

U.S. Fish & Wildlife Service

California-Nevada Fish Health Center

FY 2014 Investigational Report:

Myxosporean Parasite (*Ceratomyxa shasta* and *Parvicapsula minibicornis*) Prevalence of Infection in Klamath River Basin Juvenile Chinook Salmon, April-August 2013

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Summary

Juvenile Klamath River Chinook salmon (*Oncorhynchus tshawytscha*) were assayed from April to August 2013 by quantitative polymerase chain reaction (QPCR) and histology for myxosporean parasite infections, *Ceratomyxa 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 46%, an increase from 30% in 2012. *Parvicapsula minibicornis* in Chinook salmon above the Trinity River confluence for the same period was 88% compared to 69% in 2012. Historically, the 2013 annual *C. shasta* prevalence of infection for juvenile Chinook salmon during outmigration was relatively high compared to historical levels observed for the monitoring program (2006-2012).

Naturally produced Chinook salmon had a 25% prevalence of *C. shasta* infection by QPCR and low disease severity based on DNA copy number. The low disease severity result was also seen histologically in naturally produced Chinook salmon based on low pathology scores.

Among coded-wire tagged (CWT) juvenile Chinook salmon released from Iron Gate Hatchery (IGH), *C. shasta* was detected in 46% of fish screened by QPCR. The highest *C. shasta* prevalence of infection observed was 61-64% in IGH CWT Chinook salmon residing 4-5 Weeks At Large at time of capture. Iron Gate Hatchery Chinook salmon had a low parasite infectious load, measured as quantity of *C. shasta* DNA present in intestinal tissue, in 2013 similar to 2012. The majority of juvenile Chinook salmon examined in 2013 appeared relatively healthy; some clinical disease was observed during QPCR necropsy but in relatively few fish.

In summary, both *C. shasta* prevalence of infection by QPCR in mixed origin fish collected during peak migration and in IGH CWT Chinook salmon (both 46%) was higher in 2013 compared to 2012. In terms of parasite infection levels, Iron Gate Hatchery Chinook salmon had similarly low parasite loads in 2013 as in 2012.

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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 mainstem Klamath River, and the lower basin extends southwest to the Pacific Ocean.

The lower Klamath River supports 19 species of native fish 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, *Ceratomyxa shasta* 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 releases an infectious actinospore stage into the water column. 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). *Ceratomyxa shasta* and *P. minibicornis* are distributed throughout the mainstem 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 but increased in the mainstem Klamath River between the interstate five bridge crossing and the confluence of the Scott River (Hallet, 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).

Ceratomyxa 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 (17-24°C) in the Klamath River during peak juvenile Chinook salmon migration, April to August, 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 CWT Chinook salmon with known exposure periods in the mainstem Klamath (Nichols, et al., 2007; Nichols, et al., 2009; Bolick, et al., 2012; 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 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 discharge can also play an important role. One laboratory study found that prevalence of *C. shasta* infection was higher in a smaller volume of water when fish were exposed to the same number of parasites (Bjork, et al., 2009). Therefore, parasite concentration affected infection prevalence. Higher flows may not only dilute the infectious spore stages, but transmission efficiency may also be affected (Hallett, et al., 2012; Ray, et al., 2013).

The primary objectives of this study were to: 1) examine parasite prevalence in Klamath River juvenile Chinook salmon during the spring out-migration period; and 2) compare parasite prevalence in 2013 to previous years. The focus of the study was not on the determination of disease, but instead determining infection.

Methods

Pre-Release Examination

Prior to the release of approximately 5 million fall Chinook salmon from Iron Gate Hatchery (IGH, released May 22 through June 5) a fish health examination of 30 hatchery fish was conducted on May 23 to determine any background infections of *C. shasta* or *P. minibicornis*. A fish health examination of 30 fall Chinook salmon was also conducted at Trinity River Hatchery (TRH) on May 15, prior to approximately 1.5 million fall Chinook salmon and approximately 1.3 million spring Chinook salmon being released on June 1.

Sample Sites, Fish Groups and Number Sampled

Fish were collected in the mainstem 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.

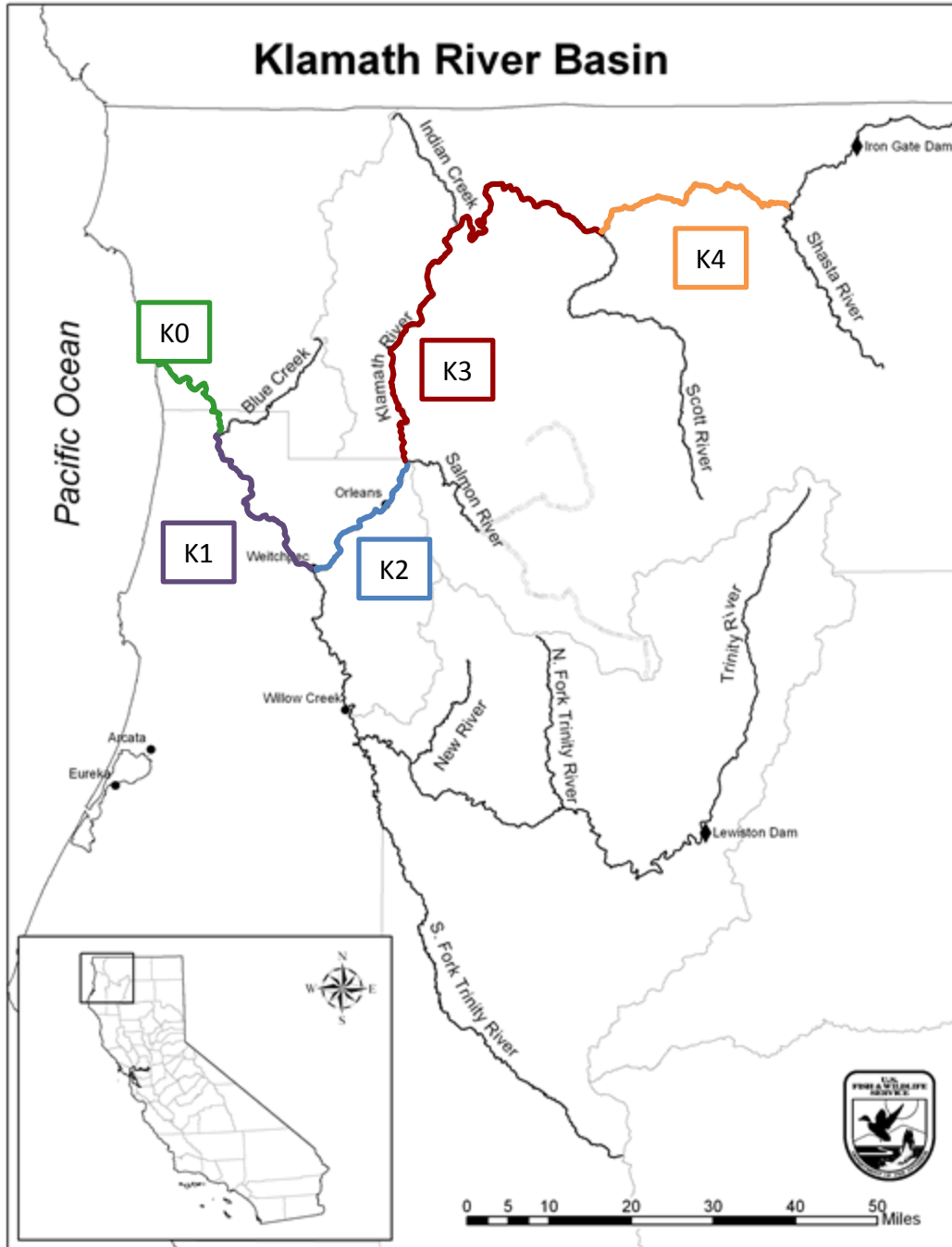


Figure 1. Klamath River watershed, major tributaries, and sample reaches: Shasta River to Scott River (K4), Scott River to Salmon River (K3), Salmon River to Trinity River confluence (K2), Trinity River to Estuary (K1), and Klamath River Estuary (K0). Map provided by the Arcata Fish and Wildlife Office.

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

Sample reach	Reach code	River miles (Upstream – Downstream)	Primary collector
Klamath River mainstem			
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 to the Klamath River estuary. Although the Trinity River was sampled from 2006 to 2012, no samples were collected in 2013. Fish were collected in the upper reaches, K4 and K3, early in the sampling season (March 28-July 14) while the lower reaches were sampled later in the season (June 16-August 11) 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. “Mixed origin” Chinook refers all group types as a whole, i.e., results for all fish collected in a particular reach, or for the entire sampling season. 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 period (Appendix A - Table1). Sampling was limited in K1 for three weeks in July (July 7-27) and in K0 for one week (July 7-13) due to concern for handling induced mortality during high water temperatures.

