

## Liver Lesions

**Animal** Health Centre, British Columbia Ministry of Agriculture and Lands

Case #: 2004-04081; 2005-00176; 2005-00622; 2005-00916; 2006-00767

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Comment [GDM1]: These type specimens and case definitions were developed as part of a multispecies collaborative project with DFO. Many of the type specimens listed here are for species other than sockeye salmon, but the lesions and scores were assigned in sockeye salmon as described here.

Summary of type specimens for liver scores used during histopathologic examination ("type specimens" are good examples of each lesion score). Abbreviations are explained on the next pages.

LIVER (Summary of type specimens)				
Lesion Abbreviation	None score = 0	Mild score = 1	Moderate score = 2	Severe score = 3
Atly	4-4081-47	4-4081-11	4-4081-202	none
Art	5-176-13	4-4081-47	6-767-237	none
PFD	4-4081-5	4-4081-77	4-4081-85	4-4081-52
GD	None	4-4081-61	4-4081-78	4-4081-47
BPC	4-4081-47	4-4081-32	6-767-410	none
PMP	4-4081-47	4-4081-196	none	none
LIP	4-4081-76	4-4081-47	4-4081-18	4-4081-144
FPL	4-4081-47	4-4081-5	7-767-12A	none
PVL	4-4081-47	4-4081-16	5-916-20	none
CPL	4-4081-47	4-4081-16	4-4081-19	none
BPH	4-4081-47	4-4081-16	4-4081-185	none
MEG	4-4081-47	4-4081-26	4-4081-152	6-767-254
SCN	4-4081-47	4-4081-19	none	none

### Quality Control/Quality Assurance

- Atly = Autolysis. Changes in membrane integrity begin immediately after death, and are often aided by leakage of bile onto cells.  
 score = 0; no membrane changes, erythrocytes stained intensely.  
 score = 1; loss of membrane integrity; hepatocytes had fragmented nuclei and pale basophilic cytoplasm; changes were probably due to autodigestion from leakage of bile.

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score = 2; cell adhesion has broken down in some areas; band of bile digestion >300  $\mu\text{m}$  thick. Moderate autolysis is evidence that the fish was dead before the tissues were preserved.

score = 3; none were severe.

2. Art = Artefact. Tissue changes that were not inherent in the tissue sampled. Sources of artefact included handling at necropsy, processing, sectioning, and staining. Artefact is scored on the basis that it impedes interpretation of tissue morphology. Examples of artefact include splits, bubbles, or knife marks in tissues. Artefact that was considered to be a result of postfixation dehydration was scored under PFD (see next category, #3).  
score = 0; sections had no tissue alterations that would impede analysis or photography of any part of the sections.  
score = 1; tissue alterations were present, but some areas could still be photographed without artefact, and analysis for lesions was unaffected. This is the most common score for paraffin-embedded sections.  
score = 2; tissue alteration prevented analysis for lesions in some areas and photography would be unacceptable anywhere.  
score = 3; tissue alterations were too extensive for histopathologic analysis.
3. PFD = postfixation dehydration. This is a specific type of artefact. The margins of affected livers have evidence of dehydration after fixation (e.g., nuclei stain dull blue; erythrocyte cytoplasm stains yellow instead of red; hepatocyte cytoplasm stains poorly or not at all). Potential causes include fixation in formalin that is too concentrated (e.g., 100% formalin instead of 10% formalin), removal of tissues from liquid for more than a few minutes, or transfer to ethanol that is too concentrated (e.g., >70% ethanol) before processing to paraffin.  
score = 0; no postfixation dehydration.  
score = 1; postfixation dehydration limited to total sectional area less than 500  $\mu\text{m}$  in diameter.  
score = 2; total sectional area of postfixation dehydration >500  $\mu\text{m}$ , but no 10 $\times$  objective lens field is completely affected by PFD.  
score = 3; total sectional area of postfixation dehydration fills at least one 10 $\times$  objective lens field.

### *Physiological condition*

1. GD = glycogen depletion. Hepatocytes in actively feeding fish have abundant cytoplasmic glycogen stores characterized by a large volume of clear, irregular, poorly demarcated vacuoles (= glycogen vacuoles). Severe hepatocellular glycogen depletion indicates that food assimilation is not adequate to supply energy needs; it occurs most commonly in fish that stop feeding, but it can also be an indicator of inadequate nutrition.  
score = 0; hepatocytes had abundant glycogen vacuoles.  
score = 1; glycogen vacuoles were smaller, but still larger than nuclei.  
score = 2; glycogen vacuoles were smaller than or about equal to nuclear diameter.

score = 3; glycogen vacuoles were absent for most hepatocytes.

2. BPC = basophilic cytoplasm (hepatocytes). Cytoplasmic staining of hepatocytes varies based on the activity of the liver, but cytoplasm is usually eosinophilic. Basophilic cytoplasm in hepatocytes is an indication of active protein synthesis (the rough endoplasmic reticulum stains basophilic). It is normal in mature females producing protein for deposition in their eggs. In juvenile salmon it might be related to increased protein needed as part an inflammatory response.  
 score = 0; hepatocytes have eosinophilic cytoplasm or abundant glycogen vacuoles.  
 score = 1; about 1/3 of hepatocyte cytoplasm is basophilic.  
 score = 2; about 2/3 of hepatocyte cytoplasm is basophilic.  
 score = 3; nearly all of hepatocyte cytoplasm is basophilic.

