

## Final Report AHC Case: 07-1327

Last Updated: 05/03/07 10:53 AM  
 Pathologist: Gary D. Marty  
 Received Date: 04/03/07  
 Collected Date: 04/03/07  
 Client Ref No: PO# CL1361, Log 6105

Veterinarian: **Diane Morrison**  
 Clinic:  
 Phone:  
 Fax:

Submitter:  
 Phone:  
 Fax:  
 Owner: **Marine Harvest Canada**  
 Phone:  
 Fax:(250) 850-3275

**Animal Data**  
 Species: Atlantic Salmon  
 Breed:  
 Sex:  
 Age:  
 Premise ID:

### Case History

Submitted 8 virology samples for PCR for IHN and VHS. Samples were collected on March 26, 2007 and frozen. Each vial sample consists of 5 fish pool.

### Molecular Diagnostics

**PCR - IHN** Resulted by: Ken Sojony Verified by: Ken Sojony on 04/10/07 @ 9:08 AM

Specimen	ID	Test	Result
Tissue	A-6105-1	PCR - IHN	Negative
Tissue	B-6105-2	PCR - IHN	Negative
Tissue	C-6105-3	PCR - IHN	Negative
Tissue	D-6105-4	PCR - IHN	Negative
Tissue	E-6105-5	PCR - IHN	Negative
Tissue	F-6105-6	PCR - IHN	Negative
Tissue	G-6105-7	PCR - IHN	Negative
Tissue	H-6105-8	PCR - IHN	Negative

**PCR - VHS** Resulted by: Ken Sojony Verified by: Ken Sojony on 04/10/07 @ 9:09 AM

Specimen	ID	Test	Result
Tissue	A-6105-1	PCR - VHSV	Negative
Tissue	B-6105-2	PCR - VHSV	Negative
Tissue	C-6105-3	PCR - VHSV	Negative
Tissue	D-6105-4	PCR - VHSV	Negative
Tissue	E-6105-5	PCR - VHSV	Negative
Tissue	F-6105-6	PCR - VHSV	Negative
Tissue	G-6105-7	PCR - VHSV	Negative
Tissue	H-6105-8	PCR - VHSV	Negative

**Tissue Culture** Resulted by: Liisa Nielsen Verified by: Liisa Nielsen on 05/03/07 @ 10:53 AM

Specimen	ID	Isolate	Result	Level
Tissue	A-6105-1		No viruses isolated	
Tissue	B-6105-2		No viruses isolated	
Tissue	C-6105-3		No viruses isolated	
Tissue	D-6105-4		No viruses isolated	
Tissue	E-6105-5		No viruses isolated	
Tissue	F-6105-6		No viruses isolated	
Tissue	G-6105-7		No viruses isolated	
Tissue	H-6105-8		No viruses isolated	



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-1353

Last Updated: 04/27/07 9:39 AM

Pathologist: Gary D. Marty

Received Date: 04/04/07

Collected Date: 04/04/07

Client Ref No: PO#2599 BM/Attriveda

Veterinarian: **Barry Milligan**

Clinic: **Milligan, Barry**

Phone: (250) 286-0838

Fax: (250) 286-1883

Submitter:

Phone:

Fax:

Owner: **Grieg Seafoods BC Ltd.**

Phone: (250) 286-0838

Fax:(250) 286-1883

### Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID:

### Case History

Submitted fresh tissue and cassettes for PCR BKD and VHS. Please prepare cassettes for histo exam by Dr. Gary Marty. No treatments administered. Two pools of kidney tissue (5 fish/pool) Two histo cassettes (7 fish) Two pools virology. Specified on bags. Fish are 250g, hemorrhaging present.

### Final Diagnosis

- 1a. Liver: sinusoidal congestion, acute, multifocal, moderate (consistent with viral hemorrhagic septicemia virus, VHSV; slide 2A, 1 piece)
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slide 1A, 2 pieces), moderate (slide 2A, 2 pieces)
- 2a. Spleen: peritonitis, granulomatous, focal, with occasional fine fibrocellular fronds, mild (slide 1A, 1 piece)
3. Intestine: luminal metazoan parasite, focal (120 x 80 µm), mild
4. Mesenteric fat: peritonitis, granulomatous, focal, with occasional fine fibrocellular fronds, mild (slide 2A)

**Final Comment:** Sinusoidal congestion in the liver is evidence of sinusoidal damage. In BC Atlantic salmon, hepatic sinusoidal congestion is an uncommon feature of infection with viral hemorrhagic septicemia virus (consistent with PCR and virology results) and *Listonella anguillarum*. Sinusoidal congestion is one of the classic lesions associated with ISAV infection, but ISAV has never been identified in British Columbia.

Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition.

These slides have no evidence of infection with *Renibacterium salmoninarum*, the cause of bacterial kidney disease.

Peritonitis involving the spleen and/or abdominal mesenteries is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

Based on the lack of digestive tract or body cavity, the metazoan parasite in the intestine probably is a cestode. Cestodes are rare in pen-reared salmon in BC; its presence in this fish is evidence that the affected fish had ingested something other than formulated feed at some time during its life. The cestode probably was of no significance to fish health.

### Histopathology

Formalin-fixed tissues were submitted in 2 cassettes in a jar of formalin for histopathology. After processing routinely into paraffin, gills were removed from the original (A) cassettes and placed in separate (B) cassettes. Tissues were not decalcified.

Slide 1A - spleen (3 pieces), liver (3 pieces), trunk kidney (3 pieces), intestinal ceca and mesenteric fat (2 pieces)

Slide 1B - gill (2 pieces)

Slide 2A - spleen (3 pieces), liver (3 pieces), trunk kidney (3 pieces), intestinal ceca and mesenteric fat (2 pieces)

Slide 2B - gill (3 pieces)

All organs were examined. Organs not listed below have no significant lesions.

