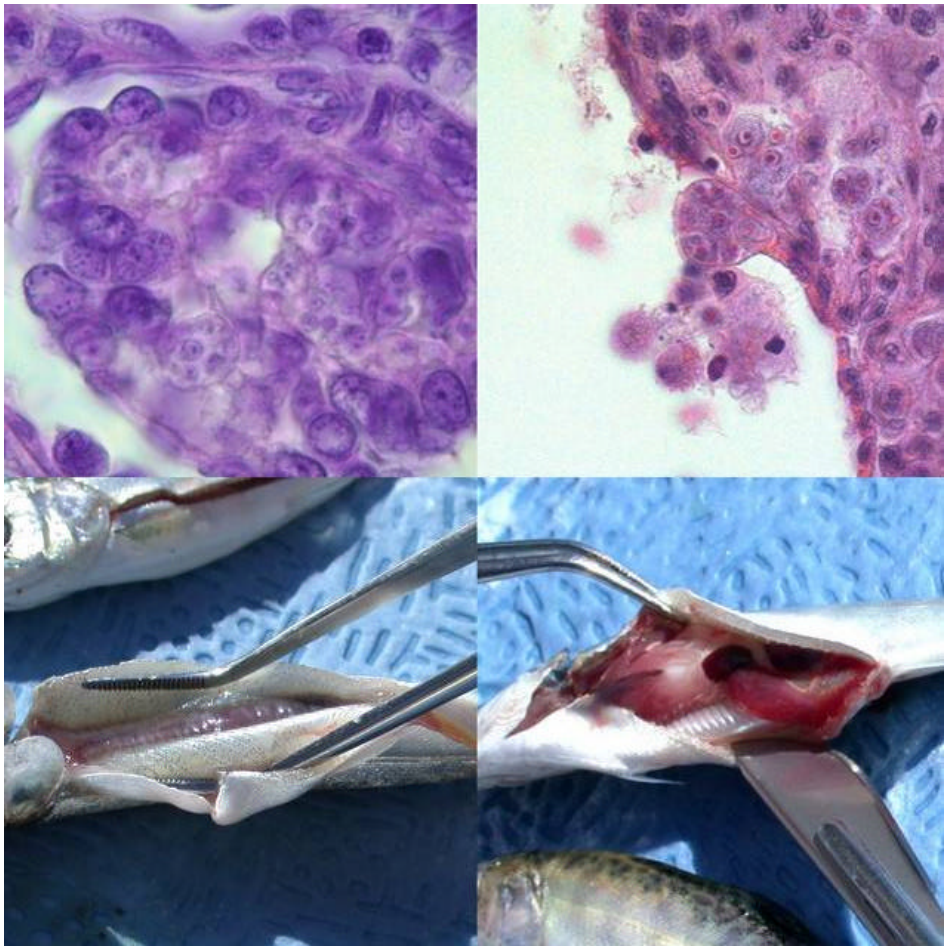
U.S. Fish & Wildlife Service

California-Nevada Fish Health Center
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

Ken Nichols and Kimberly True



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/

December 2007

SUMMARY

The weekly incidence of *Ceratomyxa shasta* and *Parvicapsula minibicornis* infection in juvenile Klamath River Chinook Salmon was monitored over 24 weeks of the spring and summer out-migration period (March – August) during 2006. The prevalence of both *C. shasta* (35% by QPCR) and *P. minibicornis* (83% by QPCR) during the height of juvenile Chinook out-migration (May, June and July) was lower in 2006 compared to previous studies in 2004 and 2005. Incidence (by QPCR) of both parasites peaked on 10 July at 93% for *C. shasta* and 100% for *P. minibicornis*. While peak prevalence of infection was still similar in magnitude to previous monitoring years, peak infection prevalence for both parasites was delayed in 2006. Among marked hatchery Chinook recovered in the Klamath incidence of *C. shasta* peaked at 65% in the third week following release from IGH while only 1% of TRH fish were infected. Infections with both parasites were observed in juvenile coho salmon, with 3% *Cs* and 48% *Pm* incidence in yearling and 7% *Cs* and 59% *Pm* incidence among naturally produced young of the year coho salmon utilizing the mainstem Klamath River.

The correct citation for this report is:
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. U.S. Fish & Wildlife Service California-Nevada Fish Health Center, Anderson, CA.

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 aeromonid bacteria), 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 studied.

Ceratomyxa shasta and *Parvicapsula 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 > 4°C (Ching and Munday 1984; Hendrickson et al. 1989; Bartholomew et al. 1989). Studies conducted in 2004 and 2005 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; Bartholomew et al. In Press).

In this study we monitored the weekly incidence of *C. shasta* and *P. minibicornis* infection in juvenile Chinook (*Oncorhynchus tshawytscha*) and coho (*Oncorhynchus kisutch*) salmon over 24 weeks of the spring and summer out-migration period (March – August) during 2006. This study utilized two complementary assays: Quantitative real-time Polymerase Chain Reaction (QPCR) for its high sensitivity and efficiency, with a small cohort assayed by histology to assess disease state and provide continuity with previous studies.

METHODS

Fish Collection

Fish collection occurred from 16 Mar through 23 Aug, 2006. Sample reaches and agencies performing collections are summarized in Table 1. Previous Klamath monitoring studies indicated target sample numbers could not be captured in any reach for the entire study period, so each reaches were only sampled when sufficient numbers of juvenile Chinook were likely to be present. 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 a full week were combined into a single sample where necessary.

A portion of the Chinook salmon released from Iron Gate Hatchery (IGH smolts) and Trinity River Hatchery (TRH smolts) were marked with an adipose fin clip indicating they were implanted with a coded-wire-tag (CWT). Heads from all marked Chinook were collected and assigned individual identification numbers. 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 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
Scott to Salmon	Klamath 143-66	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 sampled. In Klamath reaches above the confluence of the Trinity River the first 30 Chinook encountered per reach were collected each week. In Klamath reaches below the Trinity confluence all adipose clip marked fish encountered were collected, with an additional 10-20 unmarked fish collected each week from each reach. In the Trinity River, the first 30 Chinook were collected from each reach in early June and again in late July. Any coho salmon encountered in the Klamath River above the Trinity River confluence were collected under endangered species Section 10 permit 1068.

Following capture and preliminary examination by collection crews, fish were euthanized, placed in a labeled plastic bag, 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.

Each week personnel from the CA-NV Fish Health Center would accompany the samplers in one or more reaches to collect 10 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 – Fish were thawed, measured for fork length, weighed, and examined for external or internal clinical signs of disease. The intestine (pyloric ceca, small intestine and large intestine) from each fish was removed and placed into an individually numbered 1.5 ml tube. Similarly, the entire kidney was placed in another tube. Both tissue samples were then frozen until DNA extraction could be performed.