*Lesions*

1. PMP = Pigments in the liver are usually within macrophages and vary from yellow-brown to yellow-green. Pigments include lipofuscin, hemosiderin, or both.  
 Significance: Accumulation of lipofuscin in the liver is a nonspecific change that can result from a variety of insults, including rancid feed, low levels of antioxidants in the feed, chronic infections, and exposure to organic contaminants. In pen-reared salmon, hepatic lipofuscin accumulation is a common feature of netpen liver disease (microcystin-LR). Conditions that lead to moderate to abundant hepatic lipofuscin have been associated with decreased growth and survival in several studies. Hemosiderin accumulation in the liver is evidence of increased turnover of red blood cells.  
 score = 0; < 1 focus of pigment in most 20x objective lens fields.  
 score = 1; > 1 but <2 foci of pigment in most 20x objective lens fields.  
 score = 2; ≥2 foci of pigment in most 20x objective lens fields.  
 score = 3; none are severe.
2. LIP = lipidosis. A change/lesion in hepatocytes; lipid appears as clear, round, well-demarcated, cytoplasmic vacuoles (= lipid vacuoles). Pathologic change is more likely when the vacuoles are significantly larger than nuclei. When nearly all hepatocytes are uniformly affected and vacuoles are about the size of nuclei, the change may be normal form of energy storage. A PAS stain fails to stain any of the vacuoles (this staining pattern rules out glycogen in the vacuoles).  
 score = 0; hepatocytes have no lipid vacuoles.  
 score = 1; < 33% of hepatocytes in the section have lipid vacuoles larger than nuclei, or <50% have lipid vacuoles that are smaller than nuclei.  
 score = 2; 34-66% of hepatocytes in the section have lipid vacuoles larger than nuclei, or 50-100% of hepatocytes have vacuoles that are rarely larger than nuclei.  
 score = 3; more than 66% of hepatocytes in the section have lipid vacuoles that are larger than nuclei.
3. FPL = focal/multifocal parenchymal leukocytes. Leukocyte aggregates are usually less than 500 µm in diameter and composed mostly of lymphocytes.

score = 0; no focal parenchymal leukocytes.  
score = 1; <1 focus of parenchymal leukocytes per 100×field.  
score = 2; 1-2 foci of parenchymal leukocytes per 100×field.  
score = 3; none were severe

4. PVL = perivascular leukocytes (eosinophilic granular cells, lymphocytes, and plasma cells). Leukocytes infiltrate the connective tissue (adventitia) around blood vessels. Leukocytes within the tunica intima and tunica media are NOT included in this category.  
score = 0; <3 leukocytes in the adventitia of any vessel in the section.  
score = 1; 3 to many leukocytes in the adventitia of at least one vessel in the section, but leukocytes do not extend into the surrounding parenchyma or the muscular tunics of the vessel.  
score = 2; perivascular leukocytes extend into the surrounding parenchyma, and more than one vessel is involved.  
score = 3; none were severe.
  
5. CPL = cholangitis/pericholangial leukocytes (lymphocytes, plasma cells, and macrophages): a lesion of the bile ductules and the surrounding connective tissue (adventitia).  
score = 0; <3 leukocytes in the region of every bile duct in the section.  
score = 1; 3 - many leukocytes infiltrate or surround at least one bile duct in the section, but leukocytes do not extend into the surrounding parenchyma.  
score = 2; leukocytes or plasma cells extend into the surrounding parenchyma.  
score = 3; none were severe.
  
6. BPH = biliary preductular cell hyperplasia. Biliary preductular cells line the cavity that connects the short interhepatocellular canaliculi with the larger bile ductules. Hyperplasia of biliary preductular epithelial cells is evidence of exposure to toxins. The toxins could be produced inside the fish (e.g., bacterial toxins) or come from outside the fish (e.g., from the water or the feed). Normally, preductular epithelial cells are spaced so that cross sections of hepatic tubules have no more than 1 nucleus.  
score = 0; no biliary preductular cell hyperplasia;  
score = 1; >1 but < 2/3 of tubular cross sections per 40× objective lens field contain more than one preductular epithelial cell nucleus;  
score = 2; > 2/3 of tubular cross sections per 40× objective lens field contain more than one preductular epithelial cell nucleus;  
score = 3; none were severe.
  
7. MEG = hepatocellular karyomegaly and megalocytosis. Karyomegaly is the most prominent feature of hepatocellular megalocytosis. Hepatocyte nuclei are considered enlarged if they are >2.5× the diameter of normal nuclei.  
score = 0;<3 hepatocyte nuclei are >2.5× the diameter of any other hepatocyte nuclei.  
score = 1; > 3 and <20 hepatocyte nuclei are >2.5× the diameter of any other hepatocyte nuclei.

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score = 2; karyomegalic hepatocytes are in > 50% of the 40× objective lens fields but are always <10% of the hepatocytes in any 40× objective lens field.  
score = 3; karyomegalic hepatocytes are in > 50% of the 40× objective lens fields and often involve >10% of the hepatocytes in a 40× objective lens field.

8. SCN = single cell necrosis/apoptosis. Affected hepatocytes have pyknotic nuclei and condensed cytoplasm that often stains more deeply eosinophilic than normal cells. Because of cytoplasmic collapse, individual necrotic cells are sometimes surrounded by a clear ring or halo. The slide might have only remnants of nuclear material (karyorrhectic debris). SCN must be differentiated from artefact. Even slightly rough handling results in cells with dark-staining cytoplasm, but artefact altered nuclei are not pyknotic, and their cytoplasm tends to stain basophilic.
- score = 0; <3 necrotic/apoptotic cells in the section.  
score = 1; <1 necrotic/apoptotic cell per 40× objective lens field.  
score = 2; 1 to 2 necrotic/apoptotic cells per 40× objective lens field, or 20 to 50 necrotic cells per section.  
score = 3; >2 necrotic/apoptotic cells per 40× objective lens field.