**Quality control:** Basophilic structures stain very poorly, resulting in very red tissues with very little differential staining; this can happen with over-decalcification of tissues. The histotechnicians reported that the sections were difficult to cut. Liver autolysis varies from mild to severe. Large foci of erythrocytes (e.g., spleen in slide 1A) have precipitates of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic before or during fixation. Organs have no postfixation dehydration. Bones are only about 20% calcified in slide 1B and 0% calcified in slide 2B.

### Molecular Diagnostics

**PCR-Renibacterium salmoni** Resulted by: Julie Bidulka Verified by: Julie Bidulka on 04/11/07 @ 12:11 PM

Specimen	ID	Test	Result
Tissue	Kidney - BKD#1	PCR-Renibacterium salmoninaru	Negative
Tissue	Kidney - BKD#2	PCR-Renibacterium salmoninaru	Negative

**PCR - VHSV** Resulted by: Julie Bidulka Verified by: Julie Bidulka on 04/11/07 @ 12:13 PM

Specimen	ID	Test	Result
Tissue	A-Viro-1	PCR - VHSV	Positive
Tissue	B-Viro-1	PCR - VHSV	Positive

### Virology

**Tissue Culture** Resulted by: Liisa Nielsen Verified by: Liisa Nielsen on 04/11/07 @ 1:58 PM

Specimen	ID	Isolate	Result	Level
Tissue	A-Viro-1	Viral Hemorrhag Septicem Virus	Positive	
Tissue	B-Viro-1	Viral Hemorrhag Septicem Virus	Positive	



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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END OF REPORT

## Final Report AHC Case: 07-1453

Last Updated: 05/03/07 10:53 AM

Pathologist: Gary D. Marty

Received Date: 04/11/07

Collected Date: 04/11/07

Client Ref No: Log 6126, PO CL1369

Veterinarian: **Diane Morrison**

Clinic:

Phone:

Fax:

Submitter:

Phone:

Fax:

Owner: **Marine Harvest Canada**

Phone:

Fax:(250) 850-3275

### Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID:

### Case History

Two samples for PCR, 5 cassettes from 3 fish. Two samples labelled 6126-1 and 6126-2 for PCR for IHNV and VHSV. Histology (with gills) from 2 fish, gills from fish 3 only. *C. concavicornis* for the past week. Gills with mucous. *Chaetoceros* (diatoms) in gills on wet mount.

### Final Diagnosis

1a. Gill: lamellar epithelial hyperplasia and fusion of lamella and filaments, multifocal, with intralesional diatom spines/setae consistent with *Chaetoceros concavicornis*, severe (slides 1B, 2B, 3)

1b. Gill: interlamellar and interfilament filamentous bacteria, multifocal, mild (slide 1B), moderate (slide 3)

2a. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slide 1A)

2b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slides 1A, 2A)

3. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild (slide 1A)

4. Spleen: peritonitis, granulomatous, regionally diffuse, with intralesional vacuoles 100 - 500 µm in diameter, moderate (slide 2A)

**Final Comment:** The diatoms *Chaetoceros concavicornis*, *C. convolutus*, and a *Corethron sp.* have been associated with mortality of salmon reared in seawater netpens at numbers as low as 5/mL (Taylor and Harrison 2002). In these fish, the space between the base of gill filaments often contain moderate numbers of unstained structures that are consistent with the diatom *Chaetoceros*. Chains of cell bodies are about 25 µm in diameter, and individual spines/setae are about 3.5 µm in diameter. Mortality due to *Chaetoceros* can occur within a few days of exposure, as a result of physical damage to the gills and the resultant inflammatory response. Affected fish are also more vulnerable to *Vibrio* infections. Larger fish tend to be more susceptible than small fish. Characteristic gill lesions include mucous cell hyperplasia, lamellar epithelial cell hyperplasia and necrosis, and variable numbers of neutrophils. Diatoms and their processes are on the surface of the gill epithelium and sometimes entrapped by multinucleate giant cells. [Source: Kent, M.L., and T.T. Poppe. 1998. Diseases of seawater netpen-reared salmonid fishes. Quadra Printers, Ltd. Nanaimo, B.C., Canada.] Lesions due to *Corethron sp.* are similar (Spear et al. 1989).

Thin rod-shaped to filamentous bacteria are common on the gills of debilitated juvenile salmonids. Although bacterial culture or PCR is required for a definitive diagnosis, the common species in the gill include *Flavobacterium columnare* (the cause of columnaris disease), *F. psychrophilum* (the cause of coldwater disease), *F. branchiophilum* (the cause of bacterial gill disease), and in marine waters *Tenacibaculum maritimum* (one cause of necrotizing branchitis). Infections are usually associated with crowding or poor water quality. In this case, the

bacteria are associated with the diatoms.

The spleen and head kidney in slides 1A and 2A have disseminated foci of eosinophilic droplets with size and staining pattern the same as erythrocyte cytoplasm. The droplets appear to be erythrocytes that have lost their nuclei. Differentials include an artefact (perhaps associated with transfer to water before full fixation) or a disease process that I am not aware of.

Basophilic cytoplasm in hepatocytes is an indication of active protein synthesis. It is normal in mature females producing protein for deposition in their eggs. In other fish it might be related to increased protein needed as part of an inflammatory response.

Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition.

