FY 2004 Investigational Report:

Health Monitoring of Juvenile Klamath River Chinook Salmon

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Summary
Between 11 May and 27 July 2004, 745 juvenile fall-run Chinook Salmon (*Oncorhynchus tshawytscha*) were collected for pathogen and physiological assays at 4 general locations in the lower Klamath River. Pathogens of interest included the bacterium *Flavobacterium columnare*, and myxozoan parasites *Parvicapsula minibicornis* and *Ceratomyxa shasta.* Only 2.4% of fish examined were infected with *F. columnare* suggesting it was not a significant problem in these fish in 2004. Expanding from trap efficiency data, we estimated that 45% of the population was infected with *C. shasta* and 94% of the population was infected with *P. minibicornis*. The prognosis for *P. minibicornis* infection by itself is not well understood. The high incidence of dual myxozoan infection (98% of *C. shasta* infected fish), and associated pathology suggests that the majority of the *C. shasta* infected juvenile Chinook would not survive.

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Introduction

The California-Nevada Fish Health Center, as a partner in the efforts to restore salmonid populations in the Klamath River basin, conducted pathogen and physiology monitoring of juvenile Klamath River Chinook Salmon (*Oncorhynchus tshawytscha*) since 1991. Pathogens associated with diseased fish in the Klamath River include bacteria (*Flavobacterium columnare* and motile aeromonid bacteria), digenetic trematode (presumptive *Nanophyetus salmincola*) and myxozoan parasites (*Parvicapsula minibicornis* and *Ceratomyxa shasta*). 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).

Ceratomyxa shasta is a myxosporean parasite that occurs in a number of watersheds of the Pacific Northwest, and the lifecycle includes a polychaete, *Manayunkia speciosa*, and salmonid hosts (Hoffmaster et al. 1988, Bartholomew et al. 1997). Infection can occur from spring through fall at water temperatures > 7°C (Ching and Munday 1984, Hendrickson et al. 1989). Hendrickson et al. (1989) describe the parasite's distribution in California to include the San Joaquin, Sacramento, Pit, and Klamath River systems. In the Klamath basin, *C. shasta* infection has been detected in salmonids from the mouth of the Klamath River to Iron Gate dam (rm 190), Copco reservoir, both Klamath and Agency Lakes, and the lower reaches of the Williamson and Sprague Rivers (Hendrickson et al. 1989, Oregon State University 2004). No infected juvenile Chinook smolts have been detected in health monitoring work in the Scott, Shasta, and Trinity Rivers (Foott et al. 2002, Nichols et al. 2003). Infections have also not been detected by histological examination of IGH Chinook salmon sampled prior to their June release in 1992 – 1995 (Walker and Foott 1992, Foott et al. 1999).

In this study we monitored the weekly incidence of *C. shasta* and *P. minibicornis* infection in juvenile Chinook salmon during their spring emigration at several sites on the Klamath River. We expanded the observed incidence data to the juvenile Fall Chinook population using trap efficiency estimates. The utility of apparent clinical signs (pale gill, swollen abdomen and swollen kidney) for determining disease status of fish was also examined.

Methods

Sampling – During the spring and early summer of 2004, juvenile Klamath River Chinook Salmon were collected at Persido Bar (RM 81) and Big Bar (RM 51) by rotary screw trap. Fish were also collected at one beach seine site per week alternating between Big Bar and Persido Bar 11-May through 16-June. After 16 June, fish had moved into cooler water refuge sites near creek mouths, so we shifted our seine efforts from river bars to creek mouths (RM 50-81, Table 2 and Appendix I). Each week we attempted to examine 30 fish from each trap and 20 fish from the seine collection. Crews operating the traps would collect and hold live fish up to 48 hours prior to sampling depending on number of fish captured each day. Fish captured by seine were sampled immediately following capture.

Necropsy – Fish were euthanized in MS222, measured for fork length and examined for abnormalities. The degree of the abnormality was scored according to

criteria presented in Table 1. Tissue samples were collected for bacteriology, ATPase, muscle RNA:DNA and histology assays (described below).

Table 1. Abnormality scoring system used during necropsy.

Abnormality	Score
Pale Gill (Anemia)	0 = normal 1 = pale 2 = grey/white/tan – no pink
Gill Lesion	0 = normal, no lesion 1 = lesion present
Skin/Fin Hemorrhages	0 = normal, no hemorrhaging 1 = hemorrhaging of skin and/or fins
Distended Abdomen	0 = abdomen normal 1 = abdomen distended
Organ Hemorrhaging	0 = normal, no hemorrhaging 1 = hemorrhaging of one or more internal organs
Swollen Kidney (Nephritis)	0 = kidney normal concave shape 1 = kidney flat or slightly convex; some grey color 2 = kidney convex and grey