Summary of type specimens for kidney scores used during histopathologic examination (“type specimens” are good examples of each lesion score). Abbreviations are explained after the table.

KIDNEY (Summary of type specimens)				
Lesion Abbreviation	None score = 0	Mild Score = 1	Moderate score = 2	Severe score = 3
Atly	4-4081-34	4-4081-31	none	none
Art	5-176-3	4-4081-34	4-4081-75	none
PFD	4-4081-76	4-4081-109	4-4081-104	4-4081-53
ISH	4-4081-5	4-4081-138	none	none
IRT	4-4081-233	4-4081-34	4-4081-5	4-4081-177
TEP	4-4081-34	4-4081-43	none	none
GEP	4-4081-34	4-4081-185	6-767-102A, 108A	none
TEV	4-4081-167	4-4081-34	4-4081-157	none
TDI	4-4081-34	none	none	6-767-251
MIN	4-4081-34	4-4081-7	none	6-767-273
GRN	4-4081-34	4-4081-14	6-767-33A	none
EGC	4-4081-34	none	none	none
MYX	4-4081-34	4-4081-6	4-4081-15	4-4081-222
PVC	4-4081-34	4-4081-189	4-4081-144	4-4081-165
LHN	4-4081-34	4-4081-18	4-4081-13	none
RTN	4-4081-34	4-4081-30	none	none
MGN	5-916-98	5-916-97	5-916-39	none

*Quality Control/Quality Assurance*

1. Atly = Autolysis. Changes in membrane integrity begin immediately after death.  
score = 0; no membrane changes, erythrocytes stained intensely.  
score = 1; none were mild  
score = 2; none were moderate  
score = 3; none were severe
2. Art = Artefact. Tissue changes that were not inherent in the tissue sampled. Sources of artefact included handling at necropsy, processing, sectioning, and staining. Artefact is

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scored on the basis that it impedes interpretation of tissue morphology. Examples of artefact include splits, bubbles, folds, or knife marks. Artefact that was considered to be a result of postfixation dehydration was scored under PFD (see next category, #3).

score = 0; at least one section had no tissue alterations that would impede analysis or photography.

score = 1; tissue alterations were present, but most areas could still be photographed without artefact, and analysis for lesions was unaffected.

score = 2; tissue alteration prevented analysis for lesions in some areas and photography would be unacceptable anywhere.

score = 3; tissue alterations were too extensive for histopathologic analysis.

3. PFD = postfixation dehydration. This is a specific type of artefact. The margins of affected kidneys have evidence of dehydration after fixation (e.g., nuclei stain dull blue; erythrocyte cytoplasm stains yellow instead of red; tubular epithelial cytoplasm stains poorly or not at all). Potential causes include fixation in formalin that is too concentrated (e.g., 100% formalin instead of 10% formalin), removal of tissues from liquid for more than a few minutes, or transfer to ethanol that is too concentrated (e.g., >70% ethanol) before processing to paraffin.  
score = 0; no postfixation dehydration.  
score = 1; postfixation dehydration limited to total sectional area less than 500  $\mu\text{m}$  in diameter.  
score = 2; total sectional area of postfixation dehydration >500  $\mu\text{m}$ , but no 10 $\times$  objective lens field is completely affected by PFD.  
score = 3; total sectional area of postfixation dehydration fills at least one 10 $\times$  objective lens field.

#### Lesions

1. ISH = interstitial (hematopoietic) cell hyperplasia. Hematopoiesis is a normal function of the renal interstitium. An increase in the number of hematopoietic cells is an indication of immune stimulation, usually a result of an infectious organism. The relative volume of hematopoietic cells varies depending on where in the kidney the sample is taken; therefore, the scoring system comes with the assumption that each kidney was sampled from approximately the same part of each fish.  
score = 0; area of hematopoietic cells about equal to area of tubules.  
score = 1; area of hematopoietic cells > area of tubules but < 2 $\times$  area of tubules.  
score = 2; area of hematopoietic cells >2 $\times$  area of tubules  
score = 3; none were severe.
2. IRT = immature renal tubules. Immature renal tubules are small foci of basophilic epithelial cells. Each focus is usually 30-50  $\mu\text{m}$  in diameter; sometimes, sections of these foci do not contain a lumen. Immature tubular epithelial cells have a high nucleus:cytoplasm ratio (<1:3), cytoplasm is basophilic, and nuclei have vesiculated

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chromatin with prominent nucleoli. Growing fish normally have a few immature tubules, but numbers of immature tubules can also increase following tubular necrosis; therefore, comparison with control values is needed to determine significance. Lack of immature tubules might be expected in fish that are not growing or no longer have the capability to regenerate damaged tubules.

score = 0; <1 focus of immature epithelial cells per 10× objective lens field.

score = 1; >1 but ≤6 foci of immature epithelial cells per 10× objective lens field.

score = 2; >6 but ≤12 foci of immature epithelial cells per 10× objective lens field.

score = 3; >12 foci of immature epithelial cells per 10× objective lens field.

