

FY 98 Investigational Report:
**Histological Evaluation of Klamath Lake Basin Blue
Chub Tissues**



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Summary:

As part of a 1998 Klamath Lake water quality study, histological specimens of kidney, liver, pancreatic tissue, and intestinal tract from Blue Chub were evaluated by light microscopy for parasites and abnormalities. One hundred and fifty fish were collected from 7 locations in the Klamath Lake basin from June– September 1998. No significant lesions suggestive of toxic insult were observed in any of the specimens. Parasitic infections (myxosporeans, Trichodina {ciliate}, and various helminthes) of internal organs and pancreatitis were common to all sample groups.

Introduction: In 1998, the California – Nevada Fish Health Center (FHC) assisted the principal investigator, Dr. Elaine Snyder-Conn (Contaminant Specialist, Klamath Basin FWO), in a study examining the effects of Klamath Lake Basin water quality on Blue Chub (*Gila coerulea*) health and condition. The FHC performed 3 functions for the study: 1) training of 2 biological technicians in necropsy, organosomatic analysis, and tissue sampling; 2) advise on application of the organosomatic analysis technique for this study, and 3) histological evaluation of select tissues. Partial funding for supplies and technician time, totaling \$4,500, was provided to the FHC in FY98 for this study (project codes 11450-1261-1N39 and 11450-1130-1F24).

Methods: Viscera and kidney tissue was dissected from freshly killed fish and fixed for 24 hours in Davidson’s fixative, transferred to 70% ethanol, processed for 5 µm paraffin sections, and stained with hematoxylin and eosin. Tissues from a total of 150 fish from 7 locations were collected from June through September 1998. Site location data is listed in appendix 1. The kidney, liver, small intestine, pyloric caeca / adipose / pancreatic tissue of a given fish was included on one slide and the specimen was given a unique code number. The slides were read “blind” to location. Each slide was examined at both low (100X) and high magnification (400X) for the presence of tissue abnormalities and parasites. In particular, the liver and kidney were evaluated for lesions and endogenous pigments (lipofuscin, ceroid, hemosiderin) characteristic of toxic insults. Specific abnormalities screened in the liver include hepatocyte necrosis, altered basophilic foci, neoplasia, hepatocytomegaly, and biliary epithelium hyperplasia. The common occurrence of inflammation associated with the acinar cell clusters (pancreatitis) prompted the development of a qualitative severity score:

- 0 no inflammation, normal
- 1 presence of low numbers of neutrophils and monocytes, focal or multi-focal distribution
- 2 diffuse distribution of neutrophils and monocytes
- 3 massive inflammatory exudate present, often with granulomatous tissue

Results – General Comments: No significant lesions suggestive of toxic insult were observed in the tissues from any of the specimens. A number of parasites were observed in the samples, however, these infections did not appear harmful to the fish. Spores of one or more myxosporean species (presumptive identification of both *Myxidium* sp. and *Myxobolus* sp.) were observed in the kidney and occasional spleen section. Little to no inflammatory response was associated with the spores, however, small quantities of gold-brown pigment was often in the interstitium surrounding the spores (Fig. 1). In some instances the pigment was within clusters of apoptotic macrophages. It is believed that the pigment is either lipofuscin or ceroid. Granulomatous response to the infection was only seen in 3 fish. Cysts containing both sporogonic and developed spores (presumptive *Myxobolus* sp.) was seen in the glomeruli of a number of fish from most sites. A low incidence of glomerulo nephritis was associated with some of these cysts. The site with the highest incidence of myxosporean infection was Upper Klamath Lake (UK) and the lowest was Tule Lake (TL) (see site specific Tables below). The external ciliate parasite *Trichodina* sp. was seen in the collection duct portion of the kidney nephron in 10– 30% of the fish at 3 sites. It is assumed that these parasites migrated up through the ureter into kidney and that the affected fish may have had heavy external infections. Inflammation of the acinar cell clusters (pancreatitis) was seen in all groups and ranged from mild inflammatory infiltrate to severe fibrotic changes (Fig 2). This type of lesion is prevalent in out-migrant smolts in the Klamath R. basin and appears to be exacerbated by elevated temperatures. It does not seem to be dependent on the presence of parasites. The enzymatic mobilization of lipids from the mesenteric adipose cells could result in the associated release of arachidonic acid from the cell membrane phospholipids. Arachidonic acid can be converted to a number of potent chemotactic factors for phagocytes such as leukotrienes and salmonids are reported to generate such agents. Once phagocytes are recruited to the region they would release other chemotactic factors (cytokines) which would further enhance the inflammatory response. It is unclear whether pancreatic functions are significantly reduced or if this condition has a negative effect on fish health. Cross-sections of trematodes, nematodes, and possibly several cestodes were frequently observed in the viscera and, to a lesser degree, liver (Fig 3). Helminth infections were particularly prevalent in fish from Clear Lake Reservoir (CL), Klamath River (KR), and Lost River (LR).

