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Morbidity of Tagged Wild Adult Fall Chum Salmon Captured by Fish Wheel in the Yukon River, Alaska

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Morbidity of Tagged Wild Adult Fall Chum Salmon Captured by Fish Wheel in the Yukon River, Alaska

Alaska Fisheries Technical Report Number 60

by

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Abstract

A mark-recapture study using fish wheels and spaghetti tags was conducted annually on migrating Yukon River chum salmon since 1996. A substantial decrease in the relative abundance of tagged fish was observed with increased distance upstream, potentially indicating excessive mortality of tagged fish. To evaluate causes of mortality and morbidity and describe histologic changes during migration, chum salmon were sampled and examined by full necropsies that included external lesions, weight, length, hematology, bacteriology, ELISA for bacterial kidney disease antigen, and semiquantitative histopathology. To assess body condition, Fulton's condition factor, hepatosomatic and gonadosomatic, indices, were determined. None of these analyses indicated a definitive cause for mortality or morbidity specific to the tagged fish. Local damage at the tag site, higher neutrophil numbers, and lower total protein in tagged fish suggested further study into the possibility of infection as a cause of mortality associated with tagging. A single untagged fish had a systemic infection of *Aeromonas salmonicida*. These bacteriology results indicated that septicemia from tagging was not occurring during the interval examined. There was one weak positive for an antigen of the bacterial kidney disease agent indicating that this disease was not a significant factor in morbidity. Blood smears were negative for viral erythrocytic necrosis inclusion bodies. Lesions suggestive of other major infectious diseases were not evident. Lesions typical of these migrating salmon included somatic and gastrointestinal skeletal muscle degeneration, contraction band necrosis in the heart, fibrin thrombi in the heart and gills, apoptosis of the gastrointestinal epithelial cells, bone remodeling, ulcers, vacuolar change in the islets of Langerhans and zymogen granule depletion in the pancreas, and mesenteric fat atrophy. Parasites included *Ichthyophonus hoferi*, *Loma salmonae* in the gill and intra-arterial, an unidentified microsporidian in the kidney, a myxosporidian *Parvicapsula sp.* in the kidney and intestine, nematode larvae within the walls of the stomach and intestine, intestinal cestodes, and gastric trematodes.

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Introduction

Beginning in 1996, the U. S. Fish and Wildlife Service set out to test the feasibility of estimating the number of fall chum salmon in the Yukon River migrating above the confluence of the Tanana River, Alaska. A phenomena of progressively lower R/C ratios (recapture to capture ratios) with increasing distance from the marking site (Gordon et al. 1998; Underwood et al. 1998) occurred which needed further study. Nine hypotheses were proposed as possible explanations for the observed phenomena, one of which was delayed mortality caused by the capture and tagging process (Underwood et al. 1998).

The probability of recapture upstream of the recovery site was at least partially related to the number of times a fish was captured in the abundance study fish wheels. For example, a fish caught at the marking and recovery site more than three times was half as likely to be caught in the fisheries upriver than a fish caught only once (Underwood et al. 1998). These data suggested that something could be happening to tagged fish and, although it apparently was not affecting the abundance estimate, the fact that over 60,000 fish could be captured and processed each year implies that any added mortality may be significant to fish stocks. In addition, fish wheel catch-per-unit-effort has become a regular monitoring tool within the Yukon River drainage where eleven fish wheels were contracted for research or management needs in 1998. Thus, the need to establish whether there was any detrimental effects of the fish wheel capture and/or tagging process was important to fishery managers and researchers.

Fish handling, stress, and mortality have been well documented (Stichney 1983; Adams 1990; Wedemeyer 1990). The mortality caused by handling can be delayed (Stichney 1983) and the effects cumulative (Wedemeyer 1990), which could explain the decreasing R/C ratios observed upriver. Direct investigations into fish wheel stress and morbidity have not been documented. Possible causes of elevated stress include physical capture by the fish wheels, holding time in the live-box, crowded conditions within the live-box, handling procedures, and tagging. In this pilot study, we attempted to determine if there was tag-specific morbidity through complete necropsy on a small sample of fish. A second goal was to describe the histologic changes in the migrating chum salmon. A final goal to identify potential pathogens in a population that has recently experienced an overall decline.

Study Area

The Yukon River is the fifth largest drainage in North America, encompassing an area of approximately 855,000 km^2 (Bergstrom et al. 1995). Three of the tributaries that join the Yukon River are major rivers themselves, each approximately 1,000 km in length. They are the Koyukuk, Tanana, and Porcupine rivers, joining the Yukon River at 800, 1,100, and 1,600 km from the mouth, respectively. Two study sites were maintained on the main stem Yukon River upstream from the Tanana River confluence. The locations were selected to minimize capture of fall chum salmon returning to the Tanana River drainage, which constitutes the only major area of fall chum salmon spawning known to exist downstream from the study area. The marking site was located at an area known locally as “The Rapids,”

a narrow canyon 1,176 km from the mouth of the Yukon River. The recapture site was 50 km upstream from the marking site, near the village of Rampart, Alaska**.** The salmon take a mode of one day to travel between the two sites (Gordon et al. 1998).

Methods

Capture Techniques

Fall chum salmon were captured in two pairs of fish wheels, one pair at the marking site and the other at the recapture site. Each two-basket fish wheel was unique because of the builder and site fished; however, each pair of wheels shared some common features. At the marking site, fish wheel baskets were approximately 3.0 m wide and dipped to a depth of 4.5 m below the water's surface. Baskets on the recapture wheels were approximately 2.5 m wide and dipped to a depth of 3.0 m below the water's surface. Netting on the back of the baskets was composed of plastic, hardware-cloth, or chain-link fencing. Nylon seine netting was installed on the sides of the baskets. Closed-cell, foam padding to reduce impact injury to fish was placed along some chutes and ramps between the baskets and the live-boxes. Live-boxes were approximately 2.4 m long, 1.2 m deep, and 1 m wide. The walls and floors of the live boxes contained many 5 cm diameter holes to allow a continuous flow of water through the box, while preventing heavy current that could potentially stress weakened fish.

Tagged fish from the recapture site were compared to untagged fish at both sites. At the marking site, a 35-cm-long spaghetti tag was applied to each of approximately 400 fall chum salmon per day that were released to continue their upstream migration. Tagged fish that we examined at the recapture site were a sub-sample of those released at the marking site. Details regarding the tagging process are reported by Gordon et al. (1998) and Underwood et al. (1998, 2000).

Field and Laboratory Processing

Fifteen untagged fish were bled and necropsied at the downstream fish wheel. Thirty tagged and 15 untagged fish were sampled from the upstream wheel. All fish were sampled immediately upon being trapped in the fish wheel. The untagged upstream fish served as controls to the tagged fish, under the assumption that a very low percentage of these fish were stressed by going through another fish wheel and that tagging was the major stressor in the tagged fish. The lower sample sizes of 15 and 30 were chosen due to cost and because this was considered a pilot study. Larger surveys use a sample size of 60 animals per treatment group in order to determine a 5% prevalence rate with 95% confidence. With a sample size of 30, a disease prevalence of about 10% can be detected with 95% confidence (Becker and Grieb 1987).

Blood samples were collected by placing fish in a neoprene cradle with the head underwater, bled from the caudal vein using a heparinized syringe, and then placed in MS222 for euthanasia. Two blood smears were made for differential cell count and examination for viral erythrocytic necrosis virus inclusions. Two microhematocrit tubes were filled, centrifuged at 5,500 g for 5 min, and packed cell volume (PCV) was determined by a chart. A small aliquot of blood was placed in a 1.5 ml eppendorf microcentrifuge tube and stored refrigerated for red blood cell (RBC) and white blood cell (WBC) counts. Differential cell,

WBC and RBC, and enumeration of inclusion bodies were performed at Simon Fraser University in Burnaby, B.C.

A standard necropsy was performed on each fish which included body, liver and gonad weight, length, collection of vertebral bodies for aging, and fixing tissues in 10% buffered formalin. Tissues collected in formalin included the second gill arch, heart, liver, kidney, spleen, gastrointesinal (GI) tract, muscle (epaxial muscle from the area of the dorsal fin), skin, gonad, head cartilage, and brain. On the tagged fish, the external site of the tag was swabbed for bacterial culture. The caudal kidney was swabbed for bacterial culture in all fish. Loops were stabbed into trypticase soy agar (TSA) and refrigerated for the duration of the fieldwork. The kidney was frozen for later examination for the bacterial kidney disease (BKD) organism antigen, *Renibacterium salmoninarum* (Rs) using the enzyme linked immunosorbent assay (ELISA). Kidneys were submitted to Dr. Ted Meyers at the Alaska Department of Fish and Game (ADFG) Fish Pathology Laboratory in Juneau, Alaska, for the BKD-ELISA, and bacteriology cultures were examined and isolates identified using standard biochemical methods at the ADFG Pathology Laboratory in Anchorage. The ELISA was performed using the methods of Meyers et al. (1993). Any gross lesions were scored as none, mild, moderate or severe. Parasites were roughly enumerated. A section of the hind-gut was frozen for later examination for *Ceratomyxa shasta* pending review of the histopathology. Vertebral bodies were collected and analyzed for age by Kevin Boeck with the ADFG. Formalin-fixed tissues were assigned a separate number, processed routinely for paraffin sectioning at 5 μm, and stained with hematoxylin and eosin (H&E). Lesions were described and scored on a 4-point scale (Table 1). Special stains were done as needed including Prussian blue for iron, periodic acid schiff (PAS) with and without diastase to demonstrate glycogen, Gomori methenamine-silver (GMS) for fungal hypha, tissue gram stain for bacteria and microsporidia, acid fast for mycobacteria, giemsa for organisms, and trichrome to emphasize collagen.

Data Analysis

The bulk of the response variables are categorical with each fish being scored by response. The two predictor variables were category (downstream untagged, upstream untagged, upstream tagged) and sex. Age information was also available, but the sample sizes were insufficient to include age in the analyses. A generally applicable method of analyzing these data would be to fit multinomial response models, such as logit or proportional odds models (Agresti 1990). However, the large number of sampling zeros and violations of the proportionality assumption precluded that approach. Instead, chi-square tests of homogeneity (Greenwood and Nikulin 1996) were performed using a single stratification variable with six levels formed by the combinations of category (3) and sex (2). For each response variable, we tested whether the probabilities associated with each value of the response variable were equal for each of the six strata. Because of the sparseness of many of the tables, we used an exact Monte Carlo procedure to estimate significance (SAS 1999). The causes underlying significant tests were established by assessing patterns in the differences between observed cell frequencies and those expected under the hypothesis of homogeneity.

Numerical response variables' values were analyzed by analysis of variance (ANOVA; Montgomery 1984). Hematology, condition factor, hepatosomatic index, and gonadosomatic index were analyzed by two-factor factorial ANOVA, with factors category and sex. Relationships of lesions such as parasites to hematology and condition factor, spleen lesions to hematology, GI and pancreatic lesions, and ulcers to hematology, and condition factor were analyzed by a three-factor factorial ANOVA. In these analyses, category and sex were again factors, and a third predictor variable was an indicator of the presence or absence of the parasite of interest. Because of sample size limitations, we collapsed the three levels of category into tagged versus untagged groups for these analyses.

The "Frequency" and "General Linear Model" procedures of the SAS/STAT computer program (SAS 1999) were used to perform the analyses. Because this is a pilot study and, therefore, somewhat exploratory in nature, Type I errors were not consequential, and we wanted to maximize statistical power to the extent practicable given our small sample sizes (Montgomery 1984). For that reason, P_{α} less than 0.1 were considered to indicate statistical significance for all tests conducted.

Results

Necropsy Findings

External lesions.– Neither skin ulcers, fin reddening, fin fraying nor *Saprolegnia* sp. lesions varied significantly by category or sex (Table 2). Just under half (45%) of the animals had skin ulcers, including 14 animals with one ulcer, 7 with 2, and 5 with 3 or more. This is not uncommon in returning salmon and is thought to be related to trauma. Animals with ulcers had significantly higher condition factor scores than animals without ulcers ($P\alpha$ = 0.0003). Statistically significant results in the analysis of PCV and TP in relation to ulcers were not consistent with logical expectations and were, therefore, thought to be random (Type II error). Histologically, the findings were typical of an ulcer with a break in the continuity of the skin including hemorrhage, edema, and aggregates of neutrophils in the dermis, subcutis, and underlying muscle. The underlying muscle often demonstrated degenerative changes including vacuolation, centralized nuclei, loss of striation, and fragmentation. In the more chronic lesions, varying amounts of granulation tissue filled the ulcer bed. The surface was often colonized with fine, GMS-positive filaments consistent with fungal hyphae. Only two animals had grossly identifiable *Saprolegnia* sp. lesions. One animal was a 4-year-old downstream male and the other an upstream tagged 5-year-old female.