DNA extraction – Intestine and kidney tissue samples were digested in 1ml of digest reagent (1:8 proteinase K in digest buffer, Applied Biosystems, Foster City, CA) per gram tissue at 55ºC for 1 hour with constant shaking. For each fish a subsample of both digested tissues (50-200 µl digest = 25-100 mg tissue) were combined into the same well of a 96 well plate for DNA extraction (Applied Biosystems Model 6100 Nucleic Acid Prep Station). Extracted DNA was stored at -20ºC 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. Gastrointestinal tissues were tested for *C. shasta* 18S rDNA using Taqman Fam-Tamra probe and primers (Hallet

& Bartholomew 2006). Kidney tissues were tested for *P.minibicornis* 18S rDNA utilizing Taqman Minor-Grove-Binding (MGB) probe and primers (True et al., manuscript submitted). Reaction volumes of 30uL, including 5uL DNA template, were used for both assays under the following conditions: 50ºC for 2 min; 95ºC for 10 min; 40 cycles of 95ºC for 15 s and 60ºC for 1 min. Standards and no template control wells consisting of molecular grade water were included on each assay plate. Cycle threshold (C_T) values were assigned by the SDS software (v 1.3.1) (Applied Biosystems).

Assay Validation – The dynamic range and reliable endpoint define assay sensitivity. For the Pm QPCR we performed assay validation using a Pm plasmid construct and naturally infected kidney tissue (confirmed clinical infection by histology). The reliable endpoint was determined by examining C_T values of 4 replicate wells; when replicates exceeded the manufacturer's recommended standard deviation of 0.300 for quantitative assay quality control (Guide to Performing Relative Quantification of Gene Expression Using Real-time QPCR, Applied Biosystems, Inc.). We observed acceptable standard deviation among replicates at C_T 38 for both plasmid and naturally infected fish tissues. A standard curve was generated based on the Pm plasmid control which had a slope of -3.7 and a y-intercept of 44.018 such that the C_T value of 38 is equivalent to approximately 40 plasmid copies. We currently do not have a plasmid control for Cs QPCR, but performed similar assay validation with naturally infected fish tissue and determined an assay endpoint of C_T 38. For both assay, criteria for a positive test result also required test samples to produce a minimum change in normalized fluorescent signal (? Rn) of at least 10,000 fluorescent units, indicating true amplification above background fluorescent levels of the instrument. Details of the Pm QPCR assay validation and Quality Control measures are fully described in a recent publication submitted in November 2007 (True et al., manuscript submitted).

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 5 µm 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. *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).

Interannual Comparisons

Juvenile Klamath River Fall Chinook Salmon historically began out-migration in February, peaked in mid-June, and were captured in Klamath Estuary in large numbers in 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 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
- 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 22 May in 3 of 10 fish sampled in the Salmon to Trinity reach (Table 2). The peak incidence of infection was observed the week of 26 June where 80% (8/10) juvenile Chinook were *C. shasta* infected in the Salmon to Trinity reach. Overall, this parasite was detected in 18.8% (35/186) of Chinook from the Klamath River. Intestinal lesions symptomatic of clinical ceratomyxosis were observed in 83% (29/35) of infected Chinook. The peak of clinical ceratomyxosis was observed during late June. No *C. shasta* was detected in the 30 Chinook sampled 28 July in the Lower Trinity River (Table 4).

Parvicapsula minibicornis infections were first observed during the week of 8 May in one of three fish sampled in the Salmon to Trinity reach (Table 3). Incidence of infection reached 90% (9/10) by the week of 22 May. Overall, *P. minibicornis* was detected in 49.5% (92/186) of Chinook sampled in the Klamath. Clinical glomerulonephritis was observed in 54% (50/92) of the *P. minibicornis* infected Chinook. The peak incidence of clinical glomerulonephritis was 90% observed the week of 26 June. No *P. minibicornis* was detected by histology in the 30 Chinook sampled 28 July in the Lower Trinity River (Table 4).

QPCR Assay

Ceratomyxa shasta infections were detected from our first sample in the week of 13 March. The incidence of infection remained low (23% or less) until late May when we observed a sharp increase in fish sampled from most reaches. Peak incidence was 93% in Chinook captured above the Trinity confluence during the week of 10 July (Figure 1), and 71% during the week of 3 July below the Trinity confluence (Figure 2).

Ceratomyxa shasta was detected in 3% (3/87) of juvenile Trinity Chinook sampled within the Trinity River (Table 4). All were very light infections near the detection threshold of the QPCR assay. All three infected fish were captured at the Sky Ranch site in the Upper Trinity reach, and were among the earliest fish examined from the Trinity in 2006. Two of these infected fish were captured prior to hatchery release and were naturally produced. The third infected fish was captured on 6 June and may have been of either hatchery or natural origin.

Parvicapsula minibicornis infections were detected from the first Klamath samples taken the week of 13 March. Incidence remained low until early May. *Parvicapsula minibicornis* incidence peaked in the Klamath above the Trinity confluence at 100% on 10 July (Figure 3) and below the confluence at 93% on 17 July (Figure 4).

Parvicapsula minibicornis was detected in 39% (35/89) of juvenile Trinity Chinook captured in the Trinity River (Table 4). Most but not all of these infections were light. *Parvicapsula minibicornis* was detected in fish from both the Upper and Lower Trinity reaches before and after hatchery release. Peak prevalence of 64% (16/25) was observed in the Lower Trinity reach in late May.

