

Sacramento and Feather River Juvenile Chinook Pathogen Survey Spring 2013

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Summary: Asymptomatic infections of the myxosporean parasites, *Ceratomyxa shasta* (Cs) and *Parvicapsula minibicornis* (Pm), was observed in 62 natural Sacramento R. Chinook juveniles collected from Red Bluff to Tisdale. No virus was isolated nor was *Renibacterium salmoninarum* (Rs) confirmed in kidney tissues. Aeromonad bacteria were isolated from 7-62% of the collection groups and was more prevalent in the lower Sacramento fish. A similar pattern of viral and bacterial infection was observed in Feather R. juveniles. A marked difference in Cs and Pm infection and disease was seen in 77 juvenile Feather R. Chinook collected at either rm 61 (infrequent detection) compared to rm 46 (40 – 100%). High mortality due to ceratomyxosis occurred in salmon collected on 12April and held in captivity. Elevated flows in late April were associated with a marked reduction of infectivity. Sentinel Chinook held for 3 d (29April-2May) at two sites each, in the Sacramento and Feather R., developed asymptomatic Cs and Pm infections. Sentinel rainbow trout largely remained uninfected. The prognosis of myxosporean infections in natural Chinook and their effect on survival should be evaluated.

Introduction: The National Wild Fish Health Survey (**NWFS**) is a program conducted by the U.S. Fish and Wildlife Service Fish Health Centers to assess the prevalence and distribution of major fish pathogens in wild fish populations. To date, the CA-NV FHC has partnered with numerous federal and state agencies, tribal governments, universities, non-profit and educational organizations and private landowners to collect fish at over 200 collection sites. The sampling effort to date comprises a rich diversity of fish species in California and Nevada and has provided fish health information that did not exist prior to the National Wild Fish Health Survey's inception in 1997 (<http://www.fws.gov/wildfishsurvey/database/nwfhs/>, <http://www.fws.gov/wildfishsurvey/related.htm>, <http://www.fws.gov/canvfhc/reports.asp>). In 2013, one focus of the CA-NV Fish Health Center's NWFS efforts was with juvenile fall run Chinook pathogens (particularly *Ceratomyxa shasta* and *Parvicapsula minibicornis*), smolt development (Gill Na-K-ATPase activity), and response to organophosphates (Brain AChE activity) in the Sacramento and Feather Rivers. Hendrickson et al.(1989) reported that Shasta-strain rainbow trout sentinels were infected by *C. shasta* after exposure to Sacramento river water (Keswick dam and Los Molinos) and the Feather River (Marysville near rm 29 and North fork). Research in the Klamath River has documented significant juvenile Chinook mortality in some years (Foott et al. 2004) as well as a better understanding of the complex interaction of parasite's life cycle (fish and polychaete worm) with environmental factors such as temperature, flow, and nutrients (Stocking and Bartholomew 2006).

Methods:

Survey collection - All fish were collected in April and May from cooperators, under CDFW Scientific Collecting Permit SC-4085.

<u>Site</u>	<u>Cooperator</u>
RBDD rotary screw trap Rm242	USFWS Red Bluff Fish and Wildlife Office
GCID rotary screw trap	Glen-Colusa Irrigation District

Rm 206

Lower Sacramento beach seine USFWS Stockton Fish and Wildlife Office
Rm 144 – 119 (Colusa to Tisdale ramp)

Feather R. rotary screw traps Department of Water Resources
Gateway rm 61, approximately 1 mi. above the confluence of the thermolito afterbay
Herrington rm 46, approximately 2 mi. below the Gridley boat ramp

In general, the following measurements and samples were collected from each fish: Fork length (mm), weight (0.1g), bacterial (cultured and *R. salmoninarum* DFAT) and viral assay inoculum (anterior kidney and spleen), and either the entire fish (< 65mm) or specific organs (GI tract, liver, gill, and kidney) were placed into Davidson's fixative for histology. In addition to the above, gill lamellae (ATPase sample) and brain tissue (AChE) was also collected from a subset of each group. The appearance of pale gill (anemia), swollen kidney, pale or hemorrhagic intestine was recorded for each fish.

Lab methods- Bacterial and viral assays were performed as per NWFS protocols (<http://www.fws.gov/wildfishsurvey/related.htm>). Histological sections were stained with hematoxylin and eosin. Gill Na-K-ATPase (McCormick 1993) and brain Acetylcholinesterase activity (Wheelock et al. 2005) was assayed from frozen samples.

Prognosis of Feral Feather R. juveniles – Twelve feral juveniles (6 herrington and 6 gateway RST captures) were transported to the wetlab on 4/12 and held for 30d. Fish were fed frozen tubifex worms daily and mean water temperature was 16.6°C (13.2 – 24.3°C). Mortalities were frozen and their intestines tested for *C.shasta* DNA by QPCR. The four 30d survivors were sampled for histological examination.