Histology – Gastrointestinal tract (pyloric ceca and intestine) and kidney tissues were rapidly removed from the fish and immediately fixed in Davidson's fixative, transferred to 70 % ethanol after 24-48 hours, 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). The presence of the myxozoan parasites (*C. shasta* and *P. minibicornis)* and degree of tissue inflammatory response to the parasites (lesions) were rated as 0 (normal or no inflammation), 1 (parasites and inflammatory response in less than 50% of tissue section), 2 (parasite induced inflammatory response in greater than 50% of tissue section), or 3 (entire tissue section demonstrating parasite infection and inflammatory response with little or no normal tissue structure). Individual fish were categorized as uninfected, lightly infected, or severely infected according to the presence of the parasites and lesion score for the target tissue (gastrointestinal tract for *C. shasta* and kidney for *P. minibicornis).*

Bacteriology – If a fish exhibited signs of septicemia (hemorrhaging fins or skin, petechial hemorrhaging on organs) a sample of kidney tissue was inoculated onto Brain Heart Infusion agar slant tubes. Isolates were identified to genera by standard microscopic and biochemical tests (Lasee 1995). Corroboration of bacterial septicemia was performed by examining spleen imprints for large numbers of bacteria. Any fish with visible gill lesions was screened for *Flavobacterium columnare* (the causative agent

of Columnaris disease) by examining a gram stained imprint of the lesion for characteristic long filamentous Gram negative rods.

ATPase – Gill Na^+, K^+-Adenosine Triphosphatase activity was assayed by the method of McCormick and Bern (1989). Briefly, gill lamellae were dissected and frozen at -70°C in sucrose-EDTA-Imidazole (SEI) buffer on dry ice. The sample was later homogenized, centrifuged and the pellet sonicated prior to the assay. ATPase activity was determined by the decrease over time in optical density (340 nm) as NADH is converted to NAD+. This activity was reported as µmole ADP/mg protein/hour as 1 mole of NAD is produced for each mole of ADP generated in the reaction. Gill Na-K-ATPase activity is correlated with osmoregulatory ability in saltwater and is located in the chloride cells of the lamellae. This enzyme system transports salts from the blood against the concentration gradient in saltwater.

Muscle RNA:DNA – A section of caudal muscle was assayed for RNA:DNA ratio by the method of Kaplan, Leamon and Crivello (2001). Briefly, approximately 0.5g of muscle was dissected and frozen on dry ice; the sample was later homogenized and digested in a buffered digest mixture. Quantity of RNA and DNA in the sample was determined by use of fluorescent dyes compared to ribosomal RNA (16S and 23S rRNA from *E. coli*) and lambda DNA standards. RNA:DNA ratios in white muscle are highly correlated with specific growth rate and are useful in detecting growth suppression in fish (Pickering and Pottinger 1995).

Determination of pathogen prevalence – The population estimate was based on Big Bar trap efficiency data provided by the USFWS Arcata Fish and Wildlife Office (Mark Magneson, personal communication). We used estimates of daily fish passage at the trap site to calculate the percent of the total population which passed the Big Bar trap each week (Figure 1). We then multiplied the weekly prevalence of infection observed at the Big Bar trap (as a percent) by the percent of the population migrating past the trap each week, and summed these weekly estimates to produce the percentage of juvenile Klamath River Chinook Salmon passing Big Bar which were infected with either *C. shasta* or *P. minibicornis*.

Estimates of the juvenile Chinook salmon population size were difficult to quantify due to poor recapture rates and low trap efficiency. Our population infection expansions were based on these mark-recapture experiments conducted at several times during our study, and they were based on the best available information.

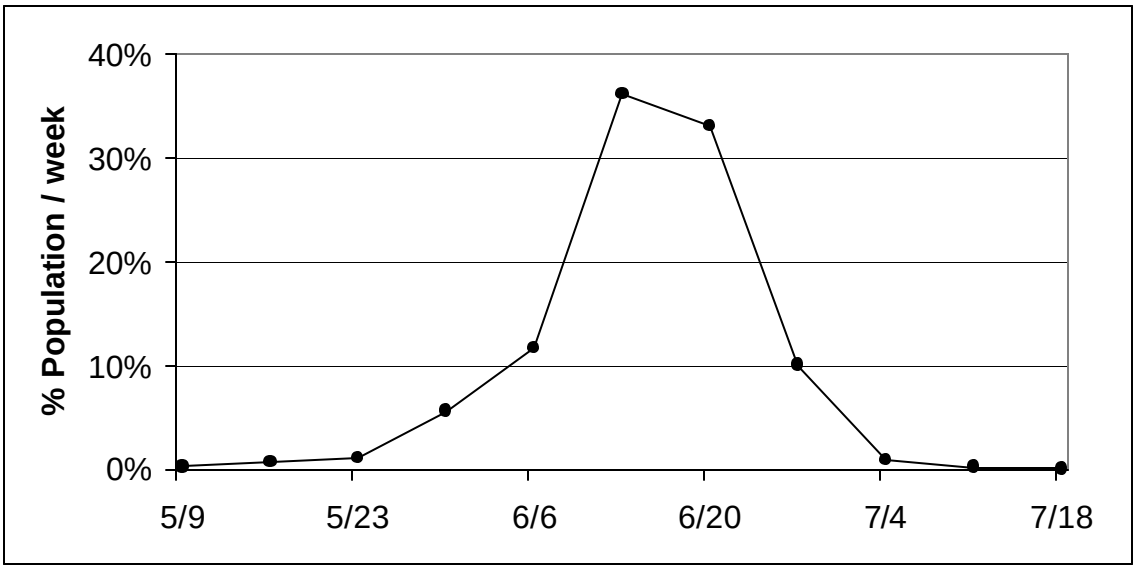

Figure 1. Percent of juvenile Klamath River Chinook Salmon emigration by week estimated at the Big Bar rotary screw trap (Mark Magneson, personal communication).