Morphometrics.– Body weight, length, liver and gonad weight, Fulton's condition factor (CF = weight/length^3), and indices of liver (hepatosomatic) and gonad (gonadosomatic) weight to body weight (Elston 1997) were not significantly different among categories (Table 3). Females had heavier livers and gonads as well as higher hepatosomatic and gonadosomatic indices.

Age.– Most of the fish were 4 or 5 years old. One fish (1.8%) was 3 years, 28 (50%) were 4 years, 24 (43%) were 5 years, and 3 (1.8%) were 6 years old. Age could not be determined on 2 animals; however, they were estimated to be 3-4 years old. Because the age

range was small and there were very small numbers of the older and younger fish, age-related lesions could not be determined statistically.

Anisakis counts.– A total of 14 of 58 animals (24%) had small numbers of anisakids. On gross examination, four animals had one anisakis in the coelomic cavity and five animals had two. No attempt was made to identify these parasites to genus or species. These were characterized as coiled nematodes covered by variable amounts of tan tissue. Five more animals had anisakis detected histologically either in the coelomic cavity (3), subcutaneous tissue (1), or kidney (1).

Tag Site Gross and Histologic Features

The tag site had relatively few grossly evident abnormalities. Typically, the tag passed cleanly through the dorsal musculature just ventral to the dorsal fin with very little evidence of reaction or infection (Figure 1). Five animals had small (5 x 2 mm) ulcers at the site of the tag knot, approximately 1.5 to 2 cm caudal to the dorsal fin on the dorsal midline. Many of the tagged animals had edema and thickening along the base of the caudal aspect of the dorsal fin and five had ulcers at this site (Figure 2). These ulcers were likely due to trauma from the back and forth movement of the tag. In some animals, there was a very thin rim of black pigment around the tag hole, most obvious in 16 of the tagged animals. Histologically, this correlated with accumulations of melanomacrophages in the area. The tag-associated ulcers in these areas were typical of acute ulcers with neutrophilic dermatitis, myositis, and edema (Figure 3). In some animals, there were accumulations of irregularly shaped refractile material consistent with scale fragments and epithelial cells within the tag tract. The skeletal muscle just adjacent to the tag demonstrated extensive degeneration characterized by fragmentation and vacuolation of the muscle fibers, centralized nuclei, loss of striation, and some contraction bands. Skeletal muscle within the tag block, but further from the tag, commonly had scattered individual fiber degeneration. These changes in inflammatory cells and muscle damage were very similar to those described in newly tagged parr (Roberts et al. 1973a).

Bacteriology

All kidney bacterial cultures were negative for significant bacterial growth except for an isolate of *Aeromonas salmonicida* from an upstream, untagged, 4-year-old female. This individual also had multiple ulcers. Histologically, this animal had a severe, multifocal ulcerative neutrophilic dermatitis with cellulitis and myositis with large intralesional colonies of fine, Gram-negative organisms (Figure 4). This lesion is typical of furunculosis. The predominant organisms from the surface swabs in all fish were representatives of the pseudomonad and aeromonad groups, both commonly present externally on fish.

Bacterial Kidney Disease (BKD) ELISA

One fish had a low positive optical density value for Rs antigens of 0.071. This was a downstream, untagged, 5-year-old female fish. A mean optical density value of at least

0.068 was considered positive for the Rs antigen. Histologically, the kidney of this fish was unremarkable.

Hematology

Values were determined for packed cell volume (PCV), total protein (TP), total white blood cell count (WBC), red blood cell count (RBC), and absolute numbers of neutrophils, thrombocytes, lymphocytes, basophils, eosinophils and monocytes, and neutrophil/lymphocyte ratio (N/L). Adjusted WBC (aWBC), which is the WBC minus the thrombocyte number, was also calculated. The means and standard deviations for these values are listed in Table 4. WBCs in teleosts typically run less than 150 x 10^3 cells/mm^3, with large variations in range between species. Red blood cell count averages 1-3 x 10^6 cells/mm^3. Therefore, these average values fall within normal limits (Bond, 1996). Salmonids usually have more lymphocytes than neutrophils; therefore, the N/L ratio of less than 1 is expected. Thirteen animals had a neutrophil/lymphocyte inversion. This usually indicates a bacterial or other acute inflammatory condition. However, the one fish with a bacterial infection did not have this inversion and a consistent reason for this change was not found. No reasonable significant associations were found for PCV, RBC, WBC, thrombocyte, basophil, or monocyte numbers. Total protein was higher in females ($P\alpha <$ 0.0001), and lower in tagged fish ($P\alpha = 0.0357$). Regarding WBC, there was an interaction between sex and category ($P\alpha = 0.0061$); however, the cause of this interaction was unclear. There was a tendency for tagged females to have higher aWBC; however, this was not significant. In males, downstream-untagged animals had the highest aWBC. Upstream-untagged males were low in value, and upstream-tagged males were intermediate. The only significant difference in males was between untagged animals with downstream animals higher than upstream. The differences in category appear to be primarily due to a few downstream males with very high WBCs. There are category differences in thrombocyte numbers with upstream-untagged fish having more thrombocytes than the downstream animals, so this may be a downstream/upstream effect. There was unequal variance in neutrophil numbers; however, there was a strong category effect ($P\alpha = 0.0005$) and tagged fish had higher neutrophil numbers than untagged fish. For lymphocyte number, there was unequal variance. Females had higher numbers of lymphocytes than males ($P\alpha = 0.084$). There was an interaction between category and sex for lymphocytes, but it appeared to be random. For the N/L ratio, there was a difference by category ($P\alpha = 0.0264$); however, this may have been more of an upstream/downstream effect with the biggest difference between downstream-untagged and upstream-tagged fish. All blood smears examined were negative for inclusions from viral erthrocytic necrosis virus.

Histopathology

Heart.– None of the lesions scored in the heart correlated with category or sex (Table 5). Zenker's necrosis was characterized by contraction bands, deeply eosinophilic transverse bands which distorted the profile of the cardiomyocyte (Figure 5). A frequently seen lesion was fibrin thrombi and mild endocarditis characterized by small accumulations of fibrin attached to the endothelial cells in the spongy part of the ventricle or atria (Figure 6). These thrombi were often surrounded, or infiltrated, by mononuclear cells consistent with

some organization, and some contained lymphocytes or neutrophils (endocarditis). Small aggregates of lymphocytes were present, commonly in the compact ventricle referred to as stromal inflammation (Figure 7). A mixture of neutrophils, lymphocytes, and other mononuclear cells were also very common within the fat surrounding the heart (epicarditis) (Figure 7). In almost all fish, there were varying degrees of vacuolation of the myocytes (Figure 8). Two of the upstream fish, one untagged fish and one tagged, had intense granulomatous inflammation in the heart associated with moderate infection by *Ichthyophonus hoferi* (Figure 9). Two fish each had a xenoma of microsporeans (most likely *Loma salmonae*) within the lumen.

Liver.– Features scored in the liver included vacuolar change, portal inflammation, biliary inflammation, and pigmentation (Table 6). The vacuolar change (Figure 10) was a combination of glycogen and fat as indicated by a PAS reaction with and without a diastase control. Males commonly had highly vacuolated hepatocytes, whereas the females often had little to no vacuolation ($P\alpha < 0.0001$). Females had higher hepatosomatic index levels (Table 2). "Normal" hepatocytes in many species of fish, including salmon, are filled with multiple, poorly delineated, clear vacuoles of glycogen, which give the cytoplasm a "moth-eaten" appearance. Thus, the females had glycogen depletion and the males had glycogen and fatty stores. Portal inflammation is characterized by inflammatory cells around bile ducts and is very common in fish. All of the fish were grade 0 for portal inflammation. Biliary inflammation was absent is all but two samples, and these two had scattered inflammatory cells within the wall of the gall bladder (score of 1). These were both 5-year-old upstream tagged females. In many fish (30), macrophage-like cells in the sinusoids were enlarged by golden, somewhat refractile, pigment. This pigment was consistent with lipofuscin because it stained with periodic acid schiff (PAS), with and without diastase treatment, but did not stain by Prussian blue. Twenty-nine were grade 1 for pigment, one was grade 2, and none were grade 3 (Table 6) .

Brain.– Almost all animals had scattered histiocytic cells within the meninges which had foamy, generally pigmented cytoplasm and were sometimes multinucleated. These were most consistent with macrophages filled with ceroid or lipofuscin because they stained strongly with PAS, were negative for mycobacteria by acid fast stain, and were negative for iron by Prussian blue stain. Two fish had focal hemorrhage in the meninges with chronicity indicated by formation of hemosiderin. One individual had a single *Ichthyophonus* spp. spore within the brain parenchyma surrounded by granulomatous inflammation. Another individual had a single glial nodule, most likely an area of parasite migration or other trauma.

Musculoskeletal system and subcutaneous tissue.– In most sections of hard tissue from the head, a dense core of cartilage was surrounded by thin trabecula of bone interspersed with fatty tissue consistent with spongy bone. The spongy bone was lined by plump, reactive osteoblasts, collagen, and mucinous ground substance, which was interpreted as bony remodeling. This was most likely related to the changes in secondary sex characteristics developing in these fish as they moved upstream. In some fish, there was almost complete replacement of the subcutaneous adipose tissue with wispy, basophilic mucinous material. The degree of bone remodeling and amount of mucinous change in the subcutaneous tissue of the head were graded (Table 7). The cranial cartilage was negative for *Myxosoma cerebralis* lesions (whirling disease) in all fish. In the somatic skeletal

muscle, contraction band necrosis and skeletal muscle degeneration were common (Table 7). Muscle degeneration was a combination of several changes. In many cases, there was a granular change to the muscle cells, which often extended to variable sized, eosinophilic globules between muscle fibers. Individual cell degeneration was characterized by loss of striation and centralization of the nuclei. None of the changes were associated with either category or sex.

Spleen.– In most animals, the ellipsoids were prominent (Table 8). There were scattered individual melanomacrophages and aggregates of immature red and white blood cells, or "hematopoiesis," organized primarily around thin walled vascular spaces. This was considered to be "normal" in these fish and was graded as 1. Thirteen animals had evidence of red blood cell phagocytosis, or erythrophagocytosis. Hematopoiesis and erythrophagocytosis are normal functions of the spleen in fish. Neither hematopoiesis nor erythrophagocytosis grades correlated with treatment group, WBC, RBC, or PCV. Two animals had scattered aggregates of reactive lymphoid cells (lymphoid hyperplasia). One of these animals had a systemic infection with *Ichthyophonus hoferi*.

Gonad.– The testes were graded according to maturity. None of the males completely lacked development, 2 had up to ¼ of the testicular tubules containing spermatozoa, 15 had ¼ - ¾ of the tubules containing spermatozoa, and 9 were ¾ to fully developed. In all the females, the eggs were mature, filled with yolk material, and surrounded by a thick membrane.

Gills.– Changes that were graded included goblet cell hyperplasia, focal epithelial cell hyperplasia, branchitis, focal apoptosis and fibrin thrombi (Figure 11), secondary lamellar clubbing, secondary lamellar fusion, and telangiectasia (Table 9). Most animals had small aggregates of goblet cells at the tips of scattered secondary lamella (grade of 1 for goblet cell hyperplasia). There were no severe cases of goblet cell hyperplasia. Under some pathologic conditions, such as pollution and parasites, goblet cells occur in increasing numbers at the distal ends of the lamellae (Ferguson 1989). Epithelial cell hyperplasia was more unusual, consisting of focal piling up of the epithelium, generally along the middle of the secondary lamella. These foci often contained 1 or 2 apoptotic cells, and some contained fibrin thrombi within the vessels. Rarely, animals had one or two small areas of lamellar clubbing or fusion. These were most likely focal areas of mechanical or parasite related trauma. Scattered animals had mild telangiectasia, or dilation of the gill vessels. This is generally associated with trauma. In one upstream-tagged fish, there was more significant epithelial hyperplasia associated with some fusion and loss of secondary lamella. This lesion was also noted grossly as a 2 mm swollen, pale area on the first gill arch. On the surface in this area, there was refractile, irregularly shaped debris. However, similar refractile material was also seen in cases without epithelial hyperplasia. In one fish (sample number 4-23) fibrin thrombi containing necrotic debris were common in vessels of the secondary lamella and at the tip of a primary filament, there was a region in which most of the vessels contained thrombi. In this area, there was blunting and fusion of the secondary lamella, epithelial cell hyperplasia, and neutrophilic infiltration. There was also edema and inflammation in the epithelium at the base of the primary lamella. In this animal, there were also organizing fibrin thrombi within the kidney. A cause for these lesions was not observed. Four animals

had microsporidian parasites within the gill most consistent with *Loma salmonae* described below (Figure 12 and 13).

