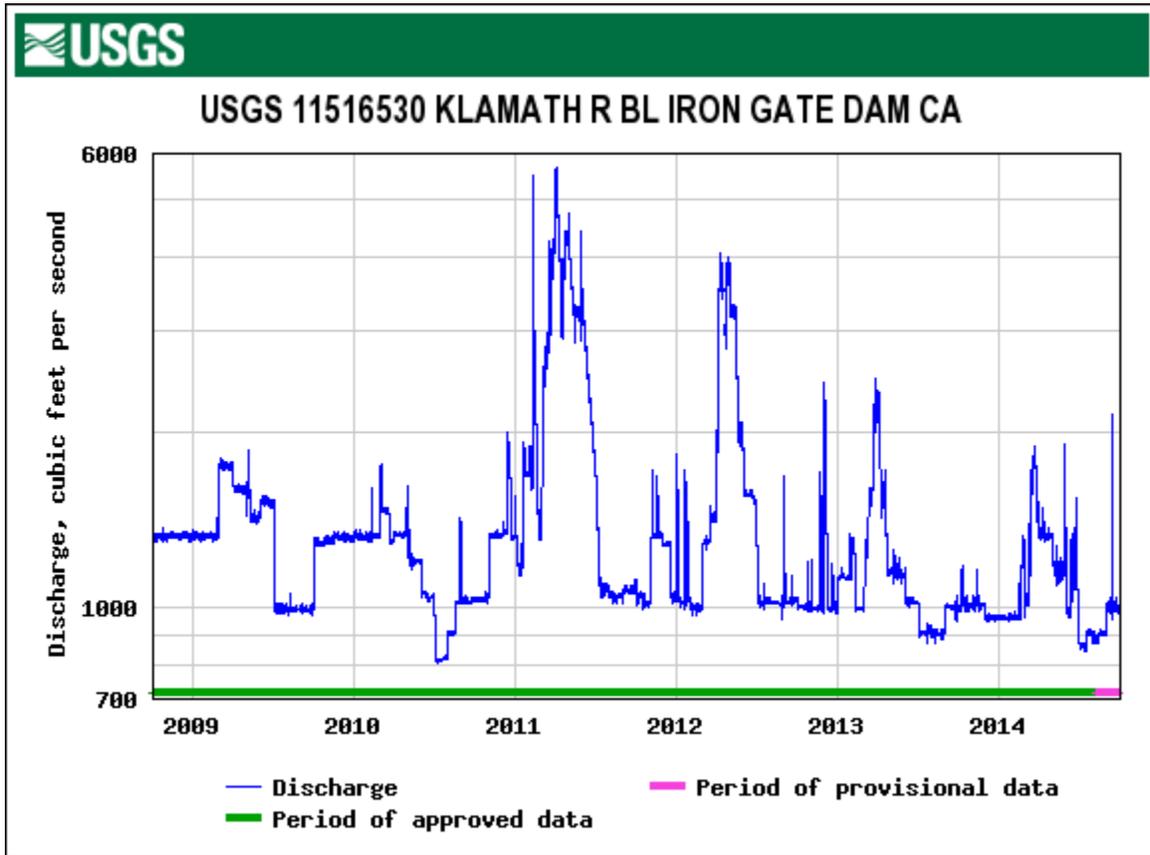


Figure 8. Daily discharge (cfs) below Iron Gate Dam from October 2008 through October 2014. Data collected after March 20, 2014 is provisional data. Data acquired from USGS waterdata.usgs.gov

Discussion

Prevalence of Infection by Fish Origin

Naturally produced Chinook salmon

Naturally produced Chinook salmon had much higher *C. shasta* prevalence of infection by QPCR in 2014 (76%) compared to 2013 (25%). Natural origin fish were collected from the end of March to the end of May in the Shasta to Scott (K4) and Scott to Salmon (K3) reaches only.

Ceratonova shasta was detected approximately 3 weeks earlier this year with the first detection occurring on April 3 compared to April 22 in 2013, and May 10 in 2012. Prevalence of infection in the first fish sampled (April 3) was 5% in the Shasta to Scott (K4) reach and rose steadily to 85% by mid-April. Warmer river temperatures in March, coupled with low spring flows, contributed to early infection, higher prevalence of infection and higher parasite loads (DNA copy number) in natural fish.