Sentinel - Juvenile rainbow trout (CDFW Darrah Spring SFH Shasta strain, mean 64 mm FL) and fall Chinook salmon (Coleman NFH, mean 70mm FL) were marked by subcutaneous injection of pink, white, green, or orange elastimere dye (Northwest Marine Technologies Inc., Shaw Island Washington) on the dorsal cranial region and used as sentinels. Groups of 30 fish were placed in 0.01m³ cages at the following locations for 3 days (4/29 – 5/2):

1. Mainstem Sacramento R. at confluence of the GCID return canal
2. Mainstem Sacramento R. immediately above the Tisdale boat ramp
3. Feather R. at the Gateway trap
4. Feather R. one mile above the Herrington trap.

Three replicate 1L water samples were collected at each location when sentinels were first placed and later at retrieval. QPCR analysis for *C.shasta* actinospore concentration was performed as per Hallott and Bartholomew (2006) with acetone extraction of the filter. Upon return to the CA-NV Fish Health Center wet lab, fish were held for up to 30d in 1.35m³ circular tanks at 15 - 17°C and fed salmon diet at 2%BW/d. A separate cohort from each group was held in a single trout and salmon tank, and sampled for histology at 10 and 21 days post-exposure (dpe). Mortality was tracked daily and carcasses assayed by QPCR for both *C.shasta* and *P.minibicornis* infection. At 30 dpe, all fish were sampled for either histology or QPCR. Wet lab effluent is disinfected with 0.3 – 0.5 ppm free chlorine in a 40 min. retention system prior to release.

Results:

Survey collection Sacramento R. Chinook Juveniles - A total of 62 juvenile Chinook salmon were collected from the Sacramento R. sites between 1 - 11 April (Table 1). None displayed signs of clinical disease. No virus was isolated from the kidney-spleen samples nor was *Renibacterium salmoninarum* confirmed in kidney (Table 1). Asymptomatic infections of common gram-negative bacteria (*Aeromonas* – *Pseudomonas* sp.) were observed in 7 – 62% of the kidney samples. The highest prevalence of bacterial infection (POI) was seen in salmon captured from the lower Sacramento R. (Table 1). Both *Ceratomyxa shasta* and *Parvicapsula minibicornis* were seen in histological specimens from all sites however moderate kidney inflammation was only observed in 7 of the 35 infected kidney sections. The *C.shasta* infections were characterized as light and early stage (Figure 9).

Table 1. Prevalence of infection (virus, *Aeromonas* / *Pseudomonas* sp.(A/P), *Renibacterium salmoninarum* (Rsal), *Ceratomyxa shasta* and *Parvicapsula minibicornis*) of juvenile Chinook collected from the Sacramento River at Red Bluff Diversion dam (RB), Glenn-Colusa Irrigation District pumps (GC), and various seine sites in the Lower Sacramento (LS). Viral samples processed as 3 – 5 fish pools.

	4/1RB	4/8RB	4/3GC	4/1LS	4/11LS
Virus pools No.	0 / 5 14	0 / 5 15	0 / 5 14	0 / 2 7	0 / 4 12
<u>Bacteria</u>					
A/P	2 / 14 (14)	1 / 15 (7)	2 / 14 (14)	4 / 7 (57)	8 / 13 (62)
Staph	0 / 14	0 / 15	1 / 14 (7)	1 / 7 (4)	0 / 13
Rsal (DFAT)	0 / 7	0 / 14	0 / 12 ^a	0 / 13 ^b	0 / 9
Cshasta - 1	0 / 14	1 / 15	9 / 14	0 / 7	7 / 13
Cshasta – 2	0 / 14	0 / 15	0 / 14	0 / 7	0 / 13
POI	0%	7 %	64%	0%	54%
Parvicapsula 1	0 / 8	7 / 15	11 / 14	2 / 7	8 / 13
Parvicapsula 2	0 / 8	1 / 15	3 / 14	0 / 7	3 / 13
POI	0%	53%	100%	29%	85%

a Single suspect bacterial cell observed in 100+ fields from 2 of 12 samples. No PCR confirmation possible.

b Single suspect bacterial cell observed in 100+ fields from 1 of 13 samples. No PCR confirmation possible.