Renal tubular epithelial protein droplets are normal in some species, or they might be an indication of glomerular disease. Renal tubular intracytoplasmic protein droplets were fairly common among fish sampled in 2006 as part of the Ministry's Fish Health Auditing and Surveillance Program: Atlantic salmon (prevalence = 22%; n = 495) and Pacific salmon (prevalence = 38%; n = 134). Ferguson ("Systemic Pathology of Fish," 1989) reports an association of renal protein droplets and high ammonia levels in salmonids. Roberts ("Fish Pathology, Third Edition," 2001) describes the change, but offers no cause.

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

#### Literature cited:

Speare DJ, Brackett J, Ferguson HW. 1989. Sequential pathology of the gills of coho salmon with a combined diatom and microsporidian gill infection. Canadian Veterinary Journal 30(7):571-575.

Taylor, F.J.R., and P.J. Harrison. 2002. Harmful algal blooms in western Canadian coastal waters. In Report #23 of the North Pacific Marine Science Organization, "Harmful algal blooms in the PICES region of the North Pacific."

## Histopathology

Formalin-fixed tissues were submitted in 5 cassettes for histopathology.

Slide 1A (6126-1) - spleen, liver, heart, head kidney, trunk kidney (2 pieces)

Slide 1B (6126-1) - gill

Slide 2A (6126-2) - spleen, liver, heart, head kidney, trunk kidney

Slide 2B (6126-2) - gill

Slide 3 (6126-3) - gill

All organs were examined. Organs not listed below have no significant lesions.

**Quality control:** Liver autolysis: mild (slides 1A, 2A); gill preservation is good. Organs have no postfixation dehydration and no acid hematin deposits.

## Molecular Diagnostics

**PCR - IHNV** Resulted by: A Scouras Verified by: A Scouras on 04/16/07 @ 10:55 AM

Specimen	ID	Test	Result
Tissue	A-6126-1	PCR - IHNV	Negative
Tissue	B-6126-2	PCR - IHNV	Negative

PCR - VHSV Resulted by: A Scouras Verified by: A Scouras on 04/16/07 @ 10:55 AM

Specimen	ID	Test	Result
Tissue	A-6126-1	PCR - VHSV	Negative
Tissue	B-6126-2	PCR - VHSV	Negative

### Virology

Tissue Culture Resulted by: Liisa Nielsen Verified by: Liisa Nielsen on 05/03/07 @ 10:53 AM

Specimen	ID	Isolate	Result	Level
Tissue	A-6126-1		No viruses isolated	
Tissue	B-6126-2		No viruses isolated	



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-1454

Last Updated: 05/03/07 10:54 AM  
Pathologist: Gary D. Marty  
Received Date: 04/11/07  
Collected Date: 04/11/07  
Client Ref No: 6120, PO CL1370

Veterinarian: **Diane Morrison**  
Clinic: **Morrison, Diane**  
Phone: (250) 850-3276  
Fax: (250) 850-3275

Submitter:  
Phone:  
Fax:  
Owner: **Marine Harvest Canada**  
Phone:  
Fax:(250) 850-3275

**Animal Data**  
Species: Atlantic Salmon  
Breed:  
Sex:  
Age:  
Premise ID:

### Case History

One sample labelled G120-1 for PCR for IHNV and VHSV. Fish with clinical signs of ERM but no bacterial growth resulting on blood agar nor TSA.

### Molecular Diagnostics

**PCR - IHNV** Resulted by: A Scouras Verified by: A Scouras on 04/16/07 @ 10:56 AM

Specimen	ID	Test	Result
Tissue	6120	PCR - IHNV	Negative

**PCR - VHSV** Resulted by: A Scouras Verified by: A Scouras on 04/16/07 @ 10:57 AM

Specimen	ID	Test	Result
Tissue	6120	PCR - VHSV	Negative

**PCR - Yersinia ruckeri** Resulted by: Julie Bidulka Verified by: Julie Bidulka on 04/24/07 @ 1:37 PM

Specimen	ID	Test	Result
Tissue	6120	PCR - Yersinia ruckeri	Negative

### Virology

**Tissue Culture** Resulted by: Liisa Nielsen Verified by: Liisa Nielsen on 05/03/07 @ 10:54 AM

Specimen	ID	Isolate	Result	Level
Tissue	6120		No viruses isolated	





Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-1455

Last Updated: 04/19/07 10:12 AM  
Pathologist: Gary D. Marty  
Received Date: 04/11/07  
Collected Date: 04/11/07  
Client Ref No: Log 6102, PO 1368

Veterinarian: **Diane Morrison**  
Clinic: **Morrison, Diane**  
Phone: (250) 850-3276  
Fax: (250) 850-3275

Submitter:  
Phone:  
Fax:  
Owner: **Marine Harvest Canada**  
Phone:  
Fax:(250) 850-3275

**Animal Data**  
Species: Atlantic Salmon  
Breed:  
Sex:  
Age:  
Premise ID:

### Case History

One blood agar plate for identification and antibiotic sensitivity. Sample collected on Mar 22/07 from fish 3. Histo and virology collected earlier and already shipped previously 2007/01256.

### Bacteriology

**Culture - Non Animal** Resulted by: Erin Zabek Verified by: Sean Byrne on 04/19/07 @ 10:12 AM

Specimen	ID	Isolate	Result	Level
Isolate		Photobacterium phosphoreum	Positive	
**: Due to poor growth of the organism, antibiotic sensitivities were unable to be performed.				