Ceratonova shasta mean DNA copy number in natural fish was higher in 2014 at 18,000 copies (Figure 4), compared to 6,000 copies in 2013 when infection prevalence in this group of fish was more moderate (25%; Bolick, et al., 2013). Clinical disease signs occurred earlier in 2014 as well: field crews reported clinical signs of ceratomyxosis in approximately 10% of juvenile Chinook sampled at the Kinsman trap (K4) in early May (sampling week of May 4).

The majority of fish tested by histology were natural origin Chinook salmon (one group collected the week of June 1 was of unknown origin). Prevalence of *C. shasta* by histology in K4 was 25% in the first group of fish sampled on April 20, and rose to 88% and 60% on May 4 and 18. Pathology scores were also indicative of higher infection levels and tissue impairment in natural fish than observed in 2013.

Likewise, the annual *P. minibicornis* prevalence of infection by QPCR in natural origin Chinook salmon was also higher in 2014 (83%) compared to 2013 (64%). *Parvicapsula minibicornis* was first detected on April 3, nearly 3 weeks earlier than 2013 or 2012 (first detection occurred April 25 in both years). First detection of this parasite occurred in natural fish sampled from the Kinsman trap located in the upper Shasta to Scott (K4) reach. *Parvicapsula minibicornis* infection by histology in natural fish collected from K4 and K3 reaches was 63% (N=27 fish).

Iron Gate Hatchery Coded-wire Tagged Chinook salmon

Ceratonova shasta POI by QPCR in IGH CWT salmon sampled in 2014 was higher (79%) compared to 2013 (46%) and 2012 (42%). Typically IGH releases fall Chinook salmon in mid-May, but this target is driven by both size at release and river temperature criteria. In 2014, Iron Gate Hatchery releases occurred from May 20 through June 13. These release periods were similar to 2013 (May 22- June 5). Exposure doses to *C. shasta* in the Shasta to Scott (K4) reach resulted in earlier infection and higher prevalence of infection in upper reaches than normally observed. Weeks At Large groups 1 - 5 all had high *C. shasta* POI, ranging from 85-96% (Figure 4).

The 1-2 WAL groups had the highest prevalence and highest mean DNA copy number. In previous monitoring years, the highest *C. shasta* POI occurs in fish residing for longer periods, generally in the 3-5 WAL range, and is related to typical periods required for disease progression.

For *P. minibicornis*, the prevalence of infection increased rapidly and remained high, a pattern consistent with previous years and especially evident in the middle to lower river reaches. *Parvicapsula minibicornis* had the highest POI (100%) observed in Salmon to Trinity (K2) reach and the lowest POI (70%) in the upper Shasta to Scott (K4) reach.

Annual Prevalence of Infection by Klamath River Reach

The monitoring program targets natural fish in the upper reaches early in the season when river temperatures are cooler; therefore we generally see lower infection levels in this group of fish. Once hatchery fish are released, we are unable to monitor natural fish exclusively and efforts are shifted to focus on IGH CWT with known exposure periods. At typical temperatures observed in the Klamath River (~18 °C in late May to early June), certarioxosis disease develops in approximately 3-4 weeks (True, et al., 2012). Therefore, the highest *C. shasta* POI occurs in lower reaches, but this is a function of the fish becoming infected in upper reaches and the time required for parasite proliferation (disease progression).

In 2014, *C. shasta* POI was higher (85-89%) in all downstream reaches (K3, K2 and K1) compared to previous years. Prevalence of *C. shasta* infection in the estuary in 2014 was 76% compared to 49% in 2013. Additionally, seasonal mean DNA copy number for all IGH CWT Chinook salmon sampled from all reaches in 2014 was 32,000 copies, compared to a mean of 4,500 copies observed in 2013. The high *C. shasta* prevalence of infection observed in all reaches indicates hatchery fish were exposed to high spore density upon release in 2014.

Another analysis of all previous monitoring years (2005-2013) confirmed *C. shasta* POI in CWT Chinook collected within the 'infection zone' in 2014 was the highest POI on record. The prevalence of infection in 2014 (77%) in these combined reaches was higher than the most severe disease years recorded for the monitoring program (2007-2008: 68% and 69%). The high POI in the infectious zone in 2014 contrasts dramatically with the low POI for CWT Chinook sampled from the same reaches during favorable environmental years (2010-2011: 16% and 13%).