3. TEP = renal tubular epithelial protein droplets. The cytoplasm of renal tubular epithelium contained protein droplets that were homogeneous, eosinophilic, and varied from 3 to 12 μm in diameter. Nuclei of affected cells were NOT undergoing degeneration.  
score = 0; no renal tubular epithelial cells contained cytoplasmic protein droplets.  
score = 1; fewer than 10% of renal tubular epithelial cells contained protein droplets.  
score = 2; more than 10% of renal tubular epithelial cells contained protein droplets.  
score = 3; none were severe.
4. GEP = golden epithelial pigment in tubules. Affected tubules contain foci of intracellular globular golden pigment (probably lipofuscin) that varies from 5 to 30 μm in diameter. Nuclei of affected cells are sometimes eccentric, with a dense chromatin pattern. Fish with this pigment often also have intratubular myxosporeans (scored separately under MYX).  
score = 0; no renal tubular epithelial cells contained globular golden pigment.  
score = 1; fewer than 10% of renal tubular epithelial cells contained globular golden pigment.  
score = 2; more than 10% of renal tubular epithelial cells contained globular golden pigment.  
score = 3; none were severe.
5. TEV = renal tubular epithelial vacuolation. The epithelium of renal tubules was considered vacuolated if it contained clear vacuoles with a cross-sectional area greater than that of the nucleus. In some cases, vacuoles may be a result of normal glycogen storage.  
score = 0; tubular epithelium was not vacuolated.  
score = 1; < 20% of proximal tubular epithelial cells were vacuolated.  
score = 2; > 20% of proximal tubular epithelial cells were vacuolated.  
score = 3; none were severe.
6. TDI = tubular dilation (of lumen). A tubule was considered dilated when luminal diameter was more than 2× the thickness of the tubular epithelium.  
score = 0; tubules were not dilated.  
score = 1; <50% of the tubules were dilated.

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score = 2; >50% of the tubules were dilated.  
score = 3; at least one tubule dilated >500  $\mu\text{m}$  in diameter.

7. **MIN** = mineralization. Calcium salts sometimes precipitate and accumulate in tubules and ducts of the trunk kidney. In more severe cases, the ducts expand and sometimes rupture; granulomatous inflammation is commonly associated with ruptured ducts.  
score = 0; tubules were not dilated.  
score = 1; <5 mineralized tubules per 10 $\times$  objective lens field and minimal fibrosis.  
score = 2; >5 but <10 mineralized tubules per 10 $\times$  objective lens field with no fibrosis, or <5 mineralized tubules per 10 $\times$  field but moderate associated fibrosis.  
score = 3; >10 mineralized tubules per 100 $\times$  objective lens field with no fibrosis, or total sectional area of fibrosis greater than 2 mm in diameter.
8. **GRN** = granulomatous nephritis. Inflammation in tubules or interstitium was composed of variable amounts of macrophages (some multinucleate) and fibroblasts, with lymphocytes and plasma cells. This type of lesion can result from chronic diseases: bacteria (e.g., *Renibacterium salmoninarum*), the mesomycetozoan *Ichthyophonus hoferi*, or *Tetracapsuloides bryosalmonae*. If specific organisms are present, they are mentioned in the comment column.  
score = 0; no granulomatous inflammation.  
score = 1; granulomatous inflammation limited to total sectional area less than 500  $\mu\text{m}$  in diameter.  
score = 2; total sectional area of granulomatous inflammation 500  $\mu\text{m}$  to 2 mm in diameter.  
score = 3; total sectional area of granulomatous inflammation greater than 2 mm in diameter.
9. **EGC** = eosinophilic granular cells/endothelial granules. Cultured fish occasionally have this lesion, but none of these wild fish had this lesion, and type specimens were not established.
10. **MYX** = intratubular or intraglomerular myxosporean species (*Sphaerospora* sp. and/or *Myxidium* sp.). Presporogonic myxosporeans were attached to the surface of the epithelium of larger renal tubules or free in the lumen; other forms were in clusters within the glomerular space. The intratubular myxosporeans were slightly larger than renal tubular epithelial cell nuclei, but not as large as the renal tubular epithelial cells. In some cases, nuclei of the infected tubules were enlarged, sometimes as large as 30  $\mu\text{m}$  in diameter.  
score = 0; no myxosporeans in the kidney.  
score = 1; large tubules and ducts contained <12 of the myxosporeans.  
score = 2; large tubules and ducts contained >12 of the myxosporeans with minimal inflammation or dilation, or the ducts had <12 myxosporeans with mild to moderate inflammation.

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score = 3; large tubules and ducts contained >12 of the myxosporeans with significant dilation or lymphocytic inflammation.

11. PVC = *Parvicapsula kabatai* (confirmed by PCR by Simon Jones). *Parvicapsula kabatai* has elongate spores with two small Gram-positive polar capsules at the anterior end. Spores and trophozoites occur in renal tubular epithelial cells and within lumina of renal tubules. The parasite is often associated with granulomatous and lymphocytic inflammation.  
score = 0; no *Parvicapsula sp.*  
score = 1; sectional area of tubules with *Parvicapsula sp.* and associated inflammation is < 500 µm in diameter.  
score = 2; sectional area of tubules with *Parvicapsula sp.* and associated inflammation is > 500 µm but < 2 mm in diameter.  
score = 3; sectional area of tubules with *Parvicapsula sp.* and associated inflammation is > 2 mm in diameter.
12. LHN = lymphohistiocytic nephritis. Affected epithelium of renal ducts and large tubules contained infiltrates of lymphocytes, plasma cells, and macrophages. Nephritis associated with *Parvicapsula sp.* is NOT scored under LHN but is scored under PVC (see above). However, moderate inflammation identical to LHN is sometimes associated with small numbers of *Parvicapsula* (e.g., 2004-04081, slide 219); therefore, mild LHN might be a result of *Parvicapsula* not included in the sections examined.  
score = 0; no significant infiltrates of lymphocytes, plasma cells, and macrophages.  
score = 1; sectional area of tubules with infiltrates of lymphocytes, plasma cells, and macrophages is < 500 µm in diameter.  
score = 2; sectional area of tubules with infiltrates of lymphocytes, plasma cells, and macrophages is > 500 µm but < 2 mm in diameter.  
score = 3; sectional area of tubules with infiltrates of lymphocytes, plasma cells, and macrophages is > 2 mm in diameter.
13. RTN = renal tubular necrosis. Necrotic tubules contained cells with pyknotic to karyorrhectic nuclei, and condensed hypereosinophilic cytoplasm. Chronic cases were sometimes surrounded by fibrosis.  
score = 0; sections had no renal tubular necrosis.  
score = 1; renal tubular necrosis present, but <4 tubules per cross section.  
score = 2; >4 or <10 foci of renal tubular necrosis per cross section.  
score = 3; >10 foci of renal tubular necrosis per cross section.
14. MGN = Membranous glomerulonephritis is fairly common in older salmonids, particularly Chinook salmon; it is often associated with infections in other parts of the fish, but a link to immune complex deposition has not been demonstrated. Membranous glomerulonephritis has been associated with cardiomyopathy

