

U.S. Fish & Wildlife Service

California-Nevada Fish Health Center

FY 2007 Investigational Report:

Klamath River Juvenile Salmonid Health Monitoring, April-August 2007

Ken Nichols, Kimberly True, Ryan Fogerty and Lisa Ratcliff



September 2008



US Fish and Wildlife Service
California-Nevada Fish Health Center
24411 Coleman Fish Hatchery Rd
Anderson, CA 96007
(530) 365-4271 Fax: (530) 365-7150
<http://www.fws.gov/canvfhc/>

SUMMARY

The California-Nevada Fish Health Center led a cooperative study to monitor the incidence of two myxozoan parasites (*Ceratomyxa shasta* and *Parvicapsula minibicornis*) in juvenile salmonids within the Klamath River during the spring and summer of 2007. This study utilized two complementary assays: Quantitative real-time Polymerase Chain Reaction (QPCR) for its high sensitivity and efficiency, and histology to assess disease state and provide continuity with previous studies. In juvenile Klamath River Chinook Salmon out-migrants, *C. shasta* incidence of infection peaked at 68% during mid June and *P. minibicornis* reached 100% during late May. In marked (coded wire tagged) hatchery Chinook smolts recaptured within the Klamath River, *C. shasta* was detected in 68% of Iron Gate Hatchery (IGH) origin smolts and 14% of Trinity River Hatchery (TRH) origin smolts; *P. minibicornis* was detected in 83% of IGH smolts and 58% of TRH smolts. Infection incidence in coded wire tagged smolts from IGH peaked the 5th week following release and subsequently declined suggesting the death of infected fish. Coho salmon also were susceptible to infection by both parasites; with 48% *C. shasta* and 65% *P. minibicornis* incidence of infection observed in naturally produced young-of-the-year. Compared to Klamath River salmonid health monitoring conducted in 2004 – 2006, incidence of *C. shasta* was below average, and incidence of *P. minibicornis* was above average.

The correct citation for this report is:

Nichols K, K True, R Fogerty and L Ratcliff. 2008. FY 2007 Investigational Report: Klamath River Juvenile Salmonid Health Monitoring, April- August 2007. U.S. Fish & Wildlife Service California – Nevada Fish Health Center, Anderson, CA. Available online: <http://www.fws.gov/canvfhc/reports.asp>.

Notice

The mention of trade names or commercial products in this report does not constitute endorsement or recommendation for use by the Federal government.

INTRODUCTION

As a partner in the efforts to restore salmonid populations in the Klamath River basin, the California-Nevada Fish Health Center has conducted pathogen monitoring of juvenile Klamath River salmonids since 1991. Pathogens associated with diseased fish in the Klamath River include bacteria (*Flavobacterium columnare* and motile aeromonads), a digenetic trematode (presumptive *Nanophyetus salmincola*), myxozoan parasites (*Parvicapsula minibicornis* and *Ceratomyxa shasta*) and external parasites (Walker and Foott 1992; Williamson and Foott 1998). Ceratomyxosis (due to *C. shasta*) has been identified as the most significant disease for juvenile salmon in the Klamath Basin (Foott et al. 1999; Foott et al. 2004). Significant kidney damage (glomerulonephritis) has been associated with *P. minibicornis* infection; however, the prognosis of such infections has not been thoroughly studied in juvenile salmonids.

Ceratomyxa shasta and *P. minibicornis* are myxosporean parasites found in a number of Pacific Northwest watersheds (Hoffmaster et al. 1988; Bartholomew et al. 1989; St.-Hilaire et al. 2002; Jones et al. 2004; Bartholomew et al. 2006). The lifecycles of both parasites include the polychaete host, *Manayunkia speciosa*, and salmonids (Bartholomew et al. 1997; Bartholomew et al. 2006). *Ceratomyxa shasta* infection can occur from spring through fall at water temperatures greater than 4°C, although is most active above 11°C (Ching and Munday 1984; Hendrickson et al. 1989; Bartholomew et al. 1989). Studies conducted in 2004, 2005 and 2006 suggest that *P. minibicornis* has seasonality similar to that of *C. shasta*, while its actinospore concentration and infectivity appears greater than *C. shasta* (Foott et al. 2006; Nichols and Foott 2006; Nichols et al. 2007; Nichols and True 2007; Bartholomew et al. 2007).

In this study we monitored the weekly incidence of *C. shasta* and *P. minibicornis* infections in juvenile Chinook (*Oncorhynchus tshawytscha*) and coho (*Oncorhynchus kisutch*) salmon over 24 weeks of the spring and summer out-migration period. Two complementary assays were utilized: Quantitative real-time Polymerase Chain Reaction (QPCR) for its high sensitivity and efficiency, and histology to assess disease state and provide continuity with previous studies.

METHODS

Fish Collection

Fish collection occurred from 19 April through 22 August, 2007, with a total of 1890 fish examined from the Klamath and Trinity Rivers. Sample reaches and cooperators performing collections are summarized in Table 1. Where possible, fish capture was performed at existing juvenile salmonid out-migration monitoring sites, but supplemental seining or electrofishing was required to achieve our target sample size in some weeks. Fish from multiple sites within each reach and captured over several days were combined into a weekly sample group.

A portion of the Chinook salmon released from the two hatcheries in the basin were marked with an adipose fin clip, and implanted with a coded-wire-tag (CWT). Iron Gate Hatchery (IGH) on the Klamath River released 5.4 million fall Chinook (5.8% CWT) from 18-31 May. Trinity River Hatchery (TRH) located near Lewiston on the Trinity River released 3.0 million spring and fall Chinook (24% CWT) in a week long volitional release from 1-8 June. Heads from any CWT Chinook recovered were assigned unique identification numbers to track lab assay results to individual CWT fish. The US Fish and Wildlife Service, Arcata FWO excised and read the CWT's. The release date for a given CWT group was used to determine weeks since release for individual marked fish. Chinook without adipose fin clips (unmarked) could have been of either hatchery or natural origin.

Table 1. Sample reach location and cooperating agencies performing collections.

Reach	River Miles	Primary collector(s)
Klamath River mainstem		
Iron Gate Dam to Shasta	Klamath 190-177	USFWS and Karuk Tribe
Shasta to Scott	Klamath 177-143	USFWS and Karuk Tribe
Salmon to Trinity	Klamath 66-44	Karuk Tribe
Trinity to Estuary	Klamath 44-4	Yurok Tribe
Klamath Estuary	Klamath 4-0	Yurok Tribe
Trinity River		
Upper – Lewiston Dam to North Fork	Trinity 111-73	Hoopa Tribe
Lower - North Fork to Klamath	Trinity 73-0	USFWS and Yurok Tribe

Target sample numbers for the QPCR assay varied depending on the reach and species sampled. In Klamath reaches above the confluence of the Trinity River the first 30 Chinook encountered per reach and all CWT Chinook were collected each week. In Klamath reaches below the Trinity confluence any adipose clip marked fish encountered were collected. Any juvenile coho salmon encountered in the Klamath River above the Trinity River confluence were collected under endangered species Section 10 permit 1068. In the Trinity River, 60 Chinook were collected in late May and again in late June.