A total of 53 man-hours were spent for this evaluation by the FHC (31 processing, 22 microscopy / report). Slide/block numbers for the study are #1281 - #1360 (Case 98-122, June & July collections) and #1447 - #1518 (case 98-150, August – September collections).

Site specific results:

Table 1 Agency Lake prevalence of infection or lesion type (number positive / total sample).

	063098AL01	081398AL02A
Intestinal abnormality / parasite	0 / 9	0 / 10
Visceral Helminth	1 / 9	3 / 9
Visceral Fat Inflamm.	9 / 10	0 / 9
Acinar cell Inflamm.		
NONE	5 / 10	6 / 10
MILD	1 / 10	3 / 10
MODERATE	4 / 10	0 / 10
SEVERE	0 / 10	1 / 10
Kidney Myxosporean		
Individual Spores	2 / 10	8 / 10
Cysts	1 / 10	1 / 10
Granuloma	1 / 10	0 / 10
Glomerulonephritis	2 / 10	0 / 10
Brown pigment foci		6 / 10
<i>Trichodina</i> sp. within Kidney Collection Duct	3 / 10	0 / 10
Liver Hepatocyte abnormalities	0 / 10	3 / 10**

** One liver with extensive hyperemia and hemorrhage showing signs of possible trauma. Two livers with inflammation associated with the bile ducts.

Table 2. Clear Lake Reservoir prevalence of infection or lesion type (number positive / total sample).

	061698CL01A	090198CL02A
Intestinal abnormality / parasite	3 / 10 *	2 / 9 +
Visceral Helminth	6 / 10 *	5 / 8 +
Visceral Fat Inflamm.	4 / 10 **	2 / 8 ++
Acinar cell Inflamm.		
NONE	9 / 10	4 / 7
MILD	1 / 10	2 / 7
MODERATE	0 / 10	1 / 7
SEVERE	0 / 10	0 / 7
Kidney Myxosporean Individual Spores	1 / 9	7 / 10
Cysts	0 / 9	0 / 10
Granuloma	0 / 9	0 / 10
Brown pigment foci	7 / 9	9 / 10
<i>Trichodina</i> sp. within Kidney Collection Duct	0 / 9	0 / 10
Liver Hepatocyte abnormalities	1 / 10***	0 / 9

* Six fish with nematodes, one fish with possible trematode, and another with a cestode.

** Inflammation associated with visible parasite (nematode) in 3 of 4 sections.

*** Inflammation associated with nematode infection of liver.

+ Nematode infections.

++ No parasites associated with inflammation site.

Table 3. Williamson River prevalence of infection or lesion type (number positive / total sample).

	061298WR01A	080598WR02A
Intestinal abnormality / parasite	0 / 10	0 / 10
Visceral Helminth	0 / 10	3 / 10 *
Visceral Fat Inflamm.	6 / 6 ++	1 / 10**
Acinar cell Inflamm.		
NONE	2 / 10	2 / 10
MILD	3 / 10	6 / 10
MODERATE	3 / 10	2 / 10
SEVERE	2 / 10	0 / 10
Kidney Myxosporean		
Individual Spores	7 / 10	7 / 10
Cysts	0 / 10	1 / 10
Granuloma	0 / 10	0 / 10
Brown pigment foci	5 / 10	5 / 10+
<i>Trichodina</i> sp. in Kidney Collection Duct	0 / 10	0 / 10
Liver Hepatocyte abnormalities	1 / 10+++	0 / 10

* Two fish with trematode infections and one with a nematode.