Spore concentration in the main stem Klamath is an important component to explain prevalence of infection in both natural and hatchery juvenile Chinook salmon. Spore densities in water samples reported by Oregon State University were high for *C. shasta* at the Kinsman trap (K4) early in the season (end of March) and remained high throughout June (*pers. comm.* Sascha Hallett, preliminary data: <http://microbiology.science.oregonstate.edu/conttne/monitoring-studies>). This period coincided with natural fish rearing and hatchery fish migration through the upper reaches. Weeks at Large data demonstrate CWT Chinook salmon recaptured after just 1-2 WAL had the highest infection prevalence and highest mean DNA copy numbers. Therefore, hatchery fish were exposed to high infectious doses throughout the May 20 – June 13 release period resulting in early and rapidly progressing infection.

Disease Severity Determined by Diagnostic Cases

Juvenile Chinook salmon mortality occurred in the Scott to Salmon River reach (K3) in early June, where clinical disease was observed in 20% of the fish observed at Ti Creek thermal refugia. These clinical fish were moribund and in end stage ceratomyxosis, confirmed by both QPCR and histology. Of the 17 CWT fish tested, all had been residing less than 3 WAL prior to recapture (the majority had been released May 23 or May 30). The most heavily infected fish had DNA copy numbers of 41,000 to 160,000, and were recaptured at 2 WAL (released May 30). These values are similar to levels measured in clinically moribund fish in previous years that had longer exposure periods. Clinical moribund juvenile Chinook salmon parasite levels have typically been in the range of 90,000 to 120,000 copies of *C. shasta* DNA (True et al., 2012, Bolick, et al., 2012).

A second examination of over 1500 juvenile Chinook, collected in July at Pecwan thermal refugia in the Trinity to Estuary (K1) reach, showed coded-wire tagged Chinook salmon captured later in the season were in relatively good condition, despite their longer exposure period. Only 13% had clinical signs of ceratomyxosis and 3% had signs of columnaris disease (bacterial lesions on gill tissue). The majority of these CWT Chinook had been released on June 2 and resided 5 WAL upon recapture. A small proportion was released June 10-13 and had a shorter exposure period of 3-4 WAL.

The diagnostic data suggest a proportion of IGH CWT examined three weeks earlier in the Scott to Salmon (K3) reach dropped out of the population due to ceratomyxosis mortality. It's difficult to determine what factors offered a survival advantage for the WAL groups collected from the lower reach in July. Spore density data was reported to drop for approximately one week after the pulse flow event of May 26, but rapidly resumed to the infectious threshold level (10 spores/L) by early June. Spore density remained in the range of >1 - >10spores/L the remainder of June at the major sampling sites (Kinsman and Beaver Creek) within the infectious zone.

Historical Comparison

The annual *C. shasta* POI (78%) in all Chinook salmon tested by QPCR in 2014 was two times higher than observed in 2013 (36%). 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 historical *C. shasta* prevalence of infection by both QPCR (81%) and histology (42%) was high in 2014 compared to all previous monitoring years (2006-2013, Table 2).

Given the continued drought conditions that started in the winter of 2013-2014 and continued through the spring and summer of 2014, the high prevalence of infection of these myxozoan parasites in both natural and hatchery juvenile Chinook salmon is not overly surprising. Low flows prior to mid-March, and an early temperature spike from early to late April, likely promoted faster development of trophozoites in polychaete worms infected with *C. shasta* in the fall of 2013 and earlier release of infectious actinospores. Increased temperatures may also have led to more rapid development of juvenile polychaete worms that would not be infected in spring but may have contributed infectious actinospores later in the season. The juvenile worm population would have to reach a specific age/size in order to ingest myxospores and become infected. Spore density data from water sampling conducted by Oregon State University corroborate exposure conditions that led to early infection and high exposure doses of *C. shasta* in 2014. Spore densities rose above the 1 spore/L threshold very early (March 31) in 2014. Furthermore, spore concentrations rose to over 10 spores/L in early April and

remained high (3-23 spores/L) throughout the month of June. In juvenile Chinook salmon, clinical disease, myxospore production, and mortality occurred in some release groups with high actinospore dose and relatively short (2 WAL) exposure periods.

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. 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 2010 and 2013, and low prevalence of infection in the more favorable environmental conditions that occurred in 2011 and 2012. Severe drought conditions in the winter of 2013 and throughout 2014 appear to have provided habitat conditions more favorable to myxozoan parasites such as *C. shasta* and *P. minibicornis*. Clinical disease, myxospore production, and mortality resulted in some release groups with high prevalence, despite relatively short exposure periods.