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syndrome (in Atlantic salmon), nephrocalcinosis, and infections with a number of bacteria and parasitic species.

score = 0; sections have no membranous glomerulonephritis.

score = 1; thickness of deposits is less than the thickness of glomerular nuclei

score = 2; thickness of deposits is greater than the thickness of glomerular nuclei.

score = 3; none were severe.

## Heart lesions

Summary of type specimens for heart scores used during histopathologic examination ("type specimens" are good examples of each lesion score). Abbreviations are explained after the table.

HEART (Summary of type specimens)				
Lesion Abbreviation	None score = 0	Mild score = 1	Moderate score = 2	Severe score = 3
Atly	4-4081-40	none	none	none
Art	4-4081-212,216	4-4081-40	none	none
ECD	4-4081-40	4-4081-134	4-4081-131	none
MKM	4-4081-40	4-4081-34	none	none
END	4-4081-40	4-4081-138	4-4081-131	none
MYX	5-176-50	5-176-51	5-176-54&69 (Chinook); 4-4081-91 (coho)	none

### Quality Control/Quality Assurance

- Atly = Autolysis. Changes in membrane integrity begin immediately after death.  
score = 0; no membrane changes, erythrocytes stained intensely.  
score = 1; loss of membrane integrity; erythrocytes were pale.  
score = 2; none were moderate.  
score = 3; none were severe.
- Art = Artefact. Tissue changes that were not inherent in the tissue sampled. Sources of artefact included handling at necropsy, processing, sectioning, and staining. Artefact is scored on the basis that it impedes interpretation of tissue morphology. Examples of artefact include splits, bubbles, folds, or knife marks. Postfixation dehydration did not seem to affect the heart as severely as other organs; therefore, PFD in the heart is included with the artefact score.  
score = 0; sections had no tissue alterations that would impede analysis or photography of any part of the sections.  
score = 1; tissue alterations were present, but most areas could still be photographed without artefact, and analysis for lesions was unaffected.  
score = 2; tissue alteration prevented analysis for lesions in some areas and photography would be unacceptable anywhere.  
score = 3; tissue alterations were too extensive for histopathologic analysis.

### Lesions

- ECD = epicarditis. Inflammation in the epicardium included eosinophilic granular cells, lymphocytes, and scattered macrophages. Small perivascular foci of

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inflammatory cells in the peripheral compact layer of the heart were also included with the ECD score.

score = 0; no epicarditis, but might include up to 6 inflammatory cells per 40× objective lens field.

score = 1; epicarditis limited to a few foci, totaling <500 μm of epicardium affected.

score = 2; epicarditis involves >500 μm but <2 mm of epicardium.

score = 3; epicarditis involves >2 mm of epicardium.

2. **MKM = myocardial karyomegaly/megalocytosis.** Nuclei of cardiac muscle cells were considered enlarged when they were at least 2× as large as normal myocardial cells. Enlarged nuclei were most common in cells at the junction of the internal spongy and peripheral compact layers of the heart. The cause and significance of myocardial karyomegaly is unknown; karyomegaly in other cell types has been associated with exposure to algal toxins (e.g., hepatocytes exposed to microcystin LR in netpen liver disease).  
score = 0; no more than 2 myocardial cells have nuclei that are > 2× as large as normal myocardial cells.  
score = 1; at least 3 myocardial cells have nuclei that are > 2× as large as normal myocardial cells. Fewer than 3 enlarged cells per 40× objective lens field affected.  
score = 2; at least 3 myocardial cells have nuclei that are > 4× as large as normal myocardial cells, or more than 3 enlarged cells per 40× objective lens field.  
score = 3; none were severe.
3. **END = endocarditis.** Inflammation of the endocardial lining of the heart often occurs as a thin layer of inflammatory cells and plump (hypertrophied) endothelial cells. The nature of the inflammation can vary from mostly lymphoplasmacytic (LEC = lymphoplasmacytic endocarditis), granulomatous (GEC = granulomatous endocarditis), to simply histiocytic (HEC = histiocytic endocarditis). When the lymphocytic form affected more than 1 or 2 foci, it was usually associated with presporogonic stages of a myxosporean (*Sphaerospora* sp.).  
score = 0; no endocarditis, but might include up to 6 inflammatory cells per 40× objective lens field.  
score = 1; endocarditis limited to a few foci; total affected sectional area <500 μm in diameter.  
score = 2; total affected sectional area of endocarditis >500 μm but <2 mm in diameter.  
score = 3; total affected sectional area of endocarditis >2 mm in diameter.
4. **MYX = presporogonic phase of a myxosporean species (probably *Sphaerospora* sp.).** Presporogonic myxosporeans were within the vascular channels of the heart. The parasites were in clusters that ranged from 15 to 40 μm in the longest dimension. Some had the classic myxosporean "cell within a cell" structure, whereas others seemed to be arranged with an outer capsule filled with multiple smaller organisms. The smallest organisms were about 5 μm in diameter. Presporogonic myxosporeans occurred only in

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hearts with lymphoplasmacytic endocarditis; however, endocarditis was scored separately in the END category (above).

score = 0; no myxosporeans in the heart.

score = 1; myxosporeans present, but  $<5$  in all 40 $\times$  objective lens fields.

score = 2;  $\geq 5$  myxosporeans in at least two 40 $\times$  objective lens fields.

score = 3; none were severe.