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). All but the first plate (88 samples) and an additional 34 samples that were too small to collect kidney tissue were tested for *P. minibicornis* (Appendix A -

Table 3). Therefore, the number of fish tested for *C. shasta* and *P. minibicornis* were not equal.

Parasite Infection Levels by Quantitative PCR Assays

Fish collection, necropsy, and DNA extraction were done according to True, et al. (2013). Assay analysis was modified in 2013 to fit all *C. shasta* QPCR data to a reference standard curve. *Parvicapsula minibicornis* data was fit to the standard curve of each individual assay plate.

The *C. shasta* reference standard curve was obtained using plasmid containing the target sequence. Specifically, 1ng of DNA, corresponding to 1.66×10^8 copies of *C. shasta* DNA was serially diluted over 10 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, range of the assay, and the limit of detection (R^2 value = 0.989):

$$y = -3.28x + 40.9 + \varepsilon$$

Quantification was determined based on 5 μ L of DNA per reaction. The reference standard curve efficiency was 102%. Generally, efficiency between 90 and 110% is required for accurate quantitation of DNA copy number (Technologies, L. 2011).

The mean standard curve for *P. minibicornis* was obtained using 1ng of DNA corresponding to 2.41×10^8 copies and the same QPCR methodology described above (R^2 value = 0.999):

$$y = -3.42x + 41.9 + \varepsilon$$

The average standard curve efficiency for *P. minibicornis* was 97%.

Parasite Infection Levels by Histology

Histological assays were done according to True, et al. (2013). In 2013, histology samples were collected in the Shasta River to Scott River reach (K4) and the Scott River to Salmon River reach (K3) between April 14 and May 26 (Appendix A -Table1).

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

Infections of *C. shasta* or *P. minibicornis* were not detected by QPCR in IGH or TRH Chinook salmon prior to release.

Number of Fish Collected by Origin

In 2013 we examined 894 juvenile Chinook salmon collected from the mainstem Klamath River. The sample consisted of 362 natural fish, and 532 fish collected after hatchery release which included 459 CWTs. Coded wire tagged Chinook salmon (marked with an adipose fin clip) accounted for 51.3% (459/894) of all fish sampled in 2013. Natural fish accounted for 40.5% (362/894) and 8.2% (73/894) of the fish are of unknown origin (unmarked hatchery fish or natural, Figure 2).

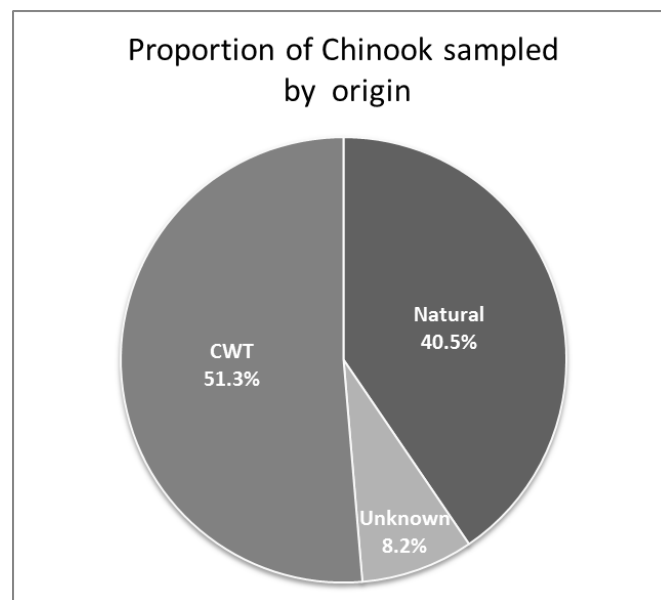


Figure 2. Proportion and origin of Chinook salmon collected (N = 894) in 2013.

Annual Prevalence of Infection by Klamath River Reach

The annual prevalence of *C. shasta* infection in mixed origin Chinook salmon in 2013 by QPCR was 36% (322/894, ci = 33-39%). *Ceratomyxa shasta* was first detected on April 22 in the Scott River to Salmon River reach (K3). *Ceratomyxa shasta* POI was highest in the Salmon to Trinity River reach (K2) at 54.9%, followed by the estuary (K0) at 49.2%. The lowest prevalence was seen in the Shasta River to Scott River reach (K4) at 12.8% (Figure 3).

The annual *P. minibicornis* POI by QPCR was 76.4% (598/782, ci = 73-79%). *Parvicapsula minibicornis* was first detected on April 25 in the Shasta River to Scott River reach (K4). Prevalence was highest in the Salmon River to Trinity River reach (K2) at 83.6%, followed closely by the Scott River to Salmon River reach (K3) at 82.1%. Lowest prevalence was seen in the Trinity River to estuary reach (K1) at 65.6% (Figure 3).

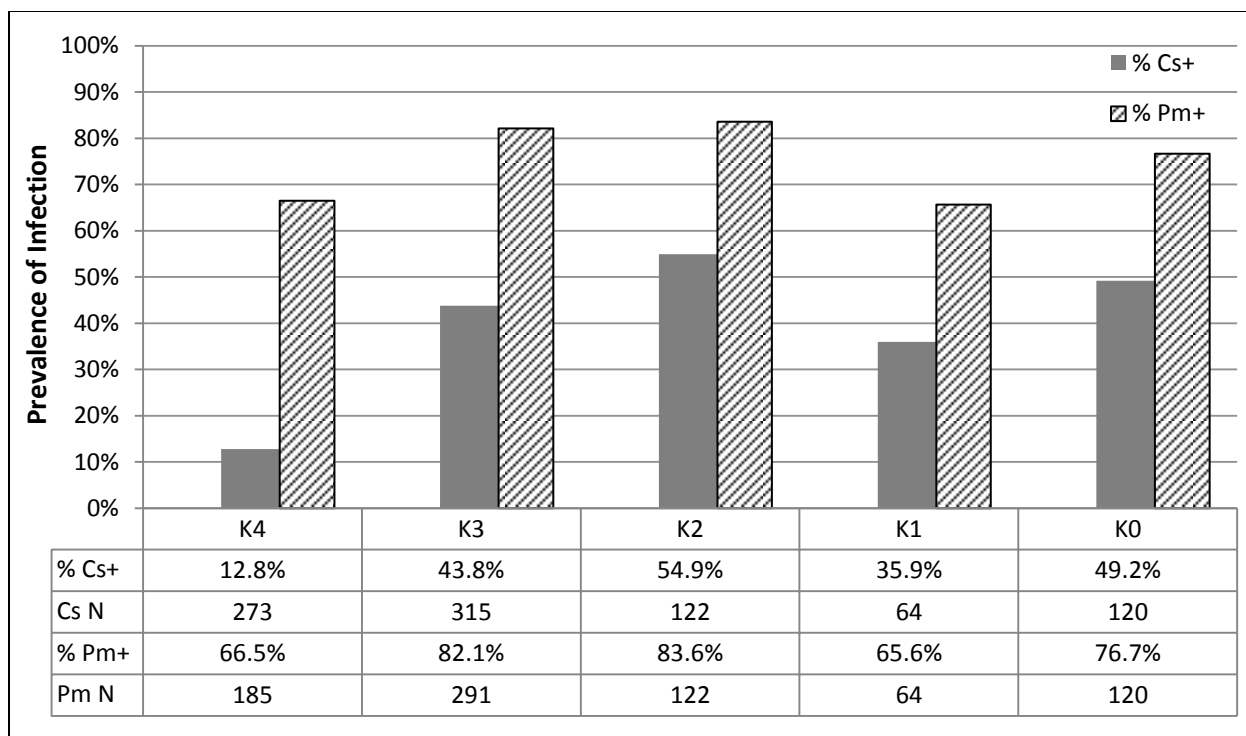


Figure 3. Prevalence of *Ceratomyxa shasta* (Cs+) and *Parvicapsula minibicornis* (Pm+) infection in juvenile Klamath River Chinook salmon by reach in 2013. K4= Shasta River to Scott River; K3= Scott River to Salmon River; K2= Salmon River to Trinity River; K1= Trinity River confluence to Estuary; K0= Klamath River Estuary. Sample numbers collected are displayed in the table below each column for both pathogens.

The annual POI in 2013 by histology for *C. shasta* was 8% (6/77, ci = 3-16%) and for *P. minibicornis* was 37% (29/78, ci = 26-49%).