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Author Roles

The contributions of each author have been summarized below.

- Kimberly True – Project coordination, data management and quality control, QPCR methodology and quality assurance, data analysis, and written report.
- Anne Bolick – 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 editorial review.

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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. All samples collected were tested for *Ceratonova shasta*. A number of samples (N=37) were too small to collect kidney tissue. Therefore, the number of fish tested for *C. shasta* and *P. minibicornis* are not equal.

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	30-Mar	20				
2	6-Apr	20				
3	13-Apr	20	20			
4	20-Apr	20 (H10)	20 (H10)			
5	27-Apr	20	20			
6	4-May	20 (H10)	20 (H10)			
7	11-May	20	20			
8	18-May	20 (H10)	20 (H10)			
9	25-May	20	21			
10	1-Jun	20	29 (H10)			
11	8-Jun	41	31			20
12	15-Jun	31	20			30
13	22-Jun		21	21	20	25
14	29-Jun		25	20	21	29
15	6-Jul		11	22	30	30
16	13-Jul		20	21	20	31
17	20-Jul		21	21	16	30
18	27-Jul			20	9	21
19	3-Aug				21	20
20	10-Aug					20
21	17-Aug					20

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	30-Mar	5% (1/20)				
2	6-Apr	15% (3/20)				
3	13-Apr	50% (10/20)	60% (12/20)			
4	20-Apr	85% (17/20)	75% (15/20)			
5	27-Apr	90% (18/20)	95% (19/20)			
6	4-May	100% (20/20)	95% (19/20)			
7	11-May	100% (20/20)	95% (19/20)			
8	18-May	90% (18/20)	95% (19/20)			
9	25-May	100% (20/20)	90% (19/21)			
10	1-Jun	5% (1/20)	76% (22/29)			
11	8-Jun	12% (5/41)	100% (31/31)			100% (20/20)
12	15-Jun	90% (28/31)	100% (20/20)			100% (30/30)
13	22-Jun		100% (21/21)	100% (21/21)	100% (20/20)	100% (25/25)
14	29-Jun		92% (23/25)	85% (17/20)	90% (19/21)	86% (25/29)
15	6-Jul		73% (8/11)	95% (21/22)	70% (21/30)	97% (29/30)
16	13-Jul		65% (13/20)	76% (16/21)	95% (19/20)	81% (25/31)
17	20-Jul		81% (17/21)	86% (18/21)	100% (16/16)	67% (20/30)
18	27-Jul			65% (13/20)	100% (9/9)	33% (7/21)
19	3-Aug				86% (18/21)	55% (11/20)
20	10-Aug					35% (7/20)
21	17-Aug					50% (10/20)
		K4 Total 59% (161/272)	K3 Total 87% (277/319)	K2 Total 85% (106/125)	K1 Total 89% (122/137)	K0 Total 76% (209/276)

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	30-Mar	5% (1/20)				
2	6-Apr	21% (3/14)				
3	13-Apr	67% (6/9)	83% (15/18)			
4	20-Apr	94% (17/18)	100% (15/15)			
5	27-Apr	83% (10/12)	100% (19/19)			
6	4-May	100% (18/18)	95% (19/20)			
7	11-May	95% (19/20)	85% (17/20)			
8	18-May	90% (18/20)	100% (20/20)			
9	25-May	95% (19/20)	100% (21/21)			
10	1-Jun	80% (16/20)	100% (29/29)			
11	8-Jun	37% (15/41)	94% (29/31)			100% (20/20)
12	15-Jun	94% (29/31)	100% (20/20)			100% (30/30)
13	22-Jun		100% (21/21)	100% (21/21)	100% (20/20)	100% (25/25)
14	29-Jun		100% (25/25)	100% (20/20)	100% (21/21)	93% (27/29)
15	6-Jul		100% (11/11)	100% (22/22)	93% (28/30)	97% (29/30)
16	13-Jul		100% (20/20)	100% (21/21)	95% (19/20)	81% (25/31)
17	20-Jul		100% (21/21)	100% (21/21)	100% (16/16)	93% (28/30)
18	27-Jul			100% (20/20)	100% (9/9)	81% (17/21)
19	3-Aug				100% (21/21)	100% (20/20)
20	10-Aug					95% (19/20)
21	17-Aug					100% (20/20)
		K4 Total 70% (171/243)	K3 Total 97% (302/311)	K2 Total 100% (125/125)	K1 Total 98% (134/137)	K0 Total 94% (260/276)

