

California-Nevada Fish Health Center

FY 2011 Investigational Report:

**Myxosporean Parasite (*Ceratomyxa shasta* and *Parvicapsula minibicornis*)  
Prevalence of Infection in Klamath River Basin Juvenile Chinook Salmon, April-  
August 2011**

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## SUMMARY

Juvenile Klamath River Chinook salmon (*Oncorhynchus tshawytscha*) were assayed by quantitative polymerase chain reaction (QPCR), and histology for infection with the myxosporean parasites *Ceratomyxa shasta* and *Parvicapsula minibicornis* from April to August, 2011. The seasonal prevalence of *C. shasta* by QPCR in Chinook salmon collected above the Trinity River confluence during the peak migration period (May-July) was 17%, the same as observed in 2010. *P. minibicornis* in Chinook salmon above the Trinity River confluence for the same period was 48% compared to 66% in 2010. The prevalence of *C. shasta* below the Trinity River was 16% by QPCR for samples collected June-August in the lower basin. *P. minibicornis* prevalence of infection below the Trinity River confluence for the same period was 43% by QPCR.

In coded-wire tagged (CWT) Iron Gate Hatchery (IGH) Chinook salmon screened by QPCR, *C. shasta* was detected in 14% of fish examined. The highest *C. shasta* prevalence of infection (39%) occurred in the IGH-CWT Chinook salmon residing 5 Weeks at Liberty (WAL) post hatchery release. *Ceratomyxa shasta* was detected in 4/49 (8%) of marked Trinity River Hatchery (TRH) Chinook salmon sampled in the Klamath River. In summary, both *C. shasta* prevalence of infection by QPCR in mixed origin and Iron Gate CWT Chinook salmon indicate that infectivity was similar to that observed in 2010 (17%) and relatively low compared to previous monitoring studies-(2006-2009). Environmentally, 2011 consisted of a manipulated pulse flow of approximately 4000 cubic feet per second (cfs) in early February as well as a cooler than normal climatic year. Spring and summer river temperatures were lower than expected, and numerous precipitation events in the basin sustained cooler temperatures well in to June. The cooler river temperature and increased flows likely reduced the density of actinospores present in the water column, which resulted in reduced exposure, and subsequent lower infection prevalence in out-migrating Chinook salmon.

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### Notice

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## INTRODUCTION

Two myxozoan parasites, *Ceratomyxa shasta* and *Parvicapsula minibicornis*, share vertebrate (salmonid) and invertebrate (*Manayunkia speciosa*) hosts and have overlapping distributions throughout the Pacific Northwest (Ching and Munday 1984; Hoffmaster et al. 1988; Hendrickson et al. 1989; Bartholomew et al. 1997; Kent et al. 1997; Jones et al. 2004; Bartholomew et al. 2006, Stocking et al. 2006). *Ceratomyxa shasta* and *P. minibicornis* are distributed throughout the 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; Stocking and Bartholomew 2007). The polychaete worm, *Manayunkia speciosa*, is most abundant in the Klamath River in an area approximately 60 river miles (RM) extending below Iron Gate Dam to Seiad Valley. *Manayunkia speciosa* populations release an infective actinospore stage into the water column, where the parasite infects juvenile Chinook salmon (*Oncorhynchus tshawytscha*) that are rearing in, or emigrating through, this section of the mid Klamath River. Klamath River 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 & Foott 2010, True et al. 2011). Ceratomyxosis in juvenile Chinook can cause significant mortality (40-50%) when warm river temperatures in late spring (15-18°C) are coupled with low flows that favor polychaete worm proliferation and maturation. Warmer temperatures also hasten disease progression within the fish host when other factors are constant (Udey 1975, Bartholomew & Foott 2010).

An annual metric for *C. shasta* prevalence of infection by histology (above the Trinity confluence and during the peak migration period of May to July) and concurrent QPCR screening has been used to provide year to year comparisons of infection prevalence. Prevalence infection by histology has ranged from 15-54% and 17-49% by QPCR in study years 2006-2011 (Table 5, page 21). *Parvicapsula minibicornis* prevalence of infection can be quite high, with infection prevalence rapidly rising to 100% by May or June in a typical year. Seasonal *P. minibicornis* prevalence has ranged from 48-91% in the same 2006-2011 time period (data not shown in Table 5). While the majority of fish are dual infected with both parasites, *C. shasta* is the most significant parasite in terms of clinical disease and associated mortality observed in both natural and hatchery origin Chinook salmon in the Klamath River.

The objectives of this study were: 1) examine *C. shasta* and *P. minibicornis* prevalence in Iron Gate Hatchery (IGH) and Trinity River Hatchery (TRH) Chinook salmon prior to and post hatchery release; 2) examine the parasite prevalence in the juvenile Chinook salmon population within specific river reaches and throughout the spring out-migration period; 3) compare parasite prevalence in 2011 to previous years; and 4) examine the diagnostic prevalence of other significant pathogens in moribund Chinook salmon in select reaches.

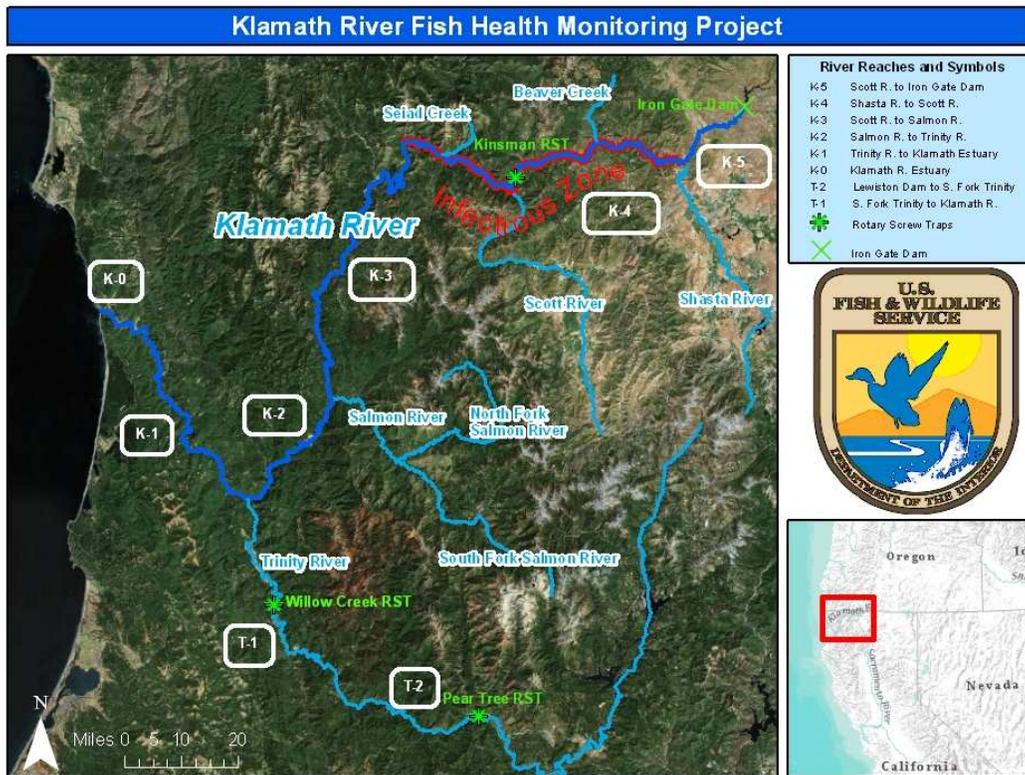
## METHODS

In 2011, changes were made to the sampling effort and therefore study design of the Klamath River Fish Health Monitoring program:

- 1) The Iron Gate to Shasta River reach (K5) was not sampled this year by QPCR, due to the relatively low prevalence of *C. shasta* observed in this reach historically and the close proximity to Iron Gate hatchery.
- 2) Histology sampling was limited to the Shasta to Scott reach (K4) and the Trinity River confluence to Estuary reach (K1). QPCR sampling in all reaches continued and will replace histology as the annual metric of parasite prevalence of infection (POI) used for comparisons between study years.
- 3) Only subsets of fish from each reach were tested for *P. minibicornis*, as prevalence of infection is often quite high (reaches and sustains 100%) in hatchery fish. Kidney tissue for *P. minibicornis* testing was collected from all fish, however only representative samples from each reach were assayed by QPCR.

**Sample Sites, Fish Groups and Number Sampled**

Fish were collected in the Klamath River from below Iron Gate Dam (Klamath River Mile [RM] 190) to the Klamath River Estuary and on the Trinity River between Lewiston Dam (Trinity RM 111) and the Trinity River confluence with the Klamath River (Klamath RM 43). Klamath and Trinity Rivers were divided into sample reaches at major tributaries, with study cooperators collecting fish in each reach (Figure 1, Table 1). When possible, existing salmonid downstream migrant trapping sites were utilized for collection, but beach seining was required to achieve the desired sample size in some weeks.



**Figure 1. Klamath River watershed, major tributaries, and sample reaches: Iron Gate dam to Shasta River (K5), Shasta River to Scott River (K4), Scott River to Salmon River (K3), Salmon River to Trinity River confluence (K2), Trinity River to Estuary (K1), Klamath River Estuary (K0). Trinity River sites: Pear Tree and Willow Creek rotary screw traps.**

**Table 1. Sample reach locations (reach code), river mile, and cooperating agencies performing fish collections on the Klamath and Trinity rivers.**

<b>Sample Reach (code)</b>	<b>River Mile</b>	<b>Primary collector(s)</b>
<b>Klamath River main stem</b>		
Shasta to Scott (K4)	Klamath 177-144	USFWS and Karuk Tribe
Scott to Salmon (K3)	Klamath 144-66	Karuk Tribe
Salmon to Trinity (K2)	Klamath 66-44	Karuk Tribe
Trinity to Estuary (K1)	Klamath 44-4	Yurok Tribe
Klamath Estuary (K0)	Klamath 4-0	Yurok Tribe
<b>Trinity River</b>		
Upper – Pear Tree Rotary Trap (T2)	Trinity 94	Hoopa Tribe
Lower - Willow Creek Rotary Trap (T1)	Trinity 21	USFWS and Yurok Tribe

All fish collected from the two rivers are categorized into three sample group types based on their origin: natural, unknown and coded wire tagged. “Mixed origin” Chinook refers to any or all group types as a whole, i.e., results for all fish collected in a particular reach, or for the entire sampling season. The sample group types are defined below.

Natural: Chinook salmon collected early in the sampling season (April-May) prior to hatchery smolt releases.

Unknown: Unmarked Chinook salmon collected after hatchery release, undifferentiated fish could be natural or hatchery origin.

Coded wire tagged (CWT): Hatchery Chinook marked with an adipose fin clip and implanted with a coded wire tag before release.

Additionally, a pre-release exam of hatchery Chinook for *C. shasta* and *P. minibicornis* infections was performed before fish were released: forty fish were sampled from the IGH on 16 May, and 62 fish were sampled from TRH on 19 May. Trinity River Hatchery volitionally releases approximately 1.5 million fall Chinook and approximately 1.3 million spring Chinook salmon each year: in 2011 this occurred from June 1-15. Iron Gate Hatchery releases approximately 5 million fall Chinook salmon in late May to early June: in 2011 the release date was June 23<sup>rd</sup>. After hatchery Chinook were released, the study focused on capture and testing of CWT fish, to assess parasite loads in relationship to Weeks At Large (WAL).

Fish numbers tested in the Klamath River varied by reach, with emphasis on natural fish in the reaches below Iron Gate Dam initially, then hatchery CWT fish for the remainder of the spring/summer migration period. In the Trinity River, we sampled the upper and lower watershed at rotary screw traps (RST) located at Pear Tree Creek and Willow Creek. Similar to the Klamath River sampling, effort focused on natural fish initially, then CWT marked hatchery Chinook as they emigrated through the lower Klamath reaches, and finally in the Klamath River Estuary. Significant recapture effort for CWT Chinook salmon occurred in the Klamath River below the Trinity River confluence and in the Estuary in the latter half of the study (Table 2).

**Table 2. Number of fish sampled for QPCR testing by Klamath River reach (reach code) and sampling week. Supplemental samples for histology (H) were collected in the Shasta to Scott reach (K4) and in the Trinity R. to Estuary reach (K0).**

Collection 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	4-Apr					
2	11-Apr	10 (H10)				
3	18-Apr	10 (H10)				
4	25-Apr	10 (H10)				
5	2-May	10 (H10)	10	10	9	
6	9-May	10 (H10)			10	
7	16-May	10 (H10)	10	10	10	
8	23-May	10 (H10)			10	
9	30-May	10 (H10)	10	10		
10	6-Jun	10 (H10)				
11	13-Jun	10 (H10)	10	10		
12	20-Jun	10 (H10)			19	19 (H10)
13	27-Jun	20 (H10)		10		
14	4-Jul	15 (H10)	10	20	20	21 (H10)
15	11-Jul	3 (H10)	25	20		
16	18-Jul	1 (H10)	30	20	20	10 (H10)
17	25-Jul		20	10		
18	1-Aug		10	10	10	20 (H10)
19	8-Aug		10	10		
20	15-Aug				7	16 (H10)

Heads from any marked IGH or TRH Chinook salmon recovered were tracked with unique identification numbers; the CWT codes were read by the USFWS Arcata Fish and Wildlife Office (AFWO). The date each group of CWT Chinook salmon was released from the hatchery and date of recapture was used to assess temporal infections levels in individual fish by comparing parasite load to Weeks at Large. Releases at TRH are volitional and occurred from 1 June through 17 (9 June was used as the mean date of release) and IGH released all Chinook on June 23<sup>rd</sup>.

Parasite Infection Levels by Quantitative PCR Assays

Fish tested by QPCR were euthanized, placed in a plastic bag labeled with date and reach, and frozen in the field. In the laboratory, fish were thawed, fork length was measured, clinical disease signs notated, and necropsy performed to collect intestine and kidney tissues for *C. shasta* and *P. minibicornis* testing, respectively. The entire intestine and kidney from each fish were removed and combined into a single well of a 96 well plate. Tissue samples were then frozen at -20 °C until DNA extraction was performed.

Combined intestine and kidney tissues were digested overnight in 100µL MagMAX Proteinase K Buffer containing 100 mg/ml proteinase K (Applied Biosystems, Foster City, CA) at 55°C with constant

shaking. A subsample of digested tissue homogenate was diluted 1:10 in molecular grade water, then 1:10 in MagMAX Multi-Sample DNA Lysis Buffer (Applied Biosystems, Foster City, CA) for a final dilution of 1:100. The diluted tissue homogenate was extracted in a 96 well magnetic bead sample processing system (Applied Biosystems MagMAX Express-96 Magnetic Particle Processor). Extracted DNA was stored at -20°C until the QPCR assays were performed.

Samples were assayed in Real Time PCR Sequence Detection Systems (SDS) using probes and primers specific to each parasite. The combined tissues were tested for *C. shasta* 18S rDNA using TaqMan Fam-Tamra probe and primers (Hallett and Bartholomew 2006) on the 7300 Sequence Detection System (Applied Biosystems, Foster City, CA). Separately, the combined tissues were tested for *P. minibicornis* 18S rDNA utilizing TaqMan Minor-Groove-Binding (MGB) probe and primers (True et al. 2009) on the StepOne Plus Sequence Detection System (Applied Biosystems Foster City, CA). Reaction volumes of 30µL, containing 5µL DNA template, were used for both assays under the following amplification conditions: 50°C for 2 min.; 95°C for 10 min; 40 cycles of 95°C for 15s and 60°C for 1 min. Plasmid standards, extraction control and no template control (NTC) wells were included on each assay plate.

Cycle threshold ( $C_T$ ) values were calculated using SDS software (7300 SDS v 1.3.1, StepOne SDS v. 2.0 Applied Biosystems) and a standard curve to transformed  $C_T$  values to parasite DNA copy number.

#### Histological Assays

Fish tested by histology were euthanized and placed in Davidson's fixative within 2 minutes of euthanasia. Fish were held in fixative for 24-48 hours. Fish tested by histology were rapidly euthanized and placed in Davidson's fixative and held for 24-48 hours. The fixative was replaced with 70% ethanol for storage until the gross examination and histological processing were performed. Each histological cassette contained kidney, intestine, and 1 to 2 gill filaments. Specimens were processed for 5µm paraffin sections and stained with hematoxylin and eosin (Humason 1979). All tissues for each fish were placed on one slide and identified by a unique number code. Each slide was examined at 40X to 400X magnification.

Histological rankings of 'clinical disease' included a pathology score: a numeric index of disease severity for kidney and intestine. The pathology score does not affect the overall prevalence of infection reported for histological assessments, but provides a numeric index of the disease state in sample groups. Pathology score is based on the degree of specific tissue abnormalities and parasite distribution (0 = normal, 1= focal, 2 = multi-focal, and 3 = diffuse distribution) listed in Table 3. A kidney pathology score was calculated by summing the score of each kidney lesion (interstitial hyperplasia, necrotic interstitium or tubule, interstitial granuloma, glomerulonephritis, and protein casts within the glomeruli or tubules). The mean kidney pathology score was reported for each collection group to demonstrate severity of disease. Similarly for the intestine, the sum of lesion scores (lamina propria hyperplasia, necrotic epithelium / sloughing, necrotic muscularis) was used to calculate a collection group's mean intestinal pathology score.