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 of this method in detecting early and/or low-level parasite infections and the ability to quantify parasite DNA copy number within fish tissue; however limited by the same data restrictions as the histology assay. 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 high (46.1%, 234/508, ci = 42-51%) in 2013, compared to previous years (Table 2). *Parvicapsula minibicornis* in Chinook salmon above the Trinity River confluence for the same period was 88% compared to 69% in 2012.

Table 2. Historic annual prevalence of *Ceratomyxa shasta* infection (% positive by assay) in all juvenile Chinook salmon collected from the mainstem Klamath River between Iron Gate Dam and Trinity River confluence during May through July, 2006-2013.

Year	Annual <i>C. shasta</i> POI ¹ All Chinook (May 1 – July 30) (% Positive by Assay)	
	Histology	QPCR
2006	21	34
2007	21	31
2008	37	49
2009	54	45
2010	15	17
2011	2 ²	17
2012	9 ³	30
2013	16 ³	46
Mean (SE)	22 (6)	34 (4)

¹ K5 reach (Iron Gate Dam to the Shasta River) was sampled each year, prior to 2011. *Ceratomyxa shasta* POI has historically been very low in this reach.

² Histology sampling was reduced and limited to 2 reaches in 2011: Shasta River to Scott River (K4) and Trinity River confluence to Estuary (K1).

³ Histology sampling was limited to two reaches in 2012 and 2013: Shasta River to Scott River (K4) and Scott River to Salmon River (K3).

Prevalence of Infection by Fish Origin

Naturally produced Chinook salmon

Naturally produced Chinook salmon represent early infection status for *C. shasta* and *P. minibicornis*, as river temperatures are generally 8-10 °C cooler in the months of April and May compared to the peak salmon migration period of May-July. A total of 362 natural fish were collected in the Klamath River above the Trinity River confluence (K4, K3, and K2) from March 28 through May 30.

Ceratomyxa shasta was detected by QPCR in 25% (90/362, ci = 20-30%) of natural fish. *Ceratomyxa shasta* POI was highest in the Scott River to Salmon River reach (K3) at 35%. The lowest prevalence was in the Salmon River to Trinity River reach (K2), where 20 fish were collected but the parasite was not detected.

Comparatively, *P. minibicornis* was detected in 64% (159/250, ci = 57-70%) of naturally produced Chinook salmon by QPCR. The highest *P. minibicornis* prevalence, 75%, was in the Shasta River to Scott River reach (K4). *Parvicapsula minibicornis* was also undetected in 20 fish collected in the Salmon River to Trinity River reach (K2).

As stated earlier, fish had an overall *C. shasta* POI of 8% (6/77, ci = 3-16%) histologically. All of these fish were collected early in the sampling season and were all naturally produced Chinook salmon. The prevalence by reach was similar for fish collected in K4 and K3 (7.6% and 7.8%); however the mean pathology score was higher in K4 (0.14) than in K3 (0.0275). All *C. shasta* pathology scores would be considered negligible; for comparison clinically affected salmon in 2009 had an intestine pathology score between 3 and 4 (True, et al., 2010).

Natural fish had an overall *P. minibicornis* POI by histology of 37% (29/78, ci = 26-49%). Prevalence was highest in K3 with a POI of 51%, compared to 23% in K4. The pathology score was also highest in K4 with a pathology score of 0.40, whereas K3 had a pathology score of zero. These pathology scores are again negligible as clinically affected populations have a kidney pathology score between 6 and 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 73 fish of unknown origin were collected throughout the Klamath River from June 3 through July 29. *Ceratomyxa shasta* was detected by QPCR in 34% (25/73, ci = 24-46%) of unknown origin Chinook salmon. *Parvicapsula minibicornis* POI in Chinook salmon of unknown origin was 92% (67/73, ci = 83-97%).

Hatchery (CWT) Chinook salmon

The 25% constant fractional mark rate at IGH (Buttars, et al., 2009) has facilitated the capture of a large proportion of IGH CWT Chinook salmon in the five past years of the monitoring program. A total of 459 CWT Chinook salmon were collected this season from the Klamath River. Iron Gate Hatchery CWT fish accounted for 78% (357/459) and TRH CWT fish accounted for 18% (84/459) of CWT fish tested. Additionally, 18 fish (4%) had lost or unreadable tags.

Iron Gate Hatchery CWT

Coded wire tagged salmon originating from IGH were collected in the Klamath River from May 29 to August 13. The largest proportion of IGH CWT (n=124 fish), were collected in the Scott River to Salmon River reach (K3). *Ceratomyxa shasta* was detected in 46% (163/357, ci = 40-51%) of all IGH CWT screened by QPCR. Prevalence of infection for *C. shasta* was highest in the Trinity River to Estuary River (K1) at 67%, followed closely by the Salmon to Trinity River reach (K2) at 66%. The lowest prevalence was in the Shasta River to Scott River reach (K4) at 2%.

Parvicapsula minibicornis was detected by QPCR in 89% (317/357, ci = 85-92%) of IGH CWT. Prevalence of infection for *P. minibicornis* was highest from fish collected in the Salmon River to the Estuary (K2 and K1); with 100% POI detected in both river reaches. The lowest prevalence was in the Shasta River to Scott River reach (K4) at 60%.

IGH CWT Weeks At Large

In the monitoring program, temporal data was derived from IGH CWT codes, with known exposure periods based on hatchery release and in-river recapture dates. The time period when fish are residing in the Klamath River post hatchery release is referred to as Weeks At Large (WAL).

Ceratomyxa shasta POI in IGH CWT Chinook salmon for WAL analysis was similar to a bell shaped curve with the highest POI in fish residing 4-5 WAL (61-64%) and lower POI at the tails; either a shorter (0-1 WAL) or longer residence time (8-11 WAL, Figure 4). The longer WAL data were combined (WAL 8-11) because sample numbers were small, with 14 fish total in those last four weeks. *Ceratomyxa shasta* POI was 0% in Weeks At Large 8 (2 fish collected) and 10 (4 fish collected).

As stated in the methods, the QPCR assay can quantify parasite DNA copies within fish tissue. Mean DNA copy number, or the parasite infection level, in IGH CWT gradually increased with WAL, with the exception of a slight decreased at 7 WAL, before reaching the highest value for salmon residing 8-11 WAL (Figure 4). An average of approximately 4500 copies (2.24 logs) of *C. shasta* DNA was present in infected fish tissue in 2013 compared to approximately 4900 copies 2012.

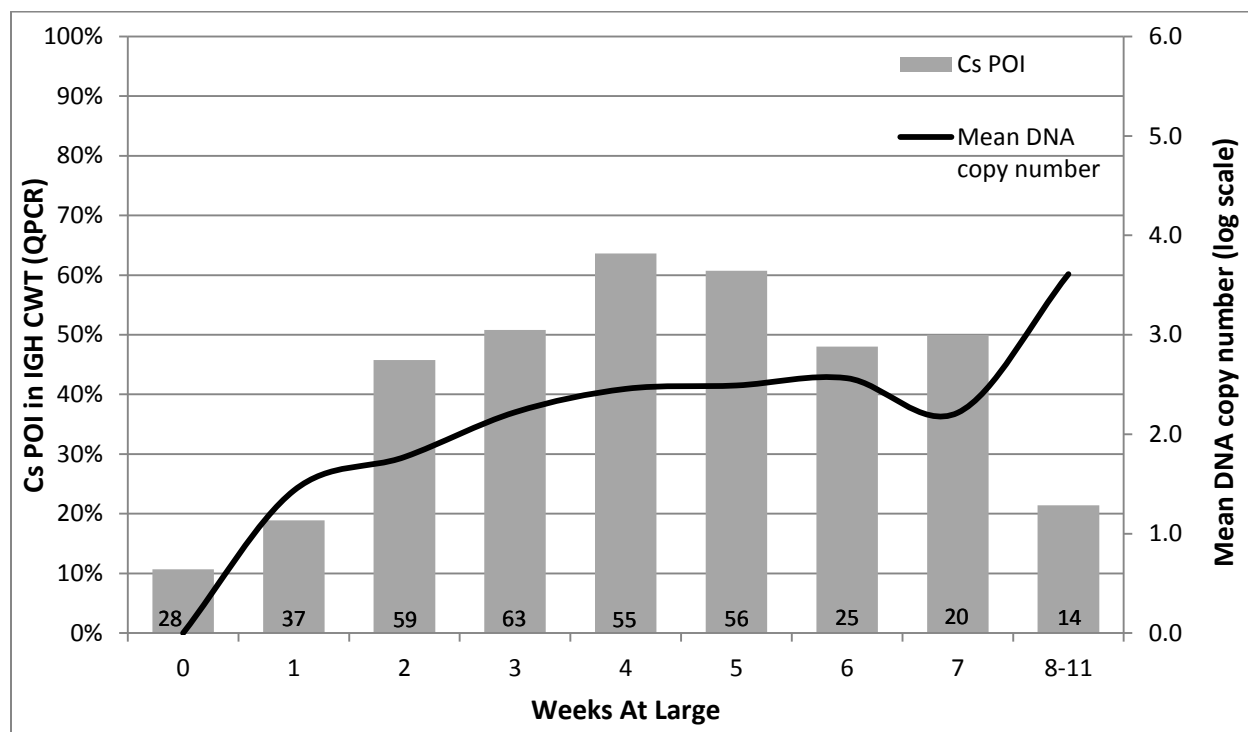


Figure 4. *Ceratomyxa shasta* prevalence of infection in IGH CWT by Weeks At Large (WAL) post hatchery release. The bar graph is prevalence of infection (%) on the primary y-axis and the line graph is the mean *C. shasta* DNA copy number (log scale) on the secondary y-axis for Chinook salmon tested by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