Mean fork length was similar among the collection groups with the exception of the larger 11 April Lower Sacramento fish (Table 2). Condition factors were considered normal (> 0.800 KFL) and gill Na-K-ATPase activities were within the smolt range (>7 $\mu\text{mole ADP/mg protein/hr}$). Brain acetylcholinesterase activity can only be viewed as relative data because of a freeze-thaw error (Fig. 1). The few samples available for a second assay run were all higher than cohort samples run in the first batch. Our values were much lower than those reported in Wheelock et al. (2005) which will prompt future work on this assay. No obvious trend for depressed brain acetylcholinesterase activity was observed in the sample groups.

Table 2. Mean (SE) morphometric and physiological data of juvenile Chinook collected from the Sacramento River at Red Bluff Diversion dam (RB), Glenn-Colusa Irrigation District pumps (GC), and various seine sites in the Lower Sacramento (LS) . Data includes condition factor (KFL= $Wt/FL^3 \times 10^5$) and gill Na-K-ATPase ($\mu\text{mole ADP/mg protein/hr}$).

	4/1RB	4/8RB	4/3GC	4/1LS	4/11LS
Fork length range	70 (1) 58 - 75	71 (1) 60 - 77	69 (1) 56 - 72	73 (3) 58 - 81	81 (1) 74 - 90
Weight (0.1g) range	3.5 (0.2) 2.0 - 4.1	3.9 (0.2) 2.1-4.6	3.6 (0.2) 1.7-4.1	4.3 (0.5) 2.0-6.1	6.1 (0.3) 5.3-8.0
KFL	1.027 (0.019)	1.069 (0.018)	1.051 (0.055)	1.091 (0.057)	1.147 (0.023)
Gill ATPase No.	11.36 (2.25) 8	11.50 (4.36) 6	10.25 (3.71) 6	9.14 (1.70) 7	11.06 (3.05) 6

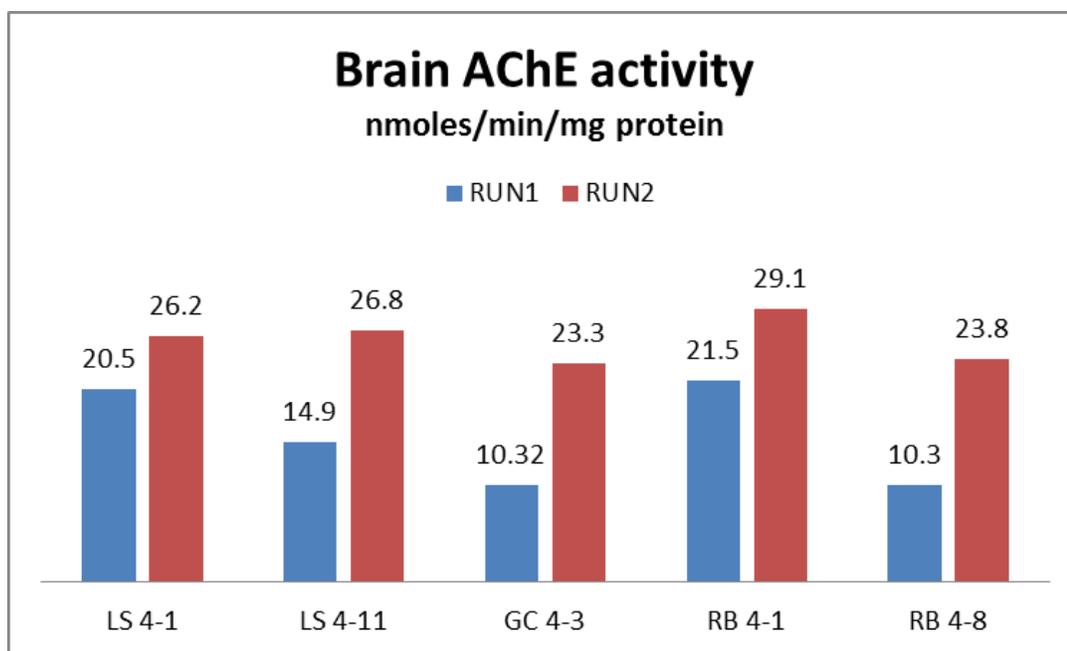


Figure 1. Brain acetylcholinesterase activity estimates (n=5) from Sacramento R. juvenile Chinook captured in lower Sac. Beach seine (LS), traps at GCID (GC) and Red Bluff Diversion Dam (RB) between 1 and 11 April. Assay run 1 values are lower due to defrost error than for single samples assayed in run 2. Run 2 values from the 1 April RB group represent the mean of 5 samples.