Following capture and preliminary examination by collection crews, fish were euthanized, placed in a plastic bag labeled with date and reach, and arranged between frozen gel pack sheets. At the end of the day, samples were transferred to a freezer until they could be shipped frozen to the CA-NV Fish Health Center laboratory.

Each week personnel from the CA-NV Fish Health Center would accompany the samplers in one or more reaches to collect 10 randomly selected juvenile Chinook for the histology assay. Following preliminary examination by the collection crew, the fish were euthanized, and target tissues were preserved in individually identified 50 ml tubes containing Davidson's fixative. Only unmarked fish were collected for the histology assay.

Laboratory Assays

Necropsy – In the laboratory fish were thawed, measured for fork length, and tissue samples were collected. The intestine (both small and large intestine) and kidney from each fish were removed and combined into an individually numbered 2 ml cluster tube. Due to limited tube volume, total sample weight was limited to 1.0g (tissue weight ranged from 0.01g to 1.0g). Tissue samples were then frozen until DNA extraction was performed.

Histology – Tissues (kidney and intestine) for histological examination were fixed for 24 hours in Davidson's fixative, transferred to 70% ethanol after 24 hours for storage, 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 code number. Each slide was examined at both low (40X) and high magnification (400X). A composite infection and disease rating was developed based on the degree of tissue inflammation associated with the presence of the parasites. A similar histology rating system has been used in Klamath monitoring studies since 2004 (Nichols and Foott 2006; Nichols et al. 2007; Nichols and True 2007). *Ceratomyxa shasta* infections were rated as clinical (parasite present and inflammatory tissue in >33% of the intestine section), subclinical (parasite present, but inflammatory tissue in <33% of intestine

section) or uninfected (no *C. shasta* detected). *Parvicapsula minibicornis* infections were rated as clinical (parasite present and glomerulonephritis in >33% of the kidney section), subclinical (parasite present, and inflammation in <33% of the kidney section) or uninfected (no *P. minibicornis* detected).

DNA extraction – Combined intestine and kidney tissues were digested in 1ml NucPrep Digest Buffer containing 1.25 mg/ml proteinase K (Applied Biosystems, Foster City, CA) at 55°C for 1 hour with constant shaking. A subsample of digested tissue homogenate was diluted 1:33 in molecular grade water (MGW) and extracted in a 96 well filter plate system (Applied Biosystems Model 6100 Nucleic Acid Prep Station). Due to dilution the weight of tissue entering extraction was limited to 3.0mg given the maximum 1.0g sample weight mentioned above. Extracted DNA was stored at -20°C until the QPCR assays were performed.

QPCR assay – Samples were assayed in a 7300 Sequence Detection System (SDS) (Applied Biosystems), 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). The combined tissues were tested for *P. minibicornis* 18S rDNA utilizing TaqMan Minor-Groove-Binding (MGB) probe and primers (True et al. in press). Reaction volumes of 30uL, containing 5uL DNA template, were used for both assays under the following conditions: 50°C for 2 min; 95°C for 10 min; 40 cycles of 95°C for 15 s and 60°C for 1 min. Standards, extraction control and no template control wells (MGW) were included on each assay plate. Cycle threshold (C_T) values were calculated by the SDS software (v 1.3.1, Applied Biosystems). Preliminary lab trials examining the dynamic range and endpoint of the assays indicated a C_T of 38 and minimum change in normalized fluorescent signal of at least 10,000 units was a reliable indicator of amplification. These thresholds were conservative and underestimate the true incidence of infection for both parasites; however, we believe that any light infections that may have been missed likely had no biologically significant impact on the survival of the animals (True et al. in press; Nichols and True 2007).

Interannual Comparisons

Using the composite histology disease rankings, a comparison of disease incidence and severity between years was possible. Juvenile Klamath River Fall Chinook Salmon historically began out-migration in February, peaked in mid-June, and were captured in large numbers within the Klamath Estuary from June through mid August (Leidy and Leidy 1984; Wallace and Collins 1997). For interannual comparisons of parasite infection, we limited the data to fish captured during the months of May, June and July and from sites in the Klamath River above the confluence with the Trinity River. Limiting the data set in this way offered several advantages:

- These months bracketed the typical peak of Fall Chinook out-migration and included the monitoring periods from previous years
- Infection incidence during the “tails” of the migration (typically lower infection rates in early spring) were not given the same weight as the peak of migration
- The Trinity population was excluded as it is largely *C. shasta* uninfected
- Our target sample size was typically met during this period reducing sample variation due to small sample size

RESULTS

Chinook Salmon

Histology Assay

Ceratomyxa shasta infections were first detected by histology the week of 29 April in 20% (2/10) of fish sampled in the Shasta to Scott reach (Table 2). The peak incidence of infection and clinical ceratomyxosis were both observed the week of 10 June in the Shasta to Scott reach where 50% (5/10) of juvenile Chinook were *C. shasta* infected with 80% (4/5) of the infections rated as clinical. Infection incidence declined in late June and no infections were detected after the week of 22 July. Overall, this parasite was detected in 16.4% (25/152) of Chinook from the Klamath River, with intestinal lesions symptomatic of clinical ceratomyxosis observed in 68% (17/25) of the infected Chinook.

Parvicapsula minibicornis infections were first observed during the week of 15 April in 60% (6/10) of fish sampled in the Shasta to Scott reach (Table 3). Incidence of infection reached 100% (10/10) by the week of 27 May. The peak incidence of clinical glomerulonephritis was 80% (8/10) observed the week of 24 June. Infection incidence remained high through the last week of sampling; however, clinical glomerulonephritis declined in late July. Overall, *P. minibicornis* was detected in 76.3% (116/152) of Chinook sampled in the Klamath, with clinical glomerulonephritis observed in 47.4% (55/116) of the *P. minibicornis* infected Chinook.

QPCR Assay

The earliest detection of *C. shasta* infections was in the week of 29 April. Prevalence remained below 40% until early June. Peak incidence was 68% in Chinook captured above the Trinity confluence during the week of 17 June (Figure 1).

Ceratomyxa shasta was detected in 3% (5/168) of juvenile Trinity Chinook sampled within the Trinity River (Table 4). All were very light infections near the detection threshold of the QPCR assay. Three of the infected fish were captured at the North Fork site in the Upper Trinity reach while the other two infected fish were captured at the Willow Creek site in the Lower Trinity. All 5 *C. shasta* infected Chinook were captured after hatchery release and were of either hatchery or natural origin.

Parvicapsula minibicornis infections were detected from the first Klamath samples taken the week of 15 April. *Parvicapsula minibicornis* incidence reached 100% in the Klamath above the Trinity confluence on 20 May and remained high through the end of the study (Figure 2).