Gastrointestinal tract.– Several different regions of the gastrointestinal tract, including esophagus/proximal stomach, pyloric stomach, pyloric caeca, and proximal and caudal intestine, were examined and scored for lesions (Table 10). Each of these sections was scored for inflammation, individual epithelial cell death or apoptosis (Figure 14), and myofiber degeneration (Figure 15). None of these features were related to category or sex. The chi-square p-values for gastritis ($P\alpha = 0.0155$) and for enteritis ($P\alpha = 0.0389$) were statistically significant; however, these patterns appeared to be random in terms of fish category and sex. Myofiber degeneration was most severe in the esophagus and least in the intestine.

Trematodes and cestodes in the lumen and nematode larvae migrating within the wall of the intestinal tract were common (Table 13) and, generally, were not associated with an inflammatory reaction. Trematodes (Figure 16) were in 51.7% (30/58) of the animals, primarily in the stomach lumen and attached to the gastric epithelium. These were oval to folded with a spiny tegument, solid or parenchymal body, an oral sucker and large ventral sucker close to the oral sucker, and post-testicular ovary. Eggs were approximately 10.4 x 22.1 -23.4 μm and had a thick, yellow, operculated shell. These are most consistent with the family Hemiuridae, of which *Brachyphallus crenatus*, *Lecithaster gibbosus*, *L. stellatus,* and *Tubulovesicula lindbergi* have been reported in the stomach and intestine of chum salmon (Hoffman 1999). In a 3-factor ANOVA, the N/L ratio was slightly greater in animals with flukes ($P\alpha = 0.0251$). Cestodes (Figure 17) were in 72.4% (42/58) of the animals in the pyloric caeca, but occasionally in the pyloric stomach and caudal intestinal lumen. These had a solid parenchymal body, a smooth cuticle, and large numbers of calcareous corpuscles. *Phyllobothrium ketae* have been described as occurring as adults and larvae in the intestine of chum salmon in Alaska (Hoffman 1999). Cross sections of nematode larvae in the lamina propria were in 63.8% (37/58) of the fish. These larvae were of variable sizes, but usually approximately 7.8 -10.4 μm in diameter with a pseudocoelom and lateral alae (Figure 18). These are consistent with larvae of the Anisakidae family, which have been reported in chum salmon and other marine and anadromous fishes (Hoffmann 1999). Small numbers of anisakids grossly evident in a few of these fish are not considered to be pathogenic. No attempt to enumerate these parasites was made. In most cases, there was no inflammatory response to these parasites. Condition factor, PCV, TP, WBC, and RBC were not affected by presence of cestodes, trematodes, or the larval nematodes. In five cases cestodes embedded within the intestinal wall or within the coelomic cavity were associated with intense inflammation and necrosis.

One fish (sample number 5-12) had multifocal necrosis and mineralization of the granular cells in the granular cell layer of the esophagus. This was associated with focal granulomas in the lamina propria. A cause could not be determined. A myxosporean parasite was in 4 cases in the pyloric caeca. The spores were linear to curved, 14.3 x 2.6 μm, with multiple, dark, internal bodies (Figure 19). The two major differential identifications for this organism would be a *Myxidium* or *Parvicapsula* sp. There appeared to be two polar capsules, most consistent with a *Parvicapsula* sp. These organisms were attached to the apical border and were not associated with inflammation.

Kidney.– Graded histologic findings included numbers of melanomacrophages in the interstitium, presence of intracytoplasmic inclusion bodies in interstitial cells, amount of interstitium, glomerulonephritis, and number of primordial structures (Figure 20) (Table 11). There were some significant sex related differences in these features; however, they occurred in a random pattern relative to category. Changes noted included intracytoplasmic inclusion bodies in the tubular epithelial cells and the presence of parasites. Melanomacrophages in chum salmon kidneys generally occur as individual cells scattered throughout the kidney, rather than in aggregates such as occurs in many other fish species. The numbers, size, and histologic appearance of melanomacrophages change with age, season, state of nutrition, and exposure to various antigens or degraded environment (Ferguson 1989). The downstream fish had significantly greater scores for melanomacrophages than the upstream animals ($P\alpha$ = 0.0056). The amount of interstitium was graded because this tissue in fish is a site of hematopoiesis and can also be an area of inflammation during kidney infections. Most fish (50/58) had a grade of 1 or 2 (Table1), which was considered to be within normal limits. Four animals had a grade of 0, and four fish had a grade of 3, which are considered to be depleted and reactive, respectively. No consistent reason could be determined for the depletion and reactivity. A very mild form of glomerulonephritis was relatively common. In these cases, there was increased mesangium in the glomerular tuft, some adhesions to Bowman's capsule, and prominent thickening of the lining of Bowman's capsule. In four fish, there were neutrophils within the glomerular tuft and/or a ring of lymphocytes around Bowman's capsule, indicating a more severe glomerulonephritis. Neither of these grades correlated with kidney parasites, category of fish, or sex. It is likely that the grade 1 and 2 is either within normal limits, or possibly an aging change. Almost all fish had scattered primordial structures within the interstitium. Again, test of homogeneity p-values were low for primordial structures; however, the pattern was inconsistent. Five animals had large eosinophilic (pink) to yellowish intracytoplasmic droplets within the epithelial cells of the mesonephric ducts. Four animals had fine, very bright intracytoplasmic inclusions within the proximal tubules that were most likely protein droplets. The most common reason to see protein droplets in the proximal tubules is from protein leakage of the glomeruli due to glomerulonephritis, or hemolysis. However, two of them had the very common mild glomerulonephritis and hematology was normal. Such droplets have been described in fish previously. They are thought to be lipoproteinaceous material, possibly reabsorbed from the tubular lumen, and are of unknown significance. These droplets in other reports have not been associated with glomerular changes. Several authors have noted an association of these droplets with toxicants and high ammonia levels in salmonids (Ferguson 1989). Because they were all females, another possibility is that it is related to vitellinogenesis.

Kidney parasites.– Fifteen fish had microsporidal xenomas within the kidney that occurred primarily within the glomerular tufts (Figure 21), less frequently within tubular epithelium, and in one case within an arteriole and in another case in the interstitium. Two fish were downstream untagged, 6 were upstream untagged, and 7 were upstream tagged. No differences in prevalence were noted by category, age or sex, and they did not appear to be related to increased interstitium or glomerulonephritis. There was also a myxosporidian parasite in the kidney of six animals (Figure 22) within the interstitium, blood vessels, tubule cells, and free within the tubule lumen. The organisms formed cysts or aggregates within

which there were multiple, deeply basophilic, immature spores. These spores were bean-shaped and had two polar capsules, most consistent with a *Parvicapsula* sp. One upstream-untagged animal had a very extensive presence of the myxosporidian within the interstitium of the kidney. There was no consistent host cell reaction to these parasites.

Pancreas.– The pancreas of the chum salmon is dispersed within the mesentery around the liver, gall bladder, and pyloric caeca. There are two major islets located by the vasculature associated with the gall bladder, and small islets of Langerhans are scattered throughout the pancreas. Vacuolation of the islet cells, zymogen granule depletion (Figure 23), and mixed cell, mild pancreatitis were all common lesions in these animals most likely related to fasting (Table 12).

Microsporidia.– Nineteen fish had microsporidial xenomas in at least one tissue. The majority had microsporidia within the kidney alone (15); one had microsporidia within the kidney (intraarterial), gill and heart; two had microsporidia within the gill alone; and one had microsporidia within the gill and the heart (Table 13). Microsporidial xenomas in the gill were present in small numbers and were 87-192 μm. Xenomas were most often within the secondary lamella, sometimes at the base of the secondary lamella (Figure 12a). In two cases, they were located within an artery of the primary lamella. In one of these fish (sample number 2-21), intra-artery xenoma was adjacent to an area of thrombosis and inflammation (Figure 13). Also adjacent to some of the cysts in this animal were increased numbers of neutrophils at the base of the secondary lamella (neutrophilic branchitis). In infected downstream fish, there were also multifocal aggregates of neutrophils within the gills, some associated with this parasite, and some not parasite associated. These parasites reacted strongly to anti-*Loma salmonae* monoclonal antibody by immunohistochemistry (Figure 12b*)*. This monoclonal antibody was quite specific and does not cross-react with closely related species such as *Loma fontinalis*. This tissue distribution (intravascular, gills, heart), salmonid host species and tendency to form xenomas, was also consistent with *L. salmonae* (Morrison and Sprague 1981, 1983). Microsporidia within the kidney most frequently formed a small cyst, or xenoma, and generally occurred within the glomerular tuft and rarely in a tubule or interstitium. There was no host cellular reaction to these xenomas in the kidney, which were also of very small numbers. Xenomas in the kidney were smaller than those in the gill, varying from 18 to 64 μm in diameter. These xenomas did not react with the monoclonal antibody for *Loma salmonae* and were most likely a different species.

Ichthyophonus.– Two fish had moderate intensities of infection with *Icthyophonus hoferi* in multiple organs (Table 14). In both cases, the heart was the most heavily affected tissue with pin-point white foci in the gross examination. Histologically, there were degenerated spores characterized by collapsed, refractile, PAS positive spore walls, or fragments of walls surrounded and filled in by variable amounts of granulomatous inflammation including neutrophils (Figure 9). Also present were multinucleated resting stage spores characterized by large round structures filled with endospores and a thick PAS positive refractile wall. Resting spores were generally surrounded by a thin rim of macrophages and collagen.

Unusual findings in individual fish.– In fish number 5-4, large numbers of hepatocytes, macrophage-like cells within the space of Disse in the liver, kidney interstitium and spleen were packed with variable sized, bright pink refractile intracytoplasmic droplets

consistent with protein droplets (Figure 24). Hepatocytes often were enlarged and had large, irregularly shaped nuclei (megalocytosis). This was an upstream-untagged female and was the only female examined that had the eggs released into the coelomic cavity. These protein droplets were most likely egg yolk that had been released into the circulation and taken up by the liver and mononuclear phagocytic system.

Discussion and Conclusions

In this pilot study, our primary goal was to determine whether there was tag-specific morbidity. By examining a cohort of tagged animals upstream from the tagging and handling site, we hoped to get an indication of whether abnormal processes were occurring in these animals that were different from the physiologic changes that occur during migration. Possible causes of tag associated morbidity and mortality include loss of osmoregulatory ability or septicemia (infection spreading through the bloodstream) from the tag site wound, trauma, or handling techniques, reduced swim efficiency and accelerated loss of reserves, or a stress-related increased susceptibility to infectious disease. In tagging salmon parr, mortaltiy due to infection by *Aeromas salmonicida* during an outbreak was 40% higher in tagged fish than untagged fish prior to use of antibiotics (Roberts et al. 1973b) suggesting that stress associated with tagging could lead to increases in stress related infectious disease. In addition, some studies indicate that some types of external tags can affect swimming performance, growth, and oxygen consumption (Serafy 1995). Migrating salmon are already going through a relatively stressful physiologic process during which body reserves are being used due to lack of feeding during the migration. Salmon appear to develop body reserves in proportion to the length of the migration with the chum salmon migrating over 2,700 km up the Yukon River needing maximal reserves (Smith 1993). Any unusual use of these reserves might therefore affect the outcome of the migration. Most stress-related responses increase during the upstream migration and peak on the spawning grounds. Pacific salmon usually die after spawning, and the death has been attributed to decreased disease resistance from the extended stress rather than any inherent mechanisms (Smith 1993). Since the development of these diseases or the agents responsible could be occurring during the migration, analysis of the prevalence of infectious diseases was important.

Diseases of particular interest to this study were those that are endemic to the area, affect adult chum salmon, and may be activated by stress. Furunculosis caused by *Aeromonas salmonicida*, enteric redmouth caused by *Yersinia ruckeri*, bacterial kidney disease caused by *Renibacterium salmoninarum*, saprolegniasis caused by a variety of fungal organisms, viral erythrocytic necrosis, and *Ceratomyxa shasta* were all candidates that fit these parameters (Shotts and Nemetz 1993, Chako 1993, McAllister 1993, Heckmann 1993, Ted Meyers, ADFG Fish, personal communication). Other significant diseases, such as whirling disease, infectious pancreatic necrosis, infectious hematopoietic necrosis, or viral hemorrhagic septicemia, either have not occurred in this area or have not been reported in chum salmon (Ted Meyers, ADFG, personal communication). Nevertheless, examination for typical lesions for these diseases was still important in case of a new disease, or unreported diseases, particularly since the population has recently experienced an overall decline.