** No parasite associated with inflammation.

+ Two of 5 kidney with apoptotic macrophage clusters.

++ Inflammation also seen in acinar cell clusters of same fish.

+++ Apoptotic macrophage clusters with brown pigment.

Table 4. Rocky Point prevalence of infection or lesion type (number positive / total sample).

	071098RP01a	081498RP02a	082798RP02b
Intestinal abnormality / parasite	0 / 8	0 / 4	0 / 6
Visceral Helminth	1 / 8 *	0 / 1	1 / 3+
Visceral Fat Inflamm.	5 / 8	0 / 1	0 / 3
Acinar cell Inflamm.			
NONE	3 / 7	0 / 4	1 / 6
MILD	1 / 7	2 / 4	1 / 6
MODERATE	2 / 7	2 / 4	3 / 6
SEVERE	1 / 7	0 / 4	1 / 6
Kidney Myxosporean			
Individual Spores	3 / 8	2 / 4	0 / 6
Cysts	2 / 8	1 / 4	0 / 6
Granuloma	0 / 8	0 / 4	0 / 6
Brown pigment foci	1 / 8	4 / 4	0 / 6
<i>Trichodina</i> sp. in Kidney Collection Duct	0 / 8	0 / 4	3 / 6
Liver Hepatocyte abnormalities	0 / 7	1 / 4**	0 / 6

* Trematode infection.

** Focal liquidifactive necrosis probably due to a bacterial infection.

+ Nematode infection.

Table 5. Tule Lake (TL) and Lost River (LR) prevalence of infection or lesion type (number positive / total sample).

	061798TL01a	090298TL02a	062598LR01a
Intestinal abnormality / parasite	0 / 10	0 / 10	0 / 10
Visceral Helminth	0 / 10	1 / 5**	5 / 10+
Visceral Fat Inflamm.	7 / 10	2 / 5	3 / 10+
Acinar cell Inflamm.			
NONE	4 / 10	1 / 10	4 / 9
MILD	1 / 10	0 / 10	2 / 9
MODERATE	5 / 10	7 / 10	3 / 9
SEVERE	0 / 10	2 / 10	0 / 9
Kidney Myxosporean			
Individual Spores	0 / 10	1 / 10	2 / 9
Cysts	0 / 10	0 / 10	3 / 9++
Granuloma	0 / 10	0 / 10	0 / 9
Brown pigment foci	1 / 10	3 / 10	2 / 9
<i>Trichodina</i> sp. in Kidney Collection Duct	0 / 10	0 / 10	2 / 8
Liver Hepatocyte abnormalities	1 / 10*	0 / 9	1 / 10+++

* Inflammation associated with septicemia.

** Focal liquidifactive necrosis probably due to a bacterial infection.

+ Trematode (5 fish) and nematode (1 fish) infection. Inflammation associated with parasites in 3 fish.

++ Moderate glomerulonephritis associated with myxosporean cysts.

+++ Focal inflammation not associated with any parasites.

Table 6. Klamath River prevalence of infection or lesion type (number positive / total sample).

	061098KR01a	080698KR02a
Intestinal abnormality / parasite	0 / 9	0 / 5
Visceral Helminth	7 / 10*	4 / 5 *
Visceral Fat Inflamm.	5 / 10**	1 / 5
Acinar cell Inflamm.		
NONE	5 / 9	1 / 5
MILD	3 / 9	3 / 5
MODERATE	0 / 9	0 / 5
SEVERE	1 / 9	1 / 5
Kidney Myxosporean		
Individual Spores	4 / 9	4 / 6
Cysts	3 / 9	0 / 6
Granuloma	0 / 9	0 / 6
Brown pigment foci	2 / 9	3 / 6
<i>Trichodina</i> sp. in Kidney Collection Duct	0 / 9	0 / 6
Liver Hepatocyte abnormalities	1 / 10	1 / 6+

* Trematode infections

** Inflammation associated with acinar cell inflammation and 2 of 7 trematode infections.

+ Inflammation associated with bile ducts.