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-1540

Last Updated: 04/20/07 3:53 PM

Pathologist: Gary D. Marty

Received Date: 04/17/07

Collected Date: 04/17/07

Client Ref No: PO #CL1374/Log 6116

Veterinarian: **Diane Morrison**

Clinic: **Morrison, Diane**

Phone: (250) 850-3276

Fax: (250) 850-3275

Submitter:

Phone:

Fax:

Owner: **Marine Harvest Canada**

Phone:

Fax:(250) 850-3275

### Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID:

### Case History

Log No. 6116. Date: April 16/07. PO #CL1374. Species: Atlantic salmon Sex: Regular Saltwater entry: 2006 Netpen/Tank ID: 12 Submitted one histo cassette. One random selected fish with large kidney granuloma. TDX: BKD.

### Final Diagnosis

1. Trunk kidney: renal tubular mineralization (nephrocalcinosis), multifocal, with dilated tubules and tubular epithelial hyperplasia, severe

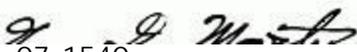
**Final Comment:** Renal mineralization is common in cultured fish species; when severe, the condition is termed nephrocalcinosis. The lesion is not considered fatal, although feed conversion may be adversely affected. The pathogenesis is not fully understood, but renal mineralization has been experimentally reproduced through high carbon dioxide levels, magnesium deficiency, selenium toxicity, and a diet low in minerals (source, "Systemic Pathology of Fish", 1989, by H. Ferguson). Clinically, renal mineralization is most commonly associated with high carbon dioxide levels. I think renal mineralization and bacterial kidney disease developed independently in these fish.

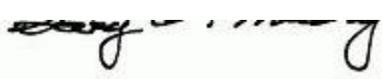
This case of nephrocalcinosis is different from most I have seen in that the volume of granulomatous inflammation is greater than 10x the volume of mineralized tubules. The primary differential for abundant granulomatous inflammation in cultured salmon is bacterial kidney disease, but lack of organisms on the Gram stain rules out this differential.

### Histopathology

Four pieces of formalin-fixed kidney (some from the margin of head and trunk kidney) plus one section of granulomatous inflammation with a margin of vascular connective tissue (kidney or swimbladder) were submitted for histopathology. One section of each piece was processed onto a single slide. Sections were stained with H&E and Twort's Gram stain.

**Quality control:** Tissue preservation is excellent. Tissues have no postfixation dehydration and no deposits of acid hematin.





Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-1541

Last Updated: 05/09/07 2:26 PM  
Pathologist: Gary D. Marty  
Received Date: 04/17/07  
Collected Date: 04/17/07  
Client Ref No: PO# CL1372/Log 6130

Veterinarian: **Diane Morrison**  
Clinic: **Morrison, Diane**  
Phone: (250) 850-3276  
Fax: (250) 850-3275

Submitter:  
Phone:  
Fax:  
Owner: **Marine Harvest Canada**  
Phone:  
Fax:(250) 850-3275

**Animal Data**  
Species: Atlantic Salmon  
Breed:  
Sex:  
Age:  
Premise ID:

### Case History

Log No. 6130 Date: April 16/07 PO CL1372 Five samples for virology, PCR for IHN and VHS. Samples labelled 5, 7, 8, 11 and 12(single fish samples). Sample 8 with petechial hem of s.b.

### Molecular Diagnostics

**PCR - IHN** Resulted by: A Scouras Verified by: A Scouras on 04/19/07 @ 4:18 PM

Specimen	ID	Test	Result
Tissue	A = #5	PCR - IHN	Negative
Tissue	B = #7	PCR - IHN	Negative
Tissue	C = #8	PCR - IHN	Negative
Tissue	D = #11	PCR - IHN	Negative
Tissue	E = #12	PCR - IHN	Negative

**PCR - VHSV** Resulted by: A Scouras Verified by: A Scouras on 04/19/07 @ 4:18 PM

Specimen	ID	Test	Result
Tissue	A = #5	PCR - VHSV	Negative
Tissue	B = #7	PCR - VHSV	Negative
Tissue	C = #8	PCR - VHSV	Negative
Tissue	D = #11	PCR - VHSV	Negative
Tissue	E = #12	PCR - VHSV	Negative

### Virology

**Tissue Culture** Resulted by: Liisa Nielsen Verified by: Liisa Nielsen on 05/09/07 @ 2:25 PM

Specimen	ID	Isolate	Result	Level
Tissue	A = #5		No viruses isolated	

Tissue	B = #7
Tissue	C = #8
Tissue	D = #11
Tissue	E = #12

No viruses isolated



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-1564

Last Updated: 04/23/07 4:33 PM

Pathologist: Gary D. Marty

Received Date: 04/19/07

Collected Date: 04/19/07

Client Ref No: PO 2599BM/Muchalat S

Veterinarian: **Barry Milligan**

Clinic: **Milligan, Barry**

Phone: (250) 286-0838

Fax: (250) 286-1883

Submitter:

Phone:

Fax:

Owner: **Grieg Seafoods BC Ltd.**

Phone: (250) 286-0838

Fax:(250) 286-1883

### Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID:

### Case History

Submitted formalized Atlantic salmon. Increase mortality. No apparent cause of death. Sent 4 histo cassettes, 7 fish. Please prepare for histo exam by Dr. Gary Marty.

### Final Diagnosis

- 1a. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate (slide 2)
- 1b. Liver: yellow-brown to yellow-green pigmented macrophage aggregates and sinusoidal macrophages, disseminated, mild (slides 2, 4)
2. Trunk kidney: fibrin thrombus, peracute, focal (1 × 0.5 mm), moderate (slide 3)
3. Spleen: parenchymal golden pigment, scattered, intracellular, mild (slide 4)

### Final Comment:

The range of changes in these organs is what I would expect from a relatively healthy group of pen-reared Atlantic salmon. The organs have no lesions that could be associated with an active infection with *Renibacterium salmoninarum*.

Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition. Pigment in the liver could be lipofuscin, hemosiderin, or both. 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. 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.