Parvicapsula minibicornis was detected in 41% (54/132) of juvenile Trinity Chinook captured in the Trinity River (Table 4). *Parvicapsula minibicornis* was detected in fish from both the Upper and Lower Trinity reaches before and after hatchery release. Peak prevalence of 88% (14/16) was observed in fish from the Lower Trinity reach in late June.

Marked Hatchery Fish

A total of 103 IGH and 332 TRH CWT marked smolts were collected between 30 May and 18 August. The IGH smolts were captured between Iron Gate Dam and the Klamath Estuary from one to 12 weeks following release. The TRH smolts were captured in the Klamath River between the Trinity River confluence and Klamath Estuary from 3 to 11 weeks following release. All CWT smolts were analyzed by QPCR assay.

Table 2. Incidence of *Ceratomyxa shasta* infection by histology in juvenile Chinook salmon captured in the Klamath River between Iron Gate Dam (IGD) and the Estuary, during spring and summer of 2007. Fish were considered infected (Cs+) if *C. shasta* was detected in histological examination of intestinal tract (pyloric ceca, small and large intestine). Fish with inflammation in >33% of the intestinal section were rated as clinically diseased (Clinical).

Sample Reach		15 Apr	29 Apr	13 May	27 May	3 Jun	10 Jun	17 Jun	24 Jun	8 Jul	15 Jul	22 Jul	5 Aug	12 Aug	19 Aug	Total
Shasta to Scott	Cs+	0/10	2/10	0/10	1/10		5/10		0/10							8/60
	Clinical	0/10	2/10	0/10	1/10		4/10		0/10							7/60
Salmon to Trinity	Cs+					3/10		4/9		1/10	1/2			0/10		9/41
	Clinical					2/10		2/9		0/10	1/2			0/10		5/41
Klamath Estuary	Cs+							4/10		2/11		2/10	0/10		0/10	8/51
	Clinical							3/10		1/11		1/10	0/10		0/10	5/51
Total	Cs+	0/10	2/10	0/10	1/10	3/10	5/10	8/19	0/10	3/21	1/2	2/10	0/10	0/10	0/10	25/152
	Clinical	0/10	2/10	0/10	1/10	2/10	4/10	5/19	0/10	1/21	1/2	1/10	0/10	0/10	0/10	17/152

Table 3. Incidence of *Parvicapsula minibicornis* infection by histology in juvenile Chinook salmon captured in the Klamath River between Iron Gate Dam (IGD) and the Estuary, during spring and summer of 2007. Fish were considered infected (Pm+) if *P. minibicornis* was detected in histological examination of the kidney. Fish with glomerulonephritis in >33% of the kidney section were rated as clinically diseased (Clinical).

Sample Reach		15 Apr	29 Apr	13 May	27 May	3 Jun	10 Jun	17 Jun	24 Jun	8 Jul	15 Jul	22 Jul	5 Aug	12 Aug	19 Aug	Total
Shasta to Scott	Pm+	6/10	7/10	6/10	10/10		10/10		9/10							48/60
	Clinical	0/10	3/10	6/10	5/10		7/10		8/10							29/60
Salmon to Trinity	Pm+					9/10		5/9		8/10	2/2			5/10		29/41
	Clinical					5/10		2/9		6/10	1/2			0/10		14/41
Klamath Estuary	Pm+							8/10		6/11		9/10	8/10		8/10	39/51
	Clinical							7/10		2/11		0/10	2/10		1/10	12/51
Total	Pm+	6/10	7/10	6/10	10/10	9/10	10/10	13/19	9/10	14/21	2/2	9/10	8/10	5/10	8/10	116/152
	Clinical	0/10	3/10	6/10	5/10	5/10	7/10	9/19	8/10	8/21	1/2	0/10	2/10	0/10	1/10	55/152

Table 4. Incidence of *C. shasta* and *P. minibicornis* infection in Chinook salmon captured in either the lower (North Fork Trinity to confluence with Klamath) or upper (Lewiston Dam to North Fork Trinity) reaches on the Trinity River. Screening for the parasites was performed by QPCR of a combined kidney and intestine sample for individual fish.

Week	27 May	27 May	3 June	3 June	24 June	8 July	Total
Reach	Lower	Upper	Lower	Upper	Lower	Lower	
Cs incidence	0/31	0/30	1/30	3/30	1/30	0/30	5/181
Pm incidence	0/16	4/17	0/16	NA	14/16	13/18	31/83

Ceratomyxa shasta was detected by QPCR in 68% (70/103) of CWT marked IGH smolts as early as 6 days post hatchery release (Figure 3). Incidence of *C. shasta* infection by QPCR in IGH smolts peaked at 100% (30/30) in the 5th week following release. Overall prevalence of *P. minibicornis* infections was 83% (69/83), reached 100% (2/2) by the third week following release, and remained high through the last IGH smolt recovery 12 weeks after hatchery release.

Ceratomyxa shasta was detected by QPCR in 13.6% (45/332) of the TRH smolts recovered in the Lower Klamath River. Infected fish were detected in the Klamath River within 3 weeks of release (Figure 4). Incidence of *C. shasta* infection by QPCR in TRH smolts peaked at 46% (12/26) 5 weeks after release and decreased beginning the 6th week. *Parvicapsula* infections were detected in 57.7% (191/331) of the TRH smolts recovered in the Lower Klamath River. Incidence of infection peaked in the 5th and 11th weeks after Trinity River Hatchery release at 85% (22/26) and 100% (5/5), respectively.

Interannual Comparisons

Compared to studies performed in 2004, 2005 and 2006 (Nichols and Foott 2006, Nichols et al. 2007, Nichols and True 2007), the incidence of *C. shasta* by histology was below average, and incidence of *P. minibicornis* was above average in juvenile Chinook (Table 5 and 6).

Coho Salmon

QPCR Assay

Ceratomyxa shasta was detected in 48% (25/52) of natural young-of-the-year (YOY) coho, and no infections (0/26) were detected in the yearling juvenile coho. The first detection of *C. shasta* occurred the week of 13 May, and the majority of *C. shasta* infected coho were captured during mid to late May in the Shasta to Scott reach (Table 7).

Parvicapsula minibicornis was detected in 65% (20/31) of natural YOY coho, and 71% (17/24) of yearling juvenile coho. The first detection of *P. minibicornis* by QPCR occurred the week of 29 April, and the majority of *P. minibicornis* infected coho were captured from early May through early June in the Shasta to Scott reach (Table 7).

Table 5. Comparison of *Ceratomyxa shasta* prevalence in juvenile Klamath River Chinook from 1994-2006 assayed by histology. Percentages indicate proportion of the total samples (N) in which the parasite was detected (Infected) or had an intestinal lesion associated with an infection (Clinical). Only fish sampled in May-July and captured above the Trinity confluence were included to aid comparisons between years.