Of the fish residing in the Klamath River the longest, 8-11 WAL, all were collected in the estuary (K0), except for two fish residing 8 WAL that were collected in the Salmon River to Trinity River reach (K2). Eleven out of fourteen fish residing 8-11 WAL were uninfected with *C. shasta*.

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) in mid to late May and increased steadily during the remainder of 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 (17% by QPCR for both years), observed to date.

Mean daily Klamath River temperatures observed in 2013 appeared to be warmer in the spring and then similar to historic temperatures in May through September, in terms of mean daily river temperature below IGD. In 2013, temperatures in March were comparable to 2012, however in April water temperature climbed steadily with mean daily temperatures approximately 2° warmer compared to 2012 (Figure 5). Mean daily temperature spiked on May 13 at 18.4°C and from late May forward followed a similar path as 2012.

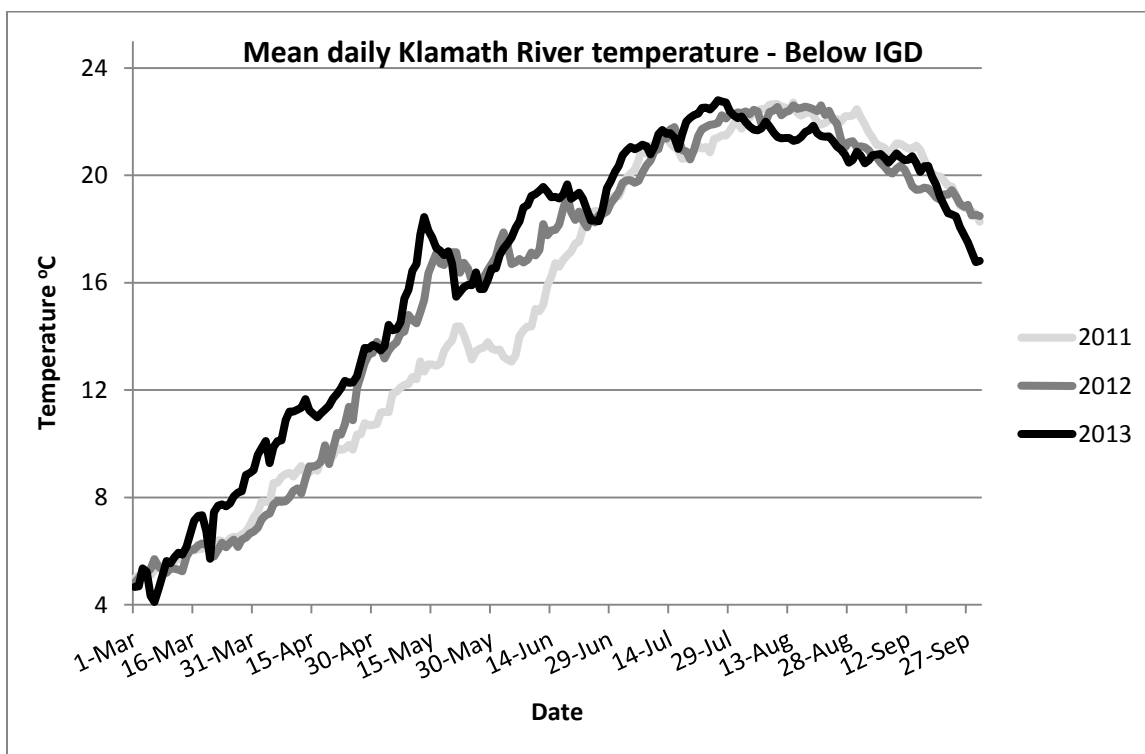


Figure 5. Mean daily Klamath River temperature below Iron Gate Dam for 2011, 2012, and 2013. Temperature data for 2011 and 2013 acquired from Arcata Fish and Wildlife Field Office. Temperature data for 2012 acquired from Iron Gate Hatchery, and taken from the mainstem Klamath River, not the hatchery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

At the Seiad Valley temperature gauge, mean daily river temperatures were more variable than IGD but higher overall than 2011-2012 from late March to late July (Figure 6). Average daily temperatures reached 22 °C on June 9 and then 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° warmer than observed in 2012.

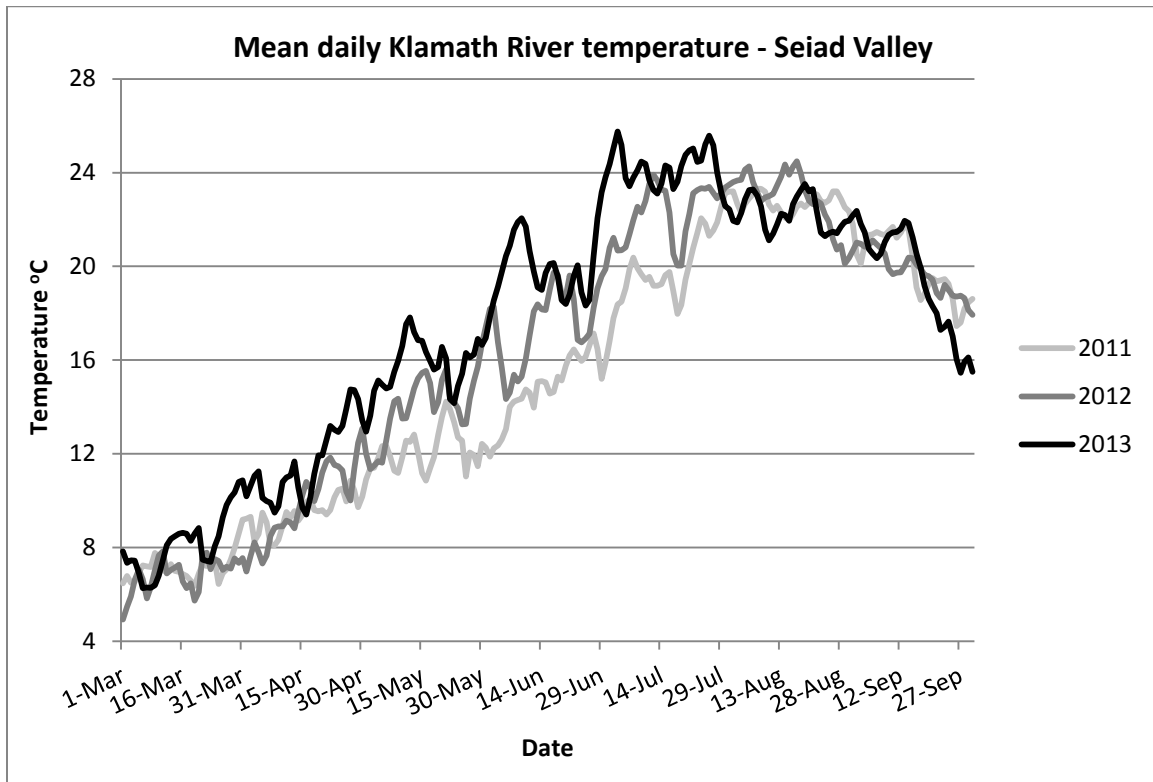


Figure 6. Mean daily Klamath River temperature from March through September 2011, 2012, and 2013 at Seiad Valley. Temperature data collected and provided by the Arcata Fish and Wildlife Field Office.