Thrombosis in the kidney is evidence of increased coagulability. This can result from endothelial damage related to virus, bacterial, or parasitic infection. The thrombus in slide 3 stains paler than usual; I interpret this as evidence that the thrombus is very acute, and it might have formed during the stress of capture and killing.

The golden pigment in the spleen most likely is lipofuscin, with the same pathogenesis and significance as described for the liver.

What other study might help determine the cause of death in these fish? I recommend sampling of brain in cases like this one where the cause of death is unknown. At the beginning of 2007, brain was added to the list of organs to sample for histopathology as part of the

Provincial government's Fish Health Auditing and Surveillance Program. Of the 168 Atlantic salmon examined during the first quarter of 2007, liver, kidney, and heart had lesions sufficient to explain the cause of death in 27% of the fish. The addition of brain histopathology allowed me to determine the cause of death of another 20% of the fish. Gill and mouth, selected only from fish with lesions in these structures, added a cause of death to another 4% of the fish. Spleen, intestine, and mesenteric adipose tissue did were examined from every fish, but they did not provide any unique information for determining the cause of death.

## Histopathology

Formalin-fixed tissues were submitted in 4 unlabelled cassettes for histopathology. Larger pieces of gill were removed from the original cassettes and placed in 2 separate cassettes. The gills were decalcified for ~2h in Protocol B and then processed routinely into paraffin. Slides from the original cassettes were arbitrarily labeled 1-4; the gill slides were labeled 5 and 6.

Slide 1 - swimbladder (2 pieces), liver, trunk kidney, spleen, gill, intestinal ceca, and mesenteric adipose tissue

Slide 2 - liver (2 pieces), trunk kidney (2 pieces), spleen (2 pieces), gill, intestinal ceca (4 pieces), and mesenteric adipose tissue

Slide 3 - trunk kidney, spleen, intestinal ceca, and mesenteric adipose tissue

Slide 4 - liver (3 pieces), trunk kidney/head kidney transition, spleen (3 pieces), intestinal ceca, and mesenteric adipose tissue

Slide 5 - gill (2 pieces)

Slide 6 - gill (3 pieces)

All organs were examined. Organs not listed below have no significant lesions.

**Quality control:** Tissue preservation is good for most organs; one gill on slide 6 has moderate autolysis. Large foci of erythrocytes in the spleen in slide 1 have precipitates of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic before or during fixation. Organs have no postfixation dehydration. Gill decalcification is nearly 100% complete.



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-1758

Last Updated: 05/08/07 3:53 PM

Pathologist: Gary D. Marty

Received Date: 05/04/07

Collected Date: 05/04/07

Client Ref No: PO# 2599BM

Veterinarian: **Barry Milligan**

Clinic:

Phone:

Fax:

Submitter:

Phone:

Fax:

Owner: **Grieg Seafoods BC Ltd.**

Phone: (250) 286-0838

Fax:(250) 286-1883

### Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID:

### Case History

Submitted formalized salmon tissue for histology (2 histo cassettes- 5 fish total). Increased mortality. Suspect plankton??

### Final Diagnosis

1a. Trunk kidney: nephritis, granulomatous, interstitial, multifocal, coalescing, (*Renibacterium salmoninarum?*), severe (slide 2A)

1b. Spleen: splenitis, granulomatous, multifocal, (*Renibacterium salmoninarum?*), mild (slide 1), severe (one piece, slide 2B; reaction includes fibrinous peritonitis)

2a. Liver: sinusoidal congestion, acute, multifocal, moderate (slides 1, 2B)

2b. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slide 2A)

2c. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate (slide 2A)

2d. Liver: hepatocellular single cell necrosis (apoptosis), disseminated, acute, mild (slide 2A)

3. Brain: capillary (vascular) congestion, diffuse, mild (slide 1)

4. Intestine: mesenteric capillary congestion and hemorrhage, diffuse, moderate (slide 2A)

5a. Mesenteric adipose tissue: peritonitis, chronic, focal, with fibrocellular fronds, mild (slide 2A)

5b. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slide 2B)

**Final Comment:** Disseminated granulomatous inflammation is consistent with infection with *Renibacterium salmoninarum*, the cause of bacterial kidney disease. Gills have no evidence of algae-associated lesions. Severely autolyzed gills have small amounts of bacteria and fungal hyphae, but I think these are postmortem invaders.

Sinusoidal congestion in the liver is evidence of sinusoidal damage. In BC Atlantic salmon, hepatic sinusoidal congestion is an uncommon feature of infection with viral hemorrhagic septicemia virus and *Listonella anguillarum*. Sinusoidal congestion is one of the classic lesions

associated with ISAV infection, but ISAV has never been identified in British Columbia. Consider bacteriology and virology and PCR for VHSV, and IHNV (if not already done). Basophilic cytoplasm in hepatocytes is an indication of active protein synthesis. It is normal in mature females producing protein for deposition in their eggs. In other fish it might be related to increased protein needed as part of an inflammatory response. Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition.

Causes of hepatocellular single cell necrosis have not been well defined in fish. Possible differentials include exposure to toxins (endogenous or exogenous), or a viral infection (VHSV). A similar change, apoptosis, occurs with remodelling of the liver in rapidly growing fish that suddenly go off feed about 24 hours before death. Apoptosis is the normal way in which hepatocyte numbers are decreased (i.e., the hepatocytes are not needed when growing fish stop feeding because few to no nutrients are being absorbed into the blood and entering the liver for processing).

Congestion of brain capillaries is evidence of circulating vasodilators; differentials include viral, bacterial, and parasitic infections. The neuropil normally contains a rich network of capillaries, but in any given section, the majority of capillaries contain no erythrocytes. By comparison, when cerebral capillaries are congested, a greater proportion of capillaries in the section will contain erythrocytes.