**Table 3. Parasite abbreviations and tissue abnormalities listed in the histological result tables.**

<p><b>Kidney</b></p> <p><i>P. minibicornis</i> Troph.  <i>P. minibicornis</i> Myxosp.  Metacercaria  <i>C. shasta</i> troph.  Chloromyxum sp</p> <p><b>Pathology Score</b></p>	<p><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</p> <p><b>Mean kidney pathology score for sample group</b></p>
<p><b>Intestine</b></p> <p><i>C. shasta</i> troph.  <i>C. shasta</i> myxosp.  Helminth</p> <p><b>Pathology Score</b></p>	<p><i>Ceratomyxa shasta</i> trophozoite stage  <i>Ceratomyxa shasta</i> myxospore stage  Trematode, nematode, or cestode</p> <p><b>Mean intestine pathology score for sample group</b></p>
<p><b>Gill</b></p> <p>Ich  Glochidia  Metacercaria  Invasive <i>C. shasta</i>  Amoeba  Multif. Hyperplasia</p>	<p><i>Ichthyophthirius multifiliis</i>  Larval mussel stage within lamellae  Immature trematode stage  Single cell trophozoite-like stage  Amoeba associated with lamellae  Multifocal hyperplastic regions on lamellae</p>
<p><b>Other</b></p> <p>Adipose steatitis  Adipose lipofuscin</p>	<p>Inflammation of visceral fat tissue  Oxidized lipopigments within adipose cells</p>

Statistical Analysis and Terms Used

Prevalence of infection and annual prevalence (defined below) for *C. shasta* and *P. minibicornis* are reported with 95% confidence intervals (denoted ci) for each sample reach. Prevalence of infection is used to describe ratios of infected Chinook salmon (numerator) in the sample (number of animals examined) for a calendar week. Annual prevalence is used to describe the overall prevalence of infection for the sampled population for the period of one calendar year. Definitions of the two terms used are as follows (Durfee 1978, USFWS Fish Health Policy FW713):

Prevalence of infection (also referred to point prevalence): 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.

Annual prevalence (also referred to as period prevalence): Measures the total number of cases known to occur during a given period. Period prevalence is often mislabeled as incidence data because the factor time enters into it. However, it should be noted that incidence describes only *new cases in a specified population, and requires knowledge of when the animals became infected* to determine the rate of infection (incidence attack rate).

## RESULTS

### Pre-release Exams of IGH and TRH Chinook Salmon

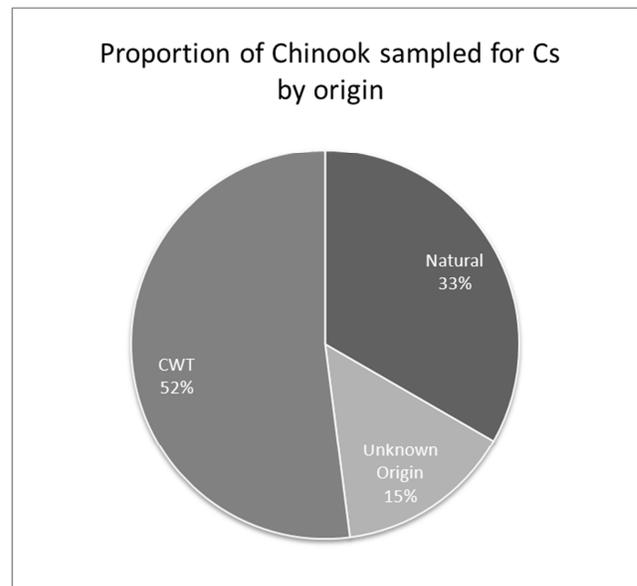
Light infections of *C. shasta* were detected by QPCR in 7.5% (3/40, ci = 2-20%) of Chinook salmon sampled 16 May at IGH, prior to hatchery release. The  $C_T$  values for the three positive samples ranged from 37.8 to 38.4 and corresponded to very low parasite copy numbers (3.9 to 6.1 DNA copy numbers respectively). This detection of *C. shasta*, near the detection threshold of the QPCR assay, indicated a very low exposure level to this parasite and was not indicative of a disease state (active or clinical infection). Infections of *P. minibicornis* were not detected by QPCR in pre-release Chinook salmon sampled at IGH. Neither *C. shasta* nor *P. minibicornis* were detected by QPCR in TRH Chinook salmon pre-release exams.

### **Parasite Prevalence of Infection by Origin of Fish Sampled**

In 2011 we examined a total of 838 Chinook salmon collected from the Klamath and Trinity Rivers, consisting of 279 natural fish, and 559 fish collected after hatchery release which included 436 CWTs. Coded wire tagged Chinook account for 52% of all fish sampled in 2011. Natural fish account for 33%, and 15% of the fish are of unknown origin (unmarked hatchery fish or natural) (Figure 2).

### Natural Production Chinook Salmon

Natural 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 hatchery salmon migration period of June-July. A total of 190 natural fish were sampled above the Trinity River confluence (K4, K3, and K2) from 15 April through 22 June, and *C. shasta* was detected by QPCR in 19% (37/190, ci = 14-26%) compared to 9% (5/58, ci = 3-19%) of fish sampled below the Trinity confluence (K1 reach) and within the Estuary (K0) (Table 4). Comparatively, *P. minibicornis* POI in natural Chinook salmon sampled above and below the Trinity confluence was 41% (70/170, ci = 34-49% and 26% (10/38, ci = 13-42%) respectively.



**Figure 2. Proportion and origin of Chinook salmon collected and sampled for *Ceratomyxa shasta* (Cs) testing.**

Historically, the prevalence of infection for *C. shasta* is lower below the Trinity River confluence, due to lower disease incidence in the Trinity River compared to Klamath River, and accretion of flows resulting in lower actinospore concentrations in the lower reaches (K1 and K0).

### Unknown Origin Chinook Salmon

As described in the methods, unknown origin Chinook are unmarked fish collected after hatchery release that cannot be differentiated from either natural fish or unmarked hatchery fish. A total of 56 fish of unknown origin were collected in the upper Klamath reaches from 27 June through 8 August. Below the Trinity River confluence, a total of 29 fish of unknown origin were collected in the Estuary (K0) on 23 June through 8 July. An additional 38 fish were sampled in the Trinity River, for a total of 123 fish sampled in 2011 (Table 4).

### Marked (CWT) Chinook Salmon

A total of 188 CWT Chinook were sampled in the upper Klamath reaches from 28 June through 8 August. An additional 123 CWT Chinook were sampled in the lower Klamath reaches from 6 July through 19 August. And 125 fish were sampled in the Trinity River, for a total of 436 fish sampled in 2011.

Prevalence of *C. shasta* infections in CWT Chinook salmon, sampled above the Trinity confluence was 14% (27/188, ci = 10-20%). Below the confluence, *C. shasta* POI was 18% (22/123, ci = 7-17%) (Table 4). Prevalence of *P. minibicornis* infection in CWT Chinook salmon sampled above the Trinity River confluence was 63% (53/84, ci = 52-73%) and 60% (20/33, ci = 42-77%) below the Trinity River confluence. Prevalence data for Coded-wire tagged Chinook salmon, and analysis of Weeks at Large data, is discussed in further detail in a separate section of the report.

### Mixed-Origin Chinook Salmon

A total of 434 mixed origin Chinook were sampled in the upper Klamath reaches from 15 April through 8 August. A total of 210 mixed origin Chinook were sampled in the lower Klamath reaches from 4 May through 19 August. An additional 194 fish were sampled in the Trinity River, for a total of 838 fish sampled in 2011.

Prevalence of *C. shasta* infections in mixed-origin Chinook salmon, sampled above the Trinity confluence was 17% (73/434, ci = 13-21%). Below the confluence, *C. shasta* POI was 16% (33/210, ci = 11-21%) (Table 4). Prevalence of *P. minibicornis* of infection in mixed origin Chinook salmon sampled above the Trinity River confluence was 48% (123/254, ci = 42-55%) and 43% (39/90, ci = 33-54%) below the Trinity River confluence. As described in the methods section, the mixed-origin sample group consists of natural, unmarked fish of unknown origin, and CWT Chinook salmon. Histological assessments were performed on random, but separate, mixed-origin fish collected from the same reach and location as fish tested by QPCR (Appendix A, Tables A1-A4).

**Table 4. *C. shasta* prevalence of infection in mixed-origin Chinook by fish origin (Natural, Unknown Origin, and CWT) and reach in which fish were collected in the Klamath River.**

	<b>Shasta to Scott (K4)</b>	<b>Scott to Salmon (K3)</b>	<b>Salmon to TR (K2)</b>	<b>TR to Estuary (K1)</b>	<b>Estuary (K0)</b>
<b>Natural Chinook salmon – Sampled 15 April through 22 June</b>					
<i>C. shasta</i> +/ N	22/110	9/40	6/40	5/58	NC
<b><i>C. shasta</i> Percent Positive</b>	<b>20% (13-29)</b>	<b>23% (11-39)</b>	<b>15% (6-30)</b>	<b>9% (3-19)</b>	NC
<b>Unknown Origin Chinook salmon (unmarked) – Sampled 27 June through 8 August</b>					
<i>C. shasta</i> +/ N	NC	5/32	4/24	NC	6/29
<b><i>C. shasta</i> Percent Positive</b>	NC	<b>16% (5-33)</b>	<b>17% (5-37)</b>	NC	<b>21% (8-40)</b>
<b>CWT Chinook salmon – Sampled 28 June through 19 August</b>					
<b>IGH-CWT <i>C. shasta</i>+/ N</b>	2/35	5/70	15/71	10/24	7/41
<b><i>C. shasta</i> Percent Positive</b>	<b>6% (0.70-19)</b>	<b>7% (2-16)</b>	<b>21% (12-32)</b>	<b>42% (22-63)</b>	<b>17% (7-32)</b>
<b>TRH-CWT <i>C. shasta</i>+/ N</b>	ND	ND	ND	2/28	2/21
<b><i>C. shasta</i> Percent Positive</b>	ND	ND	ND	<b>7% (0.88-24)</b>	<b>10% (1-30)</b>
<b>Unreadable CWT</b>	1/4	1/3	3/5	0/5	1/4
<b>ALL CWT<sup>1</sup> <i>C. shasta</i>+/ N</b>	3/39	6/73	18/76	12/57	10/66
<b><i>C. shasta</i> Percent Positive</b>	<b>8% (1-21)</b>	<b>8% (3-17)</b>	<b>24% (15-35)</b>	<b>21% (12-34)</b>	<b>15% (8-26)</b>
<b>Mixed Origin Chinook (Natural, unknown/unmarked and CWT) – Sampled 15 April through 19 August</b>					
<i>C. shasta</i> +/ N	25/149	20/145	28/140	17/115	16/95
<b><i>C. shasta</i> Percent Positive</b>	<b>17% (11-24)</b>	<b>14% (9-21)</b>	<b>20% (14-28)</b>	<b>15% (9-23)</b>	<b>17%(10-26)</b>

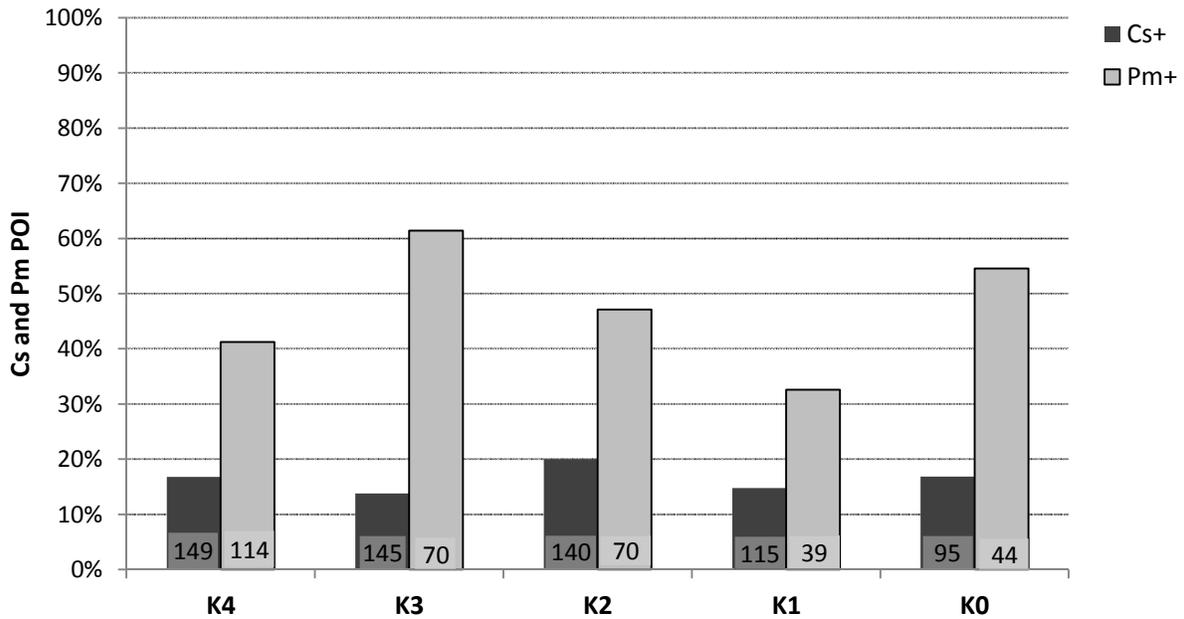
Key: N=Total sample number, ND=Not done (reach not sampled), NC = Not collected (reach was sampled, but unmarked fish were not collected in the defined time period). Percent positive is reported with 95% confidence interval in parentheses.

<sup>1</sup> Note: All CWT includes 21 CWT Chinook salmon which had unreadable tags (no tag or unreadable tag code). Therefore IGH and TRH CWT sample sizes (shown in gray highlighting) are slightly smaller than the All CWT figures given.

## Parasite Prevalence of Infection by River Reach

Prevalence of *C. shasta* infection in all Chinook salmon sampled in the Klamath River was 16% (106/644, ci = 4-20%) compared to the annual metric of 17% (restricted to above Trinity confluence and May-July peak immigration period). Prevalence was highest in K2 at 20%, followed by K4 and K0 both at 17%. Lowest prevalence was seen in K3 at 14% (Figure 3).

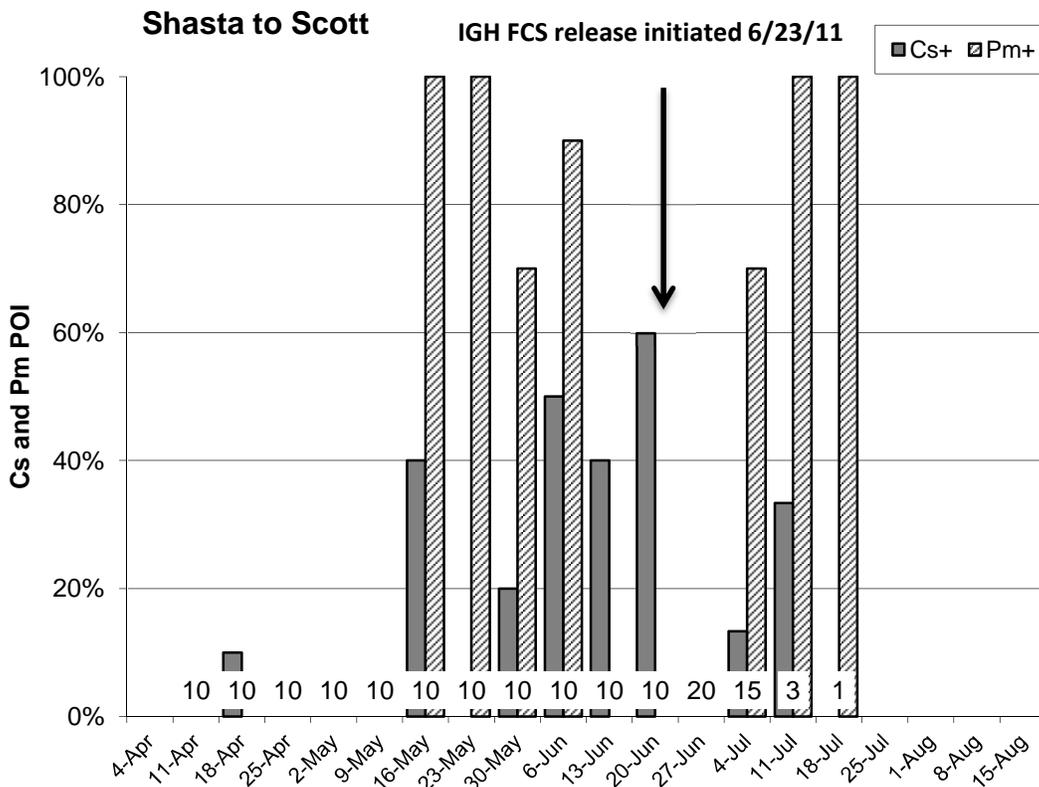
Prevalence of *P. minibicornis* infection in mixed origin Chinook salmon sampled in the Klamath River was 47% (162/344, ci = 42-53%). Prevalence was highest in K3 at 62%, followed by K0 at 55%. Lowest prevalence was seen in K1 at 33% (Figure 3).



**Figure 3. Prevalence of *Ceratomyxa shasta* (Cs+) and *Parvicapsula minibicornis* (Pm+) infection in juvenile Klamath River Chinook salmon by capture reach. Sample numbers collected are displayed at the bottom of each column for both pathogens.**

In the Shasta to Scott (K4) reach, *C. shasta* was detected by QPCR in 17% (25/149, ci =11-24%) of mixed-origin Chinook salmon which consisted primarily of natural fish. Prevalence peaked at 60% in late June and was last detected in this reach at 33% in mid-July. IGH Chinook salmon were released on June 23<sup>rd</sup> (Figure 4). In the natural fish subset collected in this reach (15 April to 22 June), *C. shasta* was first detected in mid-April, and then was not detected until four weeks later in mid-May. *Ceratomyxa shasta* prevalence of infection in natural fish was 20% (22/110, ci =13-29%). Generally natural fish have lower *C. shasta* POI, due to the lower river temperature when these fish are sampled (Apr-May). In terms of parasite load within the population, the DNA copy number observed in fish confirmed that infection levels were lower in hatchery fish released (mean of 15 copies) later in the season, than in natural fish (mean of 45 copies) sampled in May-June of 2011. Generally this trend is opposite due to cooler river temperatures when natural fish are sampled. Histologically, *C. shasta* was only detected in 2% of fish, and detected on 9 and 23 May, and again on 11 July.

In the Shasta to Scott reach, *P. minibicornis* was detected by QPCR in 41% (47/114, ci = 32-51%) of mixed-origin Chinook salmon (Figure 4). Infection prevalence reached 100% by mid-May, and decreased to 70% in late May and early July. At the end of July the prevalence had again peaked to 100%. *Parvicapsula minibicornis* POI in natural fish was similar at 40% (36/90, ci = 30-51%).