Survey collection Feather R. Chinook Juveniles - A total of 77 juvenile Chinook salmon were collected from the two Feather R. sites on 12April and 2May (Table 3). Clinical signs of ceratomyxosis (pale gill, enlarged spleen, hemorrhagic and swollen intestine) were seen in 72% of the 12April Herrington RST sample. These fish had 100% prevalence of infection for both *C.shasta* and *P. minibicornis* as per histological examination. The population in this reach was considered severely affected by parasitic disease at this time due to hemorrhagic intestines (Figure 7) and significant kidney inflammation (Figure 8). Prevalence of *C.shasta* infection at Herrington RST dropped to 40% in the 2May sample is associated with increased flows (Figure 2). No virus was isolated from the kidney-spleen samples nor was *Renibacterium salmoninarum* confirmed in kidney (Table 3). Asymptomatic infections of common gram-negative bacteria (*Aeromonas* – *Pseudomonas sp.*) were observed in 20 - 37% of the kidney samples. *Ceratomyxa shasta* and *Parvicapsula minibicornis* were only seen in histological specimens from the lower river Herrington site (below the thermolito afterbay input).

Table 3. Prevalence of infection (virus, *Aeromonas* / *Pseudomonas* sp.(A/P), *Renibacterium salmoninarum* (Rsal), *Ceratomyxa shasta* and *Parvicapsula minibicornis*) of juvenile Chinook collected from the Feather River at Herrington and Gateway rotary screw traps

	Herrington 4/12	Gateway 4/12	Herrington 5/2	Gateway 5/2
Virus 5p	0 / 6	0 / 6	ND	ND
A/P bacteria	7 / 19 (37)	4 / 20 (20)	ND	ND
Rsal (DFAT)	0 / 15	0 / 15	ND	ND
Cshasta - 1	6 / 25 (24)	0 / 29	4 / 10	0 / 10
Cshasta – 2	19 / 25 (76)	0 / 29	0 / 10	0 / 10
POI	100%	0%	40%	0%
Parvicapsula 1	0 / 28 (0)	0 / 29	0 / 10	0 / 10
Parvicapsula 2	28 / 28 (100)	0 / 29	4 / 10	0 / 10
POI	100%	0 %	40%	0%

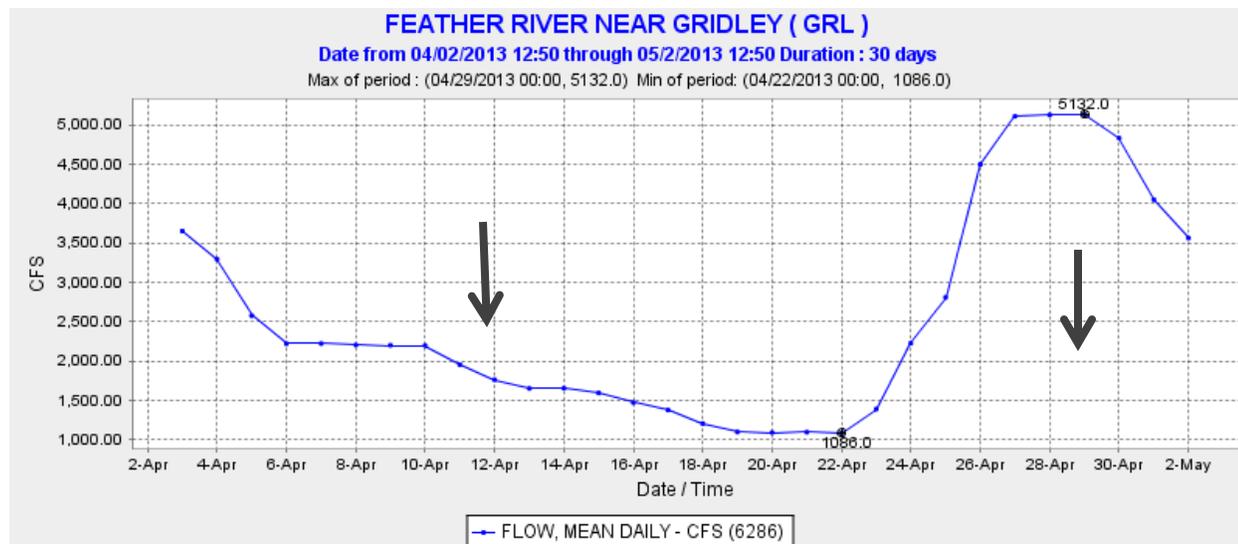


Figure 2. Mean daily flow of Feather R. near Gridley (also Herrington RST). Arrows indicate sample dates and show marked increase of flow preceding the 2May collection and sentinel fish exposure.

At both sites on 12April, fish size varied considerable and condition factors tended to be < 1.00 (Table 4). Larger fish were selected for gill Na-K-ATPase assays. Gill enzyme activities tended to be in the smolt range. As mentioned above, brain acetylcholinesterase activity can only be viewed as relative data because of a freeze-thaw error (Fig. 3). The few samples available for a second assay run were all higher than cohort samples run in the first batch. Our values were much lower than those reported in Wheelock et al. (2005) which will prompt future work on this assay. No obvious trend for depressed brain acetylcholinesterase activity was observed between the sample groups.