Distension of capillaries in the mesenteric adipose tissue is often part of the inflammatory response to many infectious diseases; hemorrhage sometimes occurs in severe cases. In British Columbia, mesenteric congestion and hemorrhage is most commonly associated with VHSV and bacterial infections.

Congestion in multiple organs can occur with *R. salmoninarum* infection, but because tissues from the 5 fish were mixed, I cannot determine the relation of lesions in one organ to lesions in another.

Chronic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

## Histopathology

Formalin-fixed tissues were submitted in 2 unlabelled cassettes for histopathology. Larger pieces of gill were removed from the original cassettes and placed in 2 separate cassettes. The gills were decalcified for ~2h in Protocol B and then processed routinely into paraffin. The remaining tissues were split into 3 cassettes.

Slide 1 - liver (2 pieces), brain, trunk kidney (2 pieces), spleen (2 pieces), intestinal ceca and mesenteric adipose tissue (2 pieces)

Slide 2 - liver, trunk kidney, intestinal ceca and mesenteric adipose tissue (2 pieces)

Slide 3 - trunk kidney, spleen, intestinal ceca, and mesenteric adipose tissue

Slide 4 - liver, trunk kidney (2 pieces), spleen (3 pieces), intestinal ceca, and mesenteric adipose tissue

Slide 3A - gill (2 pieces)

Slide 3B - gill (3 pieces)

All organs were examined. Organs not listed below have no significant lesions.

**Quality control:** Tissue preservation is highly variable, from poor to good for different pieces of most organs. Organs have no postfixation dehydration and no acid hematin deposits. Gill decalcification is complete.



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-1857

Last Updated: 05/14/07 11:38 AM  
Pathologist: Gary D. Marty  
Received Date: 05/10/07  
Collected Date: 05/10/07  
Client Ref No: Log 6130, PO CL1382

Veterinarian: **Diane Morrison**  
Clinic:  
Phone:  
Fax:

Submitter:  
Phone:  
Fax:  
Owner: **Marine Harvest Canada**  
Phone:  
Fax:(250) 850-3275

**Animal Data**  
Species: Atlantic Salmon  
Breed:  
Sex:  
Age:  
Premise ID:

### Case History

Submitted one bac-t sample for ID and antibiotic sensitivity. Log 6130, PO CL 1382

### Bacteriology

**Aerobic Culture - Prod** Resulted by: Erin Zabek Verified by: Sean Byrne on 05/14/07 @ 11:37 AM

Specimen	ID	Isolate	Result	Level
Isolate	Ref 6130 #5	Pseudomonas sp.	Positive	
**: Pseudomonas identified as Pseudomonas synxantha				

**Fish** Resulted by: Erin Zabek Verified by: Sean Byrne on 05/14/07 @ 11:38 AM

Organism	ID	e	ffc	sor	s3	sxt	ot
Pseudomonas sp.	Ref 6130 #5	r	r	r	s	r	s



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-1859

Last Updated: 06/01/07 2:33 PM  
Pathologist: Gary D. Marty  
Received Date: 05/10/07  
Collected Date: 05/10/07  
Client Ref No: Log 6164, PO CL 1380

Veterinarian: **Diane Morrison**  
Clinic:  
Phone:  
Fax:

Submitter:  
Phone:  
Fax:  
Owner: **Marine Harvest Canada**  
Phone:  
Fax:(250) 850-3275

**Animal Data**  
Species: Atlantic Salmon  
Breed:  
Sex:  
Age:  
Premise ID:

### Case History

Submitted two virology samples and one histo cassette. One fish with septicemia (viral and bact taken), virology to BCMAFF for PCR for IHN and VHSV. Histo of a second fish with liver lesions. Virology for PCR of IHN and VHSV.

### Final Diagnosis

1. Liver: sinusoidal congestion, acute, multifocal, moderate
2. Trunk kidney and head kidney: interstitial congestion and hemorrhage, diffuse, mild
- 3a. Spleen: peritonitis, granulomatous, regionally diffuse, with intralesional vacuoles about 50 µm in diameter, moderate
- 3b. Spleen: parenchymal golden pigment, scattered, intracellular, moderate

**Final Comment:** Congestion of hepatic sinusoids and renal interstitium is evidence of endothelial damage. In BC Atlantic salmon, congestion is an uncommon feature of infection with viral hemorrhagic septicemia virus and *Listonella anguillarum*. Congestion is one of the classic lesions associated with ISAV infection, but ISAV has never been identified in British Columbia. I have also seen sinusoidal congestion in farmed rainbow trout fed rancid feed with high mycotoxin concentrations (unpublished data).

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated. Vacuoles probably are a result of vaccine material lost during tissue processing.

The golden pigment in the spleen most likely is lipofuscin. Accumulation of lipofuscin 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. Conditions that lead to moderate to abundant lipofuscin have been associated with decreased growth and survival in several studies.

### Histopathology

Formalin-fixed tissues were submitted in a cassette for histopathology (heart, head kidney, trunk kidney, liver, spleen).

All organs were examined. Organs not listed below have no significant lesions.

**Quality control:** Liver autolysis is mild; trunk kidney autolysis is moderate. Large foci of erythrocytes (e.g., liver) have precipitates of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic before or during fixation. Organs have no postfixation dehydration.