**Figure 4. Weekly prevalence of *Ceratomyxa shasta* (Cs+) and *Parvicapsula minibicornis* (Pm+) infection by QPCR in juvenile Klamath River Chinook salmon captured in K4 reach on the Klamath River (Shasta River to Scott River). Sample numbers collected and tested for *Ceratomyxa shasta* each week are displayed at the bottom of each column, while sub-sample numbers for *P. minibicornis* are listed in the sample table in Appendix C. *C. shasta* was not detected on 11 Apr, 25 Apr, 2 May, 9 May, 23 May, 27 May, 27 Jun, and 18 Jul. Bold arrow indicates IGH FCS release date.**

Fifteen weekly histology collections occurred between 11 April and 18 July for a total of 150 specimens (Figure 5 and Appendix A, Table A1-A4). Collection groups between 11 April and 22 June were of natural origin while the 27 June to 18 July specimens were considered of mixed origin. Both natural and mixed origin salmon had low (2 and 3%) prevalence of *C. shasta* infection. In contrast, *P. minibicornis* infection of the kidney and metacercarial infection of the gill increased in the natural salmon beginning in late May. The kidney pathology score and gill lamellar hyperplasia also increased with time (Figure 5 and Appendix A). Kidney pathology scores are considered low given the 6 – 8 range seen in clinically affected salmon in 2009. The influence of the IGH release on infection data was suggested by the zero prevalence observed in the first potential mixed origin sample taken on 27 June. Both *P. minibicornis* and metacercaria infection prevalence and disease increased over time. Another commonly observed abnormality was steatitis (inflammation of the visceral fat) in approximately 40-50% in both natural and hatchery populations (Appendix A, Table A1).

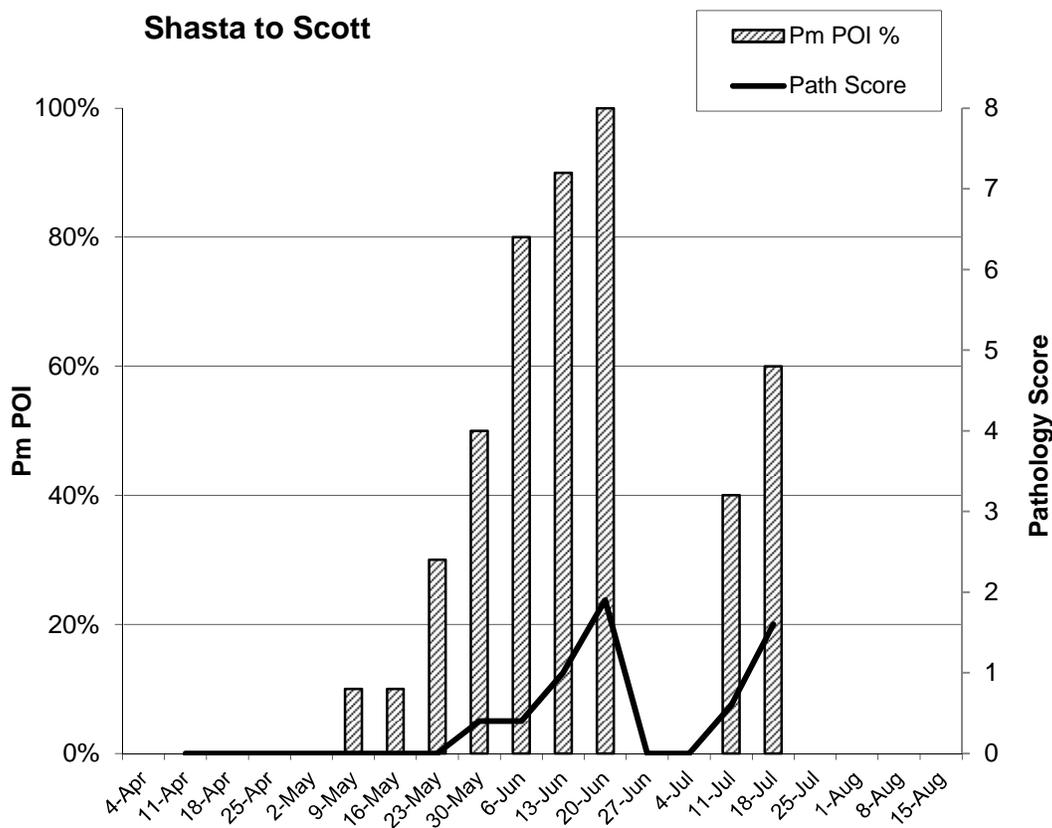
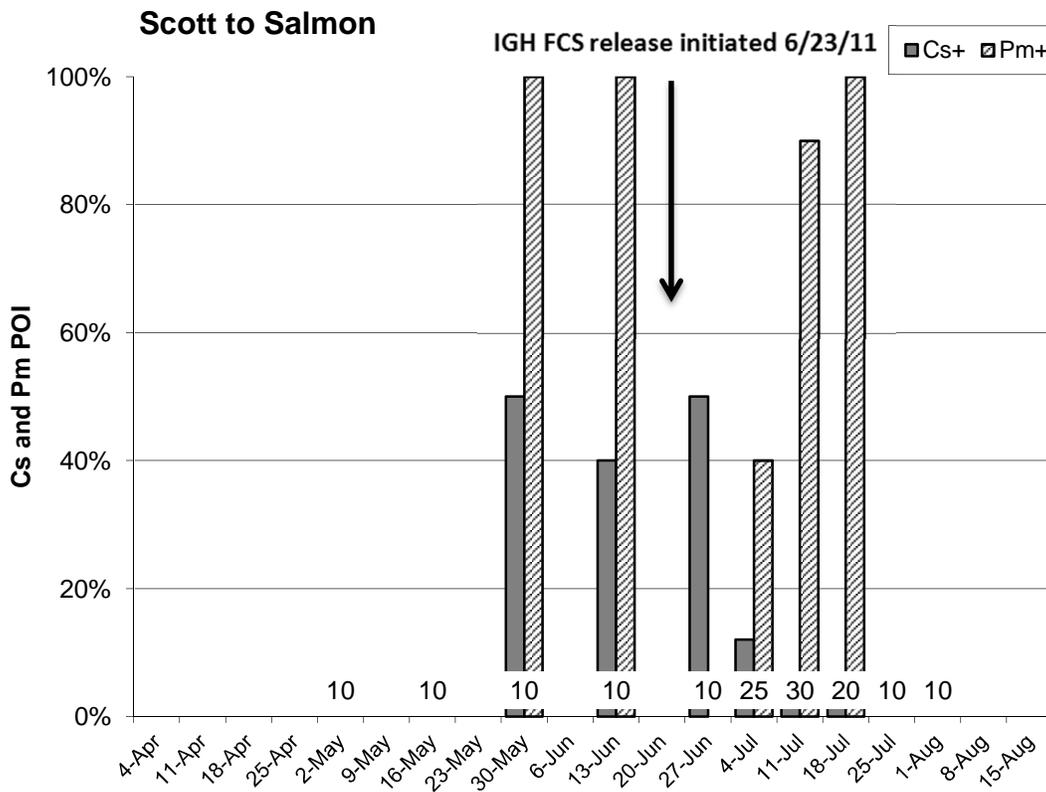


Figure 5. Weekly prevalence of infection for *Parvicapsula minibicornis* (Pm POI %) and mean pathology score (Path Score) by histology in juvenile Klamath River Chinook salmon captured in the Shasta to Scott (K4) reach.

Scott R. to Salmon R. reach (K3)

In the Scott to Salmon reach, *C. shasta* was detected by QPCR in 14% (20/145, ci = 9-21%) of mixed-origin Chinook salmon (Appendix B). Infection prevalence was first detected in late May at 50%, and ranged from 40-50% throughout June. The prevalence decreased sharply to 12% in July and remained low for the remainder of the sampling period (Figure 6). Comparatively, *C. shasta* POI in natural fish (collected 5 May to 15 June) was 23% (9/40, ci = 11-39%) in this reach.

In this reach, *P. minibicornis* was detected by QPCR in 61% (43/70, ci = 49-73%) of mixed-origin Chinook salmon. Infection prevalence was first detected at 100% in late May, and remained at 100% through mid-June. The prevalence decreased to 40% in early July and reached 100% two weeks later (Figure 6). *Parvicapsula minibicornis* POI in natural fish sampled in this reach was 50% (20/40, ci = 34-66%).



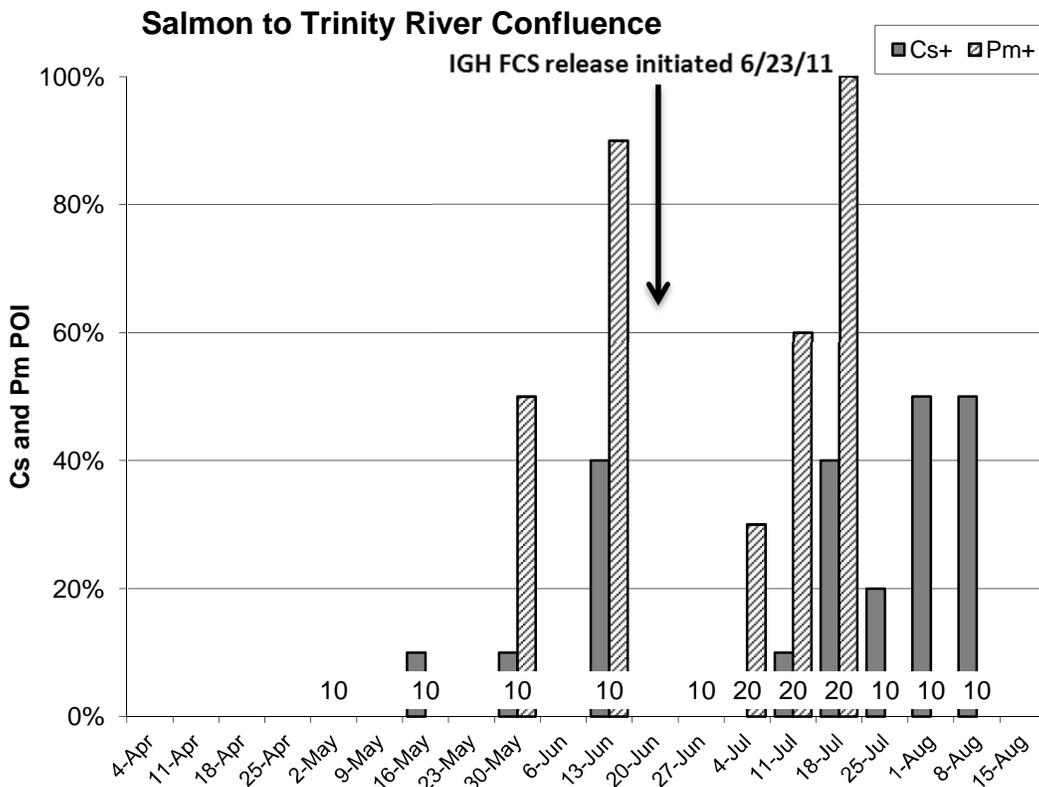
**Figure 6. Prevalence of *Ceratomyxa shasta* (Cs+) and *Parvicapsula minibicornis* (Pm+) infection by QPCR in juvenile Klamath River Chinook salmon captured in K3 reach on the Klamath River (Scott River to Salmon River). Sample numbers collected and tested for *Ceratomyxa shasta* each week are displayed at the bottom of each column, while sub-sample numbers for *Parvicapsula minibicornis* are listed in the sample table in Appendix C. *Ceratomyxa shasta* was not detected on 2 May, 16 May, 25 Jul, and 1 Aug.**

Histology sampling was not performed in this reach.

Salmon R. to Trinity R. reach (K2)

In the Salmon to Trinity reach, *C. shasta* was detected by QPCR in 20% (28/140, ci = 14-28%) of mixed-origin Chinook salmon. Infection prevalence was low (10%) in the samples collected in May, peaked in mid-June and mid-July at 40% and increased in August to 50% (Figure 7). In contrast to the larger mixed-origin group, *C. shasta* POI in natural Chinook salmon, collected 3 May to 23 June, was 15% (6/40, ci = 6-30%).

In the Scott to Trinity reach, *P. minibicornis* was detected by QPCR in 47% (33/70, ci = 35-59%) of mixed-origin Chinook salmon. Prevalence reached 90% by mid-June, decreased to 30% in early July, then rose steadily from early July (30%) to mid-July (100%) (Figure 7). *P. minibicornis* POI in natural fish, collected 3 May to 23 June, was 35% (14/40, ci =21-52%) in this reach.



**Figure 7. Prevalence of *Ceratomyxa shasta* (Cs+) and *Parvicapsula minibicornis* (Pm+) infection by QPCR in juvenile Klamath River Chinook salmon captured in K2 reach on the Klamath River (Salmon River to Trinity River). Sample numbers collected and tested for *Ceratomyxa shasta* each week are displayed at the bottom of each column, while sub-sample numbers for *Parvicapsula minibicornis* are listed in the sample table in Appendix C. *Ceratomyxa shasta* was not detected on 2 May, 27 Jun, and 4 Jul.**

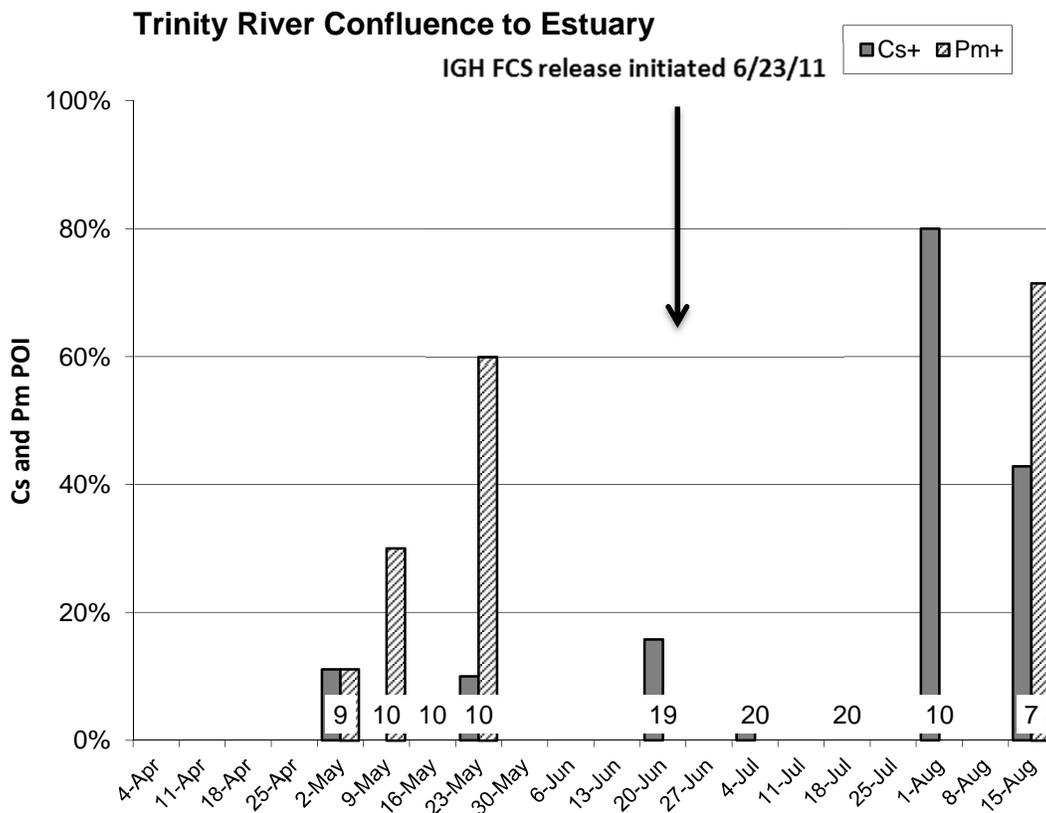
Histology sampling was not performed in this reach. A diagnostic examination was conducted in the Salmon to Trinity (K2) reach on 11 July. Moribund juvenile Chinook captured in the Big Bar rotary screw trap and beach seine were examined for diagnostic information. There was a low percentage of moribund fish in the catch with the leading causes of disease being lamprey wounds (69%, 6/9, ci = 30-

93%) and columnaris (22%, 2/9, ci =3-60%, Appendix D). Neither *C. shasta* or *P. minibicornis* were associated with morbidity.

Trinity R. to Estuary reach (K1)

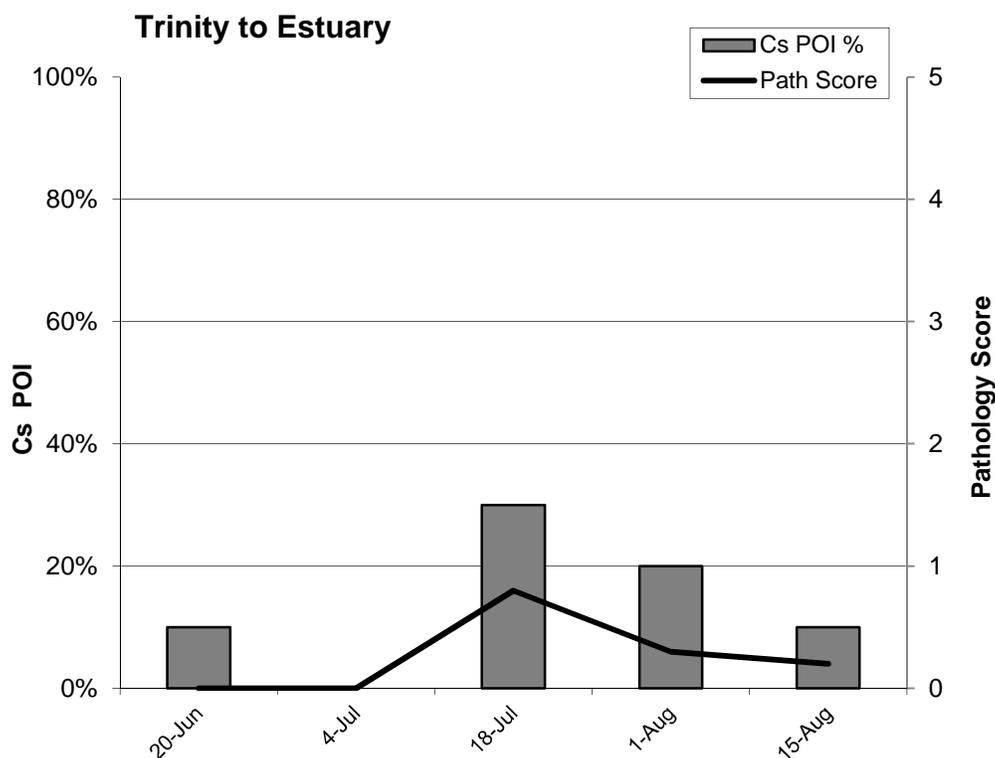
In the Trinity River to Estuary reach, *C. shasta* was detected by QPCR in 15% (17/115, ci = 9-22%) of mixed-origin Chinook salmon. Infection prevalence was low throughout most of the sampling period (10%-16%), peaked at 80% in early August, and then decreased to 43% on the last sample date of 15 August (Figure 8). *Ceratomyxa shasta* POI in natural fish sampled in this reach (4 May to 24 May) was expectedly lower at 5% (2/39, ci = 0.63-17%).