River Flows

In 2013, considered an extremely dry year (classification February through October, NOAA, 2013), Klamath River flows below IGD began to increase starting March 2 and peaked at 2480 cfs on March 26 (**Error! Reference source not found.**). Flows declined to 1100 cfs by late April. Flow stayed consistent around 1100 cfs through May, dropped close to 1000 cfs through June, and dropped once more to approximately 900 cfs from July through September. The minimum flow during the sampling season was 872 cfs on July 27. IGH fish were released May 22-June 5.

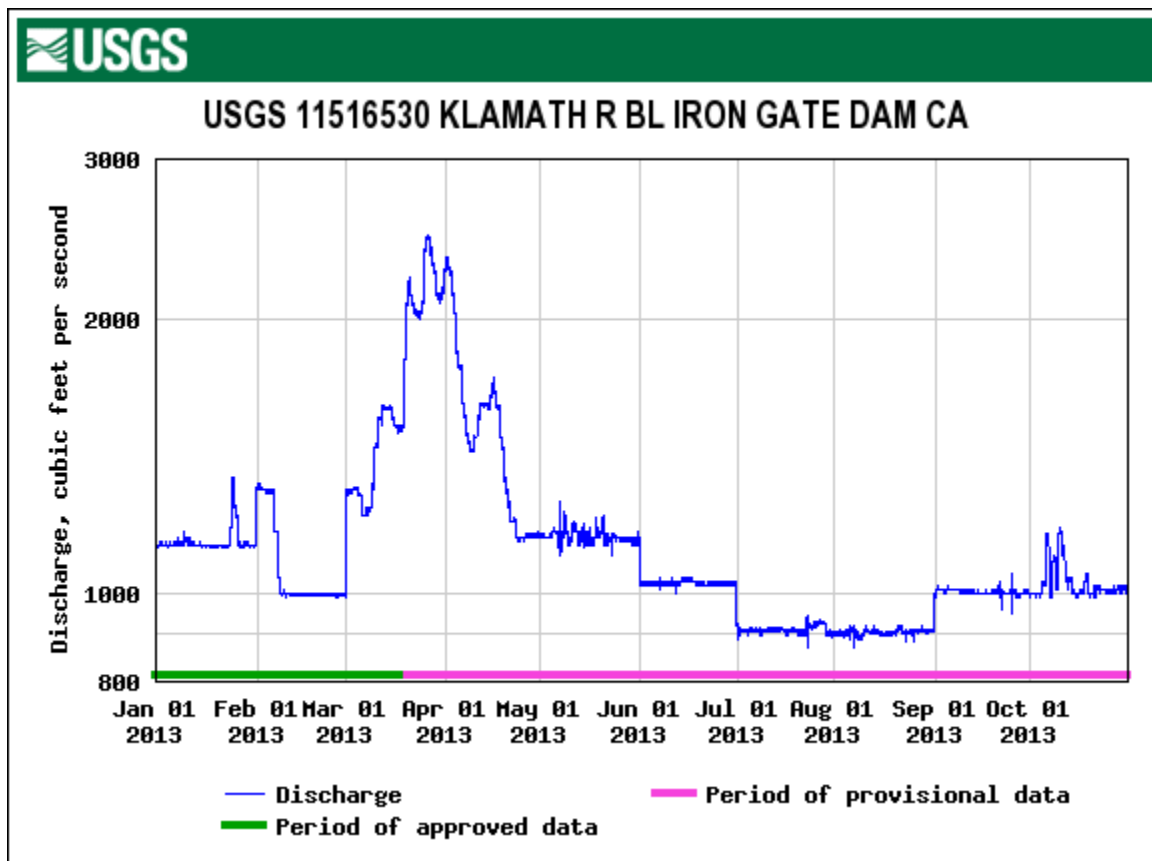


Figure 7. Daily discharge (cfs) below Iron Gate Dam from January 2013 through October 2013. Data collected after March 20, 2013 is provisional data. Data collected from USGS gaging station 11516530 at waterdata.usgs.gov.

Klamath River flows in 2013 were lower than flows seen in the previous two years (**Error! Reference source not found.**). 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). Spring flows in 2012 were close to 4000 cfs.

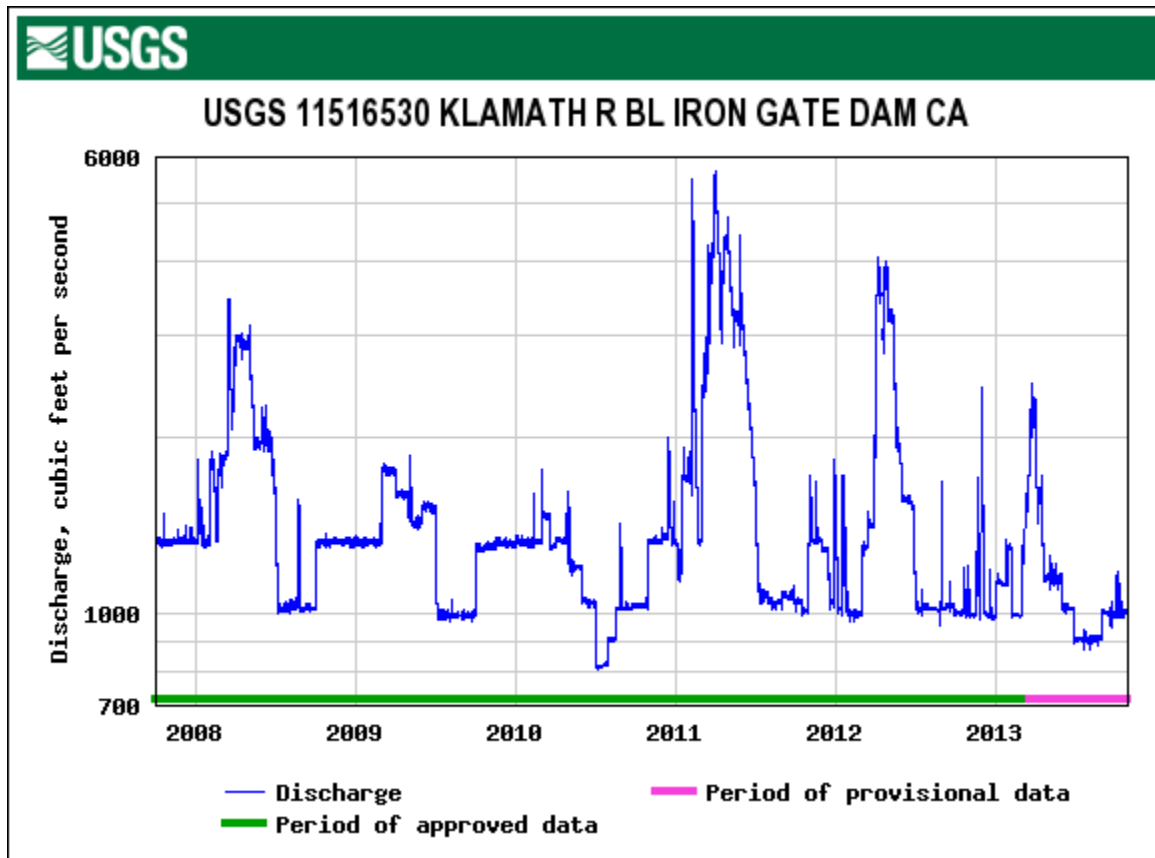


Figure 8. Daily discharge (cfs) below Iron Gate Dam from October 2008 through October 2013. Data collected after March 20, 2013 is provisional data. Data acquired from USGS waterdata.usgs.gov.

Discussion

Annual Prevalence of Infection

The annual *C. shasta* POI in mixed origin Chinook salmon by QPCR in 2013 (36%) was higher than 2012 (31%). *Ceratomyxa shasta* was also detected 2 weeks earlier in 2013, with the first detection occurring on April 22 compared to May 10 in 2012. This was not surprising given the environmental conditions in 2013. Water temperatures in 2012 and 2013 followed a similar pattern; however in 2013 spring temperatures were approximately 2° warmer compared to 2012. Higher temperatures allow faster development of polychaete worms infected with *C. shasta*, and therefore earlier maturation and/or release of infectious actinospores into the water column.

Likewise, the annual *P. minibicornis* POI by QPCR was also higher in 2013 (76%) compared to 2012 (65%). In both years *P. minibicornis* was first detected on April 25 at the Kinsman trap located in the K4 reach.