	1994-2002	2004	2005	2006	2007	Average 2004-2007
Infected	20%-50%	34%	35%	21%	21%	28%
Clinical	n/a	23%	21%	18%	15%	19%
N	156	735	134	112	81	n/a

Table 6. Comparison of *Parvicapsula minibicornis* prevalence in juvenile Klamath River Chinook from 1995-2006 assayed by histology. Percentages indicate proportion of the total samples (N) in which the parasite was detected (Infected) or had an intestinal lesion associated with an infection (Clinical). Only fish sampled in May-July and captured above the Trinity confluence were included to aid comparisons between years.

	1995-2002	2004	2005	2006	2007	Average 2004-2007
Infected	47%-88%	77%	92%	58%	81%	77%
Clinical	n/a	37%	65%	29%	53%	46%
N	176	731	134	112	81	n/a

Table 7. Incidence of *C. shasta* and *P. minibicornis* infection in young-of-the-year (YOY) and yearling Coho salmon captured in the Klamath River between the Shasta and Scott Rivers. Screening for the parasites was performed by QPCR of a combined kidney and intestine sample.

Week Beginning	<i>C. shasta</i>		<i>P. minibicornis</i>	
	YOY	yearling	YOY	yearling
15 April		0/2		0/2
22 April		0/3		0/3
29 April	0/1	0/5		1/3
6 May	0/6	0/14	2/6	14/14
13 May	11/16	0/2	2/2	1 / 2
20 May	6/7		5/5	
27 May	4/5		5/5	
3 June	2/5		4/4	
10 June	1/2		2/2	
17 June	1/6		0/6	
24 June	0/4		0/1	
Total	25/52 (48%)	0/26	20/31 (65%)	17/24 (71%)

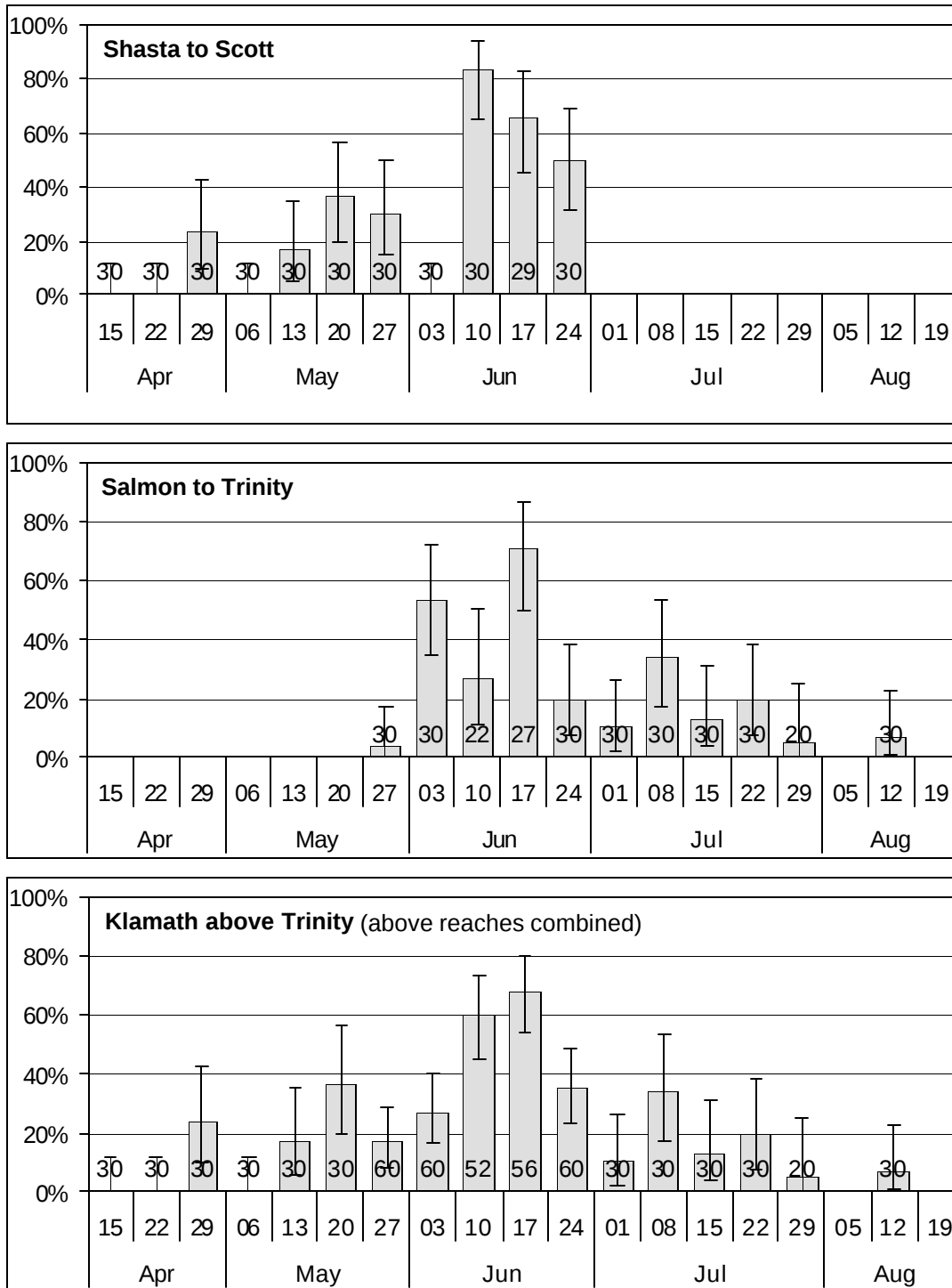


Figure 1. Incidence of *Ceratomyxa shasta* infection assayed by QPCR in juvenile Chinook salmon. Fish were captured in two reaches of the Klamath River above the Trinity River confluence (Shasta R. to Scott R., Salmon R. to Trinity R.) during the spring and summer of 2007. Sample number (n) is listed near the base of each bar. Whiskers indicate 95% confidence interval.