In the Trinity River to Estuary reach, *P. minibicornis* was detected by QPCR in 33% (15/46, ci = 20-48%) of mixed-origin Chinook salmon. Infection prevalence rose steadily in May (11-60%) and peaked at 71% in the last subset tested on 15 August (Figure 8). In natural fish, POI was 26% (10/39, ci = 13-42%).

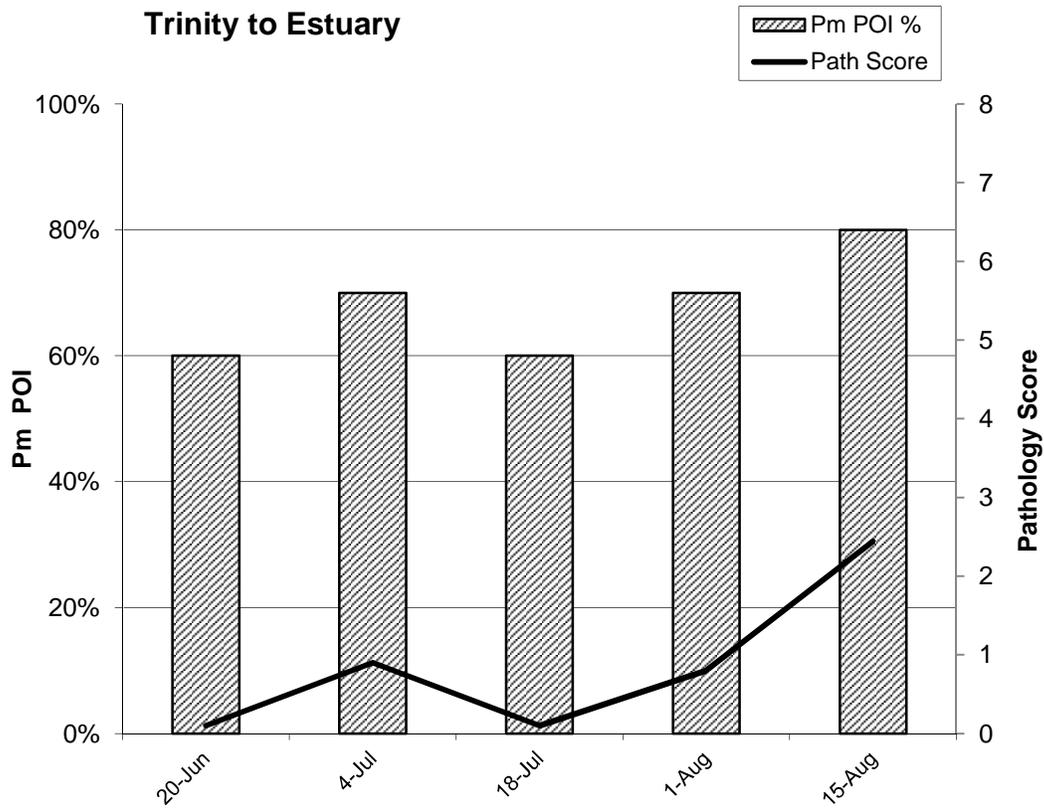


**Figure 8 . Prevalence of *Ceratomyxa shasta* (Cs+) and *Parvicapsula minibicornis* (Pm+) infection by QPCR in juvenile Klamath River Chinook salmon captured in K1 reach on the Klamath River (Trinity River to the Estuary). Sample numbers collected and tested for *C. shasta* each week are displayed at the bottom of each column, while sub-sample numbers for *P. minibicornis* are listed in the sample table in Appendix C. *Ceratomyxa shasta* was not detected on 9 May, 16 May, and 18 Jul.**

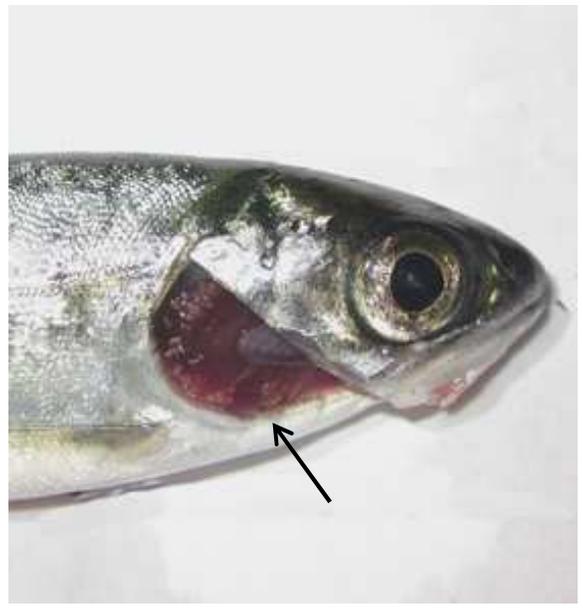
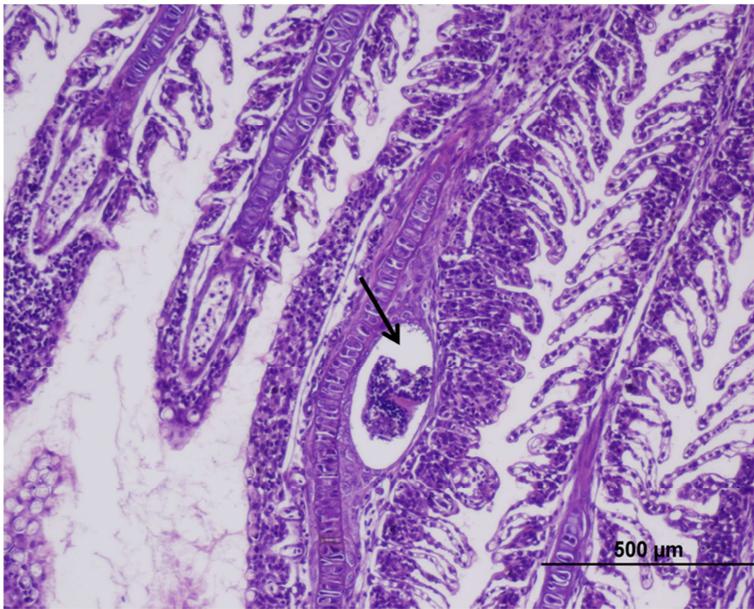
Five histology collections occurred between 20 June and 15 August for a total of 50 specimens (Figure 9 and Appendix A, Table A2). We considered these salmon to be of mixed hatchery and natural origin. *Ceratomyxa shasta* trophozoites were first observed in 10% of 20 June collection group, and peaked at 30% on 18 July (Figure 9). Overall prevalence of *C. shasta* detected by histology was 14% (7/50, ci = 6-27%). *Parvicapsula minibicornis* trophozoites were seen in 68% (34/50, ci = 33-81%) of fish collected (Figure 10). Prevalence peaked at 80% on 15 Aug, with a pathology score of 2.4. Kidney changes of affected fish included glomerular nephritis, interstitial hyperplasia, and tubular necrosis. Inflammation of visceral adipose tissue (steatitis) was a common observation (prevalence 60%). Gill sections had a 67% prevalence of metacercaria cysts (33/49, ci = 53-80%) that was associated with epithelial and cartilage hyperplasia (Figure 11). Multi-focal hyperplastic regions of gill epithelium were a common observation (80% POI) and not always associated with metacercaria.



**Figure 9. Bi-weekly prevalence of infection for *Ceratomyxa shasta* (Cs POI %) and mean pathology score (Path Score) by histology in juvenile Klamath River Chinook salmon captured in the Trinity River confluence to Estuary (K1) reach.**



**Figure 10. Bi-weekly prevalence of infection for *Parvicapsula minibicornis* (Pm POI %) by histology in juvenile Klamath River Chinook salmon captured in the Trinity River confluence to Estuary (K1) reach.**



**Figure 11. Metacercaria cyst (arrow) invoking epithelial hyperplasia and cartilage dysplasia (left image). Gross observation of metacercaria within gill lamellae (right image).**

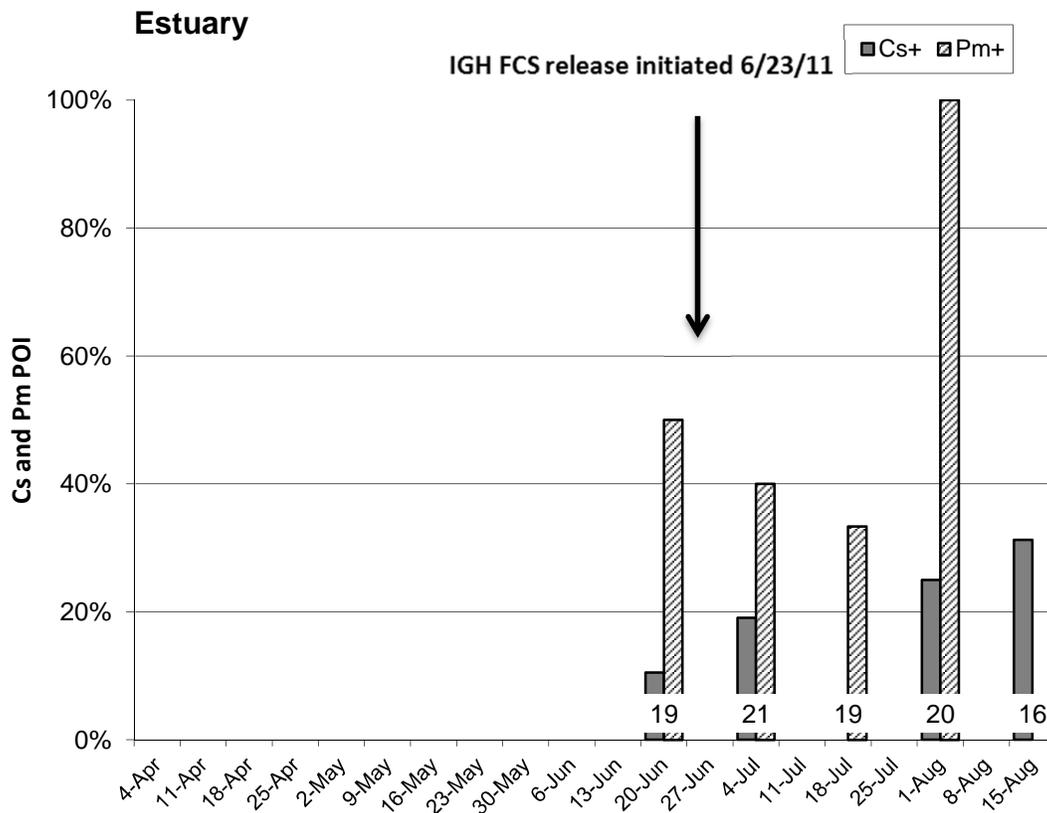
A diagnostic examination was conducted in the Trinity River to Estuary reach on 02 August. Moribund salmon collected by beach seine were diagnostically evaluated (Appendix D). Columnaris and metacercarial gill infection were the leading causes of morbidity.

**Klamath River Estuary (K0)**

In the Klamath River Estuary (K0) reach *C. shasta* was detected by QPCR in 17% (16/95, ci = 10-26%) of mixed origin Chinook salmon. Prevalence peaked over a two week period to 19% on 4 July, which included the first IGH CWT recovered from the Estuary. Prevalence decreased to 0% in mid-July, and *C. shasta* was detected again on 1 August at 25% and reached 31% by the end of the sampling period. (Figure 12).

*Parvicapsula minibicornis* was detected by QPCR in 55% (24/44, ci = 39-70%) of mixed origin Chinook salmon. Prevalence of infection decreased over a 5 week period (50%-33%), peaked at 100% on 1 Aug, and then was undetected on the last week of the sample period. *Parvicapsula minibicornis* POI in fish of unknown origin was 50% (9/18, ci = 26-74%) and similar to the larger mixed-origin group.

Histology sampling was not performed in this reach.



**Figure 12. Prevalence of *Ceratomyxa shasta* (Cs+) and *Parvicapsula minibicornis* (Pm+) infection by QPCR in juvenile Klamath River Chinook salmon captured in K0 reach on the Klamath River (Estuary). Sample numbers collected and tested for *C. shasta* each week are displayed at the bottom of each column, while sub-sample numbers for *Parvicapsula minibicornis* are listed in the sample table in Appendix C. Sample collection started the week of 20 June, therefore *C. shasta* was not detected on 18 July.**

### Marked (CWT) Chinook Salmon

The 25% constant fractional mark rate at Iron Gate Hatchery (Buttars and Knechtle, 2009) has permitted the capture of a large proportion of IGH CWT Chinook salmon in the past three years of the monitoring study. A total of 311 CWT Chinook salmon were collected this season in the Klamath River. IGH CWT fish accounted for 77% (241/311) and TRH CWT fish accounted for 16% (49/311) of all coded wire tagged fish collected (21 tags or 7% were unreadable). CWT Chinook provide a method of assessing temporal myxozoan infection level at weeks post hatchery release.

Historical data for *C. shasta* infection of IGH and TRH CWT detected by QPCR and histology are given in Table 5. Histology has been utilized as the metric for annual comparisons of disease prevalence (data confined to above the Trinity confluence and from the sampling period May-July). Concurrent testing with QPCR provides *C. shasta* POI data, quantitative assessment of parasite load within the fish in various reaches including the Estuary, and determines a relationship in CWT Chinook between WAL and infection intensity. We will transition to QPCR data as the annual metric for the monitoring program in 2011 due to the higher sensitivity by this method for early and/or low-level parasite infections and ability to quantify parasite levels in fish. Supplemental histology will still be performed for select reaches to assess tissue damage associated with clinical disease.

**Table 5. Historic annual prevalence of *Ceratomyxa shasta* infection (% positive), as diagnosed by histology and QPCR, in juvenile Chinook salmon collected from the Klamath main stem between Iron Gate Dam and Trinity River confluence during May through July, 1995-2011. Similar data is shown in columns 4 & 5 for coded-wire tagged (CWT) fish from each hatchery: Iron Gate Hatchery Chinook salmon captured in reaches above the confluence of the Trinity River (K5, K4 and K2) and Trinity Hatchery Chinook salmon (positive/total, (percent positive)) collected below the Trinity R. confluence (K1) and estuary (K0).**

Year	Chinook, May-July, Above TR Confluence (Percent Positive by Assay)		Iron Gate CWT-QPCR (Above TR confluence - reach K5, K4, and K2)	Trinity CWT- QPCR (Below TR confluence – reach K1/K0)
	Histology	QPCR		
2006	21	34	6/18 (33%)	1/67 (1%)
2007	21	31	15/22 (68%)	46/332 (14%)
2008	37	49	9/13 (69%)	8/257 (3%)
2009	54	45	82/228 (36%)	13/100 (13%)
2010	15	17	17/149 (11%)	1/45 (2%)
2011	2 <sup>2</sup>	17	22/176 (13%)	4/49(8%)
Average (SE)	25% (7)	32% (6)	25% (10)	9% (2)

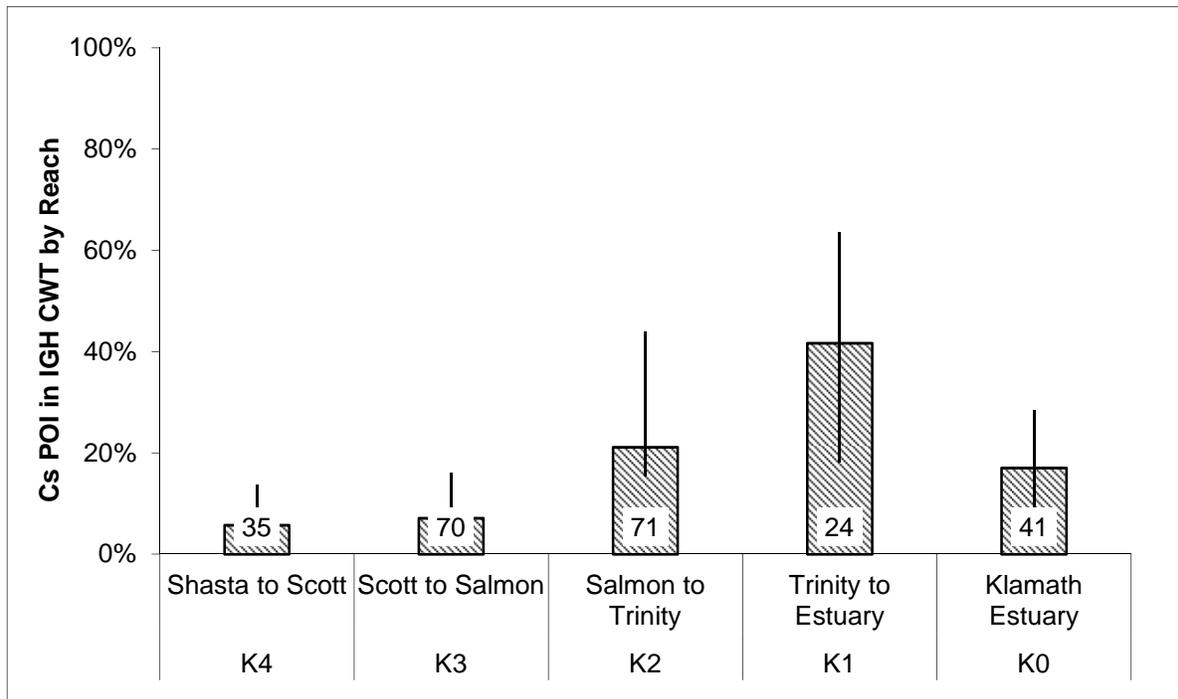
<sup>1</sup> NS= Not Sampled or QPCR data (2005) was not standardized as in subsequent study years. Only TR CWT Chinook salmon were assayed by histology in 2002 (*C. shasta* POI of 19%)

<sup>2</sup> Histology was limited to 2 reaches in 2011: Shasta to Scott (K4) and Trinity confluence to Estuary (K1).