Prevalence of Infection by Reach

The upper Klamath River reach, K4, had lowest *C. shasta* POI (13%) as expected, likely due to samples being collected earlier in the season (March 28 through June 26) when water temperatures were lower and river discharge was higher in March and April. *Ceratomyxa shasta* POI was higher downstream, with the highest prevalence (55%) observed in the Salmon to Trinity River reach (K2). In 2012, the reach with the highest POI was further downstream in the Trinity River confluence to Estuary reach (K1), possibly indicating a longer period for parasite proliferation in the fish host. In 2013, sampling effort was reduced in K1 due to concerns for handling stress and therefore this reach had the smallest sample size (N = 64). It is difficult to predict if the POI would have been higher in that reach if a larger sample size had been collected over the sampling season. Higher *C. shasta* POI in the lower river reaches was a function of disease progression from initial infection or continual exposure in the upper reaches and development of ceratomyxosis approximately 3-4 weeks post exposure at typical Klamath River temperatures (True, et al., 2012).

Parvicapsula minibicornis had a pattern similar to *C. shasta* by reach with the highest POI (84%) observed in K2 and the lowest POI (66%) in K1. Consistent with previous years of high infection, *P. minibicornis* POI increased rapidly and remained high, a pattern especially evident in the lower river reaches.

Historical Comparison

Overall, 2013 was higher in *C. shasta* prevalence by QPCR, compared to historical annual POI observed from 2006-2013 (Table 2). The results in this table show that river temperatures and flows are connected factors that influence disease in salmonids. In 2009, a year with relatively warm water temperatures and low river discharge (peak flow = 1750 cfs), *C. shasta* POI was 45%. In 2010 and 2011, environmental conditions were favorable with cooler water temperatures, but had very different hydrographs. In 2010 the river peaked at 1760 cfs, while in 2011 the peak was 5700 cfs. *Ceratomyxa shasta* POI was 17% in both years. 2012 is an

example of a more intermediate year, as it had more typical water temperatures, a peak river discharge of 4070 cfs, and *C. shasta* POI at 30%.

In 2013, water temperatures were similar to historical temperatures with 18°C reached by early May, river discharge peaked at 2480 cfs, and *C. shasta* POI was 46%. Therefore, under conditions of both warmer temperatures and lower discharge in 2013, the *C. shasta* POI was higher than 2012, and was similar to the prevalence seen in 2009.

Another factor explaining the higher annual *C. shasta* POI could be the earlier onset of infection, as observed in natural fish. Earlier infections suggest earlier development of actinospores in the polychaete worm populations, possibly higher actinospore densities in the infectious zone during the juvenile hatchery Chinook salmon emigration, and a longer exposure period for fish prior to sampling. *Ceratomyxa shasta* infection in natural fish was higher overall in 2013, but when the data is restricted for historical comparison (May-July above the Trinity River confluence), natural fish in 2013 had a POI of 53% compared to only 6% for natural fish in 2012.

Prevalence of Infection by Fish Origin

Naturally produced Chinook salmon

Naturally produced Chinook salmon had a much higher *C. shasta* prevalence of infection by QPCR in 2013 (25%) compared to 2012 (3.5%). In 2013, natural origin fish were collected from the end of March to the end of May when water temperatures were warmer early in the season, especially during the month of April, when compared to 2012 (Figure 5); this earlier warmer water could be attributed to higher POI in 2013 than in 2012. Also, in 2013 collection of natural fish started earlier in the year allowing for 13 additional sampling days compared to 2012.

Naturally produced Chinook salmon - Comparison by Assay Type

All fish collected for histology were naturally produced Chinook salmon. The prevalence of *C. shasta* infection in histology samples was approximately one third of the QPCR prevalence (8% compared to 25%). *Parvicapsula minibicornis* infection in histology samples (37%) was a little more than half of the POI by QPCR (64%).

Both *C. shasta* and *P. minibicornis* were first detected by QPCR on the fifth week of sampling (week of April 21). *Ceratomyxa shasta* was first detected by histology on sample week eight (week of May 12), and *P. minibicornis* on sample week six (week of April 28). Histology samples were collected every other week for four weeks at the beginning of the sampling season, therefore the onset of infection might have paired better with the QPCR results if the histology sampling was more frequent. Therefore, histology data did not confirm an early natural infection, but it did confirm that natural fish had low disease severity based on the histology pathology score which matched well with low DNA copy number in natural fish tested by QPCR.

Hatchery (CWT) Chinook salmon

Ceratomyxa shasta POI in CWT salmon were similar in 2013 and 2012 (46% and 42% respectively). Typically IGH releases Chinook salmon in mid-May, but this is driven by target size at release as well river temperature criteria. Fish have been released as much as 3-4 weeks later than initially scheduled in some years, as was the case in 2012 when fish were released from June 6-15 in 2012 compared to May 22-June 5 in 2013. *Ceratomyxa shasta* POI was similar in 2012 and 2013, despite the difference in release dates, as fish experienced similar water temperatures during the emigration period post release.

Comparison of Natural and CWT Chinook salmon

Throughout the Klamath River naturally produced Chinook salmon collected this season had a lower overall POI compared to IGH CWT fish. We attribute this difference to cooler water temperatures early in the season and higher temperatures during the peak migration period from May to July. However, that trend did not always hold true when looking at specific reaches within the river. In the Shasta River to Scott River reach (K4), the uppermost reach, *C. shasta* POI in natural Chinook salmon was higher (18%) than in IGH CWT (2%).

It is not surprising to see a decrease in prevalence of infection as collections switched from natural fish to CWT fish after hatchery release because large numbers of uninfected hatchery fish are entering the system. The low prevalence of *C. shasta* in CWT fish represents fish newly exposed to the parasite. Natural fish were sampled for eight weeks prior to hatchery release, whereas IGH CWT were only sampled over a three week period in the K4 reach after hatchery release.

Comparison of All Sample Groups

In all fish collected this season, *C. shasta* POI was highest in IGH CWT fish (45%), followed by fish of unknown origin (34%), and was lowest in naturally produced Chinook salmon (25%). This pattern of *C. shasta* infection was expected because of the dates these groups were sampled and the water temperatures the fish experienced. Sampling was focused on CWT fish after hatchery release and CWT fish were sampled for the longest period of time (76 days). Water temperatures were also warmer when CWT fish were collected (May 29 through August 13), therefore it was expected that the prevalence in this group would be the highest. Natural fish experienced cooler water temperatures during the time of collection (March 28 through May 30) and had the lowest POI. Fish of unknown origin were intermediate as the date of collection (June 3 through July 29) overlapped with CWT fish, however unknown fish were not the focus of collection therefore the sample number was much smaller.

In all fish collected this season, *P. minibicornis* POI was highest in fish of unknown origin (92%), followed by CWT fish (81%), and was lowest in naturally produced Chinook salmon (64%). Fish of unknown origin having a higher *P. minibicornis* POI than CWT could be due to the number of samples collected. A smaller number of unknown fish were collected, (n=73), compared to 459 CWT fish.

IGH CWT Weeks At Large

We observed the highest *C. shasta* POI of IGH CWT juvenile Chinook salmon captured 4 (POI of 64%) and 5 (POI of 60%) WAL. Again, this is the typical development period for ceratomyxosis at river temperatures generally observed in the Klamath River. In 2012, the highest POI (72%) was seen in fish that had spent 3 WAL. In terms of parasite infectious load, measured as quantity of *C. shasta* DNA present in intestinal tissue, IGH CWT Chinook salmon had similarly low mean parasite loads in 2012 and 2013 (4900 and 4500 DNA copies respectively). These values were considered low compared to levels measured from clinically moribund fish which correlates to approximately 96,000 copies of *C. shasta* DNA (Bolick, et al., 2012).

In 2013, *C. shasta* POI was similar to a bell shaped curve. However, the mean DNA copy number gradually increased over the sampling season, decreased at 7 WAL, and peaked for fish residing 8-11 WAL at 3.6 DNA copies (log scale, Figure 4). Only three out of 14 fish residing 8-11 WAL were infected with *C. shasta*, and of these two (9 and 11 WAL) were highly infected (approximately 20,000 copies of *C. shasta* DNA). These two highly infected fish were responsible for the spike in mean copy number during 8-11 WAL (Figure 4). These DNA quantities indicate that while fewer fish were infected in the 8-11 WAL group, the Chinook salmon that were infected had heavier *C. shasta* parasite infection levels than fish 4 WAL. As for the eleven uninfected fish residing 8-11 WAL, it is thought that these fish spent most of their time rearing outside of the infectious mainstem, i.e. tributaries of the Klamath River, in order to remain uninfected before migrating downstream and being collected.

The majority of CWT fish residing 4 WAL were captured in K3 and K2. Fish collected in the lower river (K1 and K0) at 3-4 WAL and were likely from the fastest migration group. Groups residing for longer periods of time (9-11 WAL) likely reared longer in the upper reaches before being captured in the estuary.