Analysis of trends between sites or methods – Wilcoxon paired-sample test was used to determine if sample site or method tended ($P<0.05$) toward higher weekly prevalence of infection. Comparisons in infection prevalence were made between Big Bar and Persido Bar traps and between Big Bar trap and seine sites to determine if the prevalence observed at Big Bar trap was representative of all sample sites. Sample sites with less than 10 samples in a single week were not used in this analysis.

Results

Fish collection – The sample date, sites and number of fish collected are summarized in Table 2. Mean fork length increased from 52 mm to 89 mm during the first half of the study then remained fairly constant through the end of our study (Figure 2).

Table 2. Number of juvenile Klamath River Fall Chinook Salmon sampled by rotary screw trap at Big Bar and Persido Bar, or beach seine (Seine). Seine location is noted for each week.

Week #	Sample Date	Big Bar	Persido Bar	Seine (location)
1	11-May	30	3	20 (Presido Bar)
2	19-May	31	12	20 (Big Bar)
3	25-May	29	29	19 (Presido Bar)
4	1-June	30	30	23 (Big Bar)
5	8-June	28	31	20 (Presido Bar)
6	16-June	27	30	23 (Big Bar)
7	22-June	30	26	18 (Camp Creek)
8	29-June	26	20	20 (Camp Creek)
9	6-July	27	25	0 (Camp Creek)
10	13-July	30	30	20 (Bluff Creek)
11	20-July	31	0	0 (Bluff Creek)
12	27-July	7	0	0
	Sub-totals	326	236	183 = 745 (total)

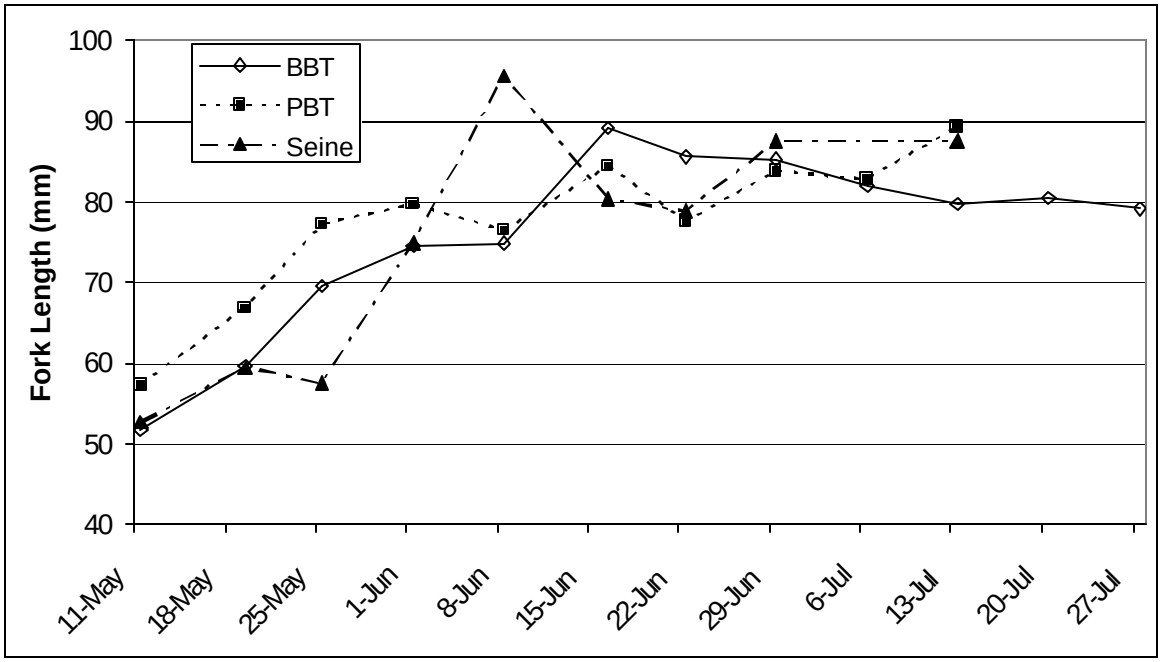

Figure 2. Mean fork length for juvenile Klamath River Chinook Salmon captured in the Big Bar trap (BBT), Presido Bar trap (PBT) and roving seine sites.