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, Spring and Summer of 2006. Fish were 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		20 Mar	3 Apr	10 Apr	17 Apr	1 May	8 May	15 May	22 May	29 May	5 Jun	12 Jun	19 Jun	26 Jun	3 Jul	10 Jul	17 Jul	24 Jul
Klamath Estuary	*Cs*+														3/10			0/10
	Clinical														2/10			0/10
Trinity to Estuary	*Cs*+													5/10		3/10	0/1	0/10
	Clinical													4/10		3/10	0/1	0/10
Salmon to Trinity	*Cs*+						0/3		3/10	3/10		1/10		8/10		0/1	0/1	
	Clinical						0/3		3/10	3/10		1/10		6/10		0/1	0/1	
Shasta to Scott	*Cs*+	0/3		0/8				0/10			1/10		7/10		1/9			
	Clinical	0/3		0/8				0/10			0/10		6/10		1/9			
IGD to Shasta	*Cs*+	0/10	0/10		0/10	0/10												
	Clinical	0/10	0/10		0/10	0/10												

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, Spring and Summer of 2006. Fish were 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		20 Mar	3 Apr	10 Apr	17 Apr	1 May	8 May	15 May	22 May	29 May	5 Jun	12 Jun	19 Jun	26 Jun	3 Jul	10 Jul	17 Jul	24 Jul
Klamath Estuary	*Pm*+														7/10			0/10
	Clinical														4/10			0/10
Trinity to Estuary	*Pm*+													9/10		9/10	1/1	1/10
	Clinical													5/10		8/10	0/1	1/10
Salmon to Trinity	*Pm*+						1/3		9/10	7/10		10/10		10/10		1/1	1/1	
	Clinical						0/3		1/10	5/10		6/10		9/10		1/1	0/1	
Shasta to Scott	*Pm*+	0/3		0/8				2/10			6/10		10/10		8/9			
	Clinical	0/3		0/8				0/10			1/10		2/10		7/9			
IGD to Shasta	*Pm*+	0/10	0/10		0/10	0/10												
	Clinical	0/10	0/10		0/10	0/10												

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 histological exam of intestine (for *Cs*) or kidney (for *Pm*) or QPCR of a combined kidney and intestine sample for individual fish.

Week	**29 May**	**29 May**	**5 June**	**12 June**	**19 June**	**21 July**	**21 July**	**Total**
Reach	Lower	Upper	Upper	Upper	Upper	Lower	Upper	
Assay	QPCR	QPCR	QPCR	QPCR	QPCR	Histology	QPCR	
Cs incidence	0/24	2/2	1/2	0/15	0/15	0/30	0/29	3/117
Pm incidence	16/25	2/2	1/2	4/15	5/15	0/30	7/30	35/119

Marked Hatchery Fish

A total of 67 IGH and 68 TRH smolts, with corresponding QPCR assay results were collected between 31 May and 23 August. All IGH smolts were captured between Iron Gate Dam and the Klamath Estuary from one to 9 weeks following release. All TRH smolts were captured in the Klamath River between the Trinity River confluence and Klamath Estuary from 4 to 12 weeks following release.

Within 5 days of release from the hatchery (entry into the Klamath) *C. shasta* DNA was detected in IGH smolts. Incidence of *C. shasta* infection by QPCR in IGH smolts peaked at 65% in the third week following release. Incidence of *P. minibicornis* infections by QPCR in IGH smolts reached 100% by the third week following release, and remained high through the last IGH smolt recovery 9 weeks after hatchery release (Figure 5).

Ceratomyxa shasta was detected by QPCR in only 1 of the 67 TRH smolts recovered in the Lower Klamath River. This infected fish was captured in the 6th week after hatchery release. No further infections fish were detected in the 49 TRH smolts recovered 7-12 weeks following Trinity River Hatchery release (Figure 6). *Parvicapsula* infections were detected in 51 of the 68 TRH smolts recovered in the Lower Klamath River. Incidence appeared to peak in the 7th week after Trinity River Hatchery release at 75% (Figure 6).

Interannual Comparisons

Compared to studies performed in 2004 and 2005 (Nichols and Foott 2005, Nichols et al. 2007) the incidence of infection peaked later in the spring of 2006 and pathogen incidence during the peak of Chinook out-migration (May, June and July) was lower than in previous years (Table 5 and 6).

Table 5. Comparison of *Ceratomyxa shasta* prevalence in juvenile Klamath River Chinook from 1994-2006. Only fish sampled in May-July are included to aid comparisons between years. Sample methods included histology in all years and quantitative real-time PCR (QPCR) in 2005 and 2006.

	1994-2002 Histology	2004 Histology	2005 Histology	2006 Histology	2005 QPCR	2006 QPCR
Infected	20%-50%	34%	35%	21%	62%	34%
Clinical (Histology only)	n/a	23%	21%	18%	n/a	n/a
N	156	735	134	112	724	822

Table 6. Comparison of *Parvicapsula minibicornis* prevalence in juvenile Klamath River Chinook from 1995-2006. Only fish sampled in May-July are included to aid comparisons between years. Sample methods included histology in all years and quantitative real-time PCR (QPCR) in 2005 and 2006.

	1995-2002 Histology	2004 Histology	2005 Histology	2006 Histology	2005 QPCR	2006 QPCR
Infected	47%-88%	77%	92%	58%	96%	83%
Clinical (Histology only)	n/a	37%	65%	29%	n/a	n/a
N	176	731	134	112	716	824

Coho Salmon

Histology Assay

Histological analysis was performed on 16 coho sampled early in the study (before May 8). No *Ceratomyxa* infections were observed, and *Parvicapsula* was detected in one fish (6%). No intestinal lesions or glomerulonephritis were observed (Table 7). Coho collected later in the study were analyzed by QPCR due to the assay's higher sensitivity.

QPCR Assay

Ceratomyxa shasta was detected by QPCR in 5% (5/96) of juvenile coho. Two (11%) of 18 natural young-of-the-year (YOY), and three (4%) of 78 yearling coho were infected. The first detection of *C. shasta* occurred the week of 8 May, and the majority of *C. shasta* infected coho were captured in early June (Table 7).
Parvicapsula minibicornis was detected in 56% (54/96) of juvenile coho. Thirteen (72%) of 18 natural YOY, and 41 (53%) of 78 yearling coho were infected. The first detection of *P. minibicornis* by QPCR occurred the week of 1 May, and the majority of *P. minibicornis* infected coho were captured in late May (Table 7).

Table 7. Incidence of *C. shasta* and *P. minibicornis* infection in Coho salmon captured in the Klamath River above the confluence with the Trinity River. Screening for the parasites was performed by histological exam of intestine (for *Cs*) or kidney (for *Pm*) or QPCR of a combined kidney and intestine sample.