Table 7. Upper Klamath Lake prevalence of infection or lesion type (number positive / total sample).

	071598UK01a	091098UK02a
Intestinal abnormality / parasite	0 / 10	0 / 9
Visceral Helminth	0 / 5	2 / 6+
Visceral Fat Inflamm.	4 / 5*	0 / 6
Acinar cell Inflamm.		
NONE	0 / 9	1 / 9
MILD	3 / 9	8 / 9
MODERATE	6 / 9	0 / 9
SEVERE	0 / 9	0 / 9
Kidney Myxosporean		
Individual Spores	3 / 9	3 / 10
Cysts	6 / 9	6 / 10
Granuloma	1 / 9	1 / 10
Brown pigment foci	4 / 9	4 / 10
<i>Trichodina</i> sp. in Kidney Collection Duct	1 / 9	0 / 10
Liver Hepatocyte abnormalities	3 / 10**	0 / 10

- * All 4 visceral adipose tissues with severe inflammation with one section showing fibrosis.
- ** Inflammation and necrotic foci may be caused by bacterial infection.
- + Trematode and nematode infections.

Figure 1 Presumptive *Myxobolus* sp. spores (arrows) and presporogonic forms in degenerative kidney tubule (H&E stain).

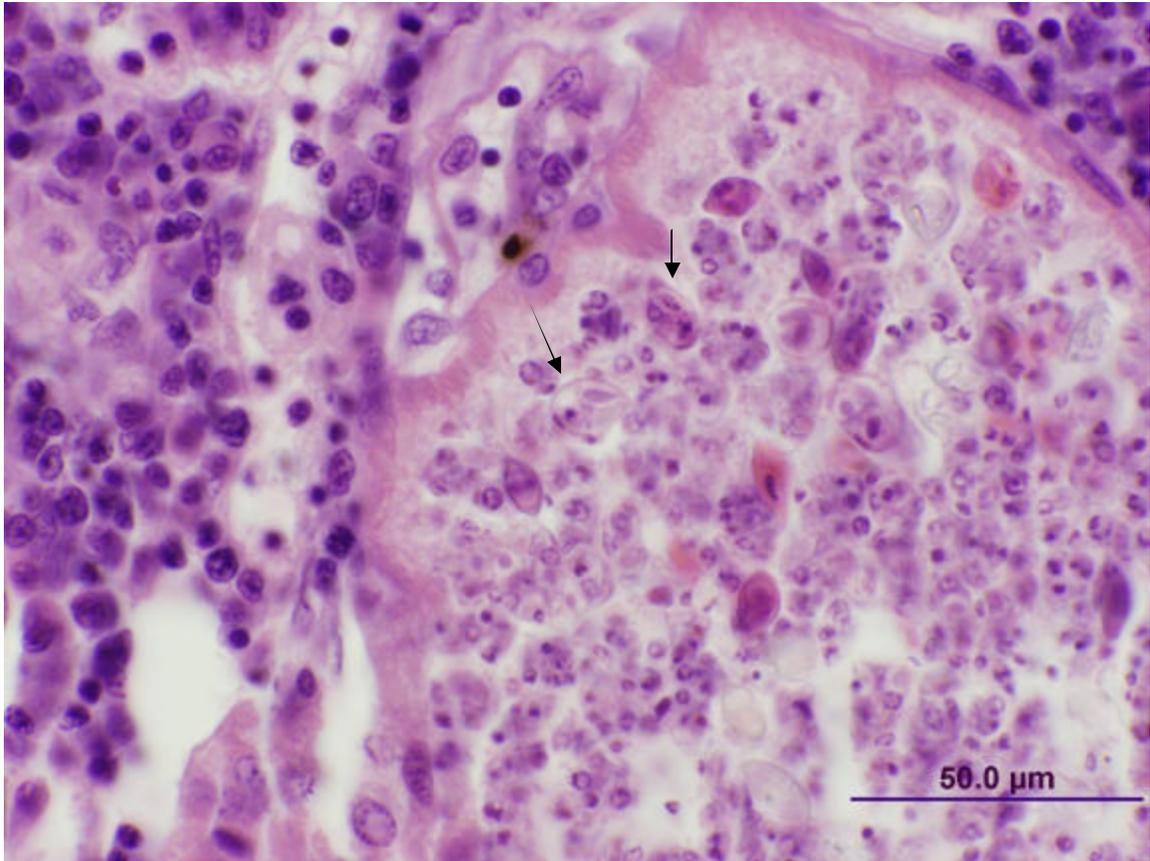


Figure 2. Inflammation (arrow = eosinophil) of adipose tissue and acinar cell clusters (periacinar steatitis). (H&E stain).

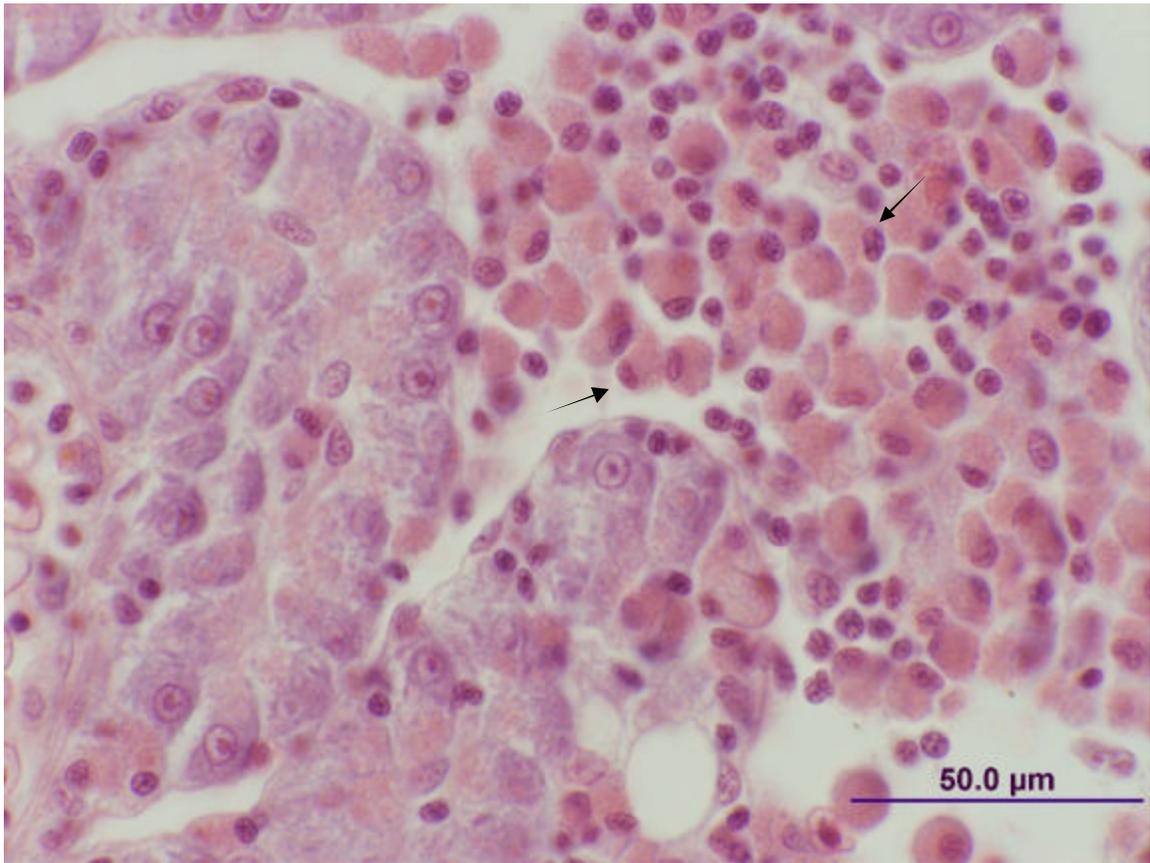


Figure 3. Cross-section of encysted trematodes (arrows) within visceral adipose tissue (H&E).