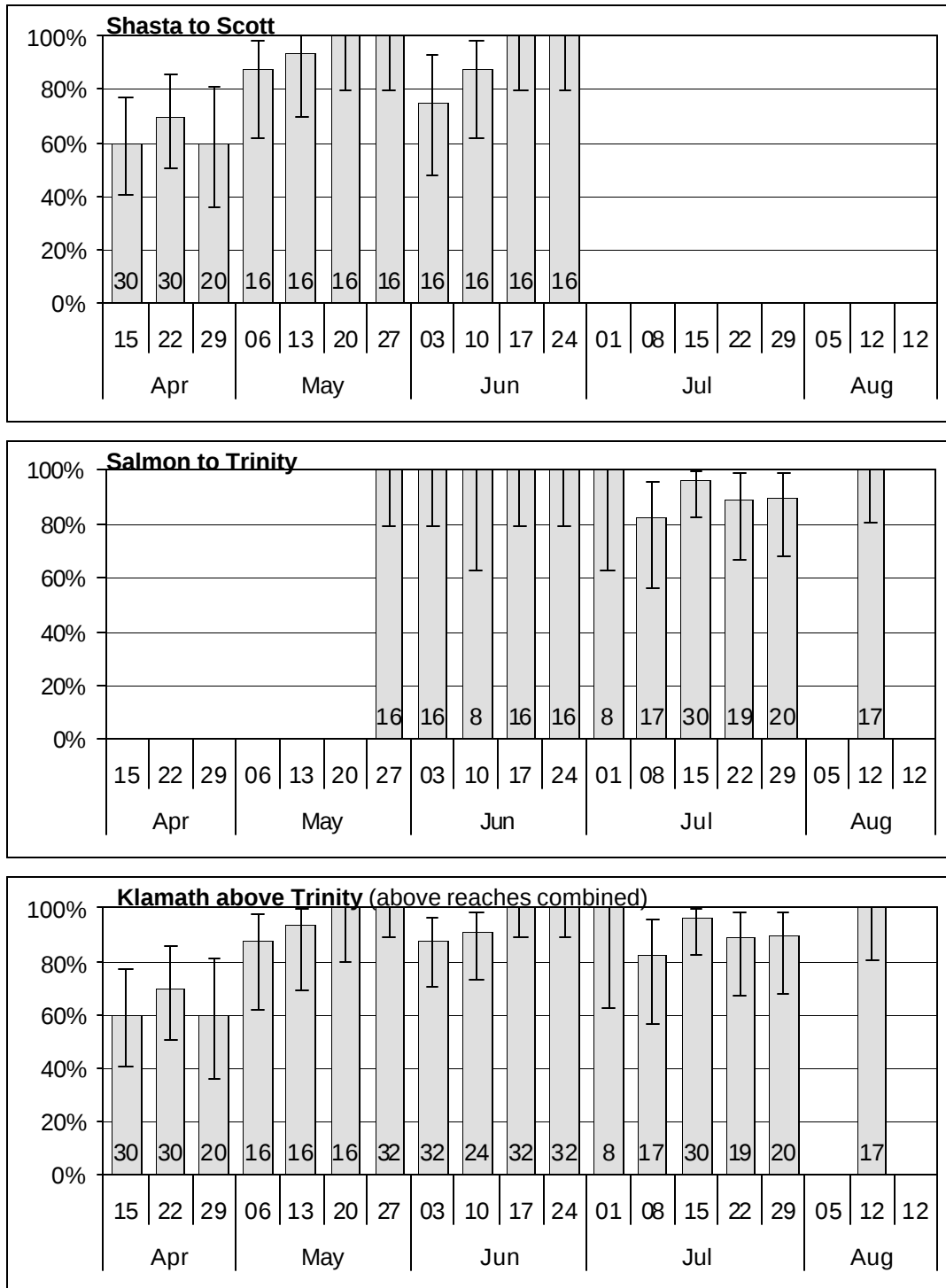


Figure 2. Incidence of *Parvicapsula minibicornis* infection assayed by QPCR in juvenile Chinook salmon. Fish were captured in two reaches of the Klamath River above the Trinity River confluence (Shasta R. to Scott R., Salmon R. to Trinity R.) during the spring and summer of 2007. Sample number (n) is listed near the base of each bar. Whiskers indicate 95% confidence interval.

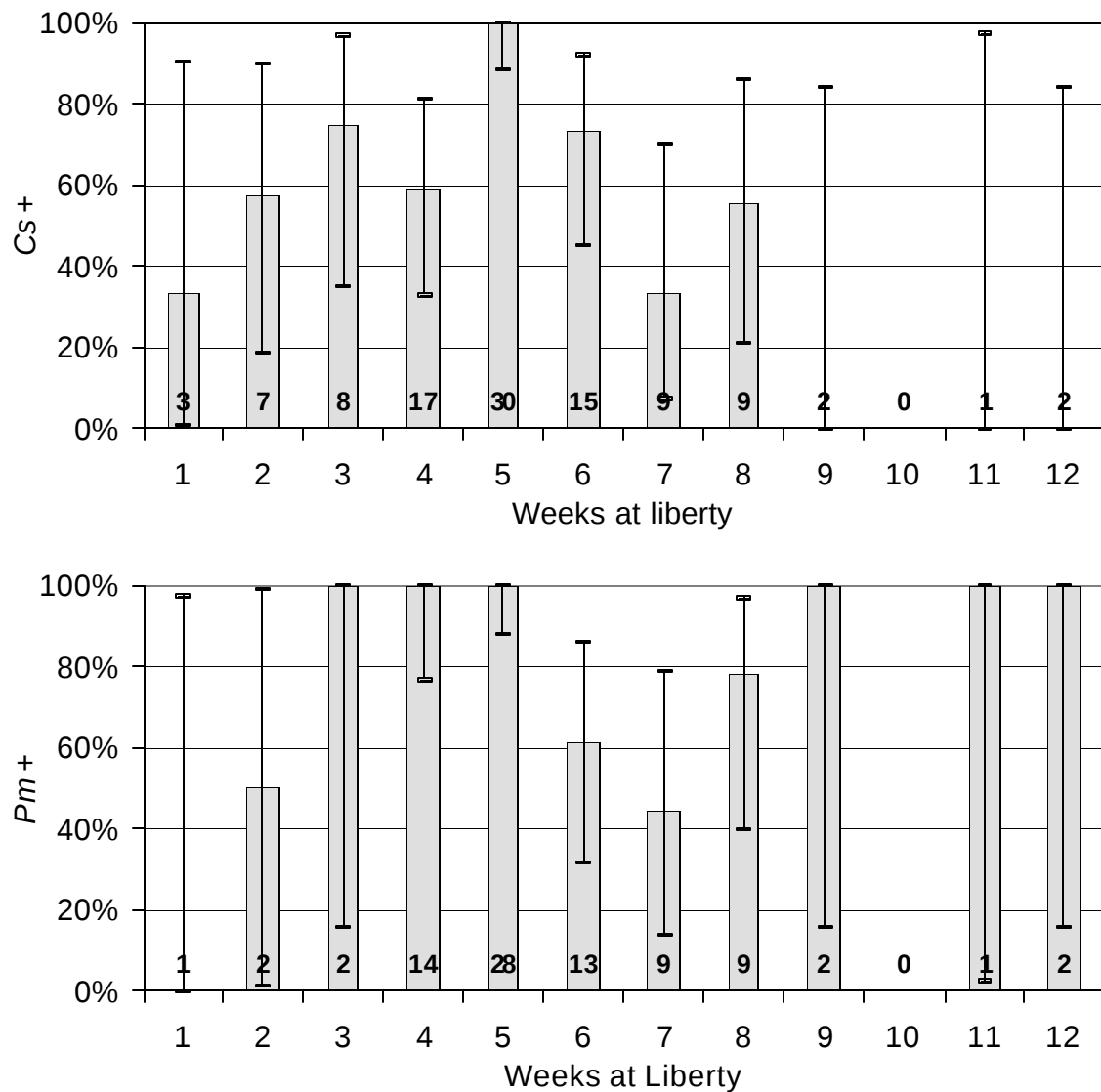


Figure 3. Prevalence of *Ceratomyxa shasta* (Cs) and *Parvicapsula minibicornis* (Pm) infections in Iron Gate Hatchery origin CWT juvenile Chinook assayed by QPCR. Fish were recaptured in the Klamath River from Iron Gate Dam to the estuary from 1-12 weeks after hatchery release (Weeks at Liberty). Sample number (n) is listed near the base of each bar. Whiskers indicate 95% confidence interval.