Week Beginning	Reach	Assay	*Cs* YOY	*Cs* 1+	*Pm* YOY	*Pm* 1+
17 Apr	IGD to Shasta	Histology		0/3		0/3
	Shasta to Scott	Histology		0/1		0/1
24 Apr	IGD to Shasta	Histology		0/3		1/3
	Shasta to Scott	Histology		0/1		0/1
1 May	IGD to Shasta	Histology	0/7	0/1	0/7	0/1
	IGD to Shasta	QPCR	0/3		3/3	
	Shasta to Scott	QPCR		0/4		2/4
8 May	IGD to Shasta	QPCR		0/2		1/2
	Shasta to Scott	QPCR	0/4	1/3	1/4	1/3
	Scott to Salmon	QPCR		0/11		2/11
	Salmon to Trinity	QPCR		0/6		2/6
15 May	IGD to Shasta	QPCR		0/1		1/1
	Shasta to Scott	QPCR	0/3	0/1	2/3	0/1
	Scott to Salmon	QPCR		0/17		8/17
22 May	Shasta to Scott	QPCR	0/1		1/1	
	Scott to Salmon	QPCR		0/6		5/6
	Salmon to Trinity	QPCR		0/14		13/14
29 May	Scott to Salmon	QPCR	0/1	0/9	1/1	3/9
5 Jun	Shasta to Scott	QPCR	1/1		1/1	
	Scott to Salmon	QPCR		1/1		1/1
	Salmon to Trinity	QPCR		0/1		0/1
12 Jun	Shasta to Scott	QPCR	0/1		1/1	
	Salmon to Trinity	QPCR		1/2		2/2
19 Jun	Shasta to Scott	QPCR	0/1		1/1	
3 Jul	Shasta to Scott	QPCR	0/1		1/1	
10 Jul	Shasta to Scott	QPCR	1/2		1/2	
Total	**All reaches**	**Histology**	**0/7**	**0/9**	**0/7**	**1/9 (11%)**
		QPCR	**2/18 (11%)**	**3/78 (4%)**	**13/18 (72%)**	**41/78 (53%)**
		Both	**2/25 (7%)**	**3/87 (3%)**	**13/25 (59%)**	**42/87 (48%)**

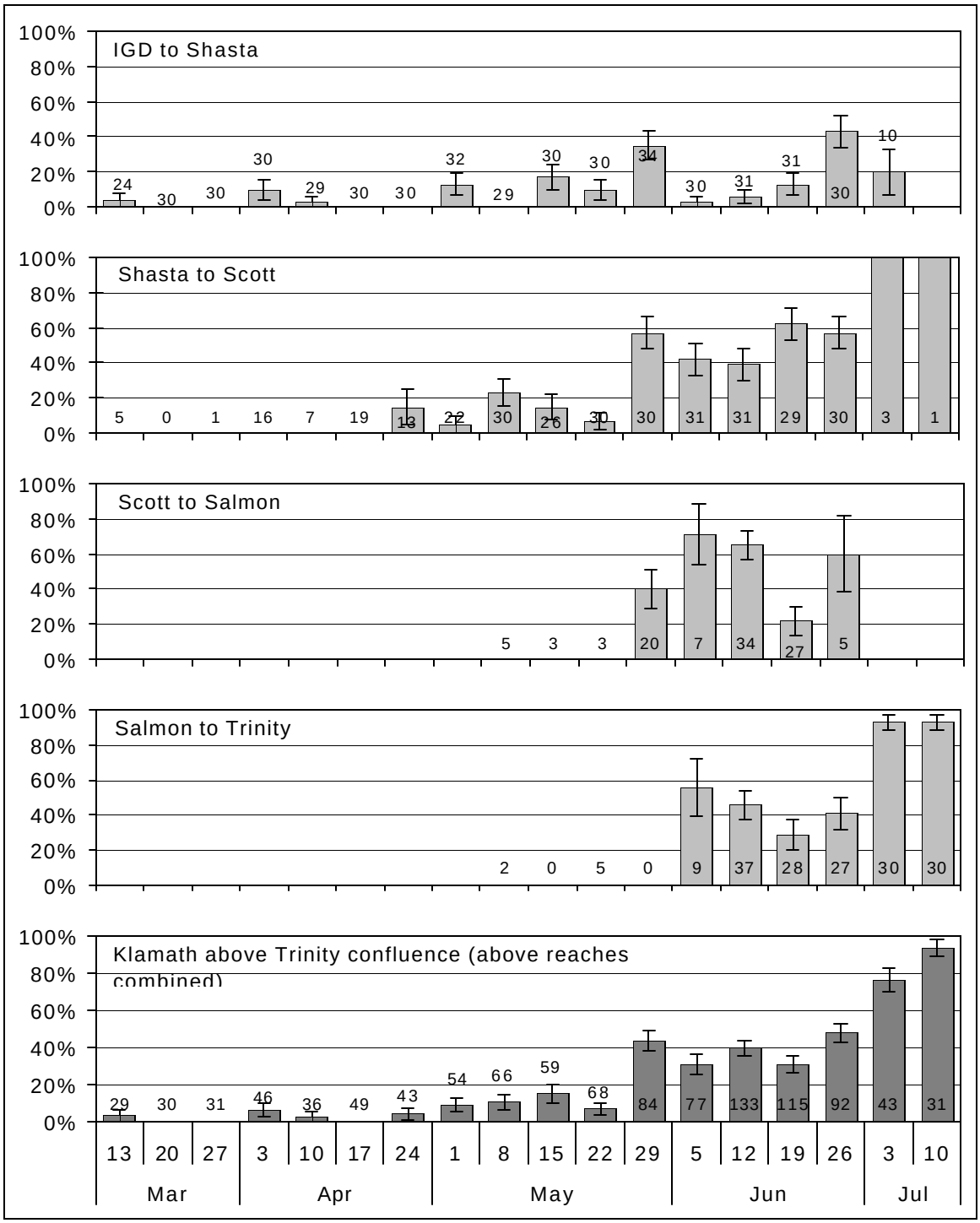


Figure 1. Incidence of *Ceratomyxa shasta* infection (± SE) in juvenile Chinook salmon captured in 4 reaches of the Klamath River above the Trinity River confluence (Iron Gate Dam to Shasta R., Shasta R. to Scott R., Scott R. to Salmon R. and Salmon R. to Trinity R.) during the spring and summer of 2006. Sample number (n) is listed near the base of each bar.