A definitive cause of morbidity and mortality was not determined in these chum salmon, however; increased susceptibility to infection and disruption to osmoregulation due to the tagging has not been ruled out. At the tag site, there was histologic evidence of moderate traumatic damage. Although there was not a significant elevation in the white blood cell counts or inversion of the neutrophil/lymphocyte ratio in the tagged fish indicating an acute systemic bacterial infection, there was a statistically significant elevation of neutrophil counts in tagged fish indicating an acute systemic reaction associated with the tagging process. This change could either be an early inflammatory reaction or an indication of stress. Also, in our study, there was a drop in total protein from the downstream-untagged versus tagged fish, possibly due to hemodilution from the tagging wound and breach of the waterproof barrier.

None of the morphometric measurements including body weight, length, condition factor, hepatosomatic ratio, gonadosomatic ratio, or histopathology findings indicated a cause for mortality or morbidity specific to the tagged fish. ELISA for Rs antigen was negative except for one weak positive fish indicating no clinical bacterial kidney disease. Kidney bacterial cultures were negative except one untagged fish that had a systemic infection with *Aeromonas salmonicida*. These data suggest that septicemia due to the tag was not occurring during the time it took the fish to travel from the downstream to upstream site. This, however, was a relatively short period of time to develop septicemia. Lesions suggestive of other major infectious disease agents were not evident in the fish examined.

There could be several reasons for this inability to determine an absolute cause of mortality and morbidity in tagged fish. First, the downstream and upstream sites were located quite close to one another, and no mortality had been recorded between these two sites in previous studies. Many causes of death are less acute than the short time it would take a fish to travel from one site to the other (approximately 24 hours). Many histologic changes also would not develop in this amount of time. Only very acute lesions, such as edema, hemorrhage, vacuolation of cells, necrosis, or neutrophil immigration, would be detectable. Certain physiologic changes that could cause acute death such as severe metabolite imbalances would not be detected using these methods. Second, certain physiologic changes that could cause acute death such as severe metabolite imbalances would not be detected using these methods. Third, this was considered a pilot project, so the number of animals sampled was relatively small, making some of the groups too small for statistical analysis. Finally, a syndrome of delayed capture mortality has been described in the skipjack tuna in which handled fish develop myopathy (muscle damage), weakness, poor vision, and incoordination (Bourke et al. 1987; Stoskopf 1993), which this study may not have fully addressed. These skipjack tuna developed a significant drop in hematocrit, total protein, loss of body weight, evidence of disseminated intravascular coagulation, and renal damage secondary to myopathy (Smith 1980). Though the tagged fish did have a drop in total protein, none of the other changes were evident in these chum salmon. Scattered, individual muscle cell necrosis or degeneration was seen in both tagged and untagged animals. At the site of the tag, there was very extensive muscle necrosis, but this may have been a local effect due to water contact and not a systemic effect such as would be required for it to be a “capture myopathy.” In some of the tagged animals, skeletal muscle observed in both the head and tag sections did not have any more muscle fiber degeneration than

untagged fish. However, if similar studies are done in the future, it would be useful to sample multiple sites of skeletal muscle in tagged fish, both at the tag site and well removed from the tag, in order to fully rule-out a myopathy in tagged fish.

A second goal of this project was to describe and establish a grading system for the histologic changes in migrating chum salmon. Further work in this area is quite important because there are many histologic features in these animals that are either normal for the species or degenerative changes probably due to the fasting and stress associated with the migration and/or to aging. Such histologic descriptions specific to migrating Pacific salmon are not present in the literature in a cohesive form. Familiarity with these "background" changes could aid in determining lesions of significance in future work in migrating chum salmon. Some histologic changes are also sex associated. We assumed that most of the degenerative changes were due to the conditions of migration or age. However, there was no strong statistical support for these conclusions because of the close proximity of the upstream and downstream sites and because the sample size precluded a range of ages of fish.

Features that were probably due to migration included the external findings of skin ulcers, fin reddening, and fin fraying (Table 2). Small numbers of skin ulcers were commonly observed and had a tendency to increase as the fish moved upstream. This was most likely from trauma, a decreased ability to repair defects due to the fasting, or possibly a decline in immune function. Ulcers have also been associated with viral infections. Viral cultures were not performed, however, there were no histologic lesions to suggest viral disease. Also, very common were fin reddening and fin fraying, again most likely due to trauma during the migration. These lesions can be associated with bacterial infection, but in this case bacterial cultures were negative. Hold time could also affect the degree of fin reddening and fraying, but this is unlikely because these fish were collected without a significant hold time.

Zenker's necrosis and fibrin thrombi (blood clots) in the heart and associated inflammation of the endocardium, as well as myocyte vacuolation, were most likely associated with migration. Mild Zenker's, or contraction band necrosis, was relatively common in fish from all categories (Table 5). Since this condition can happen quite acutely resulting in significant impairment in the animal, it was considered as a possible lesion associated with capture and tagging. However, there was no tendency for an increased occurrence of this lesion in tagged fish. This change has been observed in a variety of conditions and is thought to be related to excessive release of endogenous catecholamines. It may also be a factor of very fresh necropsies (Robinson and Maxie 1993) because this change has been observed in normal myocardium if fixed immediately after death. Nutritional cardiomyopathy is observed in a variety of fish, especially salmonids and turbot in intensive culture fed diets low in Vitamin E and selenium. Lesions have included an increase in eosinophilia and loss of striation, with myocyte fragmentation later in the course of the disease (Ferguson 1989). This condition seems unlikely in these wild fish.

Fibrin thrombi and associated endocardial inflammation were common findings in all categories, but had a slight tendency to increase in the upstream fish. Some of the fibrin thrombi appeared to be very new, while others were slightly "organized," indicating a longer presence than the time required for the fish to travel from the down to upstream sites. Thus, they were more likely features of migration. Thrombi can be very significant if vessels are

occluded, leading to tissue damage from lack of blood supply. Blood clots reported in fish have been the assumed cause of death in some cases (Smith 1980). Thrombi were also quite common in these chum salmon within the vessels of the secondary lamellae of the gills, generally without associated tissue damage (Hougie 1977). Clots can be formed intravascularly due to many factors including trauma, bacterial infection, parasitic infestation, shock, malignancy, and immunological disorders (Smith 1980). Clotting time of spawning and post-spawning silver salmon (*O. kisutch*) can be prolonged compared to that of actively feeding silver salmon (Hougie 1977), and partial thromboplastin time was significantly prolonged in spawning pink salmon. In one study, pre-spawning pink salmon with degenerative changes (fatty infiltration of the liver) also had a clotting defect. The most severely affected fish had decreased fibrinogen, poor clot retraction, hemolysis with anemia, and reduction in clotting factors indicating disseminated intravascular clotting. Although clotting wasn't studied in our chum salmon, PCV, RBC number, thrombocyte numbers, and thrombocyte percentages were not different between scores of thrombosis in the gills, suggesting that there most likely was not a systemic clotting problem. Mechanical or parasite related trauma of the gills would be likely causes of these fibrin thrombi in the gills.

In other tissues, apoptosis of the epithelial cells, degeneration of the external muscular layer of the gastrointestinal tract, zymogen granule depletion and vacuolation of the islets of Langerhans in the pancreas, and mesenteric fat atrophy were most likely due to fasting. In the case of the gastrointestinal tract, degeneration of the epithelium with individual cell necrosis has been observed in a wide variety of conditions, including starvation (Ferguson 1989). Zymogen granule atrophy is also typical of inadequate nutrition. In the kidneys, primordial structures were very common in all fish with a slight tendency for more of these structures to occur in the downstream fish. Basophilic primordia such as we observed in the chum salmon, have been described adjacent to glomeruli and are though to be the sites of origin of new glomeruli (Ellis et al. 1978) or possibly tubules. These could have been an adaptive response to the change from salt to fresh water. Fresh water teleosts have more glomeruli and more extensive tubular systems while the glomerular apparatus of some euryhaline species undergo degeneration in the marine environment.

Our chum salmon had several significant gender-related differences. Males weighed more than females, but female liver weight was greater than in males. Males had extensive vacuolar change due to a combination of glycogen and fat while the females had significantly fewer vacuoles in the liver. Predictably, gonads weighed much more in females than males. Total protein was higher in females ($P_{\text{ت}} < 0.0001$). Females had higher numbers of lymphocytes than males ($P_{\text{ت}} = 0.084$).

Features present in almost all animals that were not degenerative were considered to be normal for the species of this age group. These included a mild amount of inflammatory cells within the epicardial fat, small numbers of lymphocyte aggregates within the heart, lipofuscin accumulation within the cells lining the space of Disse in the liver and within macrophages of the meninges, a mild glomerulonephritis, and a low-number of inflammatory cells within the lamina propria of the stomach and intestines (gastritis and enteritis). A scattering of inflammatory cells in the epicardial fat and scattered aggregates of inflammatory cells, primarily lymphocytes, within the compact myocardium have also been reported for other fish species (Ferguson 1989). An increase in inflammation is most likely

related to increased inflammation elsewhere in the body, either bacterial or parasite related. For example, both fish with *Icthyophonus hoferi* had increased scores, both in epicardial and stromal inflammation. A grade of 1 or 2 for interstitium in the kidney would be normal for any fish species and was the most common in these animals. Melanomacrophages were typically scattered throughout the liver, spleen and kidney as individual cells. A mild glomerulonephritis can be observed frequently in older fish including thickening of Bowman's capsule, diffuse thickening of the glomerular basement membrane, and thickening and fibrosis of the glomerular tuft (Roberts 1978). These changes correlate very well to the common grade 1 glomerulonephritis in these chum salmon. Lipofuscin is a pigment that occurs from incomplete breakdown of lipids. Increased amounts of lipofuscin can be due to storage diseases, elevated catabolism, or accelerated break-down of lipids such as occurs with vitamin E/Selenium deficiencies.

A final goal was to identify potential pathogens in a population that has recently experienced an overall decline. Parasites detected included *Ichthyophonus hoferi* in two animals, *Loma salmonae* (a microsporidian) in the gill and vasculature, an unidentified microsporidia in the kidney, a myxosporidian (most likely *Parvicapsula* sp.) in the kidney, nematode larvae migrating in the stomach and intestinal walls, intestinal cestodes, gastric trematodes, and intestinal myxosporeans (an earlier stage of *Parvicapsula* sp.). The myxosporidian in the kidney was relatively common and did not affect body condition or cause significant lesions, except for a slight increase in glomerulonephritis. This sporozoan was most likely a *Parvicapsula* sp., described in adult sockeye salmon in British Columbia (*P. minibicornis*) and in netpen-reared coho salmon (Kent et al. 1997). In marine pen-raised coho salmon, this parasite has been a serious pathogen, causing kidney hypertrophy and tubular degeneration. In the same area, Pacific cod infected with fewer parasites and with no apparent pathogenicity were assumed to be the natural host (Hoffman 1999). The intestinal cestodes, gastric trematodes, and migrating nematode larvae were very common as they are in most wild fish populations. The intestinal myxosporean (*Parvicapsula* sp.) was not associated with a host reaction or with changes in body condition, PCV or total protein.

Ichthyophonus hoferi has a worldwide distribution appearing to infect all salmonids, with rainbow trout (*Oncorhynchus mykiss*) being particularly susceptible (Chako 1993). Previously considered to be a Phycomycete fungus, recent research suggests that *I. hoferi* belongs to a new clade of protistans (Rahimian 1998). It has been reported from over 100 species of freshwater, estuarine, and marine teleosts in temperate as well as tropical habitats (Rahimian 1998). Several epizootics of *Ichthyophonus* sp. have been linked to population declines in Atlantic herring. In Alaska, *I. hoferi* has been described in Pacific herring (*Clupea pallasi*) (Marty et al. 1998) and chinook salmon (*O. tshawytscha*) (Kocan 2001). In our two chum salmon, infection was mild to moderate, affecting a wide variety of tissues (Table 14). Infection could be recognized grossly as pinpoint white nodules in the heart. Although several tissues were involved with a granulomatous reaction to the spores, there was no loss of condition and no consistent changes in the host hematology. In experimentally infected rainbow trout, anemia and leucopenia resulted; however, body condition, hepatosomatic indices, plasma chloride, cholesterol, cortisol, creatinine, glucose, osmolarity, potassium, total protein, sodium, and T4 remained unchanged (Rand and Cone 1990). With such a low level of infection in the fish examined (2/58), it is most likely not a

major pathogen in the population. However, we do not know whether juvenile or ocean life stages would be affected more severely. Ingestion of infected fish or infected copepods are probable reservoirs of infection (Roberts 1978) and means of transmission. Because these fish are not eating during the migration, the infection rate should not increase. However, it is possible that mild infections with subclinical disease could have been missed using histopathology alone. Therefore, the ability to detect infection and fish with clinical disease may increase as the disease progresses as the fish further upriver. Such has been the case in Yukon River chinook salmon (Kocan 2001). For these reasons, studies to determine the prevalence and associated lesions of *Ichthyophonus* sp. at the different life stages of Yukon River chum salmon would be important to determine the significance of this parasite.