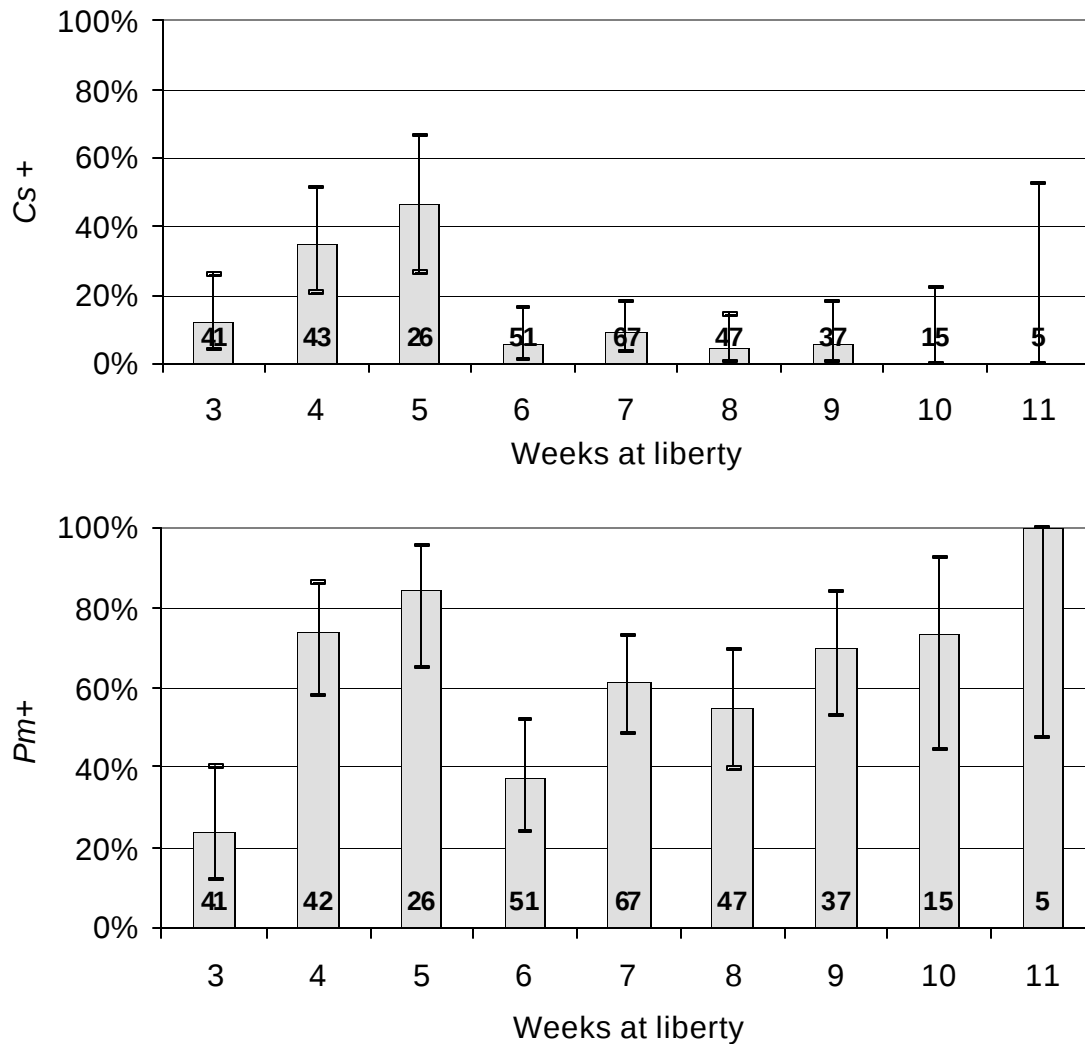


Figure 4. Prevalence of *Ceratomyxa shasta* (Cs+) and *Parvicapsula minibicornis* (Pm+) infections in Trinity River Hatchery origin CWT juvenile Chinook assayed by QPCR. Fish were recaptured in the Klamath River below the confluence with the Trinity River from 3-11 weeks after hatchery release (Weeks at Liberty). Sample number (n) is listed near the base of each bar. Whiskers indicate 95% confidence interval.

DISCUSSION

Mortality due to infection

The pattern of incidence of both *C. shasta* and *P. minibicornis* indicates moderate mortality in juvenile salmon out-migrating from the Klamath River in 2007. Infection prevalence (particularly *C. shasta*) declined following the peak of infection suggesting a loss of infected fish from the population. It was possible the decline in infection prevalence was due to the influx of uninfected fish from tributaries. However, the loss of infected fish over time was also evident in CWT marked IGH smolts. This similar pattern of infection incidence in known (CWT) and unknown (unmarked) origin fish was most likely due to disease associated mortality in both groups. Past sentinel studies where Chinook salmon were exposed for 72 hours in the Shasta to Scott reach resulted in 82% mortality in less than three weeks at 16°C, and mean survival time decreased at warmer water temperatures (Udey et al. 1975; Foott et al. 2004).

Prognosis of *Ceratomyxa* and *Parvicapsula* infections

Low survival was expected from fish diagnosed with *C. shasta* infection by histology. No signs of recovery from the *C. shasta* infections were observed in intestines examined by histology during this study (data not shown). This suggests that a significant portion of the infected fish develop debilitating disease before reaching the ocean.

The prognosis of *P. minibicornis* infection in juvenile Chinook salmon is not well understood and is an important question given the high prevalence of infection. We have observed signs of healing and recovery even in severe *P. minibicornis* infections by histology (intact nephrons in clinically infected fish, data not shown). Fish may have recovered if they survived the anemia and osmoregulation problems associated with glomerulonephritis.

The high prevalence of *P. minibicornis* infections results in nearly all *C. shasta* infected fish having dual infections. We speculate that nephron inflammation (due to *P. minibicornis*) and intestinal hemorrhage (due to *C. shasta*) would act synergistically to increase the risk of lethal disease in dual infected fish.