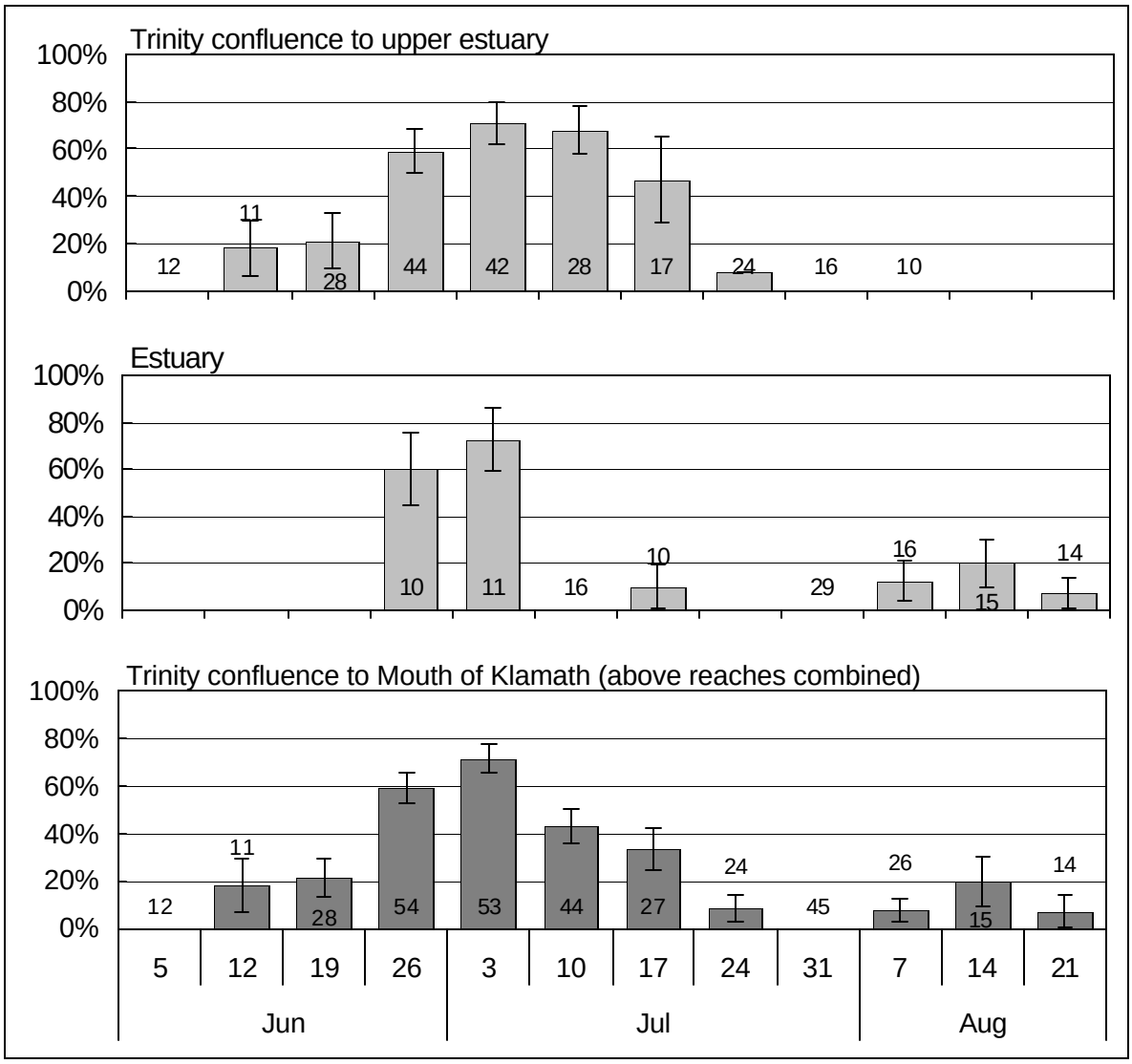


Figure 2. Incidence of *Ceratomyxa shasta* infection (± SE) in juvenile Chinook salmon captured in the Klamath River below the Trinity River confluence (Trinity R. confluence to upper Klamath Estuary and within the Klamath Estuary) during the spring and summer of 2006. Sample number (n) is listed near the base of each bar.

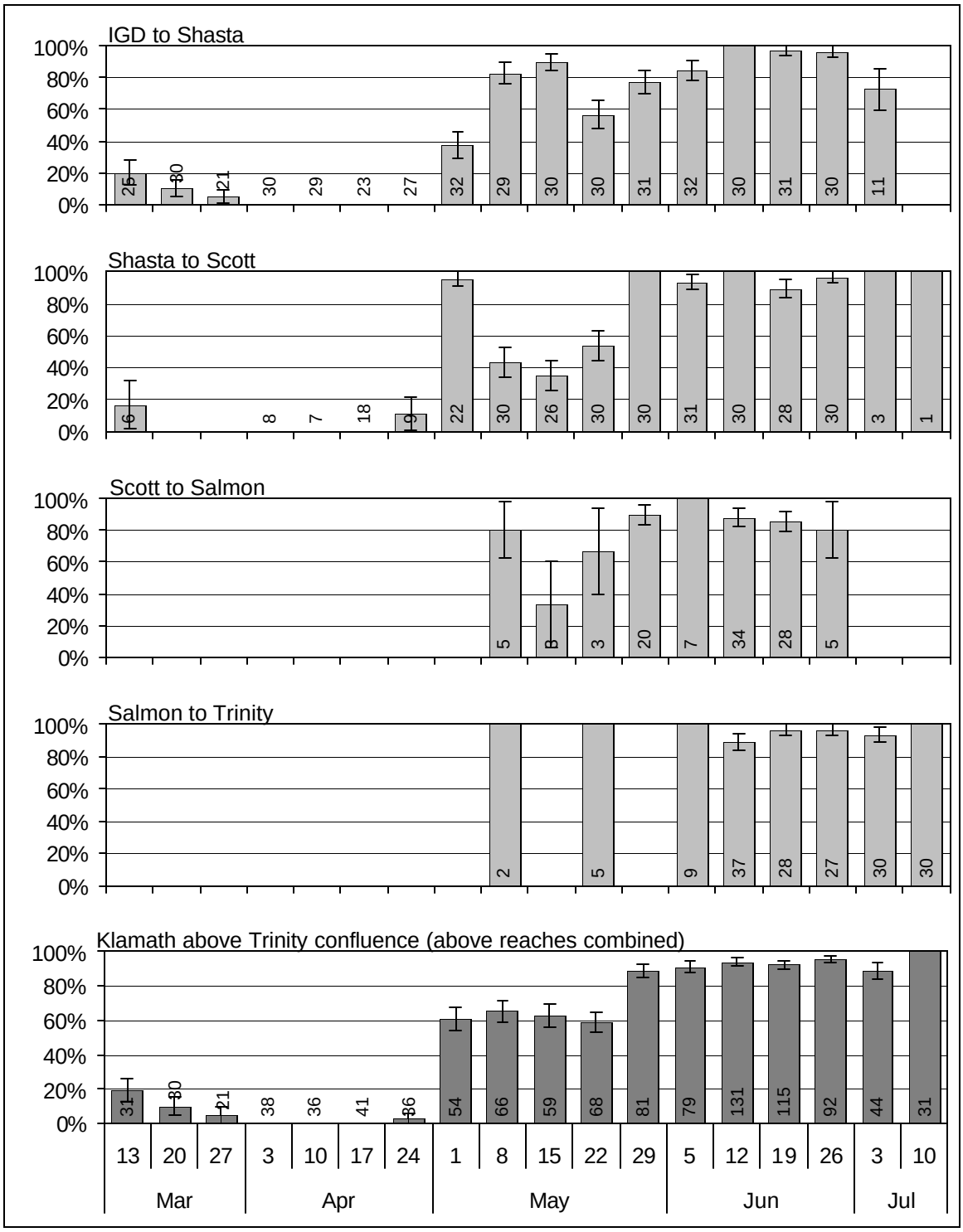


Figure 3. Incidence of *Parvicapsula minibicornis* infection (± SE) in juvenile Chinook salmon captured in 4 reaches of the Klamath River above the Trinity River confluence (Iron Gate Dam to Shasta R., Shasta R. to Scott R., Scott R. to Salmon R. and Salmon R. to Trinity R.) during the spring and summer of 2006. Sample number (n) is listed near the base of each bar.