## Spleen lesions

Summary of type specimens for spleen scores used during histopathologic examination (“type specimens” are good examples of each lesion score). Abbreviations are explained after the table.

SPLEEN (Summary of type specimens)				
Lesion Abbreviation	None Score = 0	Mild score = 1	Moderate score = 2	Severe score = 3
Atly	4-4081-9	6-767-28	none	none
Art	4-4081-9	4-4081-15	4-4081-72	none
PFD	4-4081-9	4-4081-78	4-4081-88	4-4081-53
CON	4-4081-30	4-4081-9	4-4081-60	none
PER	4-4081-9	4-4081-19	4-4081-201	6-767-420
SGR	4-4081-9	5-0916-84	none	none

### *Quality Control/Quality Assurance*

1. Atly = Autolysis. Changes in membrane integrity begin immediately after death.  
score = 0; no membrane changes, erythrocytes stained intensely.  
score = 1; dull-staining erythrocytes; dissolution of nuclei.  
score = 2; none were moderate  
score = 3; none were severe
2. Art = Artefact. Tissue changes that were not inherent in the tissue sampled. Sources of artefact included handling at necropsy, processing, sectioning, and staining. Artefact was scored on the basis that it impeded interpretation of tissue morphology. Examples of artefact include splits, bubbles, folds, and knife marks. Artefact that was considered to be a result of postfixation dehydration was scored under PFD (see next category, #3).  
score = 0; sections had no tissue alterations that impeded analysis or photography of any part of the sections.  
score = 1; tissue alterations were present, but most areas could still be photographed without artefact, and analysis for lesions was unaffected.  
score = 2; tissue alteration prevented analysis for lesions in some areas, and photography would be unacceptable anywhere.  
score = 3; tissue alterations were too extensive for histopathologic analysis.
3. PFD = postfixation dehydration. This is a specific type of artefact. The margins of affected livers have evidence of dehydration after fixation (e.g., nuclei stain dull blue; erythrocyte cytoplasm stains yellow instead of red; cytoplasm of parenchymal cells stains poorly or not at all). Potential causes include fixation in formalin that is too concentrated (e.g., 100% formalin instead of 10% formalin), removal of tissues from liquid for more

### Spleen lesions

- than a few minutes, or transfer to ethanol that is too concentrated (e.g., >70% ethanol) before processing to paraffin.
- score = 0; no postfixation dehydration.
- score = 1; postfixation dehydration limited to total sectional area less than 500  $\mu\text{m}$  in diameter.
- score = 2; total sectional area of postfixation dehydration >500  $\mu\text{m}$ , but no 10 $\times$  objective lens field is completely affected by PFD.
- score = 3; total sectional area of postfixation dehydration fills at least one 10 $\times$  objective lens field.

### Lesions

1. CON = congestion of blood vessels and red pulp. Some congestion is normal in the spleen, and lack of congestion may be an indicator of acute stress (common with fish captured before euthanasia).  
score = 0; vessels were not congested, and total vascular sectional area was  $\leq 5\%$  of spleen volume.  
score = 1; total vascular sectional area was >5% but  $\leq 25\%$  of spleen volume.  
score = 2; total vascular sectional area was >25% but  $\leq 75\%$  of spleen volume.  
score = 3; total vascular sectional area was >75% of spleen volume.
2. PER = peritonitis. Inflammation on the surface of the spleen was composed of variable amounts of granulomatous inflammation, lymphoplasmacytic infiltrates, and fine fibrocellular fronds. Peritonitis sometimes extended into surrounding exocrine pancreas and mesenteric fat, and it sometimes surrounds parasites.  
score = 0; no peritonitis, but might include up to 6 inflammatory cells per 40 $\times$  objective lens field.  
score = 1; peritonitis covers <2 mm of the surface of the spleen, or the inflammation is <300  $\mu\text{m}$  thick.  
score = 2; peritonitis covers >2 mm of the surface of the spleen, or the inflammation is >300  $\mu\text{m}$  thick.  
score = 3; none were severe.
3. SGR = granulomatous inflammation. Inflammation was composed of variable amounts of macrophages (some multinucleate) and fibroblasts, with lymphocytes and plasma cells. This type of lesion can result from a chronic bacterial infection (e.g., *Renibacterium salmoninarum*) or from infection with the protist *Ichthyophonus hoferi*.  
score = 0; no granulomatous inflammation.  
score = 1; granulomatous inflammation limited to total sectional area less than 500  $\mu\text{m}$  in diameter.  
score = 2; total sectional area of granulomatous inflammation 500  $\mu\text{m}$  to 2 mm in diameter.  
score = 3; total sectional area of granulomatous inflammation greater than 2 mm in diameter.

#### Spleen lesions

Blood smears were stained with Giemsa and examined using the 40× objective lens. Ten fields of the feathered edge of each smear were examined for inclusions of Viral Erythrocytic Necrosis virus (VEN) and Erythrocytic Inclusion Body Syndrome (EIBS). None of the blood smears had any viral inclusions.