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	Apr 20	May 4	May 18	POI
<u>Kidney</u>				
Pm Troph.	1 / 9 (11)	8 / 8 (100)	8 / 10 (80)	17 / 27 (63)
Pm Myxosp.	0 / 9 (0)	0 / 8 (0)	1 / 10 (10)	1 / 27 (4)
Metacercaria	0 / 9 (0)	0 / 8 (0)	0 / 10 (0)	0 / 27 (0)
<i>C.shasta</i> troph.	0 / 9 (0)	1 / 8 (13)	1 / 10 (10)	2 / 27 (7)
<i>Chloromyxum</i> sp	0 / 9 (0)	0 / 8 (0)	0 / 10 (0)	0 / 27 (0)
Pathology Score	0.11	1.88	1.89	
<u>Intestinal tract</u>				
<i>C.shasta</i> troph.	2 / 8 (25)	7 / 8 (88)	6 / 10 (60)	15 / 26 (58)
<i>C.shasta</i> myxosp.	0 / 8 (0)	0 / 8 (0)	0 / 10 (0)	0 / 26 (0)
Helminth	0 / 8 (0)	0 / 8 (0)	0 / 10 (0)	0 / 26 (0)
Pathology Score	0.63	5.13	4.70	
Adipose steatitis	2 / 2 (100)	3 / 5 (60)	2 / 5 (40)	7 / 12 (58)
Adipose lipofuscin	0 / 2 (0)	0 / 5 (0)	0 / 5 (0)	0 / 12 (0)
<u>Gill</u>				
Metacercaria	2 / 10 (20)	2 / 9 (22)	4 / 9 (44)	8 / 28 (29)
Multif. Hyperplasia	2 / 10 (20)	2 / 9 (22)	2 / 9 (22)	6 / 28 (21)

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	Apr 20	May 4	May 18	June 1	POI
<u>Kidney</u>					
Pm Troph.	0 / 9 (0)	7 / 8 (88)	10 / 10 (100)	8 / 8 (100)	25 / 35 (71)
Pm Myxosp.	0 / 9 (0)	0 / 8 (0)	0 / 10 (0)	0 / 8 (0)	0 / 35 (0)
Metacercaria	1 / 9 (11)	0 / 8 (0)	1 / 10 (10)	1 / 8 (13)	3 / 35 (9)
<i>C.shasta</i> troph.	0 / 9 (0)	0 / 8 (0)	0 / 10 (0)	0 / 8 (0)	0 / 35 (0)
<i>Chloromyxum</i> sp	0 / 9 (0)	0 / 8 (0)	0 / 10 (0)	0 / 4 (0)	0 / 31 (0)
Pathology Score	0.00	2.0	2.9	4.13	
<u>Intestinal tract</u>					
<i>C.shasta</i> troph.	0 / 10 (0)	5 / 10 (50)	0 / 10 (0)	2 / 10 (20)	7 / 40 (18)
<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)	1 / 10 (10)	0 / 10 (0)	1 / 40 (3)
Pathology Score	0.00	0.70	0.10	1.5	
Adipose steatitis	2 / 3 (67)	7 / 8 (88)	5 / 8 (63)	0 / 6 (0)	14 / 25 (56)
Adipose lipofuscin	0 / 3 (0)	0 / 8 (0)	1 / 8 (13)	0 / 6 (0)	1 / 25 (4)
<u>Gill</u>					
Metacercaria	0 / 10 (0)	3 / 10 (30)	7 / 10 (70)	4 / 9 (44)	14 / 39 (36)
Multif. Hyperplasia	0 / 10 (0)	5 / 10 (50)	9 / 10 (90)	6 / 9 (67)	20 / 39 (51)

Appendix C - Reviewers' comments

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

Reviewer #1

Pg 2 - Summary: How did POI in naturally produced Chinook salmon compare to previous years? – was it also the highest to date.