The microsporidian present in the gills, within the heart, and intravascularly was consistent with *L. salmonae*. Monoclonal antibodies directed against the capsule of *L. salmonae* stained these organisms strongly (Dr. Speare, Atlantic Veterinary College, University of Prince Edward Island, Canada, personal communication), and *L. salmonae* has been shown experimentally to infect chum salmon (Shaw et al. 2000). All reported microsporidia within the gill of salmonids have been placed within the *Loma* sp. (Morrison and Sprague 1981). *L. salmonae* (Syn. *Pleistophora salmonae or Plistophora salmonae*) has been reported in *O. mykiss* (steelhead/rainbow trout), *O. nerka* (sockeye salmon), *Salvelinus fontinalis* (American brook trout), *O. kisutch* (coho salmon), and *Salmo trutta* (brown trout). *Loma sp* has been reported in *O. tshawytscha* (chinook salmon) in Alaska (Hoffman 1999), *O. gairdneri (*rainbow trout, freshwater*)*, *O. masou* (masu salmon) and *Cottus* sp. (sculpin) (Canning and Lom 1986). The geographic range includes North America in California and British Columbia, and in Hokkaido, Japan, and is considered to be widespread in wild and hatchery-reared salmonids. *Loma* sp. have been associated with disease in several species of salmonid fishes reared in freshwater, producing xenomas occupying a large volume of gill tissue (Wales and Wolf 1955) in rainbow and steelhead trout. *Loma* sp. was reported in an epizootic with 10 % mortality of juvenile chinook salmon at Fort Richardson Hatchery in Alaska (Hauck 1984). In this case, lesions associated with the parasite included inflammation, necrosis, and occlusion of arteries, degeneration and necrosis of cartilage and musculature of the tail and head, pericarditis of the bulbus arteriosus, and hyperplasia of the heart tissues. In British Columbia, heavy burdens of *Loma* sp. have been noted in returning sockeye salmon combined with *Ichthyopthirius*, which together may cause pre-spawning mortality. These populations have experienced a decline, and there is considerable interest in determining the role, if any, that *Loma* sp.have in this decline (Dr. D. Speare, personal communication). Dr. Speare (1988) reported *Loma* sp. infections combined with concurrent lesions caused by *Chaetoeros* diatoms in the gills of coho salmon introduced into seawater net-pens in British Columbia. The mortality rate was 60%, but the primary cause of death was attributed to the diatoms (Kent et al. 1989). *L. salmonae* infections associated with severe inflammatory gill lesions have also been observed in coho salmon (*O. kisutch*) reared in seawater net-pens in Puget Sound, Washington, U.S.A (Kent et al. 1989). The infections were present in the gills before the fish entered seawater, but few parasites and little tissue damage were observed. After fish were transferred to seawater, significant changes occurred in the gills including mixed inflammatory infiltrate associated with ruptured xenomas of the parasite within the gill blood vessels and in the interstitium of the primary lamellae.

Although other pathogens were present in these fish, the high prevalence of *L. salmonae* and the severity of the lesions suggested that this parasite significantly contributed to the mortalities observed (Kent et al. 1989).

In our chum salmon, there was a neutrophilic response to the xenomas in the secondary lamella and, in one case, there was a xenoma within an artery within the primary lamellae associated with thrombosis and periarterial inflammation very similar to that described in the seawater net-pen coho salmon. This reaction shows the potential for serious lesions and possible mortality in these fish due to this parasite. The severity of the lesion is typically a combination of the pathogenicity of a particular microsporidia and the host immune response. This raises the question of whether the lesions associated with this parasite increase or decrease as fish move closer to the spawning grounds. The tendency for there to be more serious lesions in the coho salmon after entry into seawater also questions whether lesions caused by *L. salmonae* may be more common and serious in the Yukon River chum salmon during their salt water phase. The severity of the lesions and mortalities described in juvenile chinook salmon (Hauck 1984) also suggests that this parasite could be a serious pathogen for juvenile chum salmon and other salmonids within the Yukon River. It is not known at what life stages the current chum salmon decline is occurring (Shaw et al. 2000). Studies should be undertaken to examine the pathologic effects of *L. salmonae* during the various life stages of the Yukon River chum salmon.

Because the microsporidia in the kidney did not react to the monoclonal antibody to *L. salmonae,* it most likely is a different species of *Loma*. Xenomas caused by this parasite were smaller and were not associated with a host response or tissue damage, therefore, are considered non-pathologic in this host fish.

This study was an initial step to look for gross cause of morbidity and mortality of tagged fish. The fact that no gross cause was found is valuable in evaluating the tagging study, but does not provide a deterministic answer possibly because of sampling design. The study also provides a baseline regarding histologic conditions and pathogenic agents found at a moderate prevalence in Yukon River fall chum salmon. Additional research to determine the cause of death in tagged chum salmon would require looking at upstream and downstream sites far enough apart to observe a difference in survival. Higher numbers of fish per treatment group would increase the chances of finding any changes that may occur, and complete hematology, clinical chemistries including coagulation studies, bacterial culture, virology and histopathology would be needed.

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Table 1.— Grading systems of histologic lesions.

Lesion	Grade 0	Grade 1	Grade 2	Grade 3
		Heart		
Zenker's necrosis	None present	Rare individual fibers affected	Large patches, or multiple patches easily seen at 10x.	The majority of fibers affected.
Fibrin thrombi	None present	Up to 5 foci in section	5-10 foci in section	>10 foci in section
Lymphocytic "myocarditis"	None present	Up to 5 foci in section	5-10 foci in section	>10 foci in section
Epicardial inflammation	None present	A light sprinkling of mixed inflammatory cells	Inflammation enough to thicken the epicardial fat	Inflammation with necrosis or fibrin deposition
Vacuolation	No vacuoles	Very fine vacuoles in the central part of the cell. Up to 1/3 cytoplasm clear. Some "beading" of the cytoplasm.	1/3-2/3 vacuolated. Some larger clear vacuoles with separation of myofibrils.	Over 2/3 of cells vacuolated.

Table 1.— Continued.

Lesion	Grade 0	Grade 1	Grade 2	Grade 3
		Liver		
Vacuolar change	No vacuoles present	< 1/3 of hepatocytes in the section or <1/3 hepatocyte volume clear	1/3 – 2/3 hepatocytes vacuolated, or if generalized, 1/3 – 2/3 of volume clear	>2/3 hepatocytes vacuolated or if generalized, vacuoles filled and distended most of the cells
Pigment in Reticuloendothelial (RE) cells	No pigment present	Scattered RE cells contained pigment	(50%) of RE cells were markedly distended by pigment	Pigment involved both the RE cells and hepatocytes.
		Musculoskeletal system		
Bone remodeling	No reaction on the periosteum	Less than 1/3 of spicules involved. Thickness of reaction <100 μm	1/3 to 2/3 spicules involved. Reaction 100-150 μm	2/3 spicules involved. Very plump reactive osteoblasts and some osteoclasts. Some reaction layers >150 μm
Contraction band necrosis	None	Up to 1/3 cells	1/3 – 2/3	2/3 to total

Table 1.— Continued.

Lesion	Grade 0	Grade 1	Grade 2	Grade 3
Focal myofiber degeneration	None	Up to 1/3 cells	1/3 – 2/3	2/3 to total
SQ—mucinous change	None	Up to 1/3 SQ volume affected	1/3 – 2/3 SQ volume affected	2/3 to total SQ volume affected
		Spleen		
Ellipsoid hyperplasia or hyalinization	Thin and slightly basophilic	Some ellipsoids were eosinophilic, and <15 µm thick	Most ellipsoids eosinophilic and >12 but <25 µm thick	All ellipsoids eosinophilic and many >25 µm thick
Hematopoiesis	0-2 foci precursor cells / ten hpfs (400x)	3-10 foci of precursor cells / ten hpfs	11-35 foci of precursor cells / ten hpfs	Generalized reaction with coalescing foci of precursor cells.

Table 1.— Continued.

Lesion	Grade 0	Grade 1	Grade 2	Grade 3
Testes				
Maturity	No development of spermatozoa	¼ of the tubules contain spermatozoa	¼ - ¾ contain spermatozoa	¾ to fully developed
Gill				
Goblet cell hyperplasia (Examine only sections with 2° lamella at right angles to 1°)	None present	Small aggregates goblet cells at the tips of scattered (<1/3) secondary lamella.	regions (up to 1/3 the length of the primary lamella) of goblet cell aggregates at the tips or 1/3 – 2/3 lamella involved.	Extensive goblet cell hyperplasia (>2/3)
2° lamellar clubbing	None present	Rare clubbed 2° lamella 1 -8 / 1° lamella	Regional change(1/3-2/3) or > 8 2° lamella / 1° lamella	Greater than 2/3 2° lamella involved.
2° lamellar fusion	None present	Rare fused 2° lamella 1 -8 / 1° lamella	Regional change(1/3-2/3) or > 8 2° lamella / 1° lamella	Generalized change with greater than 2/3 2° lamella involved.

Table 1.— Continued.

Lesion	Grade 0	Grade 1	Grade 2	Grade 3
Epithelial hyperplasia	None present	Rare foci of epithelial hyperplasia on 2° lamella.(1 -8 / 1° lamella)	Regional change(1/3-2/3) or > 8 on 2° lamella / 1° lamella	Generalized change with greater than 2/3 2° lamella involved.
Fibrin thrombi/apoptosis	No foci present	Rare foci on 2° lamella.(1 -8 / 1° lamella)	Regional change(1/3-2/3) or > 8 on 2° lamella / 1° lamella	Generalized change with greater than 2/3 2° lamella involved.
Branchitis	None present	Scattered rare aggregates of neutrophils or lymphocytes	Regional inflammation and edema with 1/3 – 2/3 lamella involved	Regional to generalized change (>2/3 involved). +/- necrosis
Telangiectasia	None present	Scattered dilated vessels on the 2° lamella (up to 1/3)	Regions involved (1/3 – 2/3)	>2/3 involved
		GI tract		
Gastritis	Little to no inflammation	Single layer of inflammatory cells	2 layers between glands of inflammatory cells	3 layers of inflammatory cells between glands
Apoptosis of epithelial cells	Absent	0-5 apoptotic cells/hpf	5-15 apoptotic cells/hpf	>15 apoptotic cells/hpf

Table 1.— Continued.

Lesion	Grade 0	Grade 1	Grade 2	Grade 3
Muscle degeneration	Absent	0-1/3 of muscle fibers undergoing necrosis / vacuolation	1/3 -2 /3 fibers involved +/- fibrosis	>2/3 fibers plus fibrosis
Enteritis	Few inflammatory cells up to 2 cells thick within the villus	2 cells up to the width of the epithelium	Inflammatory cells increased width of lamina propria to width of epithelium	2 plus blunting and fusion of villi
		Kidney		
Amount of interstitium (graded in urinary kidney, not head or trunk)	< the width of proximal epithelium	< width of 1 proximal tubule, no aggregates	1 –2 tubules +/- small aggregates	< #2 +/- large aggregates +/- necrosis
Primordial structures	None present	$\leq$ 3 / 10x	$\leq$6 / 10x	> 6 / 10x
Melano-Macrophages	$\leq$10 / 40x	11-20 / 40x	21 – 30 / 40x	> 30 or with large aggregates

Table 1 Continued.

Lesion	Grade 0	Grade 1	Grade 2	Grade 3
Intracytoplasmic inclusions in the interstitium	None	Rare	Scattered	> 1 / 40x
Pancreas				
Zymogen granule depletion	Cells packed with bright pink granules	Up to 1/3 cells depleted	1/3 – 2/3 cells depleted	2/3 total depleted
Vacuolation of islets	None	Up to 1/3 cells	1/3 – 2/3	2/3 to total
Mesenteric fat	Large numbers of plump adipocytes	Slight depletion	Moderate depletion	Total depletion

Table 2.— External lesion scores.

Group and result	n	Percentage for fish within each lesion			
		0	1	2	3
Skin ulcers					
Overall	58	55	24	12	9
Downstream	15	80	20	0	0
Upstream untagged	15	67	13	7	13
Upstream tagged	28	36	32	21	11
Male	27	59	30	7	4
Female	31	52	19	16	13
Fin Reddening					
Overall	58	15	55	24	5
Downstream	15	13	53	27	7
Upstream untagged	15	20	47	27	7
Upstream tagged	28	14	61	21	4
Male	27	19	41	37	4
Female	31	13	68	13	6
Fin Fraying					
Overall	58	17	41	38	3
Downstream	15	27	40	33	0
Upstream untagged	15	27	53	20	0
Upstream tagged	28	7	36	50	7
Male	27	15	52	30	4
Female	31	19	32	4	3

Table 3.— Morphometrics results.