Table 2. Mean (SE) morphometric and physiological data of juvenile Chinook collected from the Feather River at Herrington and Gateway. Data includes condition factor (KFL= $Wt/FL^3 \times 10^5$), gill Na-K-ATPase ($\mu\text{mole ADP/mg protein/ hr}$).

	Herrington 4/12	Gateway 4/12	Herrington 5/2	Gateway 5/2
Fork length (mm) range	54 (2) 43 - 72	64 (1.7) 44 - 80	76 (3) 60 - 88	67 (3) 57 - 78
Weight (0.1g) range	1.6 (0.2) 0.6 - 3.9	2.7 (0.2) 0.8 - 5.6	ND	ND
KFL	0.895 (0.024)	0.973 (0.017)	ND	ND
Gill ATPase No.	7.56 (3.58) 5	9.60 (2.82) 6	ND	ND

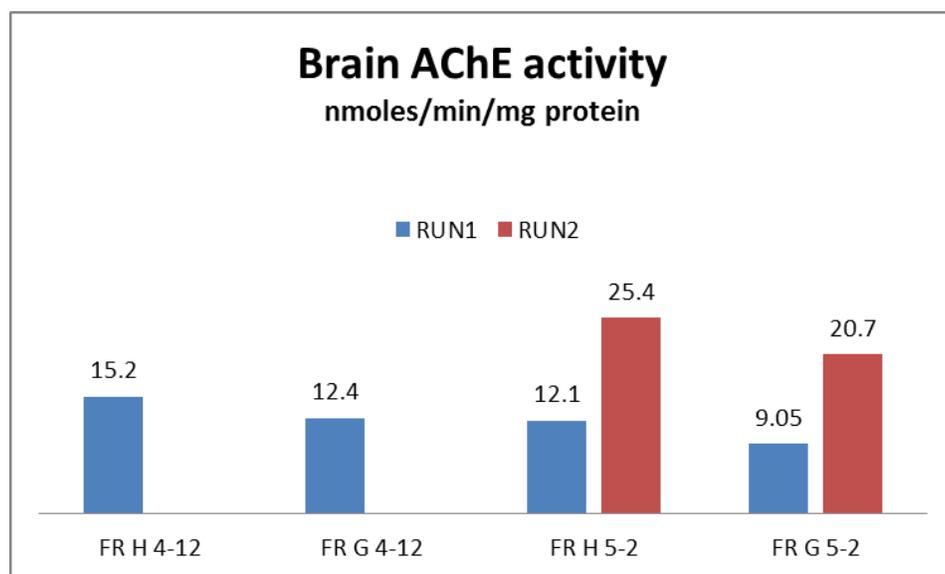


Figure 3. Brain Acetylcholinesterase activity estimates from Feather R. (FR) juvenile Chinook captured at Herrington (H) and Gateway (G) Rotary screw traps on 12April and 2May. Assay run 1 values are lower due to defrost error than for those samples assayed in run 2.

Prognosis of Feral Feather R. juveniles- Eight of the 12 salmon died between 4 and 22 days post-return. Intestinal *C.shasta* DNA content indicated clinical ceratomyxosis (C_t values ranged from 21.96 – 33.14). Similarly, *Pavicapsula* DNA was detected in the kidney from 7 of 8 mortalities. No *C.shasta* trophozoites or characteristic lesions were seen in histological sections from the four 30d survivors however one fish had a low level *Pavicapsula* infection.

The mortality pattern and high parasite DNA of juvenile Chinook obtained from the Feather R. (particularly the Herrington site) indicates that the dual infections were lethal.

Sentinel: No *Cshasta* DNA was detected in the water samples collected at each site. Given that low level infection did occur, we suspect that the negative findings are inaccurate and due to an extraction error. No significant mortality occurred in any exposure group (Chinook: 2 Herrington, 1 GCID, 5 Tisdale; Rainbow trout: 1 Tisdale and 2 Herrington) over the 30dpe observation period.