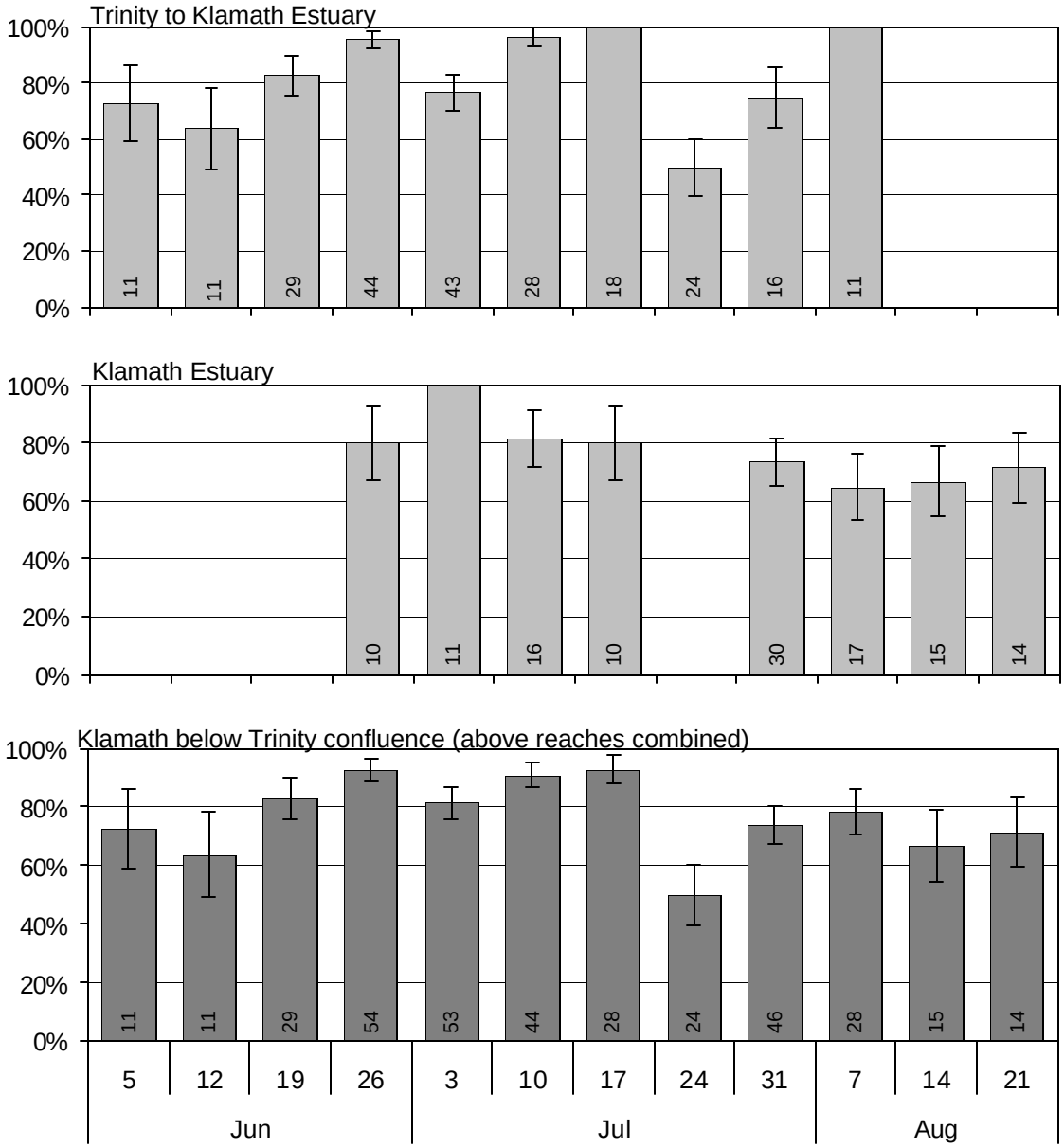


Figure 4. Incidence of *Parvicapsula minibicornis* infection (± SE) in juvenile Chinook salmon captured in the Klamath River below the Trinity River confluence (Trinity R. confluence to Klamath Estuary and within the Klamath Estuary) during the spring and summer of 2006. Sample number (n) is listed near the base of each bar.

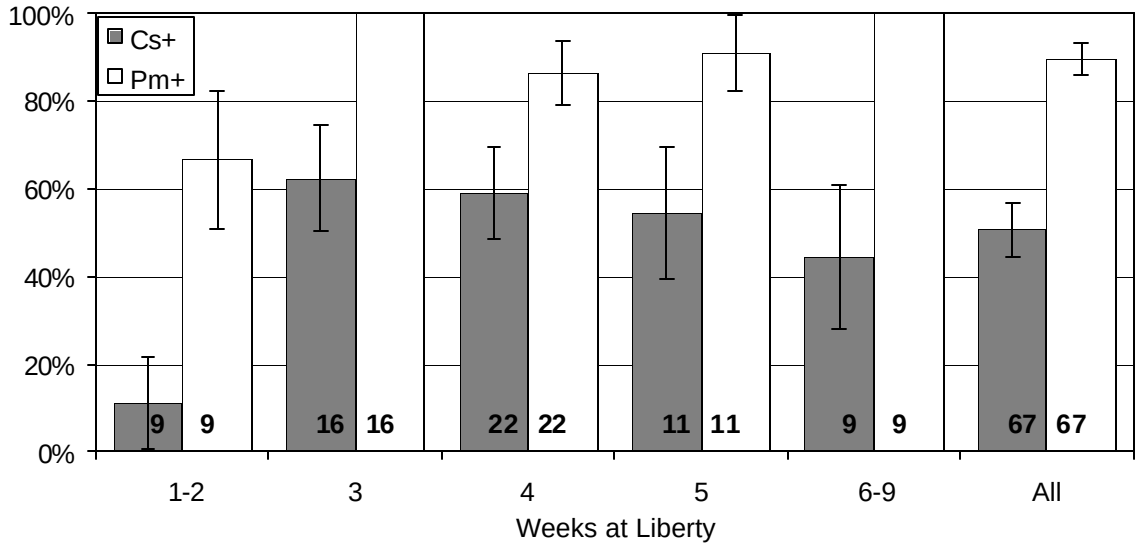


Figure 5. Incidence of *Ceratomyxa shasta* and *Parvicapsula minibicornis* infections (± SE) in Iron Gate Hatchery origin CWT juvenile Chinook recaptured in the Klamath River from Iron Gate Dam to the Estuary. Sample number (n) is listed near the base of each bar.