Iron Gate Hatchery

Coded wire tagged salmon from IGH were collected from 28 June to 19 August. The largest proportions of IGH CWT Chinook salmon were recovered from the Scott River to the Salmon River (K3) and the Salmon River to the Trinity River confluence (K2) (Figure 13).

*Ceratomyxa shasta* was detected in 16% (39/241, ci = 12-32%) of marked IGH Chinook salmon screened by QPCR and 13% (22/176, ci = 8-18%) of IGH-CWT collected above the confluence of the Trinity River. *C. shasta* POI was highest in Chinook salmon recovered from the Trinity River confluence to Estuary reach (K1) at 42% (10/24, ci = 22-63%). *Ceratomyxa shasta* POI was low (<7%) in the upper reaches (Shasta to Scott and Scott to Salmon) in 2011. A similar trend of lower *C. shasta* POI in the upper reaches was reported in 2010.



**Figure 13. *Ceratomyxa shasta* prevalence of infection (POI) by QPCR in Iron Gate Hatchery CWT by reach in which marked Chinook salmon were recovered from. Whiskers indicate 95% confidence interval; sample numbers collected and tested for *C. shasta* each week are displayed at the base of each column, while sub-sample numbers for *Parvicapsula minibicornis* are listed in the sample table in Appendix C.**

*Parvicapsula minibicornis* was detected in 68% (66/97, ci = 58-77%) of marked IGH Chinook salmon screened by QPCR and 64% (50/78, 95% ci = 52-75%) of IGH-CWT collected above the confluence of the Trinity River.

## IGH CWT - Weeks At Large

### Iron Gate Hatchery

*Ceratomyxa shasta* parasite load, as determined by parasite DNA copy number, was highest in IGH CWT Chinook salmon residing for 5 weeks post hatchery release, or WAL (Figure 14). The average parasite copy number for infected fish was ~ 625 copies when prevalence of infection was over 30%. However, the highest mean *C. shasta* DNA copy number of 625 was low compared to levels measured from clinically moribund fish: which correlates to ~96,000 *C. shasta* DNA copy number or a  $C_T$  value of approximately 25 (True unpublished data).

Parasite levels of *C. shasta* in IGH CWT increased in the 3 WAL group, followed by a decrease in the 4 WAL group, while the prevalence remained relatively low. A second rise in parasite numbers occurred in the 5 WAL group, followed by a sharp decrease in DNA copy number in the 6 WAL group. The large rise in parasite number, followed by rapid decreases suggests that highly infected Chinook salmon are dropped out of the population between 3-4 and 5-6 WAL. Note that while *C. shasta* POI remained moderate at 33-36% in the 6-7 WAL group, the parasite copy number was negligible indicating low level infections in these fish. Sample size for 6 WAL was notably small (3 fish) and only one of these three fish recovered was positive for *C. shasta*. But the trend holds true at 7 WAL with a sample size of 14 fish. We observed a similar pattern in 2010 data with bimodal peaks in parasite DNA copy number at 3 WAL, and then again at 5-6 WAL. The peak at three weeks post release is highly supported by sentinel and prognosis studies that demonstrate the temporal pathology/mortality associated with *C. shasta* at river temperatures common to the Klamath at this time of year.

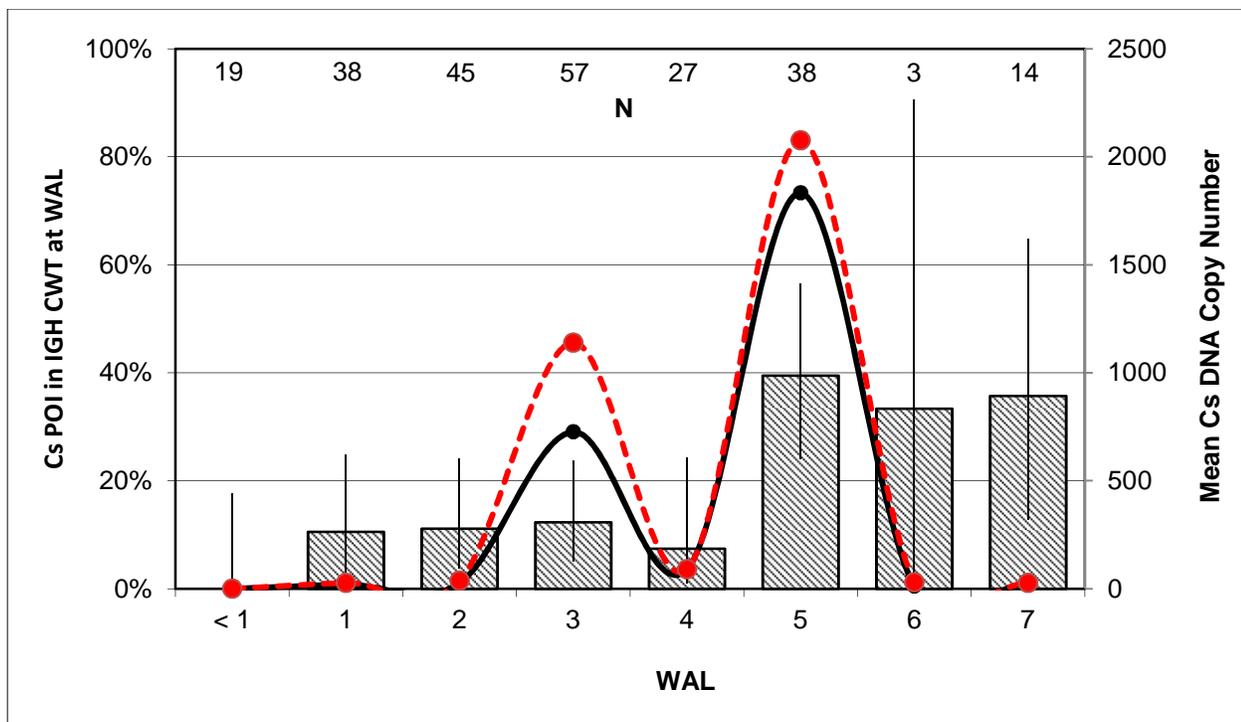


Figure 14. *Ceratomyxa shasta* prevalence of infection in IGH CWT by Weeks At Large (WAL) post hatchery release. Lines (dashed red) are the mean *C. shasta* DNA copy number for Chinook salmon testing positive by QPCR, and (solid black) mean DNA copy number for all Chinook salmon tested by QPCR.

The relatively low parasite infectious load observed for *C. shasta* in IGH CWT did not appear to hold true for *P. minibicornis* POI at WAL. For *P. minibicornis* in IGH-CWT Chinook salmon the parasite load was the highest for 3 WAL and consisted of parasite copy numbers above 22,000 copies (Figure 15). Prevalence of infection is high (95-100%) in 3, 4, and 5 WAL; however the parasite infectious load decreased after 3 WAL. We observed a lag between the peak of the parasite infectious load and infection prevalence. This could be explained by new fish becoming infected in 4 and 5 WAL, which would decrease the parasite load but increase POI to 100%.

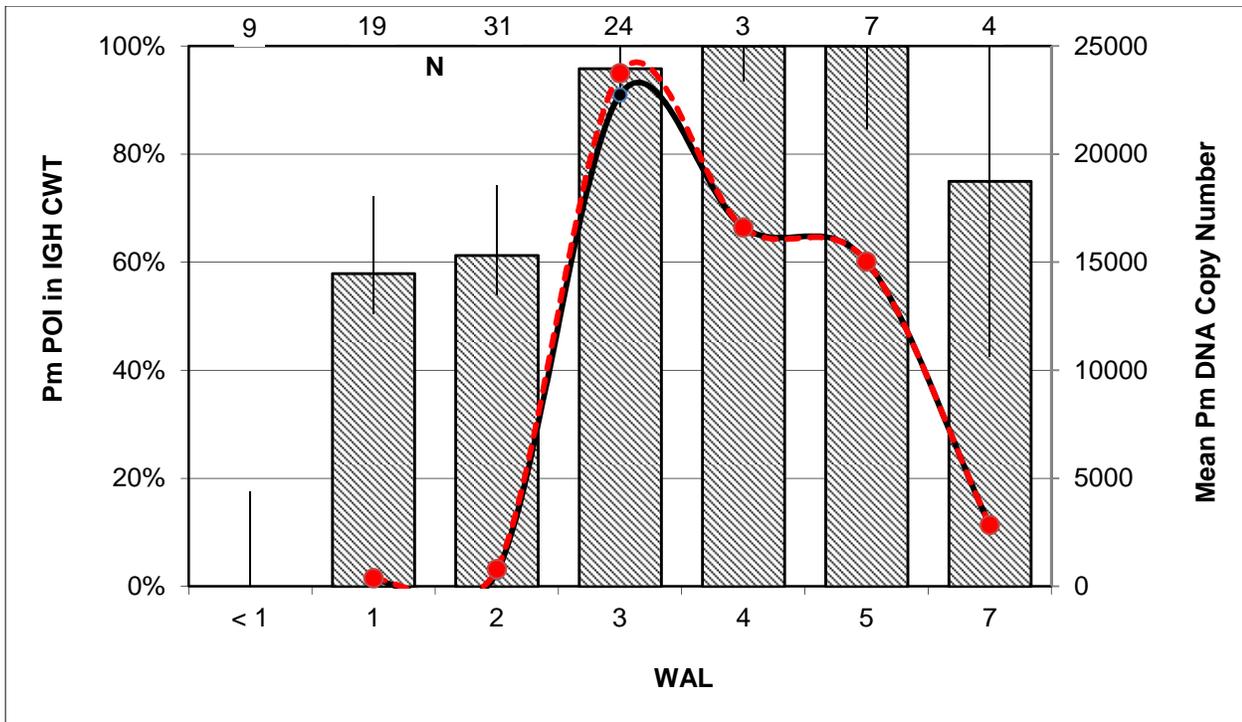


Figure 15. *P. minibicornis* prevalence of infection in IGH CWT by Weeks At Large (WAL) post hatchery release. Lines (dashed red) are the mean *P. minibicornis* DNA copy number for Chinook salmon testing positive by QPCR, and (solid black) mean DNA copy number for all Chinook salmon tested by QPCR.

## Trinity River Disease Monitoring

### Upper Trinity River (T2)

In the upper Trinity River reach (T2), *C. shasta* was detected by QPCR in 1% (1/83, ci = 0.03-7%) of mixed-origin Chinook salmon. The one positive sample was a natural fish collected 27 April with a  $C_T$  value of 38.4 and corresponds to a very low parasite copy number (3.8 DNA copy numbers respectively).

In the upper Trinity River reach, *P. minibicornis* was detected by QPCR in 4% (1/26, ci = 0.10-20%) of mixed-origin Chinook salmon. The one positive sample was a fish of unknown origin collected 17 June with a  $C_T$  value of 36.0 with a DNA copy number of 161.

Histologically, two weekly collections occurred on 25 April and 23 May for a total of 20 samples (Appendix A, Table A3). Both collection groups were considered of natural origin given TRH releases started on 1 June. *Parvicapsula minibicornis* trophozoites were seen in 15% of the fish collected (3/20 ci = 3-38%). The pathology score for both kidney and the intestinal tract in T2 were zero. A presumptive amoeba infection of the intestine was seen in one 23 May sample (Figure 16).

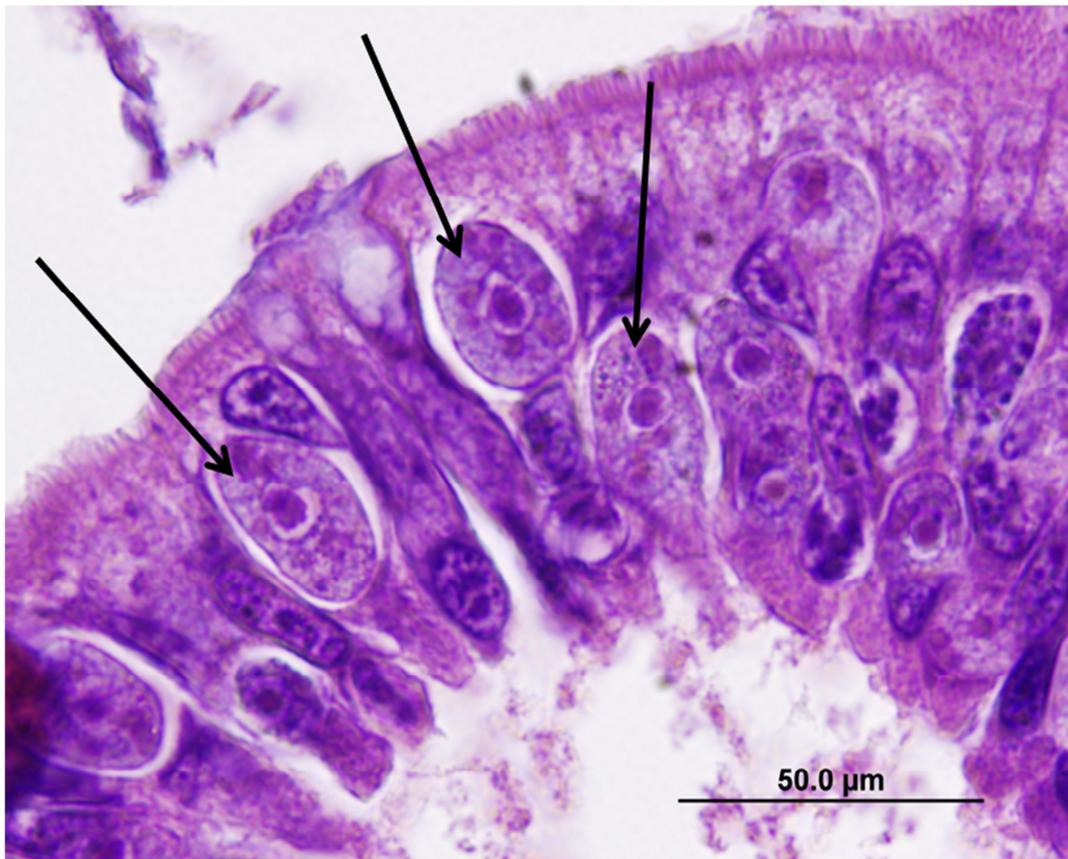


Figure 16. Presumptive amoeba infection of Trinity R. Chinook intestinal epithelium.

### Lower Trinity River (T1)

In the lower Trinity River reach (T1), *C. shasta* (0/111) and *P. minibicornis* (0/10) were not detected by QPCR or histology in mixed-origin Chinook salmon. However, *Chloromyxum* infection of the kidney (15%) and glochidia infection of the gill (25%) were detected in histology sections from both the 23 May and 01 August collections.

*Ceratomyxa shasta* was detected in 2.4% (4/166, ci = 0.6-6%) of the marked TRH Chinook salmon screened by QPCR. Parasite DNA levels (mean of ~8900 copies) were higher than those seen in IGH Chinook salmon due to two fish with high parasite loads that had been residing for 10 WAL upon recapture in the Trinity to Estuary reach. The POI for 10 WAL was 50%.

*Parvicapsula minibicornis* was detected in 17% (2/12, ci = 2-48%) of all marked TRH Chinook salmon screened by QPCR. The two positive fish were collected at 10 WAL. The POI for 10 WAL group was 50%, but the sample size was notably small with a total of four fish collected. Similar to *C. shasta* in TRH CWT Chinook salmon, the parasite load was high (mean of 4200 copies). Therefore, the same two fish had high parasite loads for both *C. shasta* and *P. minibicornis*.

### **TRH CWT - Weeks At Large**

#### Trinity River Hatchery

In the Trinity River (T1, T2), *C. shasta* was not detected. Historically, *C. shasta* POI of TRH CWT fish in the lower reaches of the Klamath River is lower than fish released from IGH because they are not exposed to the infectious zone of the Klamath River (Table 5).

CWT salmon from TRH were collected from 17 June through 18 August in the Trinity River and 6 July to 19 August in the lower Klamath River. The largest proportion of Trinity River Hatchery CWT Chinook salmon were recovered from the Willow Creek rotary screw trap in the lower Trinity River reach (T1), accounting for 57% (94/166) of all TRH CWT.

In marked TRH Chinook, *C. shasta* was detected in 2% (4/166, ci = 0.7-6%) of all salmon screened by QPCR (compared to Table 5 which is restricted to below the Trinity River confluence). *Ceratomyxa shasta* POI was similar in Chinook salmon recovered from the Trinity to Estuary reach (K1) at 7% (2/28, ci = 0.88-24%) and the Estuary reach (K0) at 10% (2/21, ci = 1-30%) (Figure 17).

*Parvicapsula minibicornis* was detected in 17% (2/12, ci = 2-48%) of marked TRH Chinook salmon screened by QPCR.