Ceratomyxa shasta – Weekly prevalence of infection for all sites combined ranged from 15% to 56%, with three peaks (>50% prevalence) occurring in May, June and July (Figure 3). Severe intestinal inflammation (inflammatory response in greater than 50% of tissue section) was observed by histology in 67% of the infected fish. Expanding from the trap efficiency data we estimated 45% of the population passing Big Bar was infected with *C. shasta.* There were no significant differences in weekly

prevalence of infection between Big Bar trap and Persido Bar trap (P=0.074) nor between Big Bar trap and seine sites (P=0.098).

Parvicapsula minibicornis – Weekly prevalence of infection for all sites combined ranged from 36% to 93%, with the peak observed in fish captured on 16-June (Figure 4). Glomerulonephritis (inflammation of the glomeruli of the kidney) was observed by histology in 48% of the infected fish. Expanding from the trap efficiency data we estimated 94% of the population passing Big Bar was infected with *P. minibicornis*. There were no significant differences in infection rates between the Big Bar trap and Persido Bar trap (P=0.203), nor between Big Bar trap and seine sites (P=1.0). The high prevalence of *P. minibicornis* infections results in nearly all (98%) of the *C. shasta* infected fish having dual (*C. shasta – P. minibicornis*) infections.

Correlation of field observations to histopathological lesions – Observations made during field collections were not a reliable diagnostic tool for predicting a fish's specific parasite infection. Three clinical signs of disease (pale gill, swollen kidney, and swollen abdomen) noted during necropsy were related to each of the four results from histological examination (*C.shasta* positive, *P.minibicornis* positive, intestinal lesion, and kidney lesion). These associations were demonstrated by the statistical significance of each pairing of clinical sign to histological condition in Table 3 ((P<0.01, one-tailed Fisher exact test). Dual parasite infections influenced the lack of diagnostic value for clinical signs.

Bacterial infections – Signs of bacterial septicemia were observed in 4 of 745 fish examined (0.5%). A motile *Aeromonas sp*. (presumptively *Aeromonas hydrophila*) was isolated from the other two of the four affected fish. Gill lesions suggestive of *F. columnare* infection were observed in 18 of 745 fish examined (2.4%). Typical *F. columnare* bacteria were observed in gill imprints from 15 of those 18 fish.

Gill ATPase –Gill Na^+, K^+-ATPase activities ranged between 1.7 and 4.3 µmole ADP/mg protein/hour and with peak values observed 19-May (Figure 5). No consistent trend was seen with time or water temperature. There were no significant differences between fish caught by trap or seine on the same sample date (P>0.05, t-test) so all samples from each day were pooled. *P. minibicornis* and *C. shasta* infections had no obvious effect on ATPase activity levels.