Chinook: *C.shasta* was detected in 8 to 25% of the 21 and 30dpe histological sample groups (Figure 4) with intestinal lesions seen in 1 GCID fish and 3 Herrington trap samples. Most trophozoites were seen within granulomas on the intestinal serosa indicating a successful host defense against a low level challenge. The more sensitive QPCR assay detected low level *Cshasta* infection in 50 – 80% of the 30dpe sentinels held at GCID, Tisdale, and Herrington (Figure 6). Except for Gateway sentinels, *Parvicapsula* infection was observed in the kidneys of most 21 and 30dpe histological sections of Chinook (Figure 7). *Parvicapsula* QPCR assays of the limited numbers of Chinook mortalities were also positive. Non exposed controls were negative for parasite infection. Actinospore concentrations at the exposure sites were apparently low and resulted in the low prevalence of asymptomatic infections. This data is particularly striking for the Herrington site (lower Feather R.) given the high infection trend seen from the 12 April sample prior to the elevated flows. It is tempting to speculate that dilution of the infective actinospores occurred because of elevated Feather River flows.

Trout: As discussed above, only 3 rainbow trout sentinel mortalities occurred over the 30d observation period. Skin infestation of Ich was seen in rainbow trout sentinel groups at the 30 dpe sample. *C. shasta* trophozoites were seen in two 21dpe trout held at Herrington and were associated with intestinal lesions. The other 42 intestinal sections from 30dpe trout sentinels did not contain the parasites. Similarly, none of the 30dpe *Cshasta* QPCR samples (10 trout per site) were positive. *Parvicapsula* infection was not observed in the sentinel trout kidneys however QPCR analysis of a Tisdale trout mortality was positive. Hydrophic vacuoles within the tubular epithelium and nephrocalcinosis was seen in trout kidneys regardless of exposure site. The nephrocalcinosis may have been a pre-exposure condition while the excess protein observed in the tubules (hyaline deposits) could be related to osmoregulatory issues with Ich infection. The lack of *C. shasta* infection observed in the Shasta strain rainbow trout suggests predominate genotype I (Chinook infective) of *C.shasta* in the Feather and Sacramento R. during the exposure (Atkinson and Bartholomew 2010).

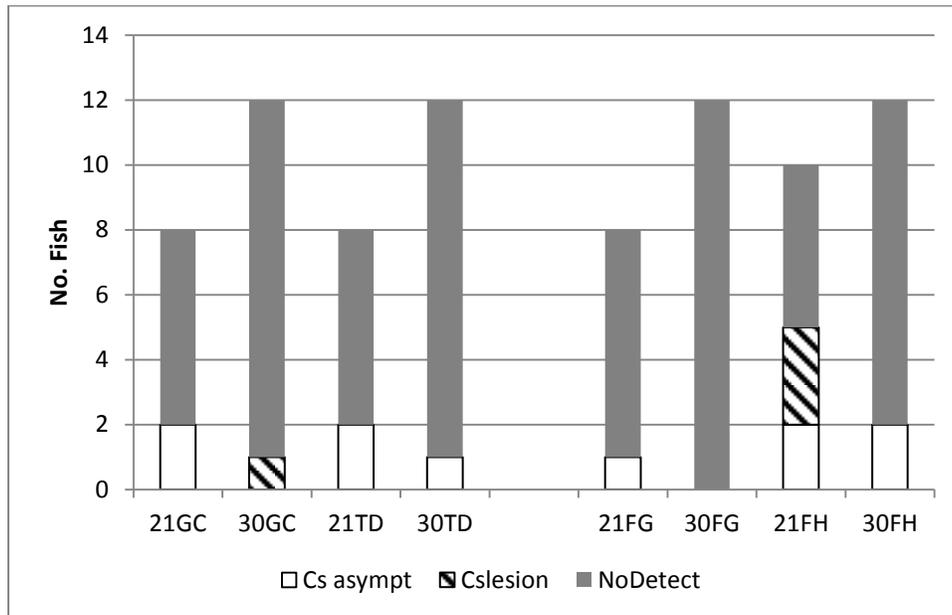


Figure 4. Number of Chinook salmon demonstrating asymptomatic (early stage) infection by *Ceratomyxa shasta* (Cs asympt), ceratomyxosis clinical signs (Cs lesion), and no parasite observed in the histological specimen (No Detect). Eight fish were sampled at 21days post-exposure (21) and twelve fish at 30dpe from the sentinel groups held at GCID (GC), Tisdale (TD), Gateway RST on the Feather R (FG), and Herrington RST on the Feather R. (FH).