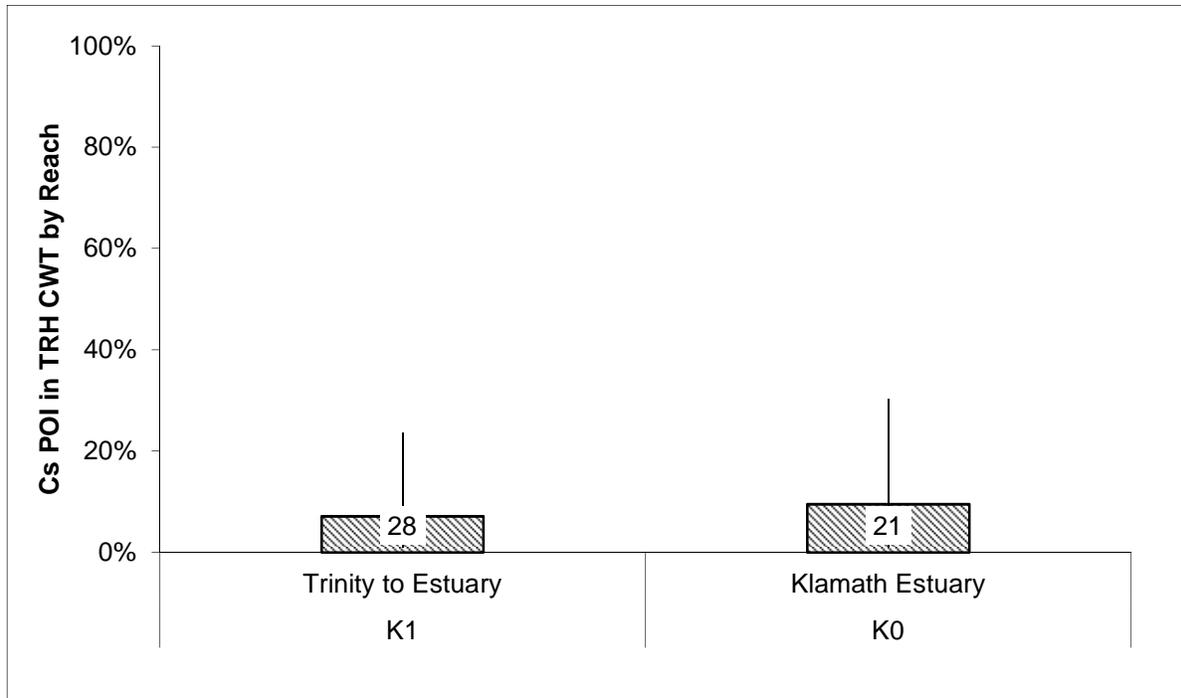


Figure 17. *Ceratomyxa shasta* prevalence of infection (POI) by QPCR in Trinity River Hatchery CWT by reach in which marked Chinook salmon were recovered from. Whiskers indicate 95% confidence intervals. Sample numbers collected and tested for *C. shasta* CWT are displayed at the base of each column. *Ceratomyxa shasta* was not detected in the Trinity River (T1 and T2 reach).

## Environmental Conditions

### Temperature

In previous study years, we typically observed temperatures above 18°C (and often as high as 22°C) approximately one month earlier than occurred in 2011. For the past two years, we have observed cooler air as well as river temperatures in the Klamath River than in previous years (2006-2009). In 2011, river temperatures in May and June ranged from 10-19°C below Iron Gate dam. From late June to early September river temperatures were consistently over 18°C (Figure 18). A similar trend was seen downstream, 60 RM, at Seiad valley. Temperatures were low in May and June (10-17 °C) and reached over 18°C in early July (Figure 19).

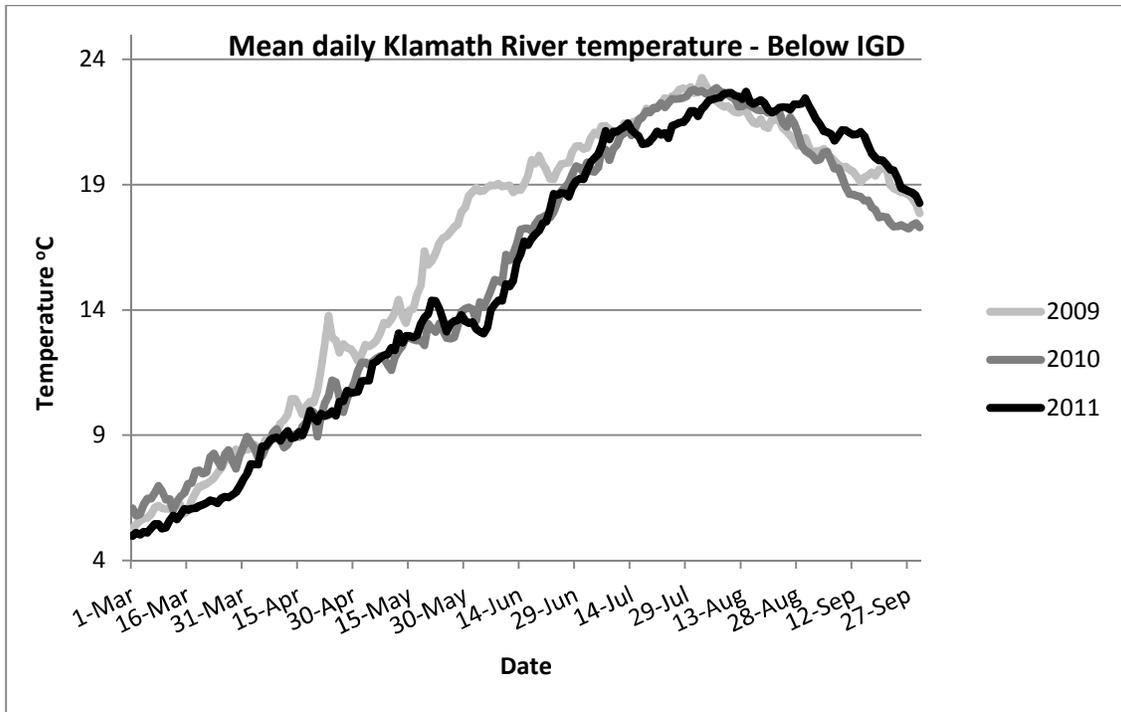


Figure 18. Mean daily temperature below Iron Gate Dam for 2009, 2010, and 2011. Temperature data acquired from Arcata Fish and Wildlife Field Office.

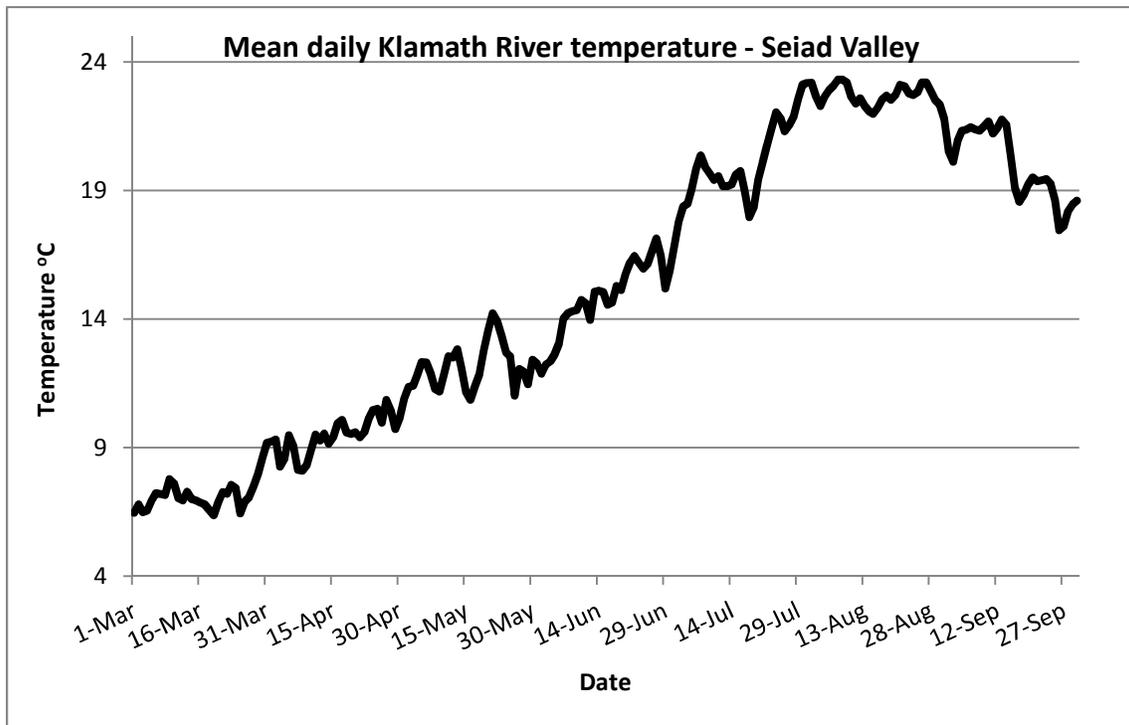


Figure 19. Mean daily temperature from March through September 2011 at Seiad Valley. Temperature data acquired from Arcata Fish and Wildlife Field Office.

## River Flows

Klamath River flows increased from approximately 1600 cubic feet per second (cfs) to over 4000 cfs as a manipulated pulse flow event in February 2011 (Figure 20). The Bureau of Reclamation increased flows on the Klamath River from Iron Gate Dam to benefit coho salmon as part of the 2010 National Marine Fisheries Service (NMFS) Biological Opinion. The flows ramped up to 4000 cfs for approximately 6 hours and then slowly ramped down to approximately 2000 cfs. The most significant increase in river flows occurred just downstream of Iron Gate Dam and occurred from noon to midnight on February 9, 2011. (BOR press release: <http://www.usbr.gov/newsroom/newsrelease/detail.cfm?RecordID=35085>).

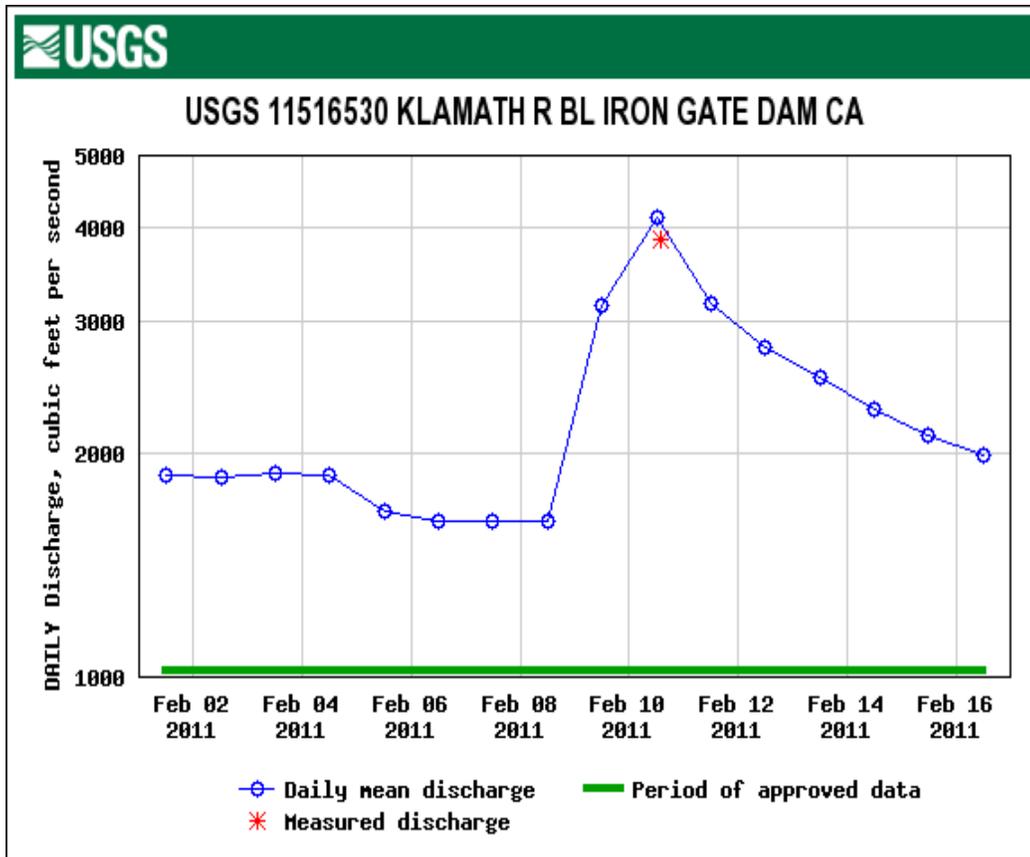


Figure 20. 2011 Pulse Flow, Feb 9-10, from below Iron Gate Dam. Data acquired from USGS [waterdata.usgs.gov](http://waterdata.usgs.gov).

This 2011 pulse flow was the highest manipulated flow event since 2006 (Figure 21), however natural precipitation events in 2011 were higher than the pulse flow in both magnitude and duration. Flows were above 3000 cfs from approximately mid-March to mid-June in 2011 (Figure 22). Weather conditions in 2011 produced not only higher river flows than typically observed in the Klamath River, but river mean and daily maximum temperatures were also lower for the majority of the period of juvenile emigration (May to July). In 2010, we also observed decreased river temperatures for an extended period into May and June. *Ceratomyxa shasta* POI, above the Trinity confluence from May to July, was similarly one of the lowest levels (17%) observed during the past 6 years of the juvenile Chinook monitoring studies.

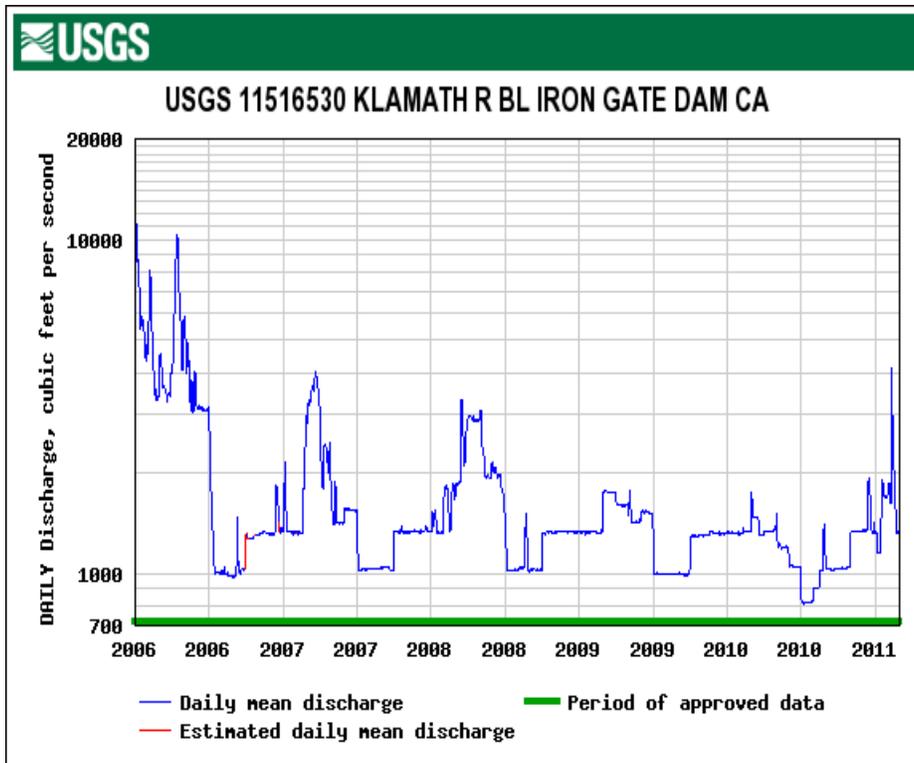


Figure 21. Daily discharge below Iron Gate Dam 2006-2011. Data acquired from USGS waterdata.usgs.gov.

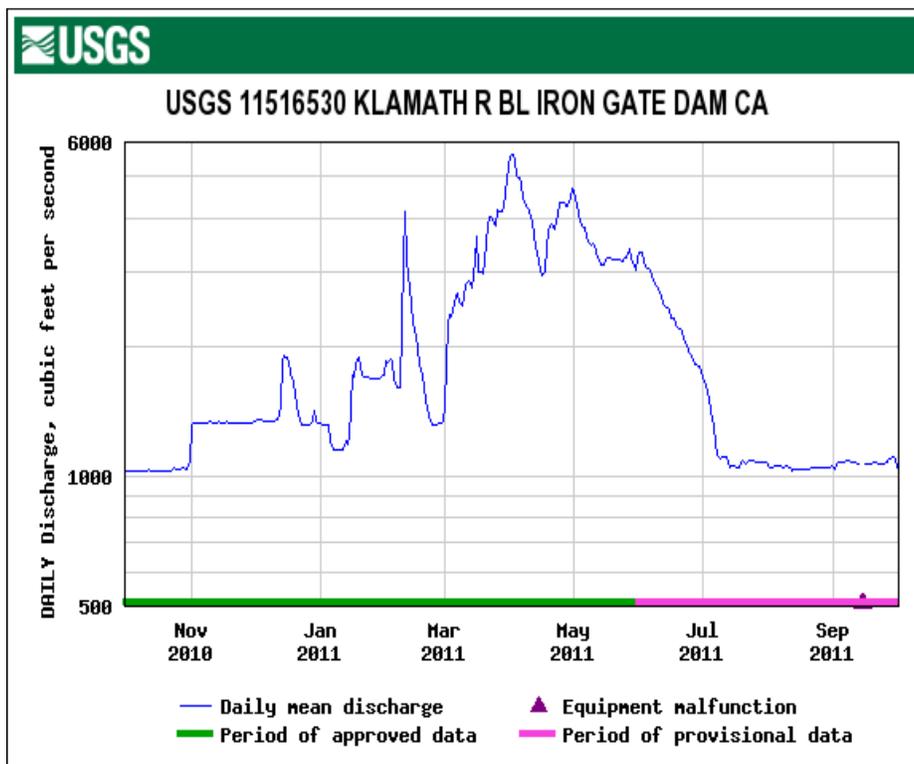


Figure 22. Daily discharge below Iron Gate Dam from Nov 2010 to September 2011 (Provisional data from mid-June through September. Data acquired from USGS waterdata.usgs.gov.

## DISCUSSION

The prevalence of *C. shasta* and *P. minibicornis* infections in juvenile Chinook salmon have been monitored in fish health studies in the Klamath River since 2004 (Nichols and Foott 2006; Nichols et al. 2007; Nichols and True 2007; Nichols et al. 2008, True et al 2010). Histological assessment of *C. shasta* POI in Chinook salmon captured above the Trinity River confluence has been the metric used to compare annual disease prevalence in the Klamath River. Annual comparisons are limited to May through July: the peak juvenile Chinook salmon migration period for Klamath River Chinook salmon (Leidy & Leidy 1984, Wallace & Collins 1997). Histological assessments, along with complimentary QPCR assays, provide a degree of temporal and spatial information for prevalence of infection over the 20 week study period, and for specific reaches of the Klamath River within the “infectious zone”. Temporal data is also derived from CWT Chinook salmon, with known exposure periods based on hatchery release and in-river recapture dates (Weeks At Large). Spatial data is provided as weekly prevalence of infection in the major reaches of the Klamath River, as juvenile Chinook salmon migrate towards the estuary.