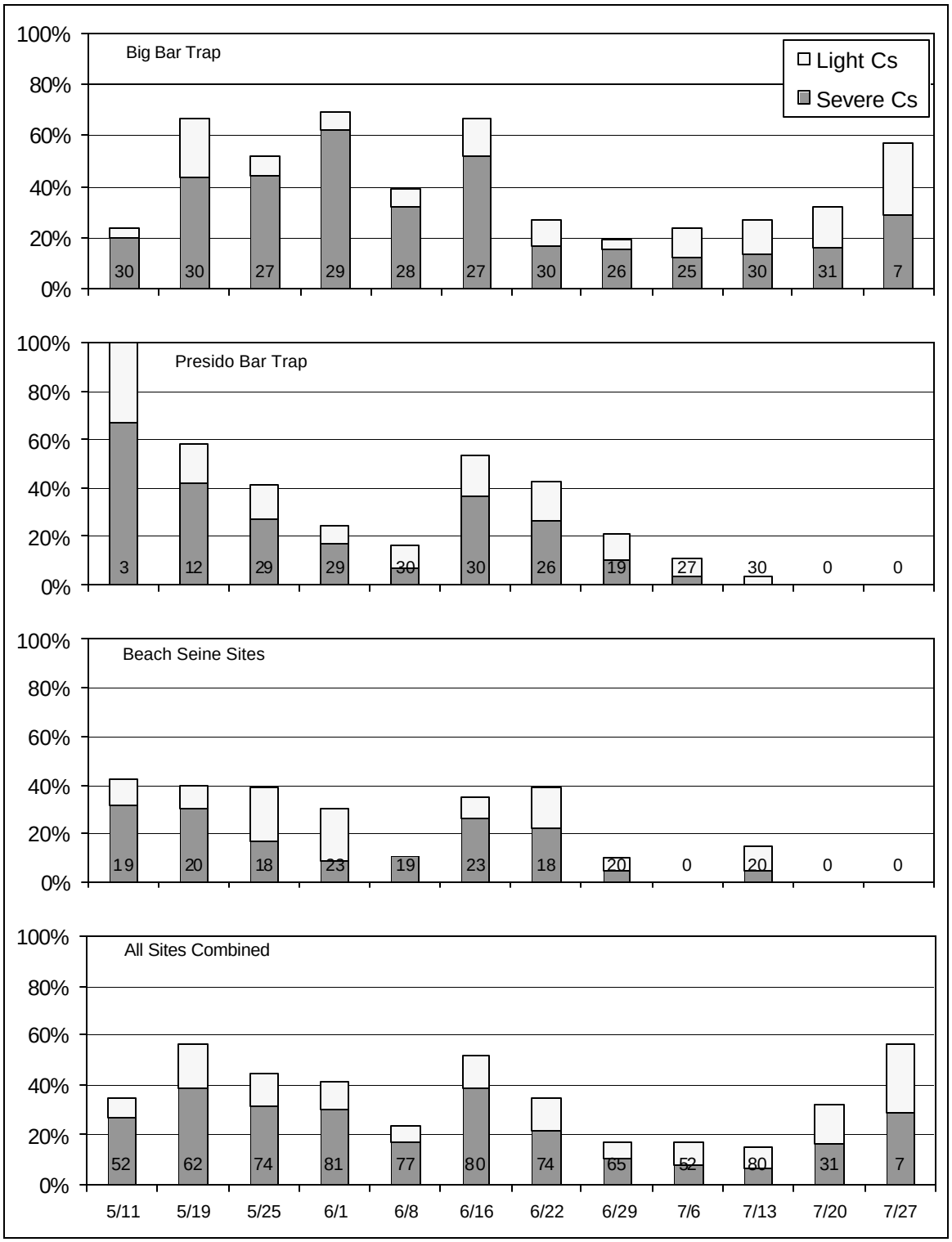

Figure 3. Percent of juvenile Klamath River Chinook Salmon with light and severe infections of *Ceratomyxa shasta*. Severe infections were indicated by greater than 50% of the intestinal section demonstrating an inflammatory response associated with the parasite. Data is presented as percent of fish infected (light + severe) with number of samples in the base of each bar.