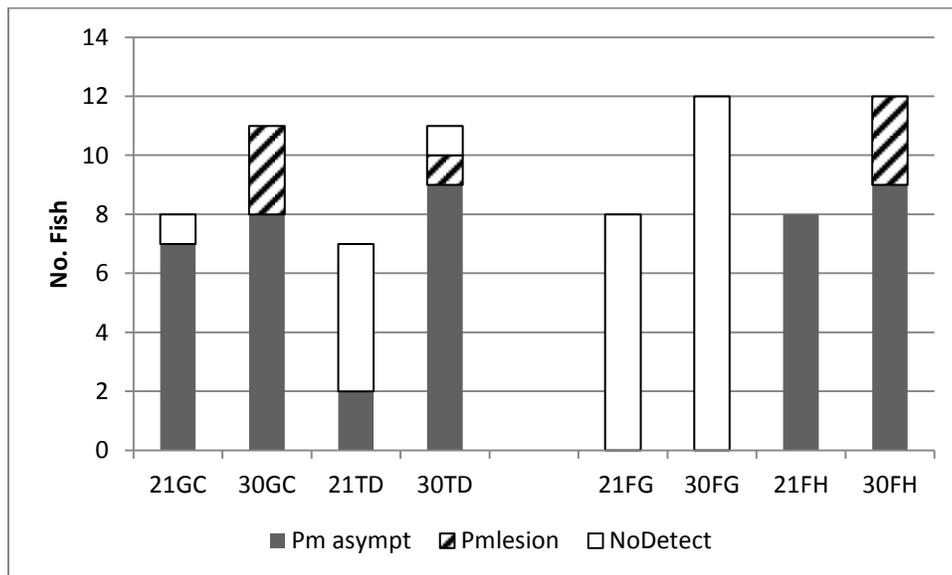


Figure 5. Number of Chinook salmon demonstrating asymptomatic (early stage) infection by *Parvicapsula minibicornis* (Pm asympt), glomerulonephritis (Pm lesion), and no parasite observed in the histological specimen (No Detect). Eight fish were sampled at 21days post-exposure (21) and twelve fish at 30dpe from the sentinel groups held at GCID (GC), Tisdale (TD), Gateway RST on the Feather R (FG), and Herrington RST on the Feather R. (FH).

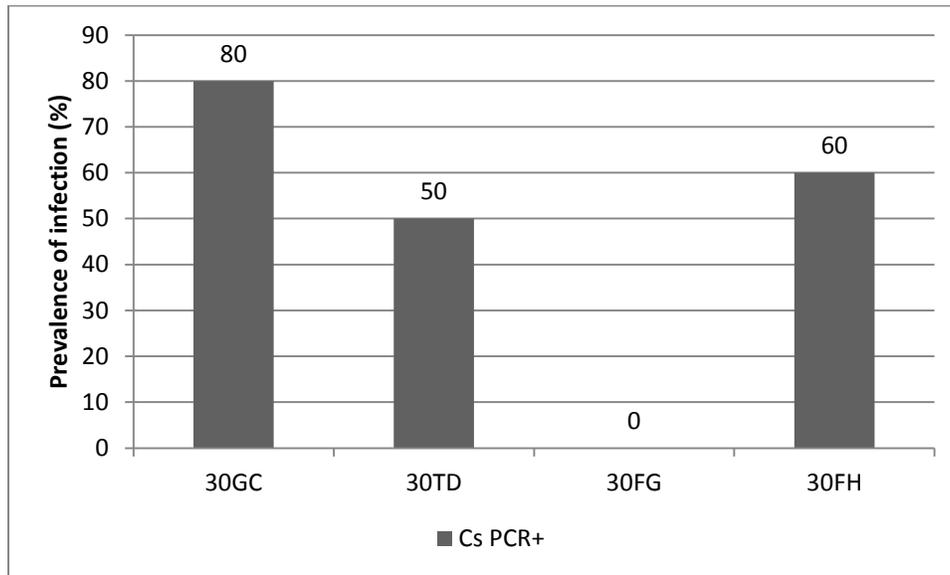


Figure6. Prevalence of *C. shasta* DNA in Chinook intestine sampled at 30dpe from the sentinel groups held at GCID (GC), Tisdale (TD), Gateway RST on the Feather R (FG), and Herrington RST on the Feather R. (FH).

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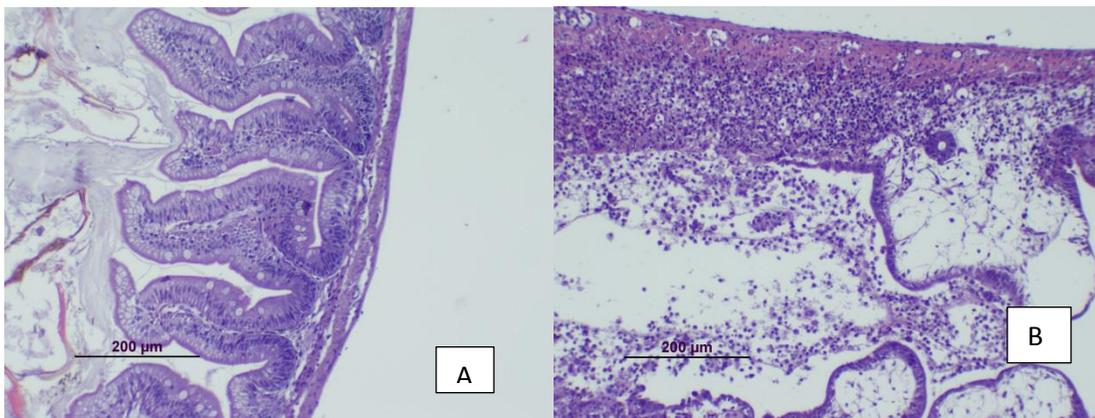