Category	n	Average	Standard deviation
		Body weight (g)	
Downstream	15	3278	445
Upstream	15	3345	1143
Upstream	28	3186	711
Male	27	3528	796
Female	31	3019	685
		Condition factor	
Untagged	30	16.09	1.78
Tagged	28	15.4	1.88
Male	27	16.18	1.63
Female	31	15.41	1.96
		Hepatosomatic index	
Downstream	15	1.74	0.43
Upstream	15	1.79	0.5
Upstream	28	1.73	0.38
Male	27	1.44	0.29
Female	31	2	0.34
		Gonadosomatic index	
Downstream	15	7.87	4.07
Upstream	15	8.21	4.74
Upstream	28	8.87	4.52
Male	27	4.16	0.79
Female	31	12.13	2.4

Table 4.— Hematology results.

Fish Category	Mean (SD)	Standard deviation	Statistical associations (ANOVAS)
	PCV (%)		
Total	51.3	5.5	None by category or sex
	Red blood cell count(cells/mm^3)		
Total	1.38 x10^6	0.21 x10^6	none
	Total protein		
Total	7.4	1.4	unequal variance ($p = 0.0114$)
Female	8.1	1.4	Female > Male: ($p <0.0001$)
Male	6.7	1.1	
Downstream untagged	7.9		Category 0 and 2 are statistically
Upstream untagged	7.6		different $p = 0.0357$
Upstream tagged	6.99		
	White blood cell count (cells/mm^3)		
Total	53,276	22,342	None
	aWBC (WBC – Thrombocyte number) (cells/mm^3)		
Total	23,949	13,040	Unequal variance ($p = 0.0605$)
Category			Category $p = 0.0098$, however
Downstream untagged	26,771		upstream untagged are different from
Upstream untagged	15,800		the other two.
Upstream tagged	26,751		
Females:			Interaction $p = 0.0061$
Downstream untagged	17,739		Female pattern was to be lower in the
Upstream untagged	17,255		untagged groups and higher in the
Upstream tagged	29,735		tagged, however this was statistically insignificant.
Males:			
Downstream untagged	35,803		Only significant difference was
Upstream untagged	14,346		between males between 0 and 1.
Upstream tagged	23,767		

Table 4.— Continued.

Thrombocyte number			
Total	29,327	(13,891)	Unequal variance (p = 0.0374)
Downstream untagged	22,782		Category p = 0.0761
Upstream untagged	34,182		Two untagged groups are different
Upstream tagged	30,353		
Neutrophil number			
Combined	7,895	(4,786)	Unequal variance (p = 0.0081)
Downstream untagged	6,319		Category p = 0.0005
Upstream untagged	5,053		
Upstream tagged	10,236		
Lymphocyte number			
Combined	14,781	10,268	Unequal variance (p = 0.0233) Can't combine untagged groups
Downstream untagged	20,178		Category p = 0.0068
Upstream untagged	9,503		
Upstream tagged	14,715		
Female	12,668		Sex: (p = 0.084)
Male	16,930		Males are higher than females
Female:			Interaction p = 0.0015 Random
Downstream untagged	11,618		No difference statistically for females.
Upstream untagged	9,513		
Upstream tagged	16,872		Downstream untagged males are
Male:			statistically higher than everything
Downstream untagged	28,738		except upstream tagged.
Upstream untagged	9,492		
Upstream tagged	12,559		
N/L ratio			
Total	0.69	0.486	p = 0.0264 by category. Possible
Downstream untagged	0.41		upstream/downstream effect
Upstream untagged	0.76		
Upstream tagged	0.82		

Table 4.— Continued.

		Basophil number	
Total	851	1,011	Unequal variance (p = 0.0563) Random associations with category (p = 0.0027)
		Monocytes	
Total	422	540	Random associations with category

Table 5.— Heart lesion results.

Category	n	Percent of samples within each lesion score			
		0	1	2	3
Zenker's necrosis					
Combined	58	52	40	9	0
Downstream untagged	15	53	33	13	0
Upstream untagged	15	60	27	13	0
Upstream tagged	28	46	50	3.6	0
Male	27	55	44	0	0
Female	31	48	36	16	0
Epicarditis					
Combined	58	9	74	17	
Downstream untagged	15	13	73	13	0
Upstream untagged	15	13	73	13	0
Upstream tagged	28	4	75	21	0
Male	27	11	70	19	0
Female	31	6	77	16	0
Stromal inflammation					
Combined	58	60	36	1.7	1.7
Downstream untagged	15	60	40	0	0
Upstream untagged	15	67	24	7	0
Upstream tagged	28	57	39	0	4
Male	27	74	22	0	4
Female	31	48	48	3	0
Fibrin thrombi / endocarditis					
Combined	58	41	46	10	2
Downstream untagged	15	67	27	7	0
Upstream untagged	15	33	53	13	0
Upstream tagged	28	32	54	11	4

Table 5.— Continued.

Category	n	Percent of samples within each lesion score			
		0	1	2	3
Male	27	44	48	4	4
Female	31	39	45	16	0
Myocyte vacuolation					
Combined	58	7	78	16	0
Downstream	15	0	73	27	0
Upstream untagged	15	13	80	7	0
Upstream tagged	28	7	79	14	0
Male	27	4	81	15	0
Female	31	10	74	16	0

Table 6.— Liver lesion results.

Category	n	Percentage of samples within each lesion			
		0	1	2	3
Vacuolar change					
Combined	58	26	31	40	3.4
Downstream	15	27	27	40	7
Upstream untagged	15	33	13	47	7
Upstream tagged	28	21	43	36	0
Male	27	0	7	85	7
Female	31	48	52	0	0
Pigment					
Combined	58	48	50	2	0
Downstream	15	33	67	0	0
Upstream untagged	15	60	33	7	0
Upstream tagged	28	50	50	0	0
Male	27	5	44	0	0
Female	31	42	55	3	0

Table 7.— Musculoskeletal system and subcutaneous tissue lesion results.

		Percentage of samples for each lesions score			
Histologic feature	n	0	1	2	3
Bone remodeling	53	8	53	26	13
Skeletal muscle contraction band necrosis	55	58	38	4	0
Skeletal muscle degeneration	54	20	50	26	4
SQ – Mucinous change	57	9	50	30	12

Table 8.— Spleen lesion results.

Histologic feature	n	Percentage of samples within each lesions score			
		0	1	2	3
Ellipsoid hyperplasia	58	2	83	15	0
Hematopoiesis	58	7	53	34	5

Table 9.— Gill lesion results.

Histologic feature	Percentage of samples within each lesion score *			
	0	1	2	3
Goblet cell hyperplasia	7	67	26	0
Secondary lamellar clubbing	88	7	5	0
Secondary lamellar fusion	74	23	4	0
Epithelial cell hyperplasia	32	44	25	0
Fibrin thrombi and apoptotic bodies	37	46	18	0
Branchitis	70	23	7	0
Telangiectasia	53	44	3	0

*57 gills were examined.

Table 10.— Gastrointestinal lesion results.

Histologic feature	n	Percentage of samples within each lesion score			
		0	1	2	3
Inflammation					
Esophagitis	50	32	62	6	0
Gastritis	55	20	47	27	5
Enteritis	58	12	64	19	5
Apoptosis of epithelial cells					
Esophagus	50	0	64	30	6
Stomach	55	2	80	18	2
Intestine	58	2	67	29	2
Muscle degeneration					
Esophagus	50	0	28	54	18
Stomach	55	13	78	9	0
Intestine	58	46	53	0	0

Table 11.— Kidney lesion results.

Category	n	Lesion score in percentage			
		0	1	2	3
Melanomacrophages					
Downstream untagged	15	0	40	20	40
Upstream untagged	15	13	73	7	7
Upstream tagged	28	14	54	25	0
Male	27	11	74	4	11
Female	31	10	39	39	13
Primordial structures					
Downstream untagged	15	0	47	40	13
Upstream untagged	15	0	73	20	7
Upstream tagged	28	7	61	29	4
Male	27	0	85	15	0
Female	31	6	39	42	13
Interstitium					
Downstream untagged	15	7	27	67	0
Upstream untagged	15	7	27	47	20
Upstream tagged	28	7	29	61	4
Male	27	4	22	70	4
Female	31	10	32	48	10

Table 11.— Continued.

Category	n	Lesion score in percentage			
		0	1	2	3
Glomerulonephritis					
Downstream untagged	15	40	47	13	0
Upstream untagged	15	67	27	7	0
Upstream tagged	28	75	21	4	0
Male	27	67	26	7	0
Female	31	61	32	6	0

Table 12.— Pancreatic lesion results.

Category	n	Percentage of samples within each lesion score			
		0	1	2	3
Vacuolar change					
Total	53	0	7	51	41
Downstream	12	0	17	42	42
Upstream untagged	14	0	0	64	36
Upstream tagged	27	0	7	48	44
Male	25	0	12	36	52
Female	28	0	4	64	32
Zymogen granules					
Total	56	7	34	48	11
Downstream	14	0	50	43	7
Upstream untagged	15	13	40	27	20
Upstream tagged	27	7	22	63	7
Male	27	11	41	41	7
Female	29	3	28	55	14
Mesenteric fat atrophy					
Total	58	41	15	24	19
Downstream	15	40	20	33	7
Upstream untagged	15	53	0	7	40
Upstream tagged	28	36	21	29	14
Male	27	56	11	19	15
Female	31	29	19	29	23

Table 13.— Parasite grading results.

Parasite present	Number affected	Overall % prevalence (n=58)	% in downstream fish (n= 15)	**%** in upstream fish (n= 43)	% in untagged (n=30)	% in tagged (n=28)	Male (%) (n = 27)	Female (%) (n= 31)
Microsporidia in kidney alone	15	27.6	13.3	32.6	26.7	28.6	29.6	25.8
Loma salmonae in gill or intravascular	4	6.9	13.3	4.6	6.7	7.1	7.4	6.4
Myxosporidia in kidney (***Parvicapsula species***)	6	10.3	26.7	4.6	16.7	3.6	7.4	12.9
Ichthyophonus hoferi	2	3.4	0	3.4	1.7	1.7	1.7	1.7
Gastrointestinal trematodes	30	51.7	46.7	53.5	46.7	57.1	40.7	61.3
Intestinal cestodes	42	72.4	66.7	74.4	73.3	75	74.1	71

Table 13.— Continued

Parasite present	Number affected	Overall % prevalence (n=58)	% in downstream fish (n= 15)	**%** in upstream fish (n= 43)	% in untagged (n=30)	% in tagged (n=28)	Male (%) (n = 27)	Female (%) (n= 31)
Larval nematodes	37	63.8	73.3	60.5	66.7	60.7	74.1	54.8
Intestinal myxosporidia	4	6.9	6.7	7.0	13.3	0	3.7	9.7

Table 14.— Ichthyophonus infected fish and organs affected in each (+ means present and - means absent).

Fish #	Age (yrs)	Sex	Heart	Gill	Liver	GI	Pancreas	Spleen	Skin / muscle	Kidney	Gonad	Brain	Gross lesion
5-1	6	F	+++	-	-	-	+	-	++	+	-	+	yes
6 -1	4	M	+++	-	+	-	+	+	++	+	-	-	yes

Figure 1

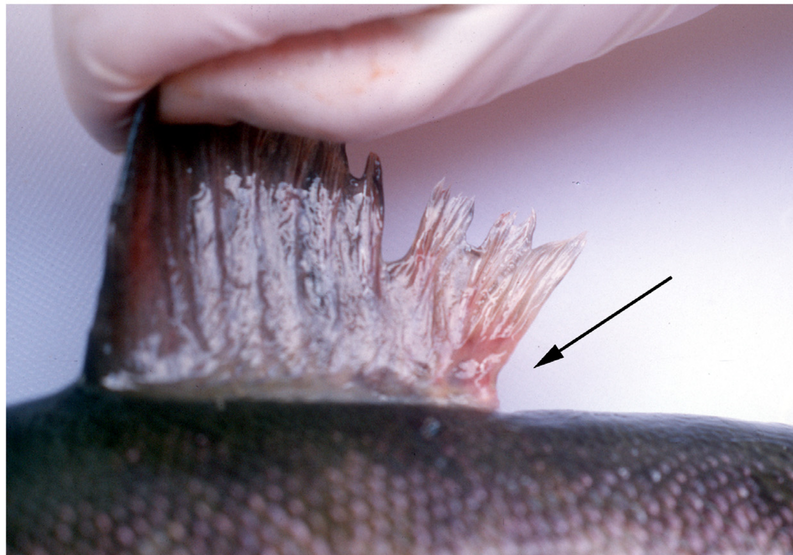


Figure 2

Figure 1.– Usual gross appearance of the tag site.