## Intestine lesions

Summary of type specimens for intestine scores used during histopathologic examination ("type specimens" are good examples of each lesion score). Abbreviations are explained after the table.

INTESTINE (Summary of type specimens)				
Lesion Abbreviation	None Score = 0	Mild score = 1	Moderate score = 2	Severe score = 3
Atly	5-916-56	5-916-6A	7-767-12A	7-767-18A
Art	none	5-916-6A	5-916-65	none
PFD	5-916-6A	5-916-64 0.5=6-767-118A	none	none
FAT	6-767-27A	6-767-12A	6-767-294	
CES	5-916-6A	5-916-99	none	5-916-39C
TMT	5-916-6A	5-916-82	none	none
ANI	5-916-6A	none	5-916-37A	none

### Quality Control/Quality Assurance

- Atly = Autolysis/autodigestion. Changes in membrane integrity begin immediately after death. The intestinal epithelium is particularly susceptible to autolysis because bile and intestinal enzymes continue to function after death while normal protective mechanisms cease to function. Bile leakage can cause severe focal autolysis (= autodigestion) despite fairly good preservation of other areas; the score for Atly allows for more severe autodigestion in a single focus (< 2 mm diameter) that is likely due to focal bile imbibition.

score = 0; no membrane changes; tips of intestinal villi intact.  
score = 1; loss of cellular structure on tips of intestinal villi.  
score = 2; loss of cellular structure on full length intestinal villi (crypts can still be intact)  
score = 3; full thickness loss of cellular structure.
- Art = Artefact. Tissue changes that were not inherent in the tissue sampled. Sources of artefact included handling at necropsy, processing, sectioning, and staining. Artefact was scored on the basis that it impeded interpretation of tissue morphology. Examples of artefact include splits, bubbles, folds, and knife marks. Artefact that was considered to be a result of postfixation dehydration was scored under PFD (see next category, #3).

score = 0; sections had no tissue alterations that impeded analysis or photography of any part of the sections.  
score = 1; tissue alterations were present, but most areas could still be photographed without artefact, and analysis for lesions was unaffected.

#### Intestine lesions

score = 2; tissue alteration prevented analysis for lesions in some areas, and photography would be unacceptable anywhere.  
score = 3; tissue alterations were too extensive for histopathologic analysis.

3. PFD = postfixation dehydration. This is a specific type of artefact. The margins of affected livers have evidence of dehydration after fixation (e.g., nuclei stain dull blue; erythrocyte cytoplasm stains yellow instead of red; cytoplasm of parenchymal cells stains poorly or not at all). Potential causes include fixation in formalin that is too concentrated (e.g., 100% formalin instead of 10% formalin), removal of tissues from liquid for more than a few minutes, or transfer to ethanol that is too concentrated (e.g., >70% ethanol) before processing to paraffin.  
score = 0; no postfixation dehydration.  
score = 1; postfixation dehydration limited to total sectional area less than 500  $\mu\text{m}$  in diameter.  
score = 2; total sectional area of postfixation dehydration >500  $\mu\text{m}$ , but no 10 $\times$  objective lens field is completely affected by PFD.  
score = 3; total sectional area of postfixation dehydration fills at least one 10 $\times$  objective lens field.
4. FAT = exocrine pancreatic fat. Lipid is stored in adipocytes that have small, dark, basophilic nuclei, with clear cytoplasm. Adipose tissue is common among the roughly triangular foci of exocrine pancreas that are between the intestinal ceca.  
score = 0; volume of peripancreatic adipocytes <2% volume of exocrine pancreas.  
score = 0.5; volume of peripancreatic adipocytes 2-20% volume of exocrine pancreas.  
score = 1; volume of peripancreatic adipocytes 20-100% of volume of exocrine pancreas.  
score = 2; volume of peripancreatic adipocytes 100-200% of volume of exocrine pancreas.  
score = 3; volume of peripancreatic adipocytes >200% of volume of exocrine pancreas.

#### Lesions

1. TMT = Trematode. Trematodes in the intestinal lumen are about 200  $\mu\text{m}$  in diameter, with oral suckers, parenchymatous body cavity, and poorly developed esophagus. Gonads are often developed, with both testes and ovaries, and embryonated eggs in some individuals. In some sections, cells with eosinophilic granules are distinctive.  
score = 0; no trematodes.  
score = 1; <5 trematodes in the section.  
score = 2; >5 trematodes in the section, but no associated inflammation.  
score = 3; > 5 trematodes in the section, with associated inflammation.
2. CES = Cestode. Cestodes in intestine are about 500  $\mu\text{m}$  in diameter, with calcareous corpuscles, no intestine, parenchymatous body cavity, and a scolex. Gonads are not included in the sections examined.

## Intestine lesions

score = 0; no cestodes.

score = 1; <5 cestodes in the section.

score = 2; >5 cestodes in the section, but no associated inflammation.

score = 3; <5 cestodes in the section, with severe inflammation, or > 5 cestodes in the section, with associated moderate inflammation.

3. ANI = Anisakis. Anisakis parasites are common in the mesenteries between loops of intestine and intestinal ceca. They are usually about 500  $\mu\text{m}$  in diameter. Common features in sections include a large-diameter intestine lined by vacuolated cells, lateral cords that attach the intestine to the body wall and suspend the intestine in the body cavity, and a large eosinophilic excretory gland cell. The parasites are commonly surrounded by a fibrous capsule that contains variable amounts of granulomatous inflammation and fibrin.  
score = 0; no Anisakis in the section.  
score = 1; sections contains Anisakis, but inflammation limited to a simple fibrous capsule.  
score = 2; sections contains Anisakis, and inflammation includes fibrin and/or granulomatous inflammation.  
score = 3; none were severe.