### Molecular Diagnostics

**PCR - IHNV** Resulted by: Julie Bidulka Verified by: Julie Bidulka on 05/14/07 @ 2:37 PM

Specimen	ID	Test	Result
Tissue	A-6164-1	PCR - IHNV	Negative
Tissue	B-6164-2	PCR - IHNV	Negative

**PCR - VHSV** Resulted by: Julie Bidulka Verified by: Julie Bidulka on 05/14/07 @ 2:39 PM

Specimen	ID	Test	Result
Tissue	A-6164-1	PCR - VHSV	Negative
Tissue	B-6164-2	PCR - VHSV	Negative

### Virology

**Tissue Culture** Resulted by: Hubert Wong Verified by: Dr. J. Robinson on 06/01/07 @ 2:33 PM

Specimen	ID	Isolate	Result	Level
Tissue	A-6164-1		No viruses isolated	
Tissue	B-6164-2		No viruses isolated	



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-1860

Last Updated: 06/01/07 2:33 PM

Pathologist: Gary D. Marty

Received Date: 05/10/07

Collected Date: 05/10/07

Client Ref No: Log 6165, PO CL1381

Veterinarian: **Diane Morrison**

Clinic:

Phone:

Fax:

Submitter:

Phone:

Fax:

Owner: **Marine Harvest Canada**

Phone:

Fax:(250) 850-3275

### Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID:

### Case History

Submitted two virology samples and one histo cassette. Histo and virology taken from one septic fish (sample 1). Virology taken from fish 2. Please run PCR for IHNV and VHSV.

### Final Diagnosis

1. Trunk kidney: renal tubular epithelial regeneration, diffuse, subacute, with epithelial necrosis and interstitial cell hypoplasia, moderate
2. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
3. Mesenteric adipose tissue: peritonitis, granulomatous, multifocal, with one intralobular vacuole about 80 µm in diameter, moderate

**Final Comment:** Lesions in the trunk kidney are evidence of repair with ongoing acute damage to renal epithelial cells; damage is reversible if the basement membrane is spared (as in this case). Causes in fish include viral hemorrhagic septicemia virus (VHSV) and exposure to toxins (e.g., bacterial toxins, or aminoglycoside antibiotics such as gentamicin). The fact that this fish is VHSV negative by PCR makes the other differentials more likely. Most of the tubules are lined by attenuated epithelium that is more basophilic than normal (i.e., evidence of regeneration); the attenuated epithelium with no change in the basement membrane diameter makes the tubules appear slightly dilated. Lack of hematopoietic cells in the interstitium could be a result of decreased production and/or increased turnover.

Basophilic cytoplasm in hepatocytes is an indication of active protein synthesis. It is normal in mature females producing protein for deposition in their eggs. In other fish it might be related to increased protein needed as part of an inflammatory response.

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated. Vacuoles probably are a result of vaccine material lost during tissue processing.

### Histopathology

Formalin-fixed tissues were submitted in a cassette for histopathology (heart, trunk kidney, liver, intestinal ceca and mesenteric adipose tissue). All organs were examined. Organs not listed below have no significant lesions.

**Quality control:** Tissue preservation is excellent for all organs. Organs have no postfixation dehydration and no acid hematin deposits.

**PCR - IHNV** Resulted by: Julie Bidulka Verified by: Julie Bidulka on 05/14/07 @ 2:39 PM

Specimen	ID	Test	Result
Tissue	A-6165-1	PCR - IHNV	Negative
Tissue	B-6165-2	PCR - IHNV	Negative

**PCR - VHSV** Resulted by: Julie Bidulka Verified by: Julie Bidulka on 05/14/07 @ 2:39 PM

Specimen	ID	Test	Result
Tissue	A-6165-1	PCR - VHSV	Negative
Tissue	B-6165-2	PCR - VHSV	Negative

**Virology**

**Tissue Culture** Resulted by: Hubert Wong Verified by: Dr. J. Robinson on 06/01/07 @ 2:33 PM

Specimen	ID	Isolate	Result	Level
Tissue	A-6165-1		No viruses isolated	
Tissue	B-6165-2		No viruses isolated	



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-1861

Last Updated: 05/14/07 4:09 PM

Pathologist: Gary D. Marty

Received Date: 05/10/07

Collected Date: 05/10/07

Client Ref No: Log 6161, PO CL1379

Veterinarian: **Diane Morrison**

Clinic:

Phone:

Fax:

Submitter:

Phone:

Fax:

Owner: **Marine Harvest Canada**

Phone:

Fax:(250) 850-3275

### Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID:

### Case History

Submitted two gills for histo. Gill samples taken from fish during jelly fish bloom. Please check for gill damage d/t bloom. Mortality is minimal at this point in time.

### Final Diagnosis

- 1a. Branchial skeletal muscle: myositis, focal, granulomatous, with intralesional *Kudoa thyrsites* spores, moderate (slide 1)
- 1b. Branchial skeletal muscle: intracellular *Kudoa thyrsites* spores, multifocal, abundant (slide 1)
- 1c. Gill: lamellar capillary thrombosis, multifocal, acute, mild (slide 2)
- 1d. Gall arch: dermatitis, bifocal, lymphocytic, mild (slide 2)

**Final Comment:** None of the lesions in these gills were sufficient to have killed the fish. The primary gill lesion described after exposure to jellyfish is lamellar edema [source: Fish Pathology, 3rd Edition. 2001. R.J. Roberts; p. 373], but neither of these gills had edema.

The most significant lesion is in slide 1, in which more than 80% of the volume of the branchial skeletal muscle is replaced by *Kudoa thyrsites* spores. This example has characteristic stellate spores with four unequal polar capsules converging on one end. Extensive inflammation is common in severe infections [source Kent, M.L., and T.T. Poppe. 1998. Diseases of seawater netpen-reared salmonid fishes. Quadra Printers, Ltd. Nanaimo, B.C., Canada.]