Figure 2.– Tag site with an ulcer at the base of the caudal aspect of the dorsal fin.

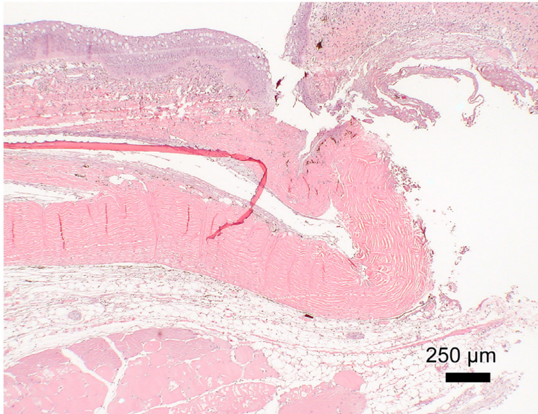


Figure 3a

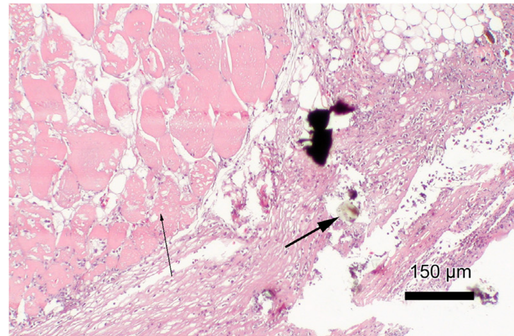
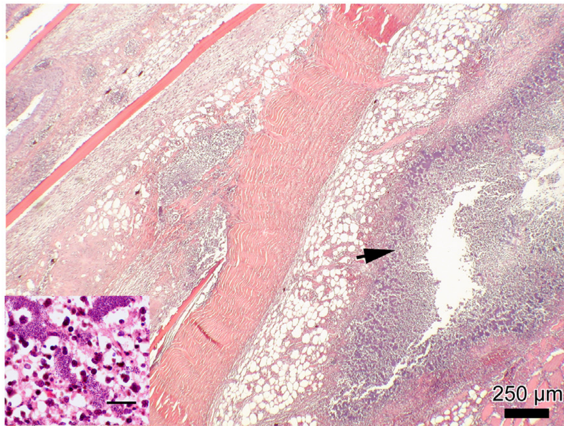


Figure 3b



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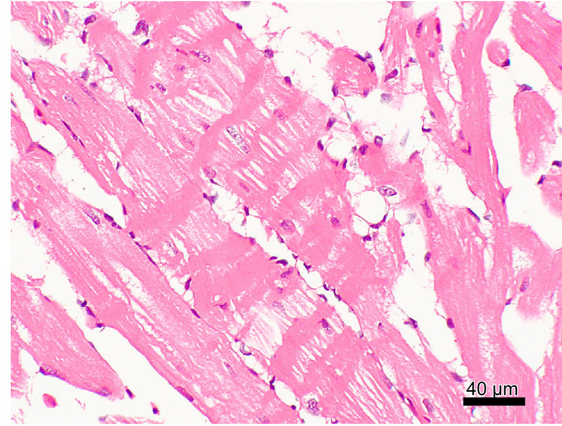


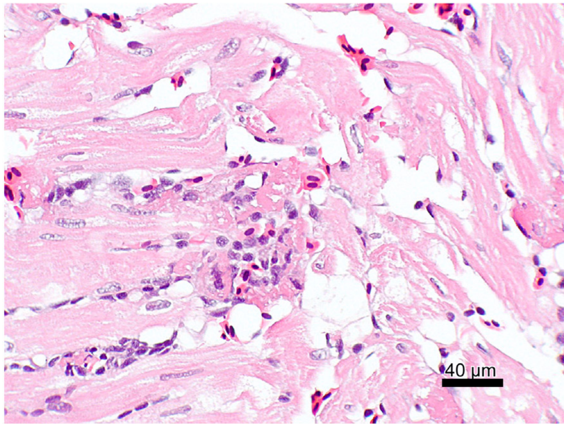
Figure 5

Figure 3a.– Typical histologic appearance of the tag site. The continuity of the epithelium was disrupted and the tag tract was filled with necrotic debris, neutrophils and occasionally refractile material consistent with scale fragments.

Figure 3b.– Irregularly shaped refractile material (large arrow) and necrotic debris present within the tag tract. There was extensive degeneration of the skeletal muscle in the area of the tag (small arrow).

Figure 4.– Ulcer and SQ bacterial colonies from a fish with *A. salmonicida* cultured from the kidney (bar in insert = 20µm). The epithelium is to the left of the field and there are dense rod-shaped bacterial colonies (arrow) surrounded by neutrophils within the subcutis.

Figure 5.– Zenker's necrosis in the heart characterized by hypereosinophilia, and presence of dark transversely placed contraction bands.



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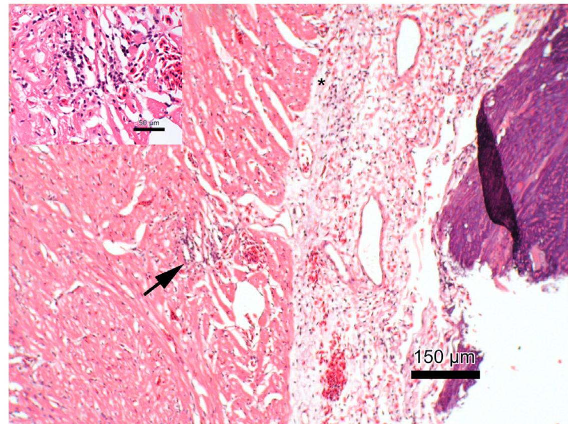

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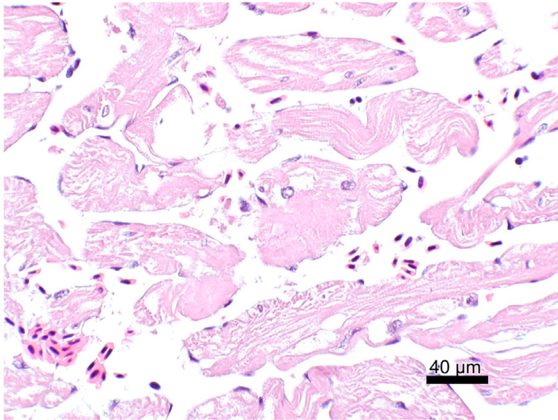

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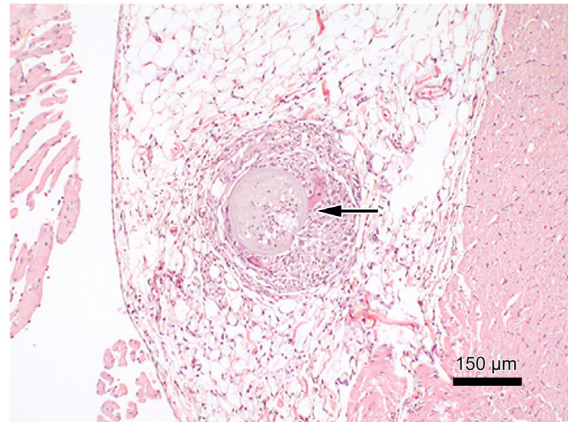


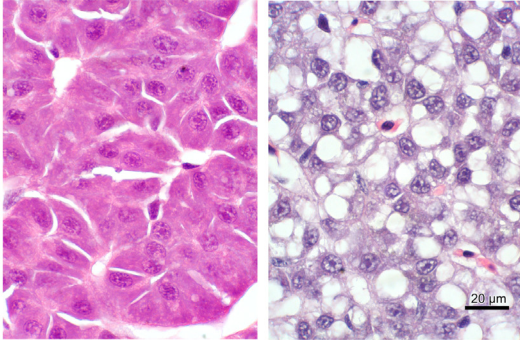
Figure 9

Figure 6.– Fibrin thrombi and endocarditis.

Figure 7.– Lymphocyte aggregates in the heart and mixed inflammatory cells in the epicardium. In the overview, there is a small aggregate of lymphocytes within the myocardium (arrow) and a sprinkling of mixed inflammatory cells within the epicardium. The insert demonstrates the lymphocytes within the myocardium.

Figure 8.– Myocyte vacuolation.

Figure 9.– A degenerated *Ichthyophonus hoferi* resting spore within the epicardium. It is surrounded by and infiltrated by neutrophils, macrophages and multinucleated giant cells.



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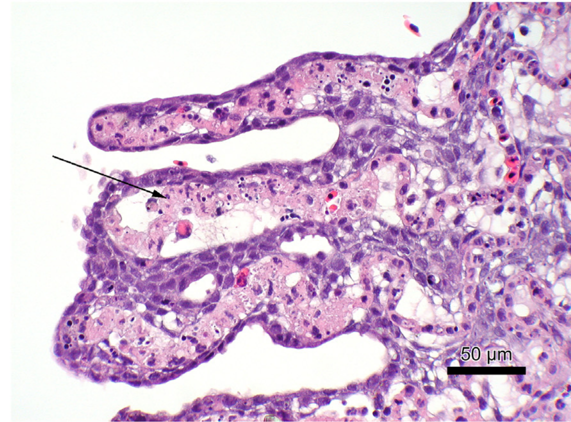

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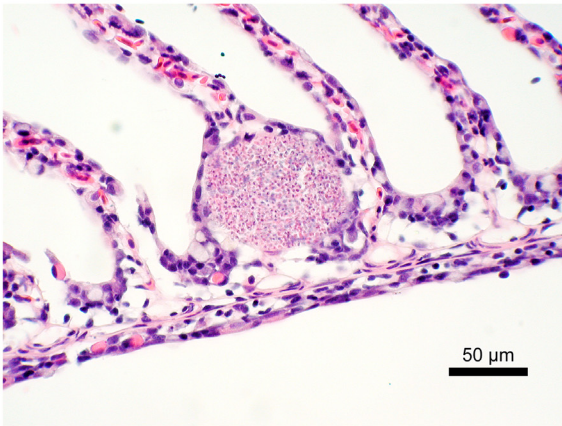


Figure 12a

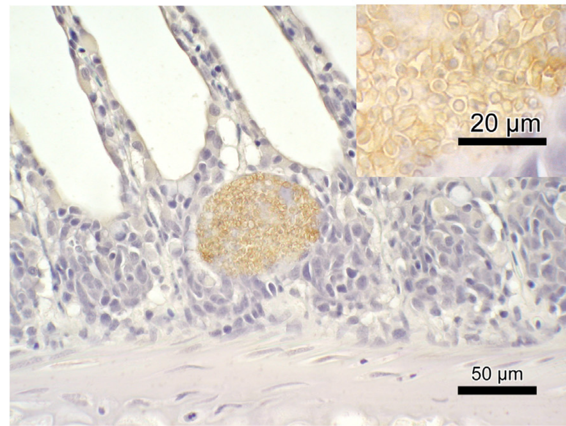


Figure 12b

Figure 10.– Typical features of the hepatocytes in the females (left side) versus the males (right side). The males had large numbers of clear vacuoles consistent with a combination of fat and glycogen.

Figure 11.– Fibrin thrombi and apoptotic cells (arrow) in the secondary lamella ofthe gill.

Figure 12a.– Microsporidia xenoma in gill tissue.

Figure 12b.– Microsporidia xenoma in gill tissue with an immunohistochemical positive reaction to monoclonal antibody for *Loma salmonae*.