Response: *Ceratonova shasta* POI in natural fish is not normally compared between years for two reasons. First of all, natural fish sampling period can vary from year and is not always comparable from year to year. Secondly, natural fish POI is only helpful in describing disease status in that group prior to hatchery releases. Once IGH Chinook are released, the sample group is an unknown mix of natural or hatchery produced fish and very little information can be gained (the Cs POI for natural fish differs from naïve IGH fish just entering the river).

Pg 9 - Figure 3 (Prevalence of Infection by Reach): I would find this graph more intuitive if the reaches were ordered as per the river ie west to east, K0-K4 (reverse order as how displayed now)

Response: Because our monitoring program is assessing myxozoan disease prevalence in outmigrating juvenile Chinook salmon, the data is presented from upstream (hatchery release) to estuary: following the temporal and spatial route of migration.

Pg 13 – Diagnostic Casework: This comes out of the blue – I suggest that the methods portion is moved to the methods section (add the same heading), leaving the results here.

Response: Concur, diagnostic methods section created and described.

Pg 21 – Discussion (Disease Severity Determined by Diagnostic Cases): No range provided...change to 'in the range of >1 - >10spores/L all that month'. Refer to <http://microbiology.science.oregonstate.edu/content/monitoring-studies>

Response: Included range, language suggested, and link to OSU data.

Pg 21 – Historical Comparison: I'm not sure that host development and parasite development are linked, except that worms may have to be a certain age/size to become infected.

Response: Sentence was clarified to describe the likely presence of diverse polychaete worm populations: adult worms which may have been infected in fall or winter of 2013 with dormant trophozoite infections that could mature once temperatures increased. And juvenile polychaete worms that would not be susceptible to infection until they reached a size capable of ingesting myxospores (but worm growth rate would also be affected by increased temperatures).

Reviewer #2

Pg 2 - Summary: What proportion of these CWT salmon experienced end stage disease in mid-June? The next sentence gives proportion info for late June-Aug.

Response: The sentence is referring to diagnostic work at the mouth of Ti Creek, conducted after Karuk Tribe field crew reports of dead juvenile Chinook salmon in this reach. . Language was added to clarify that this was diagnostic work (targeting moribund animals) and the proportion of fish residing 2 WAL (80%) that were clinically diseased.

Pg 2 - Summary (and numerous comments throughout report): Several comments requesting clarification on methodology (via QPCR?).

Response: The majority of testing was done by QPCR and these results precede histological results, reported in separate paragraphs. Histology results are stated as such in the introductory sentence of any paragraph where they are reported.

Pg 3 – Introduction: Sentence makes it sound like the host is releasing the actinospores. Maybe add clarity that the host, when diseased, releases the actinospores.

Response: Language changed to specify “if infected” for polychaete worm host and “vertebrate salmon host” to clarify secondary host.

Pg 4 - Introduction: Suggest more definitive statement, if known. Affected is very neutral (affected can lead one to wonder whether transmission efficiency increased or decreased). If Hallett et al 2012 and Ray et al 2013 found that transmission efficiency decreased with higher flows, then let’s say “decreased” instead of “affected”.

Response: Concur, language changed to ‘decreased’.

Pg 8 – Statistical Analysis: What are the thresholds for considering a salmon “infected” via QPCR vs via histology for *C. shasta* and for *P. minibicornis*?

Response: The annual report was intentionally condensed in 2014 by citing standardized methodology as reported in True et al., 2013. The QPCR methodology was validated and standardized in 2008 and has remained the same for this ongoing monitoring study.

To summarize briefly here, myxozoan parasite DNA detected above a background levels (C_T value of 38) is a positive test result. Histology is a qualitative examination of tissues for parasite presence, as well as changes to tissue associated with infection or host inflammatory response. Therefore any observation of *C. shasta* or *P. minibicornis* parasites (regardless of developmental stage) by histology is a positive (presence) result (reported as histology prevalence). Tissue response is also evaluated. Presence of other significant pathogens are reported as well. Enumeration of all of these criteria produce an overall pathology score (see Appendix B – Table 1 for list of parasites and conditions assessed by histology). QPCR and histology are completely different assays in terms of what is being measured (DNA by QPCR versus parasite presence and effects on tissue including host immune response). These assays are not comparable in terms of common threshold values.

Pg 8 – Results (Number of Fish Collected by Origin): This description of CWT fish should be at the very first use of CWT near the intro or methods.

Response: The description (adipose fin clipped) was moved up to page 5 under section Sample Sites, Fish Groups and Number Sampled.