Summary of type specimens for gill scores used during histopathologic examination (“type specimens” are good examples of each lesion score). Abbreviations are explained after the table.

GILL (Summary of type specimens)				
Lesion Abbreviation	None Score = 0	Mild score = 1	Moderate score = 2	Severe score = 3
Atly	5-916-81	5-916-44	6-767-85B	none
Art	none	5-916-81	6-767-85B	none
PFD	5-916-81	5-916-93	5-916-71	none
BRS	5-916-81	5-916-92	5-916-89	none
EPC	5-916-81	5-916-93	none	none
GLS	5-916-81	5-916-95	5-916-82	none
GLH	5-916-81		6-767-5B	

#### *Quality Control/Quality Assurance*

1. Atly = Autolysis. Changes in membrane integrity begin immediately after death.  
score = 0; no membrane changes, erythrocytes stained intensely.  
score = 1; dull-staining erythrocytes; dissolution of nuclei.  
score = 2; none were moderate  
score = 3; none were severe
2. Art = Artefact. Tissue changes that were not inherent in the tissue sampled. Sources of artefact included handling at necropsy, processing, sectioning, and staining. Artefact was scored on the basis that it impeded interpretation of tissue morphology. Examples of artefact include splits, bubbles, folds, and knife marks. Artefact that was considered to be a result of postfixation dehydration was scored under PFD (see next category, #3).  
score = 0; sections had no tissue alterations that impeded analysis or photography of any part of the sections.  
score = 1; tissue alterations were present, but most areas could still be photographed without artefact, and analysis for lesions was unaffected.  
score = 2; tissue alteration prevented analysis for lesions in some areas, and photography would be unacceptable anywhere.  
score = 3; tissue alterations were too extensive for histopathologic analysis.
3. PFD = postfixation dehydration. This is a specific type of artefact. The margins of affected livers have evidence of dehydration after fixation (e.g., nuclei stain dull blue; erythrocyte cytoplasm stains yellow instead of red; cytoplasm of parenchymal cells stains

#### Gill lesions

poorly or not at all). Potential causes include fixation in formalin that is too concentrated (e.g., 100% formalin instead of 10% formalin), removal of tissues from liquid for more than a few minutes, or transfer to ethanol that is too concentrated (e.g., >70% ethanol) before processing to paraffin.

score = 0; no postfixation dehydration.

score = 1; postfixation dehydration limited to total sectional area less than 500  $\mu\text{m}$  in diameter.

score = 2; total sectional area of postfixation dehydration >500  $\mu\text{m}$ , but no 10 $\times$  objective lens field is completely affected by PFD.

score = 3; total sectional area of postfixation dehydration fills at least one 10 $\times$  objective lens field.

#### Lesions

1. BRS = branchitis. Branchitis most commonly involved lymphocytes. In some cases, adjacent lamellae are fused. Branchitis associated with *Loma salmonae* xenomas is scored in the LOM category (below).  
score = 0; no branchitis.  
score = 1; branchitis limited to total sectional area less than 500  $\mu\text{m}$  in diameter.  
score = 2; total sectional area of branchitis >500  $\mu\text{m}$ , but no 10 $\times$  objective lens field is completely affected by branchitis.  
score = 3; total sectional area of branchitis fills at least one 10 $\times$  objective lens field.
2. EPC = branchial Epitheliocystis. Foci of Epitheliocystis (cysts) were usually about 30 to 50  $\mu\text{m}$  in diameter, surrounded by a 2- $\mu\text{m}$ -thick hyaline capsule. Contents of cysts were granular and pale basophilic.  
score = 0; no Epitheliocystis.  
score = 1; <5 Epitheliocystis foci per 10 $\times$  objective lens field.  
score = 2; >5 Epitheliocystis foci per 10 $\times$  objective lens field, but minimal inflammatory reaction.  
score = 3; >5 Epitheliocystis foci per 10 $\times$  objective lens field, with moderate to severe inflammation.
3. GLS = *Loma salmonae*. *Loma salmonae* is a microsporidian parasite that forms discreet clusters (xenomas) of spores in the circulatory system. Most xenomas are 50 to 100  $\mu\text{m}$  in diameter. Spores, about 5.5  $\times$  3  $\mu\text{m}$ , have a single polar capsule that is weakly birefringent under polarized light. Reaction to the xenomas varies from none to severe necrotizing vasculitis. Reaction to individual spores is often granulomatous.  
score = 0; no *Loma salmonae*.  
score = 1; <7 *Loma salmonae* in the section, and no significant inflammation.  
score = 2; <7 *Loma salmonae* in the section, with significant inflammation, or >7 xenomas in the section with no associated inflammation.  
score = 3; > 7 *Loma salmonae* in the section, with severe associated inflammation.

#### Gill lesions

4. GLH = gill lamellar hyperplasia/hypertrophy. The thin layer of cells lining the gill provides a vital permeable barrier between the water and the blood, allowing for exchange of oxygen, carbon dioxide, and ammonia. When these delicate cells are irritated, they often respond by increasing in number (hyperplasia) and size (hypertrophy). In some cases, the tips of lamellae fuse. Common causes of GLH include physical and chemical irritants in the water, including parasites.  
score = 0; lamellar epithelium is uniformly simple and squamous.  
score = 1; >5 but <20 thickened lamellae in a 10× objective lens field.  
score = 2; a focus of thickened lamellae extends at least 500 μm along at least one filament.  
score = 3; foci of thickened lamellae extended at least 500 μm along >10 filaments.