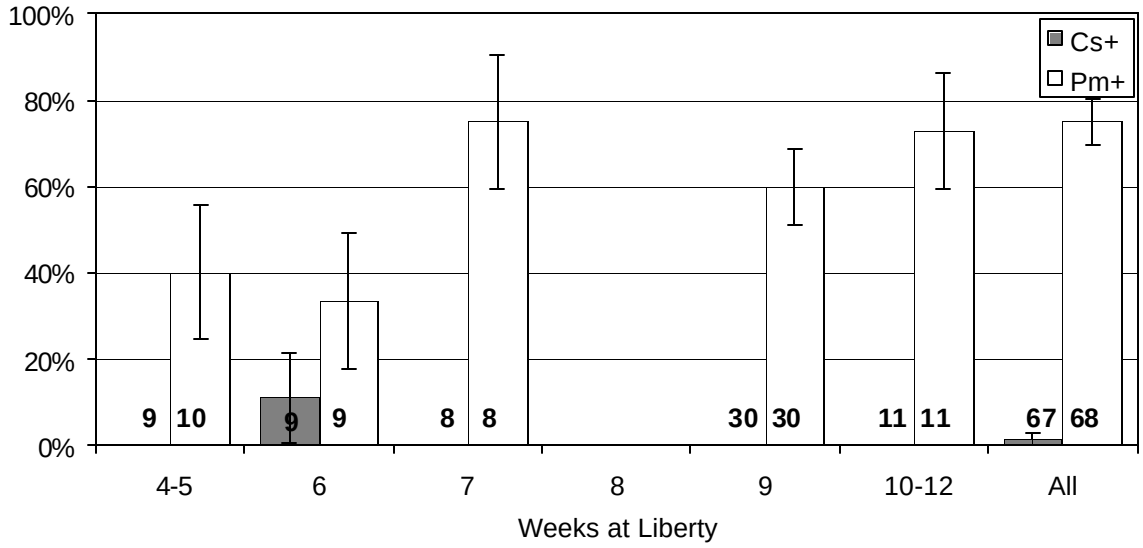


Figure 6. Incidence of *Ceratomyxa shasta* and *Parvicapsula minibicornis* infections (± SE) in Trinity River Hatchery origin CWT juvenile Chinook recaptured in the Lower Klamath River. Sample number (n) is listed near the base of each bar.

DISCUSSION

Seasonality of the Parasites

Infections of both parasites seemed to have a similar seasonality where they increased in the spring with decreasing flows and increasing water temperatures. *Parvicapsula minibicornis* tended to have an earlier rise in infection rates observed in monitoring studies in 2005 and 2006 and a higher peak incidence of infection compared to *C. shasta.* In a review of *C. shasta* literature, Bartholomew et al. (1989) cite infections occurring at water temperatures as low as 4 to 6ºC with disease progressing faster at warmer water temperatures. The first detections of *C. shasta* and *P. minibicornis* in this study were in mid March with water temperatures fluctuating daily at 4-7ºC, and peak prevalence of both parasites occurred in mid July at 20-24ºC. Average pathogen incidence during the 2006 Chinook out-migration (May, June and July) was lower than in previous years, with peak incidence observed several weeks later in 2006 compared to 2004 and 2005 (Nichols and Foott 2005, Nichols et al. 2007). This was likely due to higher than average flows during April, May and June (4000-12000 CFS at Seiad Valley in 2006 compared to the average spring range of 2200-6800 CFS). While the incidence of infection for both *C. shasta* and *P. minibicornis* was reduced, high flows in the winter and spring of 2006 did not eliminate disease problems. Possible hypothesizes for the reduced incidence of infection include:

- A reduction in the polychaete host (*Manayunkia speciosa*) involved in the life cycle of these parasites due to scouring associated with high flows
- A dilution effect on the actinospore (infectious to fish) stage of the parasites
- A reduced transmission/infection efficiency of the parasites due to environmental conditions (temperature, turbidity, velocity)
- A change in fish behavior reducing the likelihood of infection (habitat selection, migration rate or timing)

Prognosis of *Ceratomyxa* and *Parvicapsula* infections

While infection prevalence eventually did reach levels similar to previous years, the majority of juvenile fall Chinook would have out-migrated from the upper basin prior to the peak *C. shasta* incidence period. The peak of juvenile Chinook out-migration at the Big Bar trap (river mile 50) in 2006 occurred from mid to late June (Toz Soto, phone conversation 8/9/2007). In monitoring studies conducted in 2004 and 2005 the peak incidence of infection occurred in May, prior to the peak of migration in those years. During the typical May-July out-migration period average prevalence of infection was lower in 2006 compared to previous monitoring years (Nichols and Foott 2005, Nichols et al. 2007). This estimate of average prevalence may have been biased by our selected sample method. Weekly sample effort (number of fish sampled) must be proportional to the population to accurately represent the population incidence. Our method of a constant 30 fish per week did not take into account the changing smolt population within the reach. Because the peak of infection occurred prior to the peak of migration (population) in 2004 and 2005 the proportion of the population infected with *C. shasta* was likely higher than the estimated average prevalence in those years. Due to the later peak prevalence of infection in 2006 the proportion of the juvenile Chinook population infected was likely lower than the estimated average prevalence. While this bias was a concern, our study design was intended to portray the seasonal progression of the

infections. Our data could be expanded to better estimate prevalence in the population if our weekly incidence was weighted by a weekly population estimate (data currently not available).

The more sensitive QPCR assay utilized for the majority of samples allows detection of lower level infections which may not always lead to disease and mortality. In past studies using only the less sensitive histology assay, low survival was expected from fish diagnosed with *C. shasta* infection. Sentinel studies utilizing Chinook salmon in the Shasta to Scott reach resulted in 82% mortality in less than three weeks at 16ºC, with mean survival time decreasing at warmer water temperatures (Udey et al. 1975, Foott et al. 2004).