Summary

The majority of juvenile Chinook salmon examined in 2013 appeared relatively healthy; some clinical disease signs were observed during QPCR necropsy but in relatively few fish. The higher *C. shasta* and *P. minibicornis* POI observed in mixed origin Chinook salmon in the Klamath River in 2013 was likely due to warm water temperatures and decreased river discharge. The historical annual prevalence of *C. shasta* in 2013 was relatively high compared to 2012, which was more of an intermediate year. Natural fish also had a higher *C. shasta* POI compared to last year. While *C. shasta* POI was higher, disease severity was low based on the DNA copy number. The low disease severity result was also seen histologically in natural Chinook salmon based on low pathology scores. Coded wire tagged hatchery fish residing in the river for 4 to 5 weeks after hatchery release had the highest prevalence of *C. shasta* infection, however fish CWT fish residing in the river 8-11 weeks post release had the highest DNA copy number.

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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 editorial review.
- Anne Bolick – Data management and quality control, QPCR necropsy extraction and assays, pivot tables and environmental data figures, written report, and compilation of reviewers' comments.
- 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 *Ceratomyxa shasta*. The first assay plate (88 samples) was not tested for *Parvicapsula minibicornis*, as well as 34 samples that 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	28-Mar	20				
2	4-Apr	20				
3	7-Apr	20	22	20		
4	14-Apr	20 (H10)	20 (H10)			
5	21-Apr	20	20			
6	28-Apr	20 (H10)	20 (H10)			
7	5-May	20	20			
8	12-May	19 (H9)	21 (H9)			
9	19-May	20	20			
10	26-May	20 (H10)	20 (H10)			
11	2-Jun	20	40			
12	9-Jun	20	20			
13	16-Jun	16	20	1		
14	23-Jun	18	21	11	20	
15	30-Jun		19	22	20	20
16	7-Jul		19	21		
17	14-Jul		13	27		20
18	21-Jul			20		20
19	28-Jul				24	20
20	4-Aug					20
21	11-Aug					20

Table 2. *Ceratomyxa 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	28-Mar	0% (0/20)				
2	4-Apr	0% (0/20)				
3	7-Apr	0 % (0/20)	0% (0/22)	0% (0/20)		
4	14-Apr	0 % (0/20)	0% (0/20)			
5	21-Apr	25% (5/20)	5% (1/20)			
6	28-Apr	0% (0/20)	0% (0/20)			
7	5-May	45% (9/20)	70% (14/20)			
8	12-May	68% (13/19)	95% (20/21)			
9	19-May	30% (6/20)	85% (17/20)			
10	26-May	0% (0/20)	25% (5/20)			
11	2-Jun	0% (0/20)	40% (16/40)			
12	9-Jun	5% (1/20)	70% (14/20)			
13	16-Jun	6% (1/16)	60% (12/20)	100% (1/1)		
14	23-Jun	0% (0/18)	71% (15/21)	82% (9/11)	45% (9/20)	
15	30-Jun		42% (8/19)	95% (21/22)	60% (12/20)	55% (11/20)
16	7-Jul		53% (10/19)	71% (15/21)		
17	14-Jul		46% (6/13)	74% (20/27)		70% (14/20)
18	21-Jul			5% (1/20)		85% (17/20)
19	28-Jul				8% (2/24)	0% (0/20)
20	4-Aug					45% (9/20)
21	11-Aug					40% (8/20)
		K4 Total 13% (35/273)	K3 Total 44% (138/315)	K2 Total 55% (67/122)	K1 Total 36% (23/64)	K0 Total 49% (59/120)

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	28-Mar					
2	4-Apr					
3	7-Apr		0% (0/20)	0% (0/20)		
4	14-Apr					
5	21-Apr	54% (7/13)	0% (0/20)			
6	28-Apr	68 % (13/19)	63% (12/19)			
7	5-May	70% (14/20)	100% (19/19)			
8	12-May	89% (17/19)	100% (21/21)			
9	19-May	85% (17/20)	95% (19/20)			
10	26-May	10% (2/20)	100% (20/20)			
11	2-Jun	40% (8/20)	93% (37/40)			
12	9-Jun	55% (11/20)	95% (19/20)			
13	16-Jun	100% (16/16)	100% (20/20)	100% (1/1)		
14	23-Jun	100% (18/18)	100% (21/21)	100% (11/11)	70% (14/20)	
15	30-Jun		100% (19/19)	100% (22/22)	55% (11/20)	80% (16/20)
16	7-Jul		100% (19/19)	100% (21/21)		
17	14-Jul		100% (13/13)	100% (27/27)		60% (12/20)
18	21-Jul			100% (20/20)		75% (15/20)
19	28-Jul				71% (17/24)	100% (20/20)
20	4-Aug					85% (17/20)
21	11-Aug					60% (12/20)
		K4 Total 66% (123/185)	K3 Total 82% (239/291)	K2 Total 84% (102/122)	K1 Total 66% (42/64)	K0 Total 77% (92/120)

Appendix B – Histological Summary

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

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

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

Collection week	Apr 14	Apr 28	May 12	May 26	POI
<u>Kidney</u>					
Pm troph.	0 / 10 (0)	2 / 10 (20)	7 / 9 (78)	0 / 10 (0)	9 / 39 (23)
Pm myxosp.	0 / 10 (0)	0 / 10 (0)	0 / 9 (0)	0 / 10 (0)	0 / 39 (0)
Metacercaria	0 / 10 (0)	0 / 10 (0)	0 / 9 (0)	0 / 10 (0)	0 / 39 (0)
<i>C. shasta</i> troph.	0 / 10 (0)	0 / 10 (0)	0 / 9 (0)	0 / 10 (0)	0 / 39 (0)
<i>Chloromyxum</i> sp	0 / 10 (0)	0 / 10 (0)	0 / 9 (0)	0 / 10 (0)	0 / 39 (0)
Pathology Score	0.00	0.00	0.00	0.00	
<u>Intestinal tract</u>					
<i>C. shasta</i> troph.	0 / 10 (0)	0 / 10 (0)	3 / 9 (33)	0 / 10 (0)	3 / 39 (8)
<i>C. shasta</i> myxosp.	0 / 10 (0)	0 / 10 (0)	0 / 9 (0)	0 / 10 (0)	0 / 39 (0)
Helminth	0 / 10 (0)	0 / 10 (0)	0 / 9 (0)	0 / 10 (0)	0 / 39 (0)
Pathology Score	0.00	0.00	0.56	0.00	
Adipose steatitis	2 / 6 (33)	2 / 3 (67)	4 / 4 (100)	2 / 6 (33)	10 / 19 (53)
Adipose lipofuscin	0 / 6 (0)	0 / 3 (0)	0 / 4 (0)	0 / 6 (0)	0 / 19 (0)
<u>Gill</u>					
Metacercaria	2 / 10 (20)	5 / 10 (50)	5 / 9 (56)	2 / 7 (29)	14 / 36 (39)
Multif. Hyperplasia	0 / 10 (0)	3 / 10 (30)	2 / 9 (22)	1 / 7 (14)	6 / 36 (17)

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 14	Apr 28	May 12	May 26	POI
<u>Kidney</u>					
Pm troph.	0 / 10 (0)	2 / 10 (20)	9 / 9 (100)	9 / 10 (90)	20 / 39 (51)
Pm myxosp.	0 / 10 (0)	0 / 10 (0)	0 / 9 (0)	0 / 10 (0)	0 / 39 (0)
Metacercaria	0 / 10 (0)	0 / 10 (0)	0 / 9 (0)	0 / 10 (0)	0 / 39 (0)
<i>C. shasta</i> troph.	0 / 10 (0)	0 / 10 (0)	0 / 9 (0)	0 / 10 (0)	0 / 39 (0)
<i>Chloromyxum</i> sp	0 / 10 (0)	0 / 10 (0)	0 / 9 (0)	0 / 10 (0)	0 / 39 (0)
.					
Pathology Score	0.0	0.0	0.33	1.32	
<u>Intestinal tract</u>					
<i>C. shasta</i> troph.	0 / 10 (0)	0 / 10 (0)	1 / 9 (11)	2 / 9 (22)	3 / 38 (8)
<i>C. shasta</i> myxosp.	0 / 10 (0)	0 / 10 (0)	0 / 9 (0)	0 / 9 (0)	0 / 38 (0)
Helminth	0 / 10 (0)	0 / 10 (0)	0 / 9 (0)	0 / 9 (0)	0 / 38 (0)
Pathology Score	0.00	0.00	0.11	0.00	
Adipose steatitis	1 / 4 (25)	3 / 4 (75)	6 / 8 (75)	5 / 6 (83)	15 / 22 (68)
Adipose lipofuscin	0 / 4 (0)	0 / 4 (0)	0 / 8 (0)	0 / 6 (0)	0 / 22 (0)
<u>Gill</u>					
Metacercaria	0 / 10 (0)	4 / 10 (40)	6 / 9 (67)	9 / 9 (100)	19 / 38 (50)
Multif. Hyperplasia	0 / 10 (0)	4 / 10 (40)	7 / 9 (78)	6 / 9 (67)	17 / 38 (45)

Appendix C - Reviewers' comments

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

Reviewer #1

Pg. 4 – Methods. Reviewer had a general comment that the tense in the methods and results section switches back and forth.