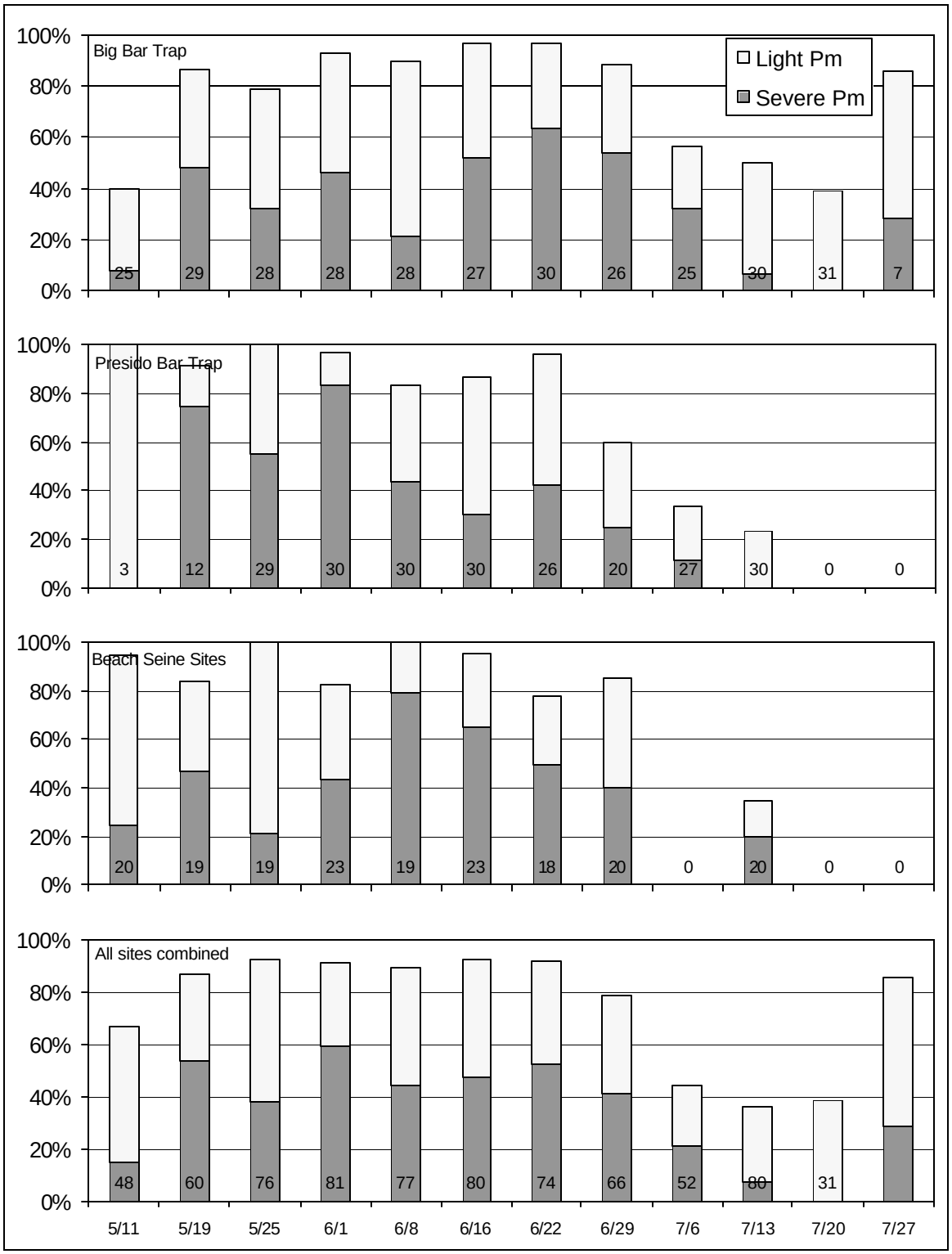

Figure 4. Percent of juvenile Klamath River Chinook Salmon with light and severe infections of *Parvicapsula minibicornis*. Severe infections were indicated by greater than 50% of the kidney section demonstrating an inflammatory response associated with the parasite. Data is presented as percent of fish infected (light + severe) with number of samples in the base of each bar.

Table 3. Frequency a clinical sign of disease (pale gill, swollen abdomen, swollen kidney) or histopathological condition (*Cs+*, IL, *Pm+* or KL) co-occurred. Numbers in bold text were significantly greater (P<0.01, one-tailed Fisher exact test) than all samples combined (bottom row). Due to fish condition or problems with lab assay we did not have complete observations of signs and conditions for every fish; therefore, sample number (N) is approximate (±2.8%). All data is reported as percent of the true sample number.

		Percent Co-occurrence with:						
Clinical Sign or Condition	N	PG	SA	SK	*Cs+*	IL	*Pm+*	KL
Pale Gill (PG)	54		**15%**	**35%**	**78%**	**59%**	**96%**	**62%**
Swollen Abdomen (SA)	30	**27%**		**40%**	**70%**	**47%**	**97%**	**77%**
Swollen Kidney (SK)	142	**13%**	8%		28%	13%	**95%**	**72%**
Cs Infected *(Cs+)*	252					**67%**	**98%**	45%
Intestine Lesion (IL)	169				**100%**		**99%**	47%
Pm Infected *(Pm+)*	561				**44%**	**30%**		**48%**
Kidney Lesion (KL)	270				42%	29%	**100%**	
All Samples	744	7%	4%	19%	34%	23%	77%	37%

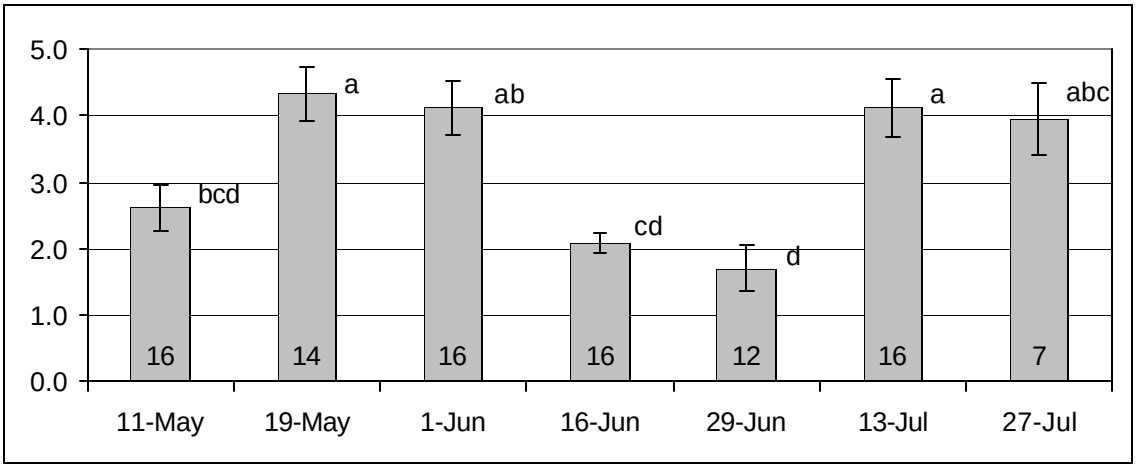

Figure 5. Gill Na+, K+-ATPase values (mmole ADP/mg protein/hour) for juvenile Klamath River Chinook Salmon collected in the Spring and Summer of 2004. Data presented as mean ±SE with sample number in base of each bar. Letters, not in common, indicate statistical differences between groups (p<0.05, ANOVA and Tukey test).

RNA:DNA – Mean muscle RNA:DNA tended to increase through the sampling period (Figure 6). This estimate of specific growth rate was not affected by *P. minibicornis* or *C. shasta* infection. Muscle RNA:DNA values correlated with sample date, fork length, and mean daily water temperature (all P<0.001, n=109). There was no correlation with gill ATPase (P=0.716, n=51).