Residence time and infection prevalence

Marked hatchery fish allowed us to relate the residence time in the river to the infection rates for both IGH and TRH origin juvenile Chinook. *Ceratomyxa shasta* was detected in recaptured IGH smolts within the first week following release from the hatchery. Iron Gate Hatchery Chinook were not inspected before release in 2007, so the pre-release infection prevalence was unknown. These early infections may represent infections acquired either in the hatchery or soon after release. The incidence of *C. shasta* infection among IGH smolts peaked within 5 weeks of release. The decline in infection prevalence beginning 6 weeks after hatchery release was likely due to mortality of infected fish. Over half of the IGH smolts sampled were *C. shasta* infected during their 190 mile out-migration. The incidence of *P. minibicornis* infections in IGH smolts jumped to 100% within 3 weeks of hatchery release and remained high through the last IGH smolt recaptures. A similar trend was observed in 2006 and 1995 IGH smolts out-migrants (Nichols and True 2007; Foott et al. 1999). These IGH smolts could be viewed as surrogates for naturally produced tributary smolts (i.e. Bogus Creek, Shasta River,

Scott River) out-migrating through the Klamath. The disease risk to parr rearing in the Klamath prior to out-migration was likely higher.

Among TRH smolts, overall incidence of *C. shasta* was low (13%) and *P. minibicornis* was moderate (58%). Infection trends were similar to that seen in IGH smolts with peak infection 5 weeks after hatchery release and a decline in incidence beginning 6 weeks following release. While this may be due to mortality of infected fish as observed in IGH smolts, this decline in incidence may also be due to large numbers of TRH smolts leaving the Trinity and migrating quickly through the Lower Klamath without having time to become infected.

Conclusions

This study indicates *C. shasta* prevalence was below average and *P. minibicornis* prevalence was above average for May-July of 2007 compared to previous Klamath fish health monitoring studies (Nichols and Foot 2006; Nichols et al. 2007; Nichols and True 2007). Naturally produced Chinook became infected with both parasites while rearing in the mainstem Klamath during March and April, but the incidence remained low during this period in 2007. Both parasites were found in naturally produced young of the year coho salmon rearing within the mainstem Klamath, and the incidence of *C. shasta* in young of the year coho appears greater than for Chinook during May. Infection prevalence in coded wire tagged smolts from both hatcheries peaked the 5th week following release and subsequently declined. This was seen as indicative of a loss of infected IGH smolts. With lower incidence of infection for both parasites and the ability to move quickly through the Lower Klamath, Trinity smolts fared better than their Klamath cohorts.

ACKNOWLEDGMENTS

We wish to thank biologists with the USFWS Arcata FWO, Yurok Tribe, Karuk Tribe and Hoopa Tribe for fish collection; Ron Stone with the CA-NV Fish Health Center for lab assistance; Anthony Scheiff with the USFWS Arcata FWO for extracting and reading the CWT's; Paul Zedonis (USFWS), Jerri Bartholomew (OSU), Sascha Hallett (OSU), Josh Strange (Yurok Tribe), Monica Hiner (Yurok Tribe), Alex Corum (Karuk Tribe) and Scott Foott (USFWS) for reviewing and commenting on a draft of this report. Partial funding for this study was provided by the Klamath River Basin Conservation Area Restoration Program and Trinity River Restoration Program.

AUTHOR ROLES

The contributions of each author have been summarized below.

- Ken Nichols – project coordination, study design, data management, histology analysis, assembly and editing of final report
- Kimberly True – QPCR methods, QPCR QA and QPCR data certification
- Ryan Fogerty – necropsy, field collection coordination, histology processing, DNA extraction, preparation and preliminary analysis of results, tables and figures
- Lisa Ratcliff – necropsy, DNA extraction, QPCR assay

REFERENCES

- Bartholomew JL, SD Atkinson, SL Hallett, CM Zielinski and JS Foott. 2007. Distribution and abundance of the salmonid parasite *Parvicapsula minibicornis* (Myxozoa) in the Klamath River Basin (Oregon-California, USA). *Diseases of Aquatic Organisms* 78(2):137-146.
- Bartholomew JL, SD Atkinson, and SL Hallett. 2006. Involvement of *Manayunkia speciosa* (Annelida: Polychaeta: Sabellidae) in the life cycle of *Parvicapsula minibicornis*, a myxozoan parasite of Pacific salmon. *Journal of Parasitology* 92:742-748.
- Bartholomew JL, JS Rohovec and JL Fryer. 1989. *Ceratomyxa shasta*, a myxosporean parasite of salmonids. US Fish and Wildlife Service. Fish Disease Leaflet 80. Available online: <http://www.lsc.usgs.gov/FHB/leaflets/index.asp>.
- Bartholomew JL, MJ Whipple, DG Stevens, and JL Fryer. 1997. The life cycle of *Ceratomyxa shasta*, a myxosporean parasite of salmonids, requires a freshwater polychaete as an alternative host. *Journal of Parasitology* 83(5):859-868.
- Ching HL and DR Munday. 1984. Geographic and seasonal distribution of the infectious stage of *Ceratomyxa shasta* Noble, 1950, a myxozoan salmonid pathogen in the Fraser River system. *Canadian Journal of Zoology* 62:1075-1080.
- Foott JS, R Harmon and R Stone. 2004. Effect of water temperature on non-specific immune function and Ceratomyxosis in juvenile Chinook salmon and steelhead from the Klamath River. *California Fish and Game* 90(2):71-84.
- Foott JS, R Stone, E Wiseman, K True and K Nichols. 2006. FY2005 Investigational report: Longevity of *Ceratomyxa shasta* and *Parvicapsula minibicornis* actinospore infectivity in the Klamath River: April – June 2005. U.S. Fish & Wildlife Service California – Nevada Fish Health Center, Anderson, CA. Available online: <http://www.fws.gov/canvfhc/reports.asp>.
- Foott JS, JD Williamson, and KC True. 1999. Health, physiology, and migrational characteristics of Iron Gate Hatchery Chinook, 1995 releases. U.S. Fish & Wildlife Service California-Nevada Fish Health Center. Anderson, CA. Available online: <http://www.fws.gov/canvfhc/reports.asp>.
- Hallett SL and JL Bartholomew. 2006. Application of real-time PCR assay to detect and quantify the myxozoan parasite *Ceratomyxa shasta* in water samples. *Diseases of Aquatic Organisms* 71:109-118.
- Henderickson GL, A Carleton, and D Manzer. 1989. Geographic and seasonal distribution of the infective stage of *Ceratomyxa shasta* (Myxozoa) in Northern California. *Diseases of Aquatic Organisms*, 7:165-169.

Hoffmaster JL, JE Sanders, JS Rohovec, JL Fyer, and DG Stevens. 1988. Geographic distribution of the myxosporean parasite, *Ceratomyxa shasta* Noble, 1950, in the Columbia River basin, USA. *Journal of Fish Diseases*. 11:97-100.

Humason GL. 1979. *Animal tissue techniques*. 4th ed., WH Freeman and Co., San Francisco.