Response: Corrected the report so it is written in the past tense and consistent throughout the report.

Pg. 6 – Methods. In reference to the Trinity River not being sampled in 2013, the reviewer would like to know how many years the Trinity River has been sampled.

Response: The sentence has been changed to reflect that the Trinity River has been sampled from 2006 through 2012.

Pg. 7 – Methods. The reviewer would like clarification – do we use point prevalence?

Response: Yes, we use point prevalence. Prevalence of infection is also referred to as point prevalence. This is the number of cases of a disease which are detected in a population *at a designated point in time*. This is usually expressed as a ratio where the numerator is the number of cases detected at a point in time and the denominator is the sample from a population from which the cases were drawn.

Pg. 8 – Results. Reviewer would like to see consistency with use of abbreviations.

Response: We provide the reader with the abbreviation of a term when it is first used; after that we feel it is appropriate to use either the abbreviation or the full name of a term (e.g. at the beginning of a sentence).

Pg. 9 – Results. Reviewer suggests that the historical comparison introduction might belong in the method section.

Response: We think it is appropriate to leave the paragraph in the results as we are using it to explain the results in Table 2. We were explaining that histology was the screening method in the earlier years, but screening has transitioned to QPCR in recent years. This paragraph is an introduction to the table.

Pg. 17 – Discussion. Reviewer points out that annual prevalence of *C. shasta* in the discussion is reported as 36%, but in the abstract it is reported as 46%.

Response: This is reported correctly. The annual prevalence is for all fish sampled this season, and the *C. shasta* POI was 36%. For fish collected during peak migration, the POI was 46%. Data was restricted to peak migration dates of May through July (our annual metric), and therefore the results were different.

Pg. 17 – Discussion. In regards to *P. minibicornis* POI by reach, the reviewer asks what the *P. minibicornis* pattern is similar to.

Response: The sentence has been changed to read clearly that the pattern is similar to the pattern by reach seen in *C. shasta* POI. “*Parvicapsula minibicornis* had a pattern similar to *C. shasta* by reach with the highest POI (84%) observed in K2 and the lowest POI (66%) in K1.”

Pg. 17 – Discussion. When talking about the *C. shasta* historical comparison, the reviewer suggested that it is not correct to state that 2013 was higher overall in ceratomyxosis when it was conveyed that infection prevalence was higher, but disease severity was similar to previous years (pathology score <1).

Response: That is true, and the sentence was changed to clarify that *C. shasta* POI was higher by QPCR assay in 2013. Therefore, the term “ceratomyxosis disease” was taken out of the sentence. “Overall, 2013 was higher in *C. shasta* prevalence by QPCR, compared to historical annual prevalence of infection observed from 2006-2013.”

Pg. 17 – Summary. Reviewer states that an additional sentence is needed in the summary to emphasize that although POI was higher, parasite load was low.

Response: Sentence was added to the summary to make sure that DNA copy number was emphasized as one of the main take away points of the report.

Reviewer #2

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

Response: Minor edits were done throughout the report to incorporate these suggestions.

Pg. 3 – Introduction. Reviewer had a general comment that the description of the parasite life cycle is not very clear and there was some confusion about the release of the myxospore stage or if the actinospore transformed into the myxospore.

Response: Sentenced modified in the report to convey that the actinospore is released from the invertebrate host. Once a fish is infected, the spore develops into a myxospore in the fish host. The myxospore is released from the fish host and can infect the invertebrate host to continue the life cycle.

Pg. 3 – Introduction. In reference to the sentence about the infectious zone, the reviewer stated that the sentence reads that as the zone grows, density decreases and as the zone shrinks, density increases. All while abundance stays the same. Is that correct?

Response: The sentence was referring to the pattern of abundance remaining stable (i.e., low at the outflow of IGD and higher parasite abundance between the I-5 bridge crossing and the Scott River) as reference from Bartholomew et al 2010. We believe Bartholomew was stating that pattern was stable, not parasite abundance. Therefore it would not be correct to say that if the infectious zone was larger the density would decrease because actinospore density depends on other factors such as polychaete abundance, river temperature during development and release of actinospores, and river discharge/flows.

Pg. 7 – Methods. Reviewer suggests that the equation of a line should be given instead of writing out the slope and y-intercept in the text for the standard curve.

Response: We agree that this is a more appropriate way of presenting the data. The equation of a line was added for both the *C. shasta* and *P. minibicornis* standard curves.

Pg. 7 – Methods. Reviewer suggests that methods should be expanded when discussing how histology samples were collected.

Response: In order to make the report more concise, we referenced the report from the previous year as the collection methods for histology have been consistent and similar to last year.

Pg. 8 – Results. In reference to number of fish collected by origin, the reviewer points out that the CWT fish have an adipose fin clip as well, and that might need to be stated.

Response: That is correct. All hatchery fish that are tagged with a CWT also have adipose fin clip. Sentence modified to make this point clear.

Pg. 9 – Results. Reviewer suggests that the discussion on the transition from histology to QPCR as the historical metric should be moved to the methods.

Response: We think it is appropriate to leave the paragraph in the results as we are using it to explain the results in Table 2. We were explaining that histology was the screening method in the earlier years, but that has transitioned to QPCR in recent years. This paragraph is an introduction to the table.

Pg. 9 – Results. Reviewer asks why *C. shasta* has an historical table and is compared to all years, whereas *P. minibicornis* is only compared to the previous year.

Response: While fish are dual infected with both parasites, *C. shasta* is the most significant parasite in terms of clinical disease and associated mortality. *Ceratomyxa shasta* is the parasite that tends to drive mortality, and therefore we only emphasize a historical comparison for *C. shasta*.

Pg. 11 – Results. Reviewer wanted more context of the histology pathology score. The range and significance of score is not provided.

Response: Score numbers added to text and range of scores for clinically infected fish gives the reader information that pathology scores in 2013 were very low.

Pg. 11 – Results. Reviewer had a general comment and wanted to know if POI data could be referred to in a table or appendix. Might be better to refer to a summarized data table then to put all the data in the text.

Response: Will take this into consideration for the report next year. Would consist of many more tables or appendices (POI by reach, by origin, etc.), but still might be a better way to present the data.

Pg. 13 – Results. In terms of environmental conditions, the reviewer asked why the last 3 years were chosen as opposed to comparing 2013 to a 10 year mean.

Response: If this report was an historical review, then yes we would look at mean daily temperatures over many years (such as 10 year mean), but we are doing an annual comparison of 2013 to the previous year. One more year was added (2011) for visual comparison as 2011 was an environmentally favorable year.

Pg. 17 – Discussion. When comparing POI in 2013 to 2012, the author wants to know if POI for other years is available (pre-2012).

Response: Again, since we are doing an annual comparison, we discuss POI compared to the previous year. Using the annual metric (the restricted data for peak migration), the reader can see the POI pattern of *C. shasta* POI over the years (Table 2, page 10).

Pg. 18 – Discussion. When discussing POI by fish origin and specifically naturally produced Chinook salmon, the reader asks if sampling fish for 13 additional days in 2013 biases the comparison of 2013 to 2012.

Response: Yes, that is why the topic of additional sampling days was stated. *Ceratomyxa shasta* POI in 2013 was much higher than 2012 and additional sampling days could be one explanation of why we saw that result.

Pg. 19 – Discussion. In reference to the comparison of all sample groups, the reviewer suggests that this paragraph might not be necessary and that it contradicts the paragraph above regarding natural and CWT fish and a lower POI after hatchery release.

Response: We decided to leave this comparison in the report because it gives the reader information on all the samples groups and how they compare to each other. Natural fish and CWT fish were compared in the paragraph above, but this section of the report informs the reader how fish of unknown origin fit into the data. This paragraph also states that CWT fish overall had a higher POI than natural fish; therefore we don't feel that this is contradictory. The paragraph above is specifically talking about natural fish and CWT fish in a specific reach (K4).