Figure 7. A) normal intestine and B) severe erosion of intestinal epithelium due to ceratomyxosis in Feather R. salmon collected 4/12 from Herrington trap.

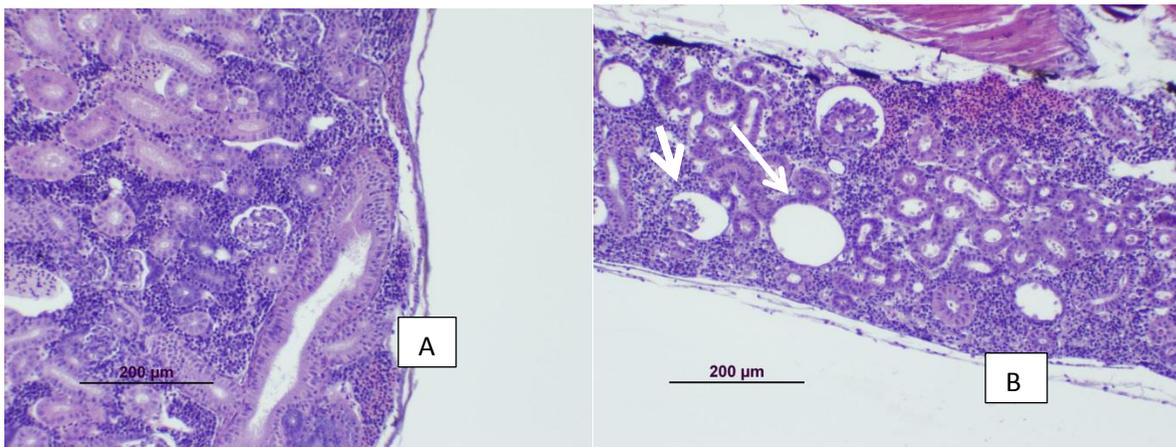


Figure 8. A) Normal kidney and B) glomerulonephritis and enlarged Bowman Capsules (arrows) due to infection by *Parvicapsula* in Feather R. salmon collected 4/12 from Herrington trap.

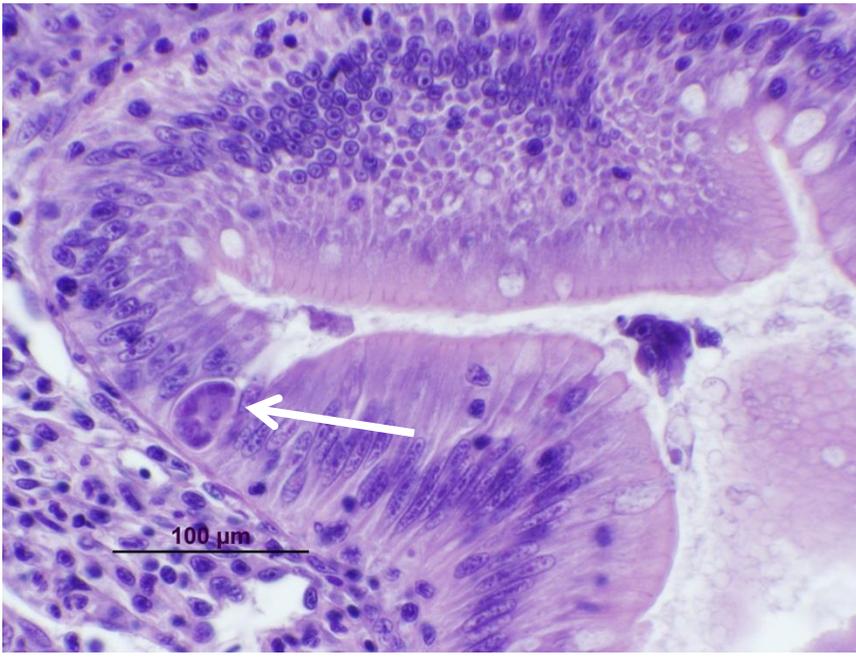


Figure 9. *C. shasta* trophozoite within (GCID) Chinook intestinal epithelium characteristic of an asymptomatic “early” infection.