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. The high prevalence of *P. minibicornis* infections results in nearly all *C. shasta* infected fish having dual infections. We speculate that nephrons 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.

Naturally produced Chinook became infected with both parasites during March and April, but the incidence remained low during this period in 2006. Juvenile coho salmon also became infected during out-migration, but the overall incidence of infection appears lower than the later migrating Chinook.

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. Within only 5 days of entering the Klamath, IGH smolts had become infected with *C. shasta.* The incidence of *C. shasta* infection among IGH smolts peaked within a few weeks of release. Over half of the IGH smolts were *C. shasta* infected during their 190 mile out-migration. A similar trend in *C. shasta* infection was observed in 1995 IGH smolts out-migrants (Foott et al. 1999). These IGH smolts could be viewed as surrogates for naturally produced tributary smolts (i.e. Shasta River, Scott River) out-migrating through the Klamath. The disease risk to parr rearing in the Klamath prior to out-migration is likely higher. No signs of recovery from the infections were observed in intestines examined by histology during this study (data not shown). This suggests that a significant portion of the infected fish succumb before reaching the ocean.

The incidence of *P. minibicornis* infections in IGH smolts jumped to 100% within 3 weeks of hatchery release and remained near 100% until the last IGH smolt recaptures 9 weeks post release. While *P. minibicornis* incidence in IGH smolts remained high, the incidence of heavy infections observed by QPCR decreased between 3 and 9 weeks post release (data not shown). 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 recover if they survived the anemia and osmoregulation problems associated with glomerulonephritis.

Among TRH smolts, *C. shasta* incidence was very low with only 1% of the TRH smolts infected. A study by the CA-NV Fish Health Center conducted in 2002 found that, by histology, 19% of TRH smolts captured in the Klamath estuary were infected with *C. shasta,* and 23% were infected with *P. minibicornis* (Nichols et al. 2003).

Directly comparing the results of the histology assay used in 2002 with the more sensitive QPCR used in this study is not appropriate. However, QPCR results indicate that while *P. minibicornis* prevalence was still high, TRH smolts escaped *C. shasta* infection during their passage through the Lower Klamath in 2006.

Conclusions

We were provided an opportunity in 2006 to observe the effect of high winter and spring flows on infection and disease progression in juvenile Klamath River Salmonids. The prevalence of both *C. shasta* and *P. minibicornis* during May, June and July was lower in 2006 compared to previous studies in 2004 and 2005. The higher flows appeared to delay the peak of infection for both parasites, but peak prevalence of infection was still similar in magnitude to previous monitoring studies. Histological observations of Chinook captured in late June show that a majority were in a clinical disease state. Juvenile coho salmon become infected with both parasites including naturally produced young of the year coho salmon utilizing the mainstem Klamath River.

ACKNOWLEDGMENTS

We wish to thank biologists with the USFWS Arcata FWO, Yurok Tribe, Karuk Tribe and Hoopa Tribe for fish collection; Eric Wiseman and 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; Jerri Bartholomew, Scott Foott, Nina Hemphill and Paul Zedonis 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.

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APPENDIX
Reviewer's Comments

Listed below are comments on a draft this report provided by reviewers and the author's responses.

1. Were temperatures the same? I think you need a sentence or two to discuss other possible variables

Response: Flows were mentioned in this report because we felt they were significantly different than the typical year. A short list of environmental variables we feel may have influence infection prevalence has been added to the discussion. However, the major focus of this study was to report the pathogen incidence. We feel it would be useful to explore the relationship between this data and environmental variables in a collaborative study, but that is beyond scope of this report.

2. I think that the summary is the weak point of the report. There is a lot of good information in this report that I believe should be brought forward. Give mean and peak prevalence and dates here as this is interesting.

Response: Agreed, more data added to summary.

3. Better to divide the Trinity into upper and lower at North Fork rather than South Fork.

Response: We agree. Text has been changed to reflect division of Trinity River into upper and lower at the confluence of the North Fork rather than South Fork.

4. Coho origin: Were any of these Trinity coho?

Response: Text in has been changed to emphasize all coho were Klamath origin.

5. How you did come to the conclusion that infection incidence in the population was lower than reflected in the results? I would like to see under results or methods how you arrived at his conclusion or the rationale for this.

Response: This is a discussion point and speaks in relative terms. This section of the discussion has been rewritten to clarify logic behind our assertion.

6. Did the movement of fish downriver indicate a loss as they moved downriver in the years where the peak occurred early?

Response: Do to the influx of presumably healthy fish from tributaries the population is constantly changing. We have no way of knowing the history of unmarked fish. Large numbers of marked fish would be needed to assess mortality during out-migration. No changes to the text of the report were made in response.

7. Does anything in the literature suggest that the timing of the peak can be crucial because of the susceptibility on the upward swing of migration?

Response: Salmonids may be more susceptible to stress during smoltification causing immunosupression. This study sought to monitor the prevalence and not model susceptibility (which would be interesting).

8. Is there info on the susceptibility or morbidity with dual as opposed to single infections?

Response: Some language has been added to reflect the effects of kidney infection, but are still working to understand the prognosis of *P. minibicornis* infection.

9. Relating the results to environmental conditions is a worthy consideration but a much more thorough presentation of flow/temp/ migration timing from tributaries etc should be integrated and assessed to help discern any relationships.

Response: We agree. A close look at environmental variables (flow, temp, etc.) is needed and is beyond the scope of this report. Flows were mentioned in this report because we felt they were significantly different from a typical year (4000-12000 CFS at Seiad Valley in 2006 compared to the average spring range of 2200-6800 CFS).

10. I would find it valuable to present the quality control results of the QPCR. If results are boarder line for CS or PM what are the chances of false positives due to inhibitions/ contamination issues. I think that quality control data is very important to include and that a section of this report should be devoted to such a topic.

Response: Our assay validation of the *P. minibicornis* QPCR has been submitted for publication so we have decided not to present the data here. Much of the language suggesting results of QPCR validation have been removed and the draft paper has been referenced (True et al. manuscript submitted 11/20/2007).

11. Infections of both parasites were detected in Chinook captured in the Trinity River. It is assumed these were light infections, is that true?

Response: The text has been changed to include some indication of severity where appropriate.

12. What other environmental conditions could affect disease transmission in 2006?

Response: Several environmental variables were added to better define the term. Analysis of environmental conditions was not done in this report.