

Memorandum

TO: interested parties

DATE: March 21, 2007

FROM: J. Scott Foott
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SUBJECT: 2006 survey of Klamath River adult Fall- run Chinook for *Ceratomyxa shasta* myxospores and kidney infection by *Parvicapsula minibicornis*.

Methods

Thirty adult Fall-run Chinook salmon were sampled at Iron Gate Hatchery on both 18 October and 01 November 2006. These fish had either been spawned or were fresh mortalities from the holding ponds on the day of collection. The entire gastrointestinal tract (esophagus to rectum) was removed from the fish, placed into individual ziplock plastic bags, held on ice in the field and stored at -20°C until processed. Liver and kidney tissue from the first 10 adults of each collection was placed in Davidson's fixative for 48 h and later processed for 5µm paraffin sections stained with hematoxylin and eosin. The intestine was dissected from the defrosted GI tract at the small intestine – pyloric caecae junction and the interior scraped with a #21 scalpel. All material scraped from the intestine was mixed vigorously in 5 mL of PBS. The suspension was poured through a single layer of cheese-cloth that removed large fibers and cestodes from the suspension. The cloth was washed once with an additional 5 mL PBS and the 10mL suspension was centrifuged at 3200xg for 15 min. The supernatant was carefully removed with a pipette until approximately 2 mL remained above the pellet. The pellet was resuspended with a disposable pipette and 2 drops placed onto separate 30x 40mm #1 coverslips. After inverting the coverslips onto microscope slides, both wetmounts were examined for the characteristic *C.shasta* myxospore at both 20x and 40x phase for a total of 100 fields.

Case 07-002, 18 October 2006, histo block 5280 – 5289

Case 07 -006, 01 November 2006, histo block 5324 – 5333

Results:

Low numbers of *Ceratomyxa shasta* myxospores were observed in only 1 intestine (01 Nov fish). A hemocytometer count indicated that there were approximately 2500 spores / mL. Trophozoite stages of the parasite are not detected with any degree of confidence in wet mount preparations from defrosted tissue. All intestines contained high numbers of cestodes. One kidney section contained *C.shasta* trophozoites indicating a systemic infection for the fish. A pre-sporogonic myxozoan, morphologically different from either *Ceratomyxa shasta* or *Parvicapsula minibicornis*, was observed within hepatic arterioles in 5 of 20 liver sections (Fig. 1). There were no inflammatory cells associated with the liver arteriole myxozoans. In comparison to the low *C. shasta* myxospore stage detection rate, histological survey of IGH adults Fall-run in 2005 had an 80% prevalence of infection for the trophozoite stage.

Parvicapsula minibicornis was observed in 95% of the kidney sections with large numbers of pre-sporogonic forms in the tubules (Table 1 and Figure 2). Glomerulonephritis was judged to be relatively minor. Low numbers of metacercaria were detected in 60% of the samples.

Conclusions:

These findings raise questions about the role adult Fall-run Chinook play in providing *Cshasta* myxospores to the Klamath River but do reinforce their role for *Parvicapsula* myxospore input. One bias to the study was the collection and rapid freezing of the gut samples. If these samples are to represent carcass myxospore input, it may be essential to allow time (days?) for *Cshasta* pre-sporogonic stages to form myxospores prior to evaluation. Future survey efforts will include a 3-5d incubation step at 10°C prior to long-term frozen storage of the intestinal samples as well as matching histological section of intestine for trophozoite counts.

Figure 1. Myxosporean (pre-sporogonic form) observed in arteriole of liver (600x mag., H&E).

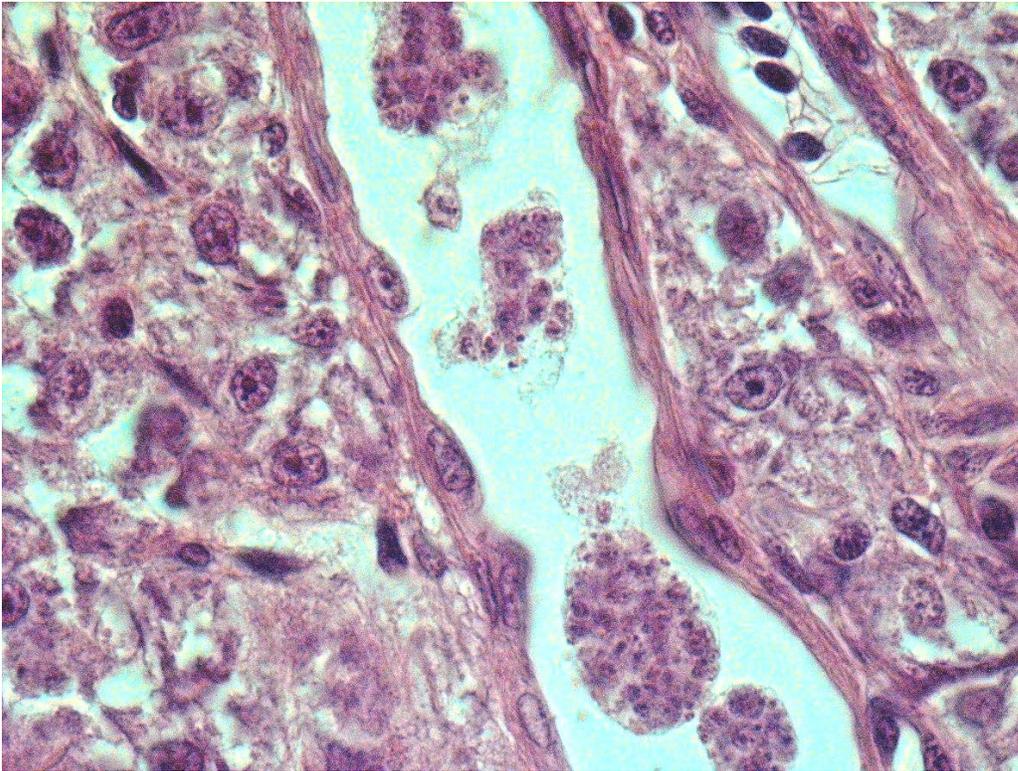


Figure 2. *Parvicapsula minibicornis* sporogonic stages and possible myxospore (arrow) in kidney tubules (600x mag., H&E).



Table 1. Histological evaluation of liver and kidney (Kd) from adult Chinook collected at Iron Gate Hatchery on 18 October and 01 November 2006. *Parvicapsula minibicornis* (Pm) presence and numbers in the glomeruli (Glom.) and tubules were used to set qualitative Pm infection score of 1 (moderate) or 2 (heavy).

	18OCT	01 NOV	Incidence
<u>Kd Glom. Pm</u>	10 / 10	9 / 10	19 / 20 (95)
<u>Kd Tubule Pm</u>			
low- moderate	2 / 10	2 / 10	4 / 20 (20)
High	7 / 10	7 / 10	<u>14 / 20 (70)</u>
			18 / 20 (90)
<u>Pm Score</u>			
1	3 / 10	4 / 10	7 / 20 (35)
2	7 / 10	6 / 10	<u>13 / 20 (65)</u>
			20 / 20 (100)
Kd metacercaria	4 / 10	8 / 10	12 / 20 (60)
LIVER			
Liver Cshasta	0 / 10**	0 / 10	0 / 20
Liver Non-Cs/Pm myxosporean	2 / 10	3 / 10	5 / 20 (25)

** One kidney with focal *Cshasta* trophozoite infection