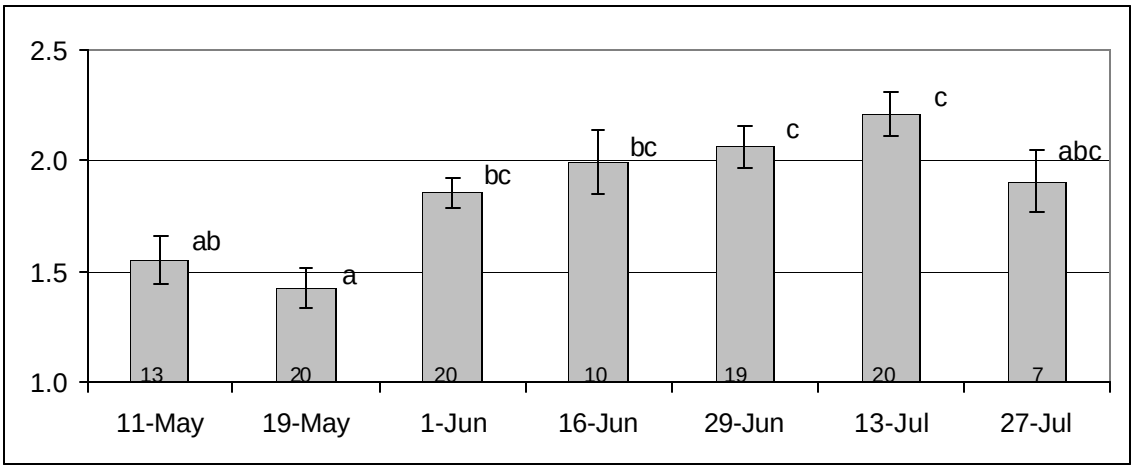

Figure 6. Mean muscle RNA:DNA values from juvenile Klamath River Chinook Salmon. Data is presented as mean ±SE and samples number in the base of each bar. Letters, not in common, indicate statistical differences between groups (p<0.05, ANOVA and Tukey test).

Discussion

Unknown influence of tributary populations on disease observations – Since only five marked hatchery salmon were collected in study, the history of each sampled fish was largely unknown. Previous examinations of juvenile salmon from the Shasta, Scott, and Trinity River have not detected *Ceratomyxa shasta* infections (National Wild Fish Health Survey 2005, Foott et al. 2002, and Nichols et al. 2003). If we assumed that parasite infection was primarily focused in the mainstem Klamath River, then the time of entry and duration of exposure to the mainstem river were a major determinant in disease. We noted a distinct group of fish captured in the 6-July sample (all sites). Histologically these fish demonstrated no inflammation of the adipose tissue or other characteristics associated with the stress of rearing at warm water temperatures. We hypothesize that many of salmon collected on 6-July had recently reared in a cool water environment.

Drops in the pathogen incidence data in late May and again in late June may represent pulses of fish which had spent little time in the mainstem Klamath River. The sudden dip in gill ATPase activity with no correlation with disease incidence or water quality also supports this theory. These pulses of fish and corresponding changes in infection rates demonstrate the potential bias towards one segment of the population by sampling over a limited time frame. The expanded population infection rates for both parasites were heavily weighed towards the infection rates during the month of June, as this is when the majority of smolts passed the Big Bar trap (Figure 1). Since Iron Gate Hatchery had released their juvenile Chinook salmon only a few weeks prior to this peak, we conclude a significant portion of the fish observed during this migration peak were of hatchery origin. Our estimated population infection rates could therefore be heavily influenced by these hatchery fish. Prior to Iron Gate Hatchery smolt releases in mid-May, the infection rates in naturally produced Chinook for *C. shasta* and *P. minibicornis* were 20-60% and 40-100% respectively. Increased marking effort of both the hatchery and tributary populations would allow for analysis of the disease risk as a function of river entry and days of exposure.

Potential bias – A collection bias towards healthier fish at the trap sites is a possibility as dead fish were not included in these samples. Fish in a severe disease state are more likely to die if held even for only few hours particularly in warm water. It was common for fish captured in a trap to sit in the live box for over 12 hours before the trap was checked. It was necessary for the trap crew to hold fish in a live box before we arrived for sampling, and we routinely observed 10-20% mortality during this holding period. Seine fish were sampled immediately following capture and were not affected by this practice.

Limited diagnostic value of clinical signs - In observations of clinical signs and histopathological conditions we introduced an intentional bias. Only those fish which clearly demonstrated abnormalities were considered "sick". Examples of this include observations of pale gills, swollen kidneys and histological lesions where we scored the abnormalities on a zero-two or zero-three scale with zero being normal. We only considered the scores of 2 or more to be abnormal even through those fish with a rating of one were showing some signs of abnormality. In these cases we considered a score of one to be transitional to a more severe disease state.

The clinical signs of disease we tracked (pale gills, swollen abdomen, and swollen kidney) demonstrated only marginal utility in identifying sick fish. Pale gill is a result of anemia which could be produced by intestinal hemorrhage (ceratomyxosis) or insufficient erythropoiesis due to kidney inflammation (*Parvicapsula* infection). Similarly, swollen abdomen occurs when the fish is unable to maintain its water balance and the peritoneum fills with ascitic fluid. Damage to kidney or the intestine can induce this condition. Dual infections further complicate the diagnostic picture. There may be some benefit in monitoring clinical signs to track population health over time, but the researcher should be aware that many fish without clinical signs were infected and would later progress into a disease state.