Jones S, G Prosperi-Porta, S Dawe, K Taylor and B Goh. 2004. *Parvicapsula minibicornis* in anadromous Sockeye (*Oncorhynchus nerka*) and Coho (*Oncorhynchus kisutch*) Salmon from tributaries of the Columbia River. *Journal of Parasitology* 90(4): 822-885.

Leidy RA and GL Leidy. 1984. Life stage periodicities of anadromous salmonids in the Klamath River basin, northwestern California. US Fish and Wildlife Service Division of Ecological Services. Sacramento, CA. Available online: http://www.fws.gov/arcata/fisheries/reports/krflow/life_stage_periodicities_of_anadromous_salmonids_1984.pdf.

Nichols K, JS Foott. 2006. FY 2004 Investigational Report: Health monitoring of juvenile Klamath River Chinook Salmon. US Fish and Wildlife Service California-Nevada Fish Health Center. Anderson, CA. Available online: <http://www.fws.gov/canvfhc/reports.asp>.

Nichols K, K True, E Wiseman and JS Foott. 2007. FY 2005 Investigational Report: Incidence of *Ceratomyxa shasta* and *Parvicapsula minibicornis* infections by QPCR and Histology in juvenile Klamath River Chinook Salmon. US Fish and Wildlife Service California-Nevada Fish Health Center. Anderson, CA. Available online: <http://www.fws.gov/canvfhc/reports.asp>.

Nichols K and K True. 2007. FY 2006 Investigational Report: Monitoring incidence and severity of *Ceratomyxa shasta* and *Parvicapsula minibicornis* infections in juvenile Chinook salmon (*Oncorhynchus tshawytscha*) and coho salmon (*Oncorhynchus kisutch*) in the Klamath River, 2006. US Fish and Wildlife Service California-Nevada Fish Health Center. Anderson, CA. Available online: <http://www.fws.gov/canvfhc/reports.asp>.

St-Hilaire S, M Boichuk, D Barnes, M Higgins, R Devlin, R Withler, J Khattra, S Jones and D Kieser. 2002. Epizootiology of *Parvicapsula minibicornis* in Fraser River sockeye salmon, *Oncorhynchus nerka* (Walbaum). *Journal of Fish Diseases* 25:107-120.

True K, MK Purcell and JS Foott. in press. Development and validation of a quantitative PCR to detect *Parvicapsula minibicornis* and comparison to histologically ranked juvenile Chinook salmon (*Oncorhynchus tshawytscha*) from the Klamath River, USA. *Journal of Fish Disease*.

Udey LR, JL Fryer, and KS Pilcher. 1975. Relation of water temperature to ceratomyxosis in rainbow trout (*Salmo gairdneri*) and coho salmon (*Oncorhynchus kisutch*). Journal of the Fisheries Research Board of Canada 32:1545-1551.

Walker RL and JS Foott. 1992. Disease survey of Klamath River salmonid smolt populations. U.S. Fish & Wildlife Service California – Nevada Fish Health Center, Anderson, CA. Available online: <http://www.fws.gov/canvfhc/reports.asp>.

Wallace M and BW Collins. 1997. Variation in use of the Klamath River Estuary by juvenile Chinook salmon. California Fish and Game 83(4):132-142.

Williamson JD and JS Foott. 1998. FY98 Investigational Report: Diagnostic evaluation of moribund juvenile salmonids in the Trinity and Klamath Rivers (June-September 1998). US Fish and Wildlife Service, California-Nevada Fish Health Center, Anderson, CA. Available online: <http://www.fws.gov/canvfhc/reports.asp>.

APPENDIX

Paraphrased Reviewer's Comments

Comment: Both parasites were detected in the first week after hatchery release from IGH. Was pathogen screening conducted at IGH or TRH prior to release.

Authors' Reply: No pathogen screening was conducted in either hatchery population prior to release; however, from previous studies and ongoing work conducted in 2008 it is known that hatchery fish can have light infections of both parasites. The text was changed to reflect this possibility.

Comment: Figures 1 and 2 present weekly pathogen incidence above the Trinity confluence. Is there similar data for fish captured below the Trinity?

Authors' Reply: Only CWT Chinook were collected in the Klamath below the Trinity. Above the Trinity both CWT and random unmarked Chinook were collected. Only the random Chinook were included in estimates of parasite incidence.

Comment: Previous sentinel studies were discussed. It should be noted how long fish were exposed so readers can get a sense of how long it takes for fish to become infected with lethal doses of the pathogens.

Authors' Reply: The study referenced in the discussion used a 72 hour exposure period. This has been added to the text.

Comment: Any explanation for the huge difference in *Cs* vs *Pm* infection prevalence in TRH CWT smolts?

Authors' Reply: The prevalence of *Pm* was higher than *Cs* in all groups. This difference may have been larger in the Trinity origin Chinook, but we do not have data to indicate why at this time. No changes were made to the text.

Comments: Any thoughts on why there was such a steep decline in infection prevalence 5 weeks after hatchery release in TRH fish compared to IGH fish?

Authors' Reply: The text was changed to suggest TRH smolts could escape infections if they moved quickly through the Lower Klamath.

Comment: The QPCR assay is semi-quantitative. Why have you chosen to report the incidence of infection but not the severity of the infections by QPCR?

Authors' Reply: The histology assay has been rated using a similar system for the last 4 years and that data was presented to describe the severity of the infection. The methods used in tissue collection and digestion were modified each year to optimize the QPCR assay. More work is needed to identify the levels of infection by QPCR associated with disease and mortality. As the assay is developed we plan to report the levels and prognosis of the infections.

Comment: Was the same histology methodology used in previous years? It would be useful to reference it.

Authors' Reply: Yes, a similar methodology has been used for histology since 2004. The text of the methods section has been changed to reflect this and reference the earlier studies.

Comment: Results report trends at sites, but no discussion of differences or trends across sites was presented.

Authors' Reply: Since the fish were migrating downstream comparisons between sites would essentially be a discussion of trends over time. For trends over time the best data available was CWT marked Chinook since these fish had a common origin and known release date. No changes were made to the text.

Comment: Text refers to the average incidence of infection and references tables 5 and 6. The tables do not support easy interpretation of this.

Authors' reply: The tables have been changed to include a simple average of 2004-2007.

Comment: Are you inferring that the histology fish are useful to examine proportions of the population that are clinical? Should you recognize that fish captured for histology may have been those that are the easiest to capture?

Authors' reply: It was necessary to keep sample collection simple to avoid unnecessary burdens on field crews; the collection of fish for the histology and QPCR assays was performed randomly. Any bias was likely due to our stated capture methods. The capture methods did not change significantly, and any bias would remain throughout the study.

Comment: Using OSU's water sampling results might strengthen the conclusion that infected fish were dying in June resulting in the declining prevalence of infection and clinical disease.

Author's reply: The focus of this report was to describe the data we collected. The OSU spore count data would be interesting to correlate with our disease data. The OSU's data speaks to a specific time and place where the fish became infected. Mortality would follow by several weeks and we do not know where the fish spent that time which would complicate any correlation of the data sets.