Infectivity patterns for *C. shasta* infections 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, there is generally a three week period from initial parasite exposure to development of clinical disease that results in moderate to high levels of mortality. This infectivity pattern has been established through sentinel susceptibility studies (Bartholomew 2010, Bjork and Bartholomew 2010, Stone et al. 2008, True et al. unpublished data) and annual monitoring of CWT Chinook salmon with known exposure periods in the main stem Klamath (Nichols and Foott 2006, Nichols et al. 2007, Nichols and True 2007, Nichols et al. 2009, True et al 2010). This infectivity pattern is usually apparent in the majority of reaches as a bimodal distribution in bi-weekly prevalence of infection data: natural Chinook salmon sampled prior to hatchery releases, and in mixed-origin Chinook salmon collected from June to August. This *C. shasta* infectivity pattern is also observed in Iron Gate Hatchery CWT Chinook salmon, Weeks At Large data.

Despite well-defined infection patterns for Klamath River salmonids, predictions for quantitative myxozoan disease impacts on the population level are difficult to make in an actively emigrating juvenile Chinook population particularly when environmental factors, such as river temperature and flows, influence disease progression and severity. For ceratomyxosis, the primary factors for the fish host include: species and individual fish susceptibility (Zinn 1977, Buchanan 1983, Ibarra et al. 1992, Bartholomew 1998,), parasite exposure dose (frequency and duration) (Ratcliff 1981, Bjork & Bartholomew 2009b), and water temperature (Ray et al. 2011, Udey et al. 1975, Bartholow 2005). Temperature is extremely important in regulating fish metabolism (immune response and energy metabolism), as well as polychaete and parasite development (Ratcliff 1983, Foott et al. 2004, Bartholomew 2006, Meaders and Hedrickson 2009). Main stem and tributary flows influence polychaete abundance and maturation, and the level of parasite infection within the worm ( Bjork and Bartholomew 2009b, Stocking et al. 2006,) as well as migration behavior of juvenile Chinook salmon (timing, rate, utilization of tributaries and/or thermal refugia) (Harmon et al. 2001).

In 2011, *C. shasta* prevalence of infection detected by histology was 2% and QPCR was 17%. Histology was limited to select reaches and therefore is not representative of the overall Chinook population sampled during the peak migration period, or of fish sampled above the Trinity River confluence. QPCR data will replace histology as an annual prevalence of infection metric in future monitoring studies as it offers two important advantages of higher sensitivity to early or low level infections and a quantitative assessment of parasite levels within the fish. We will continue to supplement QPCR testing with histology for key reaches, such as the Shasta to Scott (K4) and Trinity to Estuary (K1), and also to utilize the strength of this assay to assess tissue damage leading to a disease state in juvenile Chinook salmon.

In the Shasta to Scott (K4) reach, we primarily sampled natural fish due to the later release of Iron Gate Hatchery fish (23 June) that occurred in 2011. Natural fish are sampled in cooler months (Apr-May) and therefore are generally expected to have lower disease progression/prevalence compared to hatchery Chinook sampled in June and July. Normally we observe a bimodal distribution of *C. shasta* POI in natural and hatchery fish, but the natural component becomes masked once IGH releases 5 million smolts in late May to early June. In 2011, we were able to observe the true peak of natural Chinook infection that occurred 20 June (60%) as well as the peak for hatchery Chinook that occurred 11 July (33% at 3 WAL). IGH Chinook actually had lower *C. shasta* POI than natural fish in 2011, despite sampling later in the season. *Ceratomyxa shasta* POI by histology was also clearly bimodal with peaks in pathology scores occurring on 20 June (natural Chinook) and 18 July (3 weeks post IGH Chinook releases).

In the Scott to Salmon reach (K3), *C. shasta* was first detected in late May; POI increased to 40-50% through June, and then decreased sharply to 12% in July following hatchery releases. *Ceratomyxa shasta* POI remained low for the remaining 4 weeks of sampling that occurred in this reach, which is normally highly infectious. In the Salmon to Trinity confluence reach (K2) reach, *C. shasta* POI was 20% and closely resembled *C. shasta* POI in adjoining reaches. Bimodal distribution was apparent for natural Chinook with a peak *C. shasta* POI on 13 June, and 18 July for hatchery Chinook (4 WAL). In the Trinity River confluence to Estuary (K1) reach, *C. shasta* POI was low (15%) throughout the sampling period of May- July. Only on 1 Aug and 15 Aug did prevalence of infection rise to 80% and 45% respectively. Prevalence of *C. shasta* in this reach often is indicative of the time required for this parasite infection to fully develop as clinical disease in juvenile Chinook (rather than indicative of infectivity occurring within the reach itself). The reduction in *C. shasta* prevalence of infection in 2011 was not simply a result of delayed disease onset, as we observed previously in 2006 and 2008 monitoring studies, but rather represents both a reduction of parasite abundance in the environment and infection levels within juvenile fish. Histology confirmed the onset, and low *C. shasta* prevalence of infection (14%) as well as low severity of infection for fish captured in this reach. The observed peak of infection in early to mid-August was at least one month later than normally observed in emigrating juvenile Chinook and pathology scores observed (0.5) were very low.

Coded-wire tagged Chinook comprised 52% of all fish sampled, and provide our best source of infectivity data regarding fish groups. CWT fish with known residence period post hatchery release showed a similar trend of low infectivity and infection prevalence in 2011. *Ceratomyxa shasta* IGH CWT prevalence of infection, as well as parasite infection load (DNA copy number) was highest at 5 WAL. Prevalence remained moderate in the following weeks, while parasite infection load was minimal. We observed a similar pattern in 2010 data with bimodal peaks in parasite DNA copy number at 3

WAL, and then again at 5-6 WAL. The peak at three weeks post release is highly supported by sentinel and prognosis studies that demonstrate the temporal pathology/mortality associated with *C. shasta* at river temperatures common to the Klamath at this time of year. The second peak at 5 WAL is more difficult to assess. It is possible that fish captured at 6-7 WAL may have reared in the tributaries and then out-migrated to the Klamath River main stem later than their cohorts. Past monitoring of tributary Chinook salmon has shown negligible *C. shasta* infections in fish that do not rear in the Klamath main stem. If subsets of juvenile Chinook truly are rearing in tributaries and migrating to the Estuary later than their cohorts, this produces a complex epidemiological picture for the basin's Chinook salmon populations, with regard to main stem exposure period(s).

The *C. shasta* POI was 14% above the Trinity River confluence and 18% below (K1 reach). Normally, the Trinity River has a moderating effect on *C. shasta* prevalence of infection for two reasons: non-infected TRH Chinook may be included in fish sampled and Trinity River flow accretions dilute the concentration of infectious actinospores in the water column. It is notable that the trend was not observed 2011; *C. shasta* prevalence of infection above the confluence (14%) and below (18%) appears to be similar, or possibly reversed. Because not all fish are randomly collected from rotary screw traps, t-tests to assess POI differences between reaches are not appropriate. Also, the recent findings (past two years) of high *C. shasta* spore concentrations near Tully Creek may not sustain this trend of lower infectivity below the Trinity River confluence in future monitoring studies.

*Ceratomyxa shasta* prevalence of infection observed in Klamath River juvenile Chinook corroborated Oregon State University's water testing results for 2011, where the majority of sampling sites were found to have less than 1 spore/L concentrations of the infectious stage present in the water column (the exception was Tully Creek). Past OSU studies have demonstrated an exposure dose of ~ 10 spores/L results in significant (>50%) mortality in 72 hour sentinel fish. Sentinel studies conducted above Beaver Creek in June 2011 resulted in mortality of 17% (fish held at 18°C post exposure) which is much lower than typically observed in sentinel exposures at this index site (Bartholomew et al. 2012). Other supportive evidence of reduced myxozoan infectivity and disease prevalence was observed for *Parvicapsula minibicornis*, which was at notably lower prevalence in 2011, ranging from 41-61% depending on reach, compared to 80-100% in previous monitoring years (2006-2010).

2011 marked the second consecutive year where climatic/environmental conditions in the Klamath basin were quite favorable for juvenile Chinook in terms of ceratomyxosis. In 2010, we observed reduced river temperatures and subsequently low *C. shasta* prevalence of infection (17%). In 2011, river conditions were characterized by reduced air and river temperatures, in addition to a manipulated pulse flow of ~ 4000 cfs in February, followed by continued heavy precipitation and high flows (>3000cfs) that extended well into mid-June. The resulting mean river temperatures were below 14°C through mid-June, did not reach 18°C until the end of June, and were typically 2-4 degrees below average temperatures for the basin through the end of July. Not only was the prevalence of *C. shasta* infection low, but the actual parasite levels within fish were lower in DNA copy number, even in the most infectious reaches below Iron Gate Dam.

River temperatures and flows are both important considerations in assessing disease impacts on juvenile Chinook salmon in a given study year. In 2006, we observed that a large precipitation and flow event shifted the disease onset and peak by approximately 2-3 weeks towards the later migration period. However, *C. shasta* prevalence of infection decreased temporarily and the overall annual prevalence of

infection was not substantially lower in 2006 compared 2007 when no significant flow events occurred. Despite a 3 week later release date in 2011, juvenile IGH Chinook salmon experienced lower *C. shasta* infection levels in all reaches sampled and lower annual prevalence of infection than previously observed. It is apparent that reduced river temperatures, coupled with higher and sustained flows in May to June of 2011 produced beneficial conditions for juvenile Chinook salmon.

Cooler spring/early summer temperatures and higher flows through mid-June, likely reduced the number of polychaetes, the number of actinospores released, or the effectiveness of the spore in attaching and proliferating within the fish host. 2011 represented an environmental year where both temperature and flows were favorable for Chinook salmon, compared to 2010 where temperature alone appeared to reduce disease prevalence significantly. It will be interesting to see how disease prevalence is affected in future years where one or both of these key environmental variables (cooler river temps and increased flows) are in play; and if we can discern and begin to quantify which environmental factor is more important in disease development and mortality.

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## **Author Roles**

The contributions of each author have been summarized below.

- Anne Bolick – Data management, necropsy, DNA extraction, QPCR assay, pivot tables and report figures, finalizing reviewer comments.
- Kimberly True – Project coordination, data management and quality control, QPCR methods and quality assurance, data analysis and final editing of report.
- Scott Foott – Histology, diagnostic assessments, and histological report sections.

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## APPENDIX A – Histological Summary Table

**Table A1. Parasite prevalence of infection (POI) and tissue (no. positive / total (%)) and pathology score for kidney and intestine 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. Period prevalence (POI) is also reported for the natural, unknown origin, and mixed origin groups (all).**

	4/11	4/18	4/25	5/02	5/09	5/16	5/23	5/30	6/06
<b>Kidney</b>									
Pm Troph.	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	1 / 10 (10)	1 / 10 (10)	3 / 10 (30)	5 / 10 (50)	8 / 10 (80)
Pm Myxosp.	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)
Metacercaria	1 / 10 (10)	1 / 10 (10)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)
<i>C. shasta</i> troph.	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)
<i>Chloromyxum</i> sp	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)
<b>Pathology Score</b>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.40	0.40
<b>Intestinal tract</b>									
<i>C. shasta</i> troph.	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	1 / 10 (10)	0 / 10 (0)	1 / 10 (10)	0 / 10 (0)	0 / 9 (0)
<i>C. shasta</i> myxosp.	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 9 (0)
Helminth	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 9 (0)
<b>Pathology Score</b>	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.00
Adipose steatitis	1 / 9 (11)	2 / 7 (29)	3 / 7 (43)		2 / 4 (50)	4 / 10 (40)	4 / 10 (40)	4 / 8 (50)	
Adipose lipofuscin	0 / 9 (0)	0 / 7 (0)	0 / 7 (0)		0 / 4 (0)	0 / 10 (0)	0 / 10 (0)	0 / 8 (0)	2 / 9 (22)
Liver <i>C. shasta</i>	ND	ND	ND		ND	ND	ND	ND	0 / 9 (0)
<b>Gill</b>									
Ich	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 9 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)
Glochidia	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 9 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)
Miracidia	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 9 (0)	0 / 10 (0)	0 / 10 (0)	1 / 10 (10)
Metacercaria	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	1 / 9 (11)	2 / 10 (20)	1 / 10 (10)	4 / 10 (40)
Invasive <i>C. shasta</i>	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 9 (0)	0 / 10 (0)	1 / 10 (10)	0 / 10 (0)
Multif. Hyperplasia	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	4 / 10 (40)	2 / 9 (22)	4 / 10 (40)	2 / 10 (20)	7 / 10 (70)

**Table A1 continued**

	6/13	6/20	6/27	7/04	7/11	7/18	POI of Natural (11 Apr-20 Jun)	POI of Unknown Origin (27 Jun – 18 Jul)	POI of Mixed Origin (all)
<u>Kidney</u>									
Pm Troph.	9 / 10 (90)	10 / 10 (100)	0 / 10 (0)	0 / 10 (0)	5 / 10 (50)	6 / 10 (60)	36 / 110 (33)	11 / 40 (28)	48 / 150 (32)
Pm Myxosp.	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 110 (0)	0 / 40 (0)	0 / 150 (0)
Metacercaria	1 / 10 (10)	0 / 10 (0)	0 / 10 (0)	2 / 10 (20)	0 / 10 (0)	2 / 10 (20)	3 / 110 (3)	4 / 40 (10)	7 / 150 (5)
<i>C. shasta</i> troph.	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	1 / 10 (10)	0 / 10 (0)	0 / 110 (0)	1 / 40 (3)	1 / 150 (1)
<i>Chloromyxum</i> sp	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 110 (0)	0 / 40 (0)	0 / 150 (0)
<b>Pathology Score</b>	1.00	1.90	0.00	0.00	0.60	1.60			
<u>Intestinal tract</u>									
<i>C. shasta</i> troph.	0 / 9 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	1 / 10 (10)	0 / 10 (0)	2 / 108 (2)	1 / 40 (3)	3 / 148 (2)
<i>C. shasta</i> myxosp.	0 / 9 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 108 (0)	0 / 40 (0)	0 / 148 (0)
Helminth	1 / 9 (11)	0 / 10 (0)	0 / 10 (0)	1 / 10 (10)	1 / 10 (10)	4 / 10 (40)	1 / 108 (1)	6 / 40 (15)	7 / 148 (5)
<b>Pathology Score</b>	0.00	0.00	0.00	0.60	0.60	0.00			
Adipose steatitis	4 / 8 (50)	7 / 8 (88)	1 / 10 (10)	2 / 3 (67)	5 / 6 (83)	4 / 5 (80)	33 / 80 (41)	12 / 24 (50)	45 / 104 (43)
Adipose lipofuscin	0 / 8 (0)	1 / 8 (13)	0 / 10 (0)	1 / 3 (33)	0 / 6 (0)	1 / 5 (20)	1 / 80 (1)	2 / 24 (8)	3 / 104 (3)
<u>Gill</u>									
Ich	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 109 (0)	0 / 40 (0)	0 / 149 (0)
Glochidia	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 109 (0)	0 / 40 (0)	0 / 149 (0)
Miracidia	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 109 (0)	0 / 40 (0)	1 / 149 (1)
Metacercaria	7 / 10 (70)	10 / 10 (100)	0 / 10 (0)	6 / 10 (60)	5 / 10 (50)	7 / 10 (70)	25 / 109 (23)	18 / 40 (45)	43 / 149 (29)
Invasive <i>C. shasta</i>	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	1 / 10 (10)	0 / 10 (0)	0 / 109 (0)	1 / 40 (3)	2 / 149 (1)
Multif. Hyperplasia	9 / 10 (90)	10 / 10 (100)	4 / 10 (40)	9 / 10 (90)	8 / 10 (80)	9 / 10 (90)	38 / 109 (35)	30 / 40 (75)	68 / 149 (46)

**Table A2. Parasite prevalence of infection (POI) and tissue (no. positive / total (%)) and pathology score for kidney and intestine observed in histological sections of juvenile Klamath River Chinook salmon collected from the Trinity River confluence to Estuary reach (K1). Collection dates are reported as Monday of given week. Overall incidence of infection also reported.**

	6/20	7/04	7/18	8/01	8/15	POI
<u>Kidney</u>						
Pm Troph.	6 / 10 (60)	7 / 10 (70)	6 / 10 (60)	7 / 10 (70)	8 / 10 (80)	34 / 50 (68)
Pm Myxosp.	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 50 (0)
Metacercaria	0 / 10 (0)	2 / 10 (20)	5 / 10 (50)	3 / 10 (30)	2 / 10 (20)	12 / 50 (24)
<i>C. shasta</i> troph.	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 50 (0)
<i>Chloromyxum</i> sp	1 / 10 (10)	1 / 10 (10)	3 / 10 (30)	1 / 10 (10)	1 / 10 (10)	7 / 50 (14)
<b>Pathology Score</b>	0.10	0.90	0.10	1.8	2.44	
<u>Intestinal tract</u>						
<i>C. shasta</i> troph.	1 / 10 (10)	0 / 10 (0)	3 / 10 (30)	2 / 10 (20)	1 / 10 (10)	7 / 50 (14)
<i>C. shasta</i> myxosp.	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 50 (0)
Helminth	0 / 10 (0)	1 / 10 (10)	0 / 10 (0)	2 / 10 (20)	1 / 10 (10)	4 / 50 (8)
<b>Pathology Score</b>	0.00	0.00	0.80	0.30	0.20	
Adipose steatitis	3 / 7 (43)	4 / 10 (40)	5 / 8 (63)	7 / 8 (88)	2 / 2 (100)	21 / 35 (60)
Adipose lipofuscin	0 / 7 (0)	0 / 10 (0)	2 / 8 (25)	2 / 8 (25)	1 / 2 (50)	5 / 35 (14)
<u>Gill</u>						
Ich	0 / 9 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	1 / 10 (10)	1 / 49 (2)
Glochidia	0 / 9 (0)	2 / 10 (20)	1 / 10 (10)	0 / 10 (0)	0 / 10 (0)	3 / 49 (6)
Miracidia	0 / 9 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 49 (0)
Metacercaria	2 / 9 (22)	9 / 10 (90)	7 / 10 (70)	8 / 10 (80)	7 / 10 (70)	33 / 49 (67)
Invasive <i>C. shasta</i>	0 / 9 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 10 (0)	0 / 49 (0)
Multif. Hyperplasia	4 / 9 (44)	10 / 10 (100)	9 / 10 (90)	10 / 10 (100)	6 / 10 (60)	39 / 49 (80)