Pg 9 – Results (Annual Prevalence by River Reach): Which reach(es), what was the time period of these sampling? (histology)

Response: Histology date range and reaches sampled were added to results section (were previously described in methods).

Pg 9 – Results (Annual Prevalence by River Reach): Pls clarify for the reader what CS N is in plain language? Then delete the caption description.

Also, recommend replacing K4-K0 with reach names in the “Figure” such as “Shasta to Scott”, “Scott to Salmon”, etc. Then delete the caption description.

Response: Cs N and Pm N are sample number collected in each reach. Explanation was added to caption. Recommendation to replace reach code with names was not taken, as reach names will not fit in the table graph and are provided in the table caption.

Pg 9 – Results (Figure 3): Unclear whether these data represent QPCR or histology.

Response: Moved histology section below Figure 3 (which is QPCR data only) and included language in methods to clarify QPCR data is presented first and histology second (in separate paragraph that includes an introductory sentence stating results are for histology).

Pg 14 – Historical Comparison: This section seems premature. We haven't covered all of 2014 data yet and we're already comparing with past POI. Suggest moving to later sections.

Response: Concur. Section moved from page 10 end of Results Section on page 14.

Pg 10 – Prevalence of Infection by Fish Origin: Unclear why only 1 out of 10 is considered mix origin. What was the criteria for categorizing fish collected after May 20 as “natural” Maybe a brief discussion in the methods section would help.

Response: The sentence is referring to one histological sample set (not 1 of 10 fish). Language added to clarify histological sample set collected after May 20 could include a mix of natural or hatchery fish and therefore are categorized as unknown origin. Removed term ‘mixed origin’ as it seems to confuse readers.

Pg 10- Prevalence of Infection by Fish Origin: What does the pathology score mean? I didn't see any discussion or recall any at least. A table would be helpful to reader.

Response: Pathology score is defined in the methods section as described below. Also see Appendix B – Table 1 for list of parasites and conditions assessed by histology.

‘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’

Pg 13 – Diagnostic Casework: Would be nice to discuss the C_T , DNA copy numbers = what clinical disease or parasite infection levels in the methods section. These numbers come out of the blue to the reader.

Response: There is no single specific C_T value for the threshold of clinical disease compared to parasite infectious load. Therefore DNA copy number ranges are given for those readers who are interested in this level of detail. While DNA copy number is related to ‘infectious load’ in the specific tissue tested, it does not represent a linear relationship with overall disease ‘state’ of the animal. For example, immunological tissue response of the host (the common inflammatory response elicited by *C. shasta* or *P. minibicornis*) can vary from fish to fish with varying numbers of parasites present. There can also be synergistic interactions when fish are dual infected with *C. shasta* and *P. minibicornis*. C_T value and DNA copy number should be not be interpreted as a linear representation of disease state. Histology is the best assessment for overall disease state of a specific fish or group of fish.

Pg 19 – Discussion (Iron Gate Hatchery Coded-wire Tagged Chinook salmon): Similar to what? (referring to WAL groups 1-5 had similar Cs POI in Figure 4).

Response: Sentence was clarified to make this point clear and Figure 4 was referenced. among

Pg 19 – Discussion (Annual Prevalence of Infection by River Reach): May want to discuss that it's not that these reaches contribute to high infection rates, but that fish are getting infected in the infectious zone upstream and are then migrating downstream to these reaches. Without such clarifying discussion, people unfamiliar with the pathogen might think these lower reaches are the problem.

Response: Language added to clarify this point.

Pg 21 – Discussion (Historical Comparison): It's not that the worms had faster development that resulted in earlier maturation and release of actinospores, right? It's that the trophozoites matured faster in the worms. Probably both, but more importantly with the trophozoite.

Response: We believe increased temperature in early spring would have both affects: more rapid maturation of worms that overwintered and may be infected, and in young worms that likely are uninfected until they reach an appropriate age/size (ability to ingest myxospores). This area of research is ongoing and not conclusive at this time. Language was edited to make this point more clearly.

Pg 21 – Discussion (Historical Comparison): I didn't realize 1 spore/L was considered significant. Thought it was 10 spores/L.

Response: 1 spore/L is biologically significant for susceptible rainbow trout used in sentinel studies. 10 spores/L is significant for juvenile Chinook salmon. Reworded sentence (removed term biological significant) to avoid confusion on spore thresholds for different species.