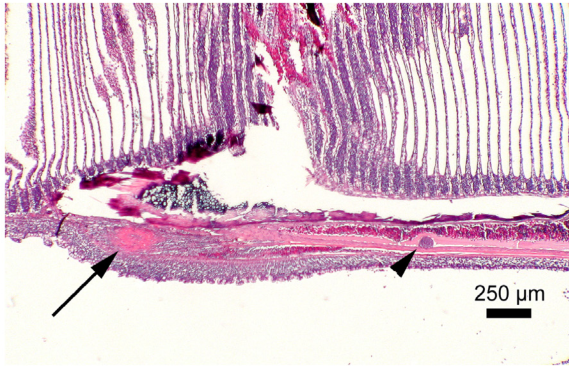
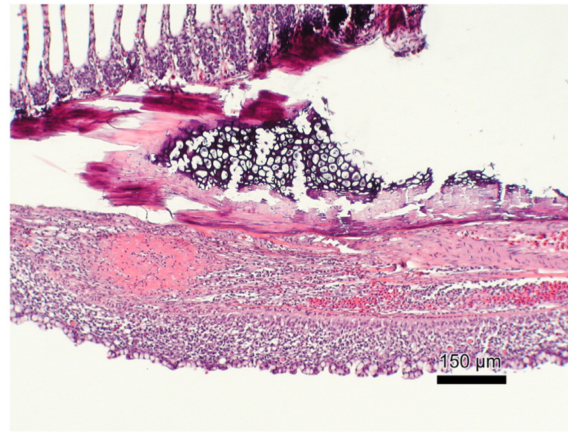


Figure 13a



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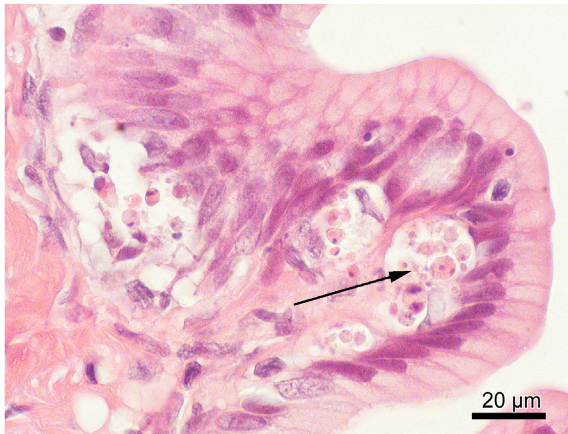


Figure 14

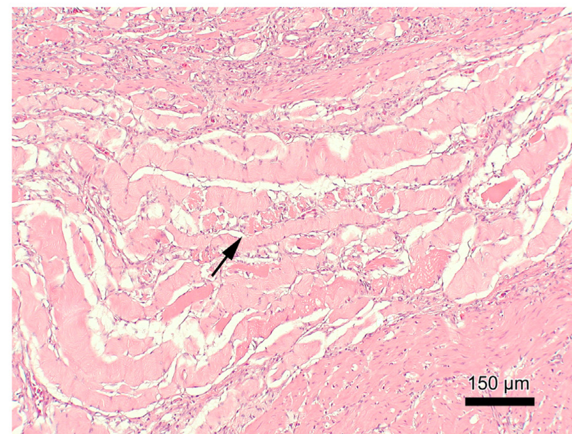


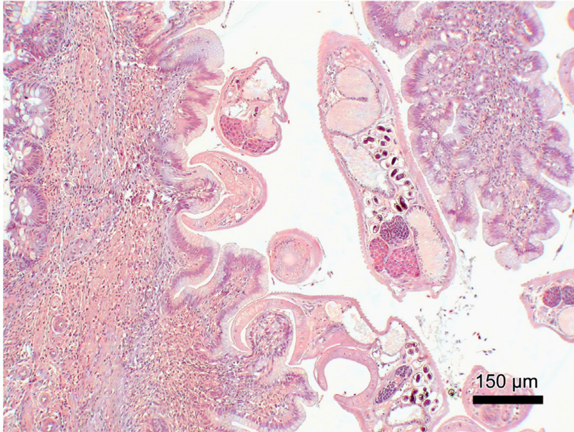
Figure 15

Figure 13a.– Intravascular *Loma salmonae* xenoma in the primary lamella (arrowhead) with a thrombus and vasculitis in the distal blood vessel (arrow).

Figure 13b.– Higher power view of the thrombus and associated neutrophilic vasculitis.

Figure 14.– Apoptosis of epithelial cells (arrow) in the gastrointestinal tract.

Figure 15.– Muscle degeneration (arrow) in the skeletal muscle of the external muscle layer of the esophagus.



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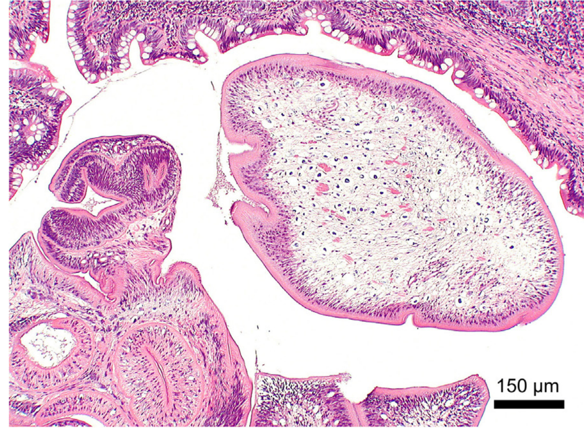

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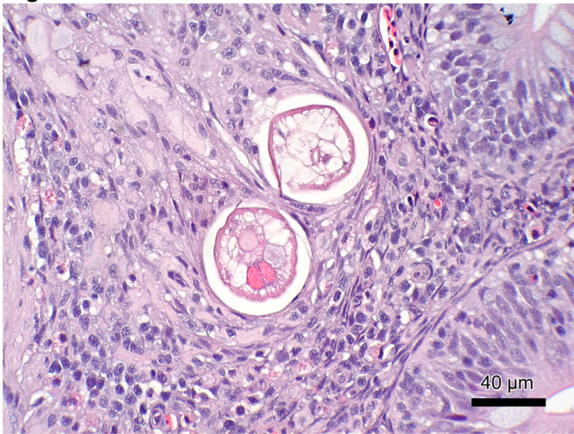

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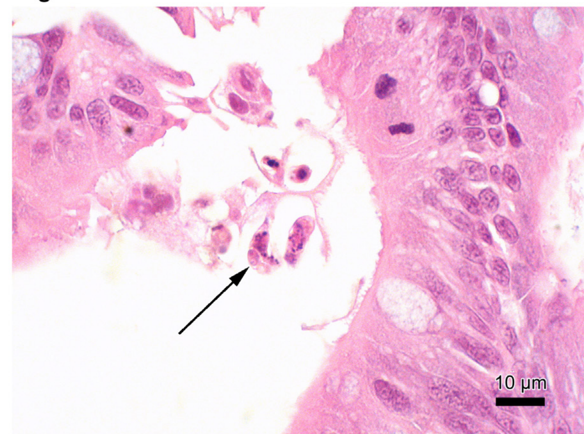


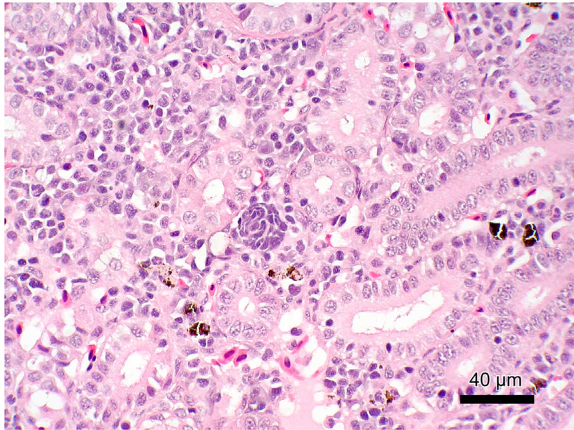
Figure 19

Figure 16.– Trematodes within the stomach lumen.

Figure 17.– Cestodes within the pyloric caeca and intestine.

Figure 18.– Nematode larva within the wall of the intestine.

Figure 19.– Myxosporeans in the intestinal lumen. The arrow indicates two polar capsules.



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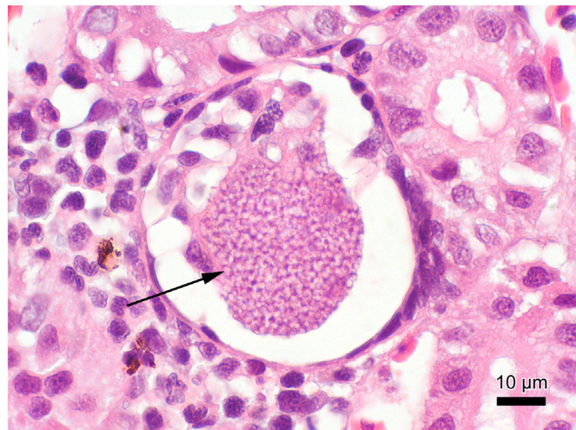


Figure 21a

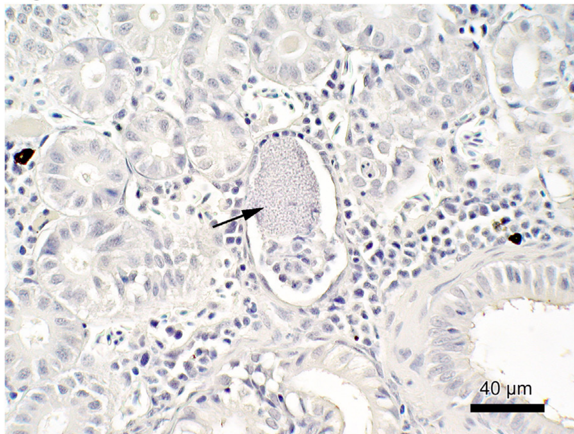


Figure 21b

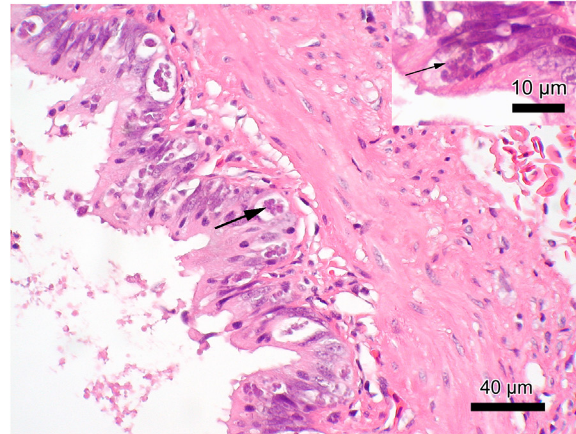


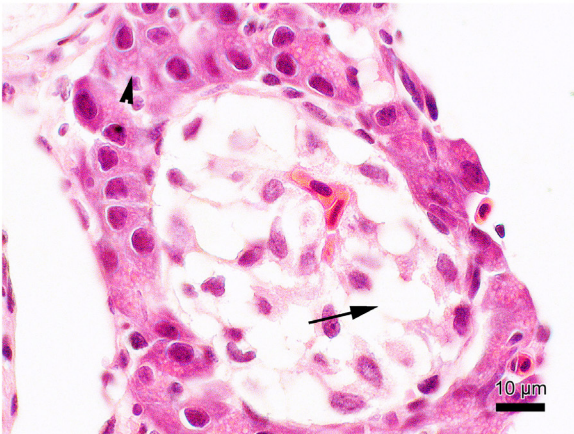
Figure 22

Figure 20.– The arrow indicates the primordial structures within the kidney interstitium.

Figure 21a.– Microsporidial xenomas present within the glomerular tuft in the kidney.

Figure 21b.– Xenomas (Figure 21a) were negative by immunohistochemistry using a monoclonal antibody to *L salmonae.*

Figure 22.– Myxosporidia parasites consistent with *Parvicapsula sp*. were present both within the epithelium of the renal tubules, the lumen and in rare cases within the interstitium. The arrow indicates two polar capsules.



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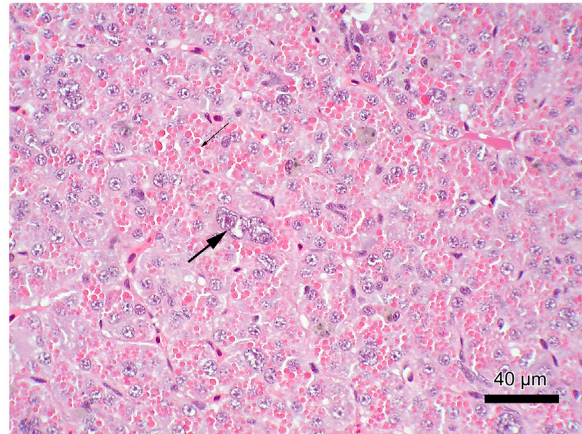

Figure 24a

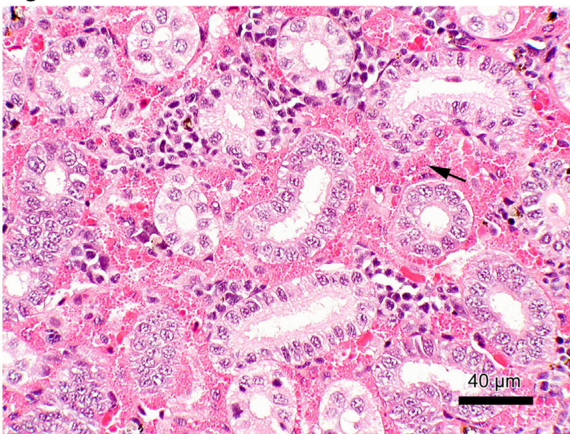

Figure 24b

Figure 23.– In the pancreas, vacuolar change within the islets of Langerhans cells (arrow) was very common as well as zymogen granule depletion (arrowhead).

Figure 24a.– Liver from a fish with eosinophilic inclusions (small arrows)and hepatic karyomegaly (large arrow).

Figure 24b.– Kidney from a fish with hepatic karyomegaly. Eosinophilic inclusions are packed in interstitial cells (large arrow).