**Table A3. Parasite prevalence of infection (POI) and tissue (no. positive / total (%)) and pathology score for kidney and intestine observed in histological sections of juvenile Klamath River Chinook salmon collected from the Upper Trinity River (T2) rotary screw trap at Pear tree trap (RM 94). Collection dates are reported as Monday of given week. Overall incidence of infection also reported.**

	4/25	5/23	POI
<u>Kidney</u>			
Pm Troph.	0 / 10 (0)	3 / 10 (33)	3 / 20 (15)
Pm Myxosp.	0 / 10 (0)	0 / 10 (0)	0 / 20 (0)
Metacercaria	0 / 10 (0)	0 / 10 (0)	0 / 20 (0)
<i>C. shasta</i> troph.	0 / 10 (0)	0 / 10 (0)	0 / 20 (0)
<i>Chloromyxum</i> sp	0 / 10 (0)	1 / 10 (10)	1 / 20 (5)
<b>Pathology Score</b>	0.00	0.00	
<u>Intestinal tract</u>			
<i>C. shasta</i> troph.	0 / 10 (0)	0 / 10 (10)	0 / 20 (0)
<i>C. shasta</i> myxosp.	0 / 10 (0)	0 / 10 (0)	0 / 20 (0)
Helminth	0 / 10 (0)	0 / 10 (0)	0 / 20 (0)
<b>Pathology Score</b>	0.00	0.00	
Adipose steatitis	0 / 6 (0)		0 / 6 (0)
Adipose lipofuscin	0 / 6 (0)		0 / 6 (0)
<u>Gill</u>			
Ich	0 / 10 (0)	0 / 10 (0)	0 / 20 (0)
Glochidia	0 / 10 (0)	0 / 10 (0)	0 / 20 (0)
Miracidia	0 / 10 (0)	0 / 10 (0)	0 / 20 (0)
Metacercaria	0 / 10 (0)	0 / 10 (0)	0 / 20 (0)
Invasive <i>C. shasta</i>	0 / 10 (0)	0 / 10 (0)	0 / 20 (0)
Multif. Hyperplasia	0 / 10 (0)	0 / 10 (0)	0 / 20 (0)

**Table A4. Parasite prevalence of infection (POI) and tissue (no. positive / total (%)) and pathology score for kidney and intestine observed in histological sections of juvenile Klamath River Chinook salmon collected from the Lower Trinity River (T1) rotary screw trap at Willow Creek trap (RM 14). Collection dates are reported as Monday of given week. Overall incidence of infection also reported.**

	5/23	8/01	POI
<u>Kidney</u>			
Pm Troph.	0 / 10 (0)	0 / 3 (0)	0 / 13 (0)
Pm Myxosp.	0 / 10 (0)	0 / 3 (0)	0 / 13 (0)
Metacercaria	1 / 10 (10)	1 / 3 (33)	2 / 13 (15)
<i>C. shasta</i> troph.	0 / 10 (0)	0 / 3 (0)	0 / 13 (0)
<i>Chloromyxum</i> sp	2 / 10 (20)	0 / 3 (0)	2 / 13 (15)
<b>Pathology Score</b>	0.1	0.00	
<u>Intestinal tract</u>			
<i>C. shasta</i> troph.	0 / 10 (0)	0 / 2 (0)	0 / 12 (0)
<i>C. shasta</i> myxosp.	0 / 10 (0)	0 / 2 (0)	0 / 12 (0)
Helminth	1 / 10 (10)	0 / 2 (0)	1 / 12 (8)
<b>Pathology Score</b>	0.00	0.00	
Adipose steatitis	2 / 6 (33)		2 / 6 (33)
Adipose lipofuscin	1 / 6 (17)		1 / 6 (17)
<u>Gill</u>			
Ich	0 / 9 (0)	0 / 3 (0)	0 / 12 (0)
Glochidia	0 / 9 (0)	3 / 3 (100)	3 / 12 (25)
Miracidia	0 / 9 (0)	0 / 3 (0)	0 / 12 (0)
Metacercaria	0 / 9 (0)	0 / 3 (0)	0 / 12 (0)
Invasive <i>C. shasta</i>	0 / 9 (0)	0 / 3 (0)	0 / 12 (0)
Multif. Hyperplasia	0 / 9 (0)	3 / 3 (100)	3 / 12 (25)

**APPENDIX B – Summary table of *Ceratomyxa shasta* infection by QPCR in juvenile Chinook salmon sampled from 5 reaches within the Klamath River and the upper and lower Trinity River. The prevalence (#positive/#sampled) is presented for each sample reach by collection week and sample date.**

Collection Week	Sample Date	Shasta R. to Scott R.	Scott R. to Salmon R.	Salmon R. to Trinity R.	Trinity R. to Estuary	Estuary	Upper Trinity R.	Lower Trinity R.
1	4-Apr							
2	11-Apr	0% (0/10)						
3	18-Apr	10 % (1/10)						
4	25-Apr	0 % (0/10)					10% (1/10)	
5	2-May	0% (0/10)	0% (0/10)	0% (0/10)	11% (1/9)			
6	9-May	0 % (0/10)			0% (0/10)			
7	16-May	40% (4/10)	0% (0/10)	10% (1/10)	0% (0/10)			
8	23-May	0% (0/10)			1% (1/10)		0% (0/11)	0% (0/10)
9	30-May	20% (2/10)	50% (5/10)	10% (1/10)				
10	6-Jun	50% (5/10)						
11	13-Jun	40% (4/10)	40% (4/10)	40% (4/10)			0% (0/25)	0% (0/20)
12	20-Jun	60% (6/10)			16% (3/19)	17% (2/19)		
13	27-Jun	0% (0/20)	50% (5/10)	0% (0/10)				
14	4-Jul	13% (2/15)	12% (3/25)	0% (0/20)	5% (1/20)	19% (4/21)	0% (0/20)	0% (0/20)
15	11-Jul	33% (1/3)	7% (2/30)	10% (2/20)				
16	18-Jul	0% (0/1)	5% (1/20)	40% (8/20)	0% (0/20)	0% (0/10)	0% (0/17)	0% (0/21)
17	25-Jul		0% (0/10)	20% (2/10)				
18	1-Aug		0% (0/10)	50% (5/10)	80% *8/10)	25% (5/20)		0% (0/20)
19	8-Aug			50% (5/10)				
20	15-Aug				43% (3/7)	31% (5/16)		0% (0/20)
		<b>K4 Total 17% (25/149)</b>	<b>K3 Total 14% (20/145)</b>	<b>K2 Total 20% (28/140)</b>	<b>K1 Total 15% (17/115)</b>	<b>K0 Total 17% (16/95)</b>	<b>T2 Total 1% (1/83)</b>	<b>T1 Total 0% (0/111)</b>

**APPENDIX C – Summary table of *Parvicapsula minibicornis* infection by QPCR in juvenile Chinook salmon sampled from 5 reaches within the Klamath River and lower Trinity River. The prevalence (#positive/#sampled) is presented for each sample reach by collection week and sample date.**

<b>Collection Week</b>	<b>Sample Date</b>	<b>Shasta R. to Scott R.</b>	<b>Scott R. to Salmon R.</b>	<b>Salmon R. to Trinity R.</b>	<b>Trinity R. to Estuary</b>	<b>Estuary</b>	<b>Upper Trinity R.</b>	<b>Lower Trinity R.</b>
1	4-Apr							
2	11-Apr	0% (0/10)						
3	18-Apr	0% (0/10)						
4	25-Apr	0% (0/10)					0% (0/10)	
5	2-May	0% (0/10)	0% (0/10)	0% (0/10)	11% (1/9)			
6	9-May	0% (0/10)			30% (3/10)			
7	16-May	100% (10/10)	0% (0/10)	0% (0/10)	0% (0/10)			
8	23-May	100% (10/10)			60% (6/10)		0% (0/11)	0% (0/10)
9	30-May	70% (7/10)	100% (10/10)	50% (5/10)				
10	6-Jun	90% (9/10)						
11	13-Jun		100% (10/10)	90% (9/10)			20% (1/5)	
12	20-Jun					50% (9/18)		
13	27-Jun	0% (0/10)						
14	4-Jul	70% (7/10)	40% (4/10)	30% (3/10)		40% (4/10)		
15	11-Jul	100% (3/3)	90% (9/10)	60% (6/10)				
16	18-Jul	100% (1/1)	100% (10/10)	100% (10/10)		33% (2/6)		
17	25-Jul							
18	1-Aug					100% (9/9)		
19	8-Aug							
20	15-Aug				71% (5/7)	0% (0/1)		
		<b>K4 Total 41% (47/114)</b>	<b>K3 Total 61% (43/70)</b>	<b>K2 Total 47% (33/70)</b>	<b>K1 Total 33% (15/46)</b>	<b>K0 Total 55% (24/44)</b>	<b>T2 Total 4% (1/26)</b>	<b>T1 Total 0% (0/10)</b>

## APPENDIX D – Summary of diagnostic examinations.

### K2 – Big Bar rotary screw trap

Summary: Low percentage of catch moribund (<13%) with external lesions a leading cause

Nine moribund or fresh mortalities examined.

### K1 – Beach seine

Summary: Low percentage of catch moribund, columnaris and gill metacercarial infections leading causes of morbidity.

Fourteen salmon examined.

Collection Site	Sample Date	Lamprey wound	Columnaris lesion	Intestine section with <i>C. shasta</i>	Kidney section with <i>P. minibicornis</i>	Gill sections with metacercaria	Gill with epithelial hyperplasia
K2 - Big Bar rotary screw trap and beach seine	11-Jul	67% (6/9)	22% (2/9)	20% (1/5)	60% (3/5)*	60% (3/5)	60% (3/5)
K1 – Beach seine at Pecwan, Tech tah, and Blue Creek	2-Aug	0% (0/14)	36% (5/14)	10% (1/10)	40% (4/10)**	36% (4/11)	64% (7/11)

\* No pathology was associated with *P. minibicornis*

\*\* 2/10 sections had pathology associated with *P. minibicornis*

## APPENDIX E - Reviewers' comments

Listed below are verbatim (in quotes) or paraphrased comments provided by reviewers of a draft of this report. The primary author's reply is given unless noted otherwise (additional authors name and responses are provided for specific sections of this report).

### Reviewer #1

**Pg. 3 - Introduction:** In reference to reporting data from previous study years, the reviewer commented, "Should you only go up to 2010 as you are reporting on 2011?"

Response: 2011 is included in Table 5 (page 21) to use for comparison to historical data for annual prevalence of infection.

**Pg. 7 – Methods:** In reference to fish sampled for histology, the reviewer commented, "Were these fish euthanized differently than those fish collected for QPCR?"

Response: No, all fish were euthanized in the same manner (MS-222). It was meant to convey that histology fish were fixed quickly. Sentence changed to the following:

Fish tested by histology were euthanized and placed in Davidson's fixative within 2 minutes of euthanasia.

**Pg. 9 – Results:** Reviewer commented that the total of number fish samples (by origin) did not add up.

Response: There were 838 fish total samples (from both Klamath and Trinity Rivers); 279 naturals, 123 unknown, and 436 CWT. 21 CWT fish that had unreadable tags, but you are correct that they need to be included in the total number of fish sampled. Refer to Table 4 for sample numbers from the Klamath River. Numbers in sentence corrected.

**Pg. 10 – Results:** Regarding the Unknown Origin Chinook Salmon section, the reviewer wanted to know "Why is there no discussion about the POI for the unknown origin fish?"

Response: The POI data for the unknown origin fish (N=123 total, N = 85 in Klamath R.) is reported in Table 4.

**Pg. 13 – Results.** Reviewer inquired if a mean of 45 DNA copies is dangerous to native fish

Response:

The authors would not consider 45 copies (or 15 copies in hatchery fish) to be 'dangerous' and this was not stated or implied in the text. The comparison was intended to illustrate that natural fish had higher levels of parasite present in 2011 when compared to hatchery fish. This is the opposite of what we observed in all other study years (2006-2010) because natural fish are sampled earlier in the season when river temperatures are cooler, and fish infectivity is not as high as later in the season when hatchery fish are typically released (late May to early June). Lower *C. shasta* POI in hatchery fish sampled later in the season illustrates the uncommon environmental conditions (river temperature, higher flows and extended precipitation) that existed in the Klamath River in 2011.

**Pg. 14 – Results.** Reviewer commented that we should report the kidney pathology scores we refer to in the text.

Response: Pathology scores are reported in Figure 5 as 2 or less, and the figure number has been added to the sentence to direct the reader to that figure. Kidney pathology scores are considered low (Figure 5) given the 6.0 – 8.0 range observed in *P. minibicornis* clinically affected salmon in 2009, and the 3.0-5.0 range observed in 2010.

**Pg. 16, 20, 26 – Results.** Reviewer wants to see statistical analysis when comparing data between groups.

Response: Fish tested under this monitoring program are not selected randomly from the out-migrating juvenile Chinook population, but are collected from Rotary Screw Traps. In order to use statistics appropriately, the fish must be collected randomly, or at least have an equal opportunity to be included in the sample group being tested. When we make general comparisons in the text between fish groups (natural, mixed origin, or coded-wire tagged fish), or reaches, these are only intended to be descriptive comparisons of trends observed, not a statistical analysis between groups or geographical areas.

**Pg. 22 – Marked (CWT) Chinook.** Reviewer commented that the overall POI of CWT is confusing, “Total Cs POI in CWT Chinook, right?”

Then 13 % above confluence, 42% below, but where are the remaining 41 fish sampled?”

Response: The 42% POI is prevalence for K1 only, and not the prevalence below the confluence. Cs POI in IGH CWT above is 13% (22/176), and below is 26% (17/65). Leading to the overall POI of 16% (39/241).

**Pg. 26 – Trinity River Disease Monitoring.** In reference to the lower Trinity River (T1), the reviewer suggested that the Pm section needs clarification, “The same two fish had high copy numbers of both Cs and Pm?”

Response: Yes, two TRH CWT fish collected in K1 had high copy numbers for both Cs and Pm. Sentence restructured:

*Parvicapsula minibicornis* was detected in 17% (2/12, ci = 2-48%) of all marked TRH Chinook salmon screened by QPCR. The two positive fish were collected at 10 WAL. The prevalence of infection for 10 WAL group was 50%, but the sample size was notably small with a total of four fish collected. Similar to *C. shasta* in TRH CWT Chinook salmon, the parasite load was high (mean of 4200 copies). Therefore, the same two fish had high parasite loads for both Cs and Pm.

**Pg. 29 – Environmental Conditions.** When referring to natural precipitation in 2011, the review suggests clarification is needed when referring to precipitation as higher; “Higher than normal or higher than the event?”

Response: Higher than the pulse flow event, sentence clarified in text.

## Reviewer #2

**Pg. 3 - Introduction:** Reviewer wanted clarification on what we refer to as the Lower Klamath River as the term might not mean the same for all audiences

Response: Changed sentence to inform the reader that we were referring to “the Klamath River from Iron Gate Dam to the estuary.”

**Pg. 6 – Methods:** In reference to Table 2 the reviewer asked “Does K1 include Trinity River Sampling? I don’t think it does so maybe include another column for Trinity.”

Response: No, K1 does not include the Trinity River. Table 2 caption changed to clarify that Table 2 is only for the main stem of the Klamath River. Appendix B includes the sampling schedule for the Trinity River sites.

**Pg. 6 – Methods:** In regards to data collection that accompany samples, the reviewer asked if data should “be done by sample site, capture method, and reach so someone coming back to this could possibly look at intra-reach or sampling differences?”

Response: Yes, data collected with fish include the site of collection, the collector, and method of collection if not a RST. Somebody would be able to go back and look at intra-reach prevalence by collection site if needed.

**Pg. 10 – Results:** In the Unknown Origin Chinook Salmon, the reviewer would like to see the date reported when it is stated that fish “were collected in 2011”

Response: Table 2 and Appendix B outline that fish sampled in 2011 were collected April through August.

**Pg. 14, 24 – Results:** In the Parasite Prevalence of Infection by River Reach, the reviewer commented that there is too much commentary in the results section and that it should be moved to the discussion.

Response: A few redundant points were removed from the results to discussion, however we feel that some commentary is needed when the data is first reported to put it in context. We don’t want to wait until the discussion to bring up a key point illustrated by the data, and presented many pages earlier, for the first time.

**Pg. 33 – Discussion:** In reference to the section on river temperature and flow, the reader suggested that we clarify that paragraph and the differences between flow in 2006 and 2011 and what that meant for disease that year.

Response: The sentence is stating that flow alone might not be the only factor contributing to the reduction in infection prevalence. The 2006 flow event did not cause a lower annual prevalence, whereas the 2011 flow event did reduce annual infection prevalence.

### **Reviewer #3**

**Pg. 5 – Methods:** In the Sample Sites, Fish Groups, and Number Sampled section, the reviewer asked in reference to fish of unknown origin, “Do you use the CWT fraction to estimate which of these unknown are natural and which are hatchery?”

Response: No

**Pg. 8 – Methods:** In the Statistical Analysis and Terms Used section, the reviewer commented that the term sample population is confusing because a sample is taken from a population.

Response: The sentence has been changed to make the statement clearer.

**Pg. 9, 11 – Results:** Reviewer commented that throughout the report, any time raw data is used a confidence interval needs to accompany the data as well. The reviewer also added that confidence intervals should be added to Table 4 to make the data easier to compare.

Response: Corrected throughout report.