Flavobacterium columnare - The one clinical sign with diagnostic value was gill erosion that is often associated with *Flavobacterium columnare* infection. *Flavobacterium columnare* was not a significant health issue in this section of river during 2004 (2.4% incidence of infection). Past fish health examinations at Big Bar has found *F. columnare* to be a more significant problem with up to 20% incidence of infection (Nichols et al. 2003). It was associated with fish kills on the Klamath River most notably during an adult salmon fish die-off which occurred in 2002 (Guillen 2003).

Low survival is expected in the estimated 45% of the juvenile Klamath River Chinook Salmon infected with *C. shasta.* In controlled studies with Chinook salmon, exposure in the Klamath River lead to 82% mortality in less than 3 weeks at 16ºC and warmer water temperatures can further decrease mean survival time (Udey et al. 1975, Foott et al. 2004). The progress of *P. minibicornis* infection in juvenile Chinook salmon is not well understood and this is an important question given the high prevalence of infection (94%) observed in this study. The high prevalence of *P. minibicornis* infections results in nearly all (98%) of the *C. shasta* infected fish having dual (*C. shasta – P. minibicornis*) infections. We conclude that the juvenile Klamath River Chinook population experienced a high mortality prior to their migration to the ocean below our

sample reach. There could also be some level of mortality above our sample reach which went undetected in this study.

Recommendations for future studies:

- Determine the prognosis of *Parvicapsula* infection.
- Determine the effects of disease on specific tributary populations.
- Determine the infection and mortality rates in specific reaches of the Klamath River.

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Notes

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Appendix I

Sample site, date, number and clinical signs of disease observed in juvenile Klamath River Fall Chinook Salmon during the Spring and Summer of 2004.

Site	Date	n	Pale Gill[1]	Gill Les.[2]	Dist. Ab.[3]	Ext. Hem.[4]	Int. Hem.[5]	Sw. Kid.[6]
Big Bar Trap	5/11	30	3%	0%	0%	0%	N/A	N/A
	5/19	31	7%	0%	3%	0%	N/A	N/A
	5/25	29	3%	3%	0%	0%	3%	7%
	6/1	30	30%	0%	3%	0%	0%	21%
	6/8	28	18%	11%	0%	4%	4%	32%
	6/16	27	11%	4%	15%	0%	7%	26%
	6/22	30	17%	3%	7%	7%	7%	57%
	6/29	26	4%	0%	12%	0%	8%	27%
	7/6	27	0%	0%	0%	0%	0%	28%
	7/13	30	0%	7%	0%	0%	0%	0%
	7/20	31	0%	10%	0%	3%	0%	0%
	7/27	7	0%	0%	0%	0%	29%	0%
Presido Bar Trap	5/11	3	66%	0%	0%	0%	N/A	N/A
	5/19	12	17%	0%	0%	0%	N/A	N/A
	5/25	29	7%	3%	0%	0%	0%	14%
	6/1	30	10%	0%	3%	0%	0%	30%
	6/8	31	3%	0%	3%	0%	0%	39%
	6/16	30	0%	3%	3%	0%	3%	40%
	6/22	26	7%	4%	12%	0%	15%	38%
	6/29	20	20%	5%	10%	0%	25%	25%
	7/6	25	0%	0%	0%	4%	0%	0%
	7/13	30	7%	0%	0%	7%	0%	0%
Presido Bar Seine	5/11	20	0%	0%	0%	0%	N/A	N/A
Big Bar Seine	5/19	20	5%	0%	0%	5%	N/A	N/A
Presido Bar Seine	5/25	19	11%	0%	0%	0%	0%	0%
Big Bar Seine	6/1	23	13%	4%	0%	0%	0%	13%
Presido Bar Seine	6/8	20	10%	0%	5%	0%	5%	70%
Big Bar Seine	6/16	23	9%	4%	13%	0%	13%	30%
Camp Creek Seine	6/22	18	6%	0%	17%	6%	6%	50%
Camp Creek Seine	6/29	20	0%	5%	20%	5%	10%	10%
Camp Creek Seine	7/6	0	N/A	N/A	N/A	N/A	N/A	N/A
Bluff Creek Seine	7/13	20	0%	0%	0%	0%	0%	0%

Notes:

1. Pale gill = gills had lost typical red color. Gills were tan or grey color. Gills with were pink or red coloration were considered normal.
2. Gill lesion = focal discoloration on gill possibly due to *Flavobacterium columnare* infection.
3. Distended abdomen = Abdomen notably swollen or inflated.
4. External hemorrhaging = pinpoint hemorrhaging on skin or at base of fins
5. Internal hemorrhaging = pinpoint hemorrhaging on visceral fat, organs or peritoneum
6. Swollen kidney = kidney notably inflated (nephritis)