Thrombosis in the gill is evidence of increased coagulability. This can result from endothelial damage related to virus, bacterial, or parasitic infection.

Lymphocytic dermatitis in the gill is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine.

### Histopathology

Two formalin-fixed parts of gill arches (with filaments and lamellae) were submitted for histopathology; they were processed onto slides 1 and 2.

**Quality control:** Autolysis is severe in slide 1; mild in slide 2. Organs have no postfixation dehydration and no acid hematin deposits.



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D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-1915

Last Updated: 06/13/07 1:40 PM  
Pathologist: Gary D. Marty  
Received Date: 05/15/07  
Collected Date: 05/15/07  
Client Ref No: Log 6168, PO CL1383

Veterinarian: **Diane Morrison**  
Clinic:  
Phone:  
Fax:

Submitter:  
Phone:  
Fax:  
Owner: **Marine Harvest Canada**  
Phone:  
Fax:(250) 850-3275

**Animal Data**  
Species: Atlantic Salmon  
Breed:  
Sex:  
Age:  
Premise ID:

### Case History

Submitted two samples for virology labeled #2 and #3 for PCR IHNV and VHSV. Samples were collected on May 9/07 and frozen.

### Molecular Diagnostics

**PCR - IHNV** Resulted by: A Scouras Verified by: A Scouras on 05/18/07 @ 10:39 AM

Specimen	ID	Test	Result
Tissue	A-#2	PCR - IHNV	Negative
Tissue	B-#3	PCR - IHNV	Negative

**PCR - VHSV** Resulted by: A Scouras Verified by: A Scouras on 05/18/07 @ 10:40 AM

Specimen	ID	Test	Result
Tissue	A-#2	PCR - VHSV	Negative
Tissue	B-#3	PCR - VHSV	Negative

### Virology

**Tissue Culture** Resulted by: Liisa Nielsen Verified by: Dr. J. Robinson on 06/13/07 @ 1:40 PM

Specimen	ID	Isolate	Result	Level
Tissue	A-#2		No viruses isolated	
Tissue	B-#3		No viruses isolated	



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-1916

Last Updated: 05/22/07 9:58 AM  
Pathologist: Gary D. Marty  
Received Date: 05/15/07  
Collected Date: 05/15/07  
Client Ref No: Log 6165, PO CL1381

Veterinarian: **Diane Morrison**  
Clinic:  
Phone:  
Fax:

Submitter:  
Phone:  
Fax:  
Owner: **Marine Harvest Canada**  
Phone:  
Fax:(250) 850-3275

**Animal Data**  
Species: Atlantic Salmon  
Breed:  
Sex:  
Age:  
Premise ID:

### Case History

Submitted one culture for bacteriology ID and sensitivity.

### Bacteriology

**Aerobic Culture - Prod** Resulted by: Erin Zabek Verified by: Sean Byrne on 05/22/07 @ 9:58 AM

Specimen	ID	Isolate	Result	Level
Isolate		Photobacterium damsela	Positive	

**Fish** Resulted by: Erin Zabek Verified by: Sean Byrne on 05/22/07 @ 9:58 AM

Organism	ID	e	ffc	sor	s3	sxt	ot
Photobacterium damsela		s	s	s	s	s	s



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-1917

Last Updated: 05/22/07 9:58 AM  
Pathologist: Gary D. Marty  
Received Date: 05/15/07  
Collected Date: 05/15/07  
Client Ref No: Log 6164, PO CL1380

Veterinarian: **Diane Morrison**  
Clinic:  
Phone:  
Fax:

Submitter:  
Phone:  
Fax:  
Owner: **Marine Harvest Canada**  
Phone:  
Fax:(250) 850-3275

**Animal Data**  
Species: Atlantic Salmon  
Breed:  
Sex:  
Age:  
Premise ID:

### Case History

Submitted one culture plate for bacteriology ID and sensitivities.

### Bacteriology

**Aerobic Culture - Prod** Resulted by: Erin Zabek Verified by: Sean Byrne on 05/22/07 @ 9:57 AM

Specimen	ID	Isolate	Result	Level
Isolate		Photobacterium damsela	Positive	

**Fish** Resulted by: Erin Zabek Verified by: Sean Byrne on 05/22/07 @ 9:58 AM

Organism	ID	e	ffc	sor	s3	sxt	ot
Photobacterium damsela		s	s	s	s	s	s



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-2112

Last Updated: 06/29/07 1:37 PM  
Pathologist: Gary D. Marty  
Received Date: 05/30/07  
Collected Date: 05/30/07  
Client Ref No: Log 6180, PO CL1389

Veterinarian: **Diane Morrison**  
Clinic:  
Phone:  
Fax:

Submitter:  
Phone:  
Fax:  
Owner: **Marine Harvest Canada**  
Phone:  
Fax:(250) 850-3275

**Animal Data**  
Species: Atlantic Salmon  
Breed:  
Sex:  
Age:  
Premise ID:

### Case History

Samples collected May 22, 2007 and frozen. Please do PCR for IHN and VHS.

### Molecular Diagnostics

**PCR - IHN** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/01/07 @ 4:47 PM

Specimen	ID	Test	Result
Tissue	#1	PCR - IHNV	Negative

**PCR - VHSV** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/01/07 @ 4:47 PM

Specimen	ID	Test	Result
Tissue	#1	PCR - VHSV	Negative

### Virology

**Tissue Culture** Resulted by: Hubert Wong Verified by: Liisa Nielsen on 06/29/07 @ 1:37 PM

Specimen	ID	Isolate	Result	Level
Tissue	#1		No viruses isolated	



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-2113

Last Updated: 06/29/07 1:36 PM  
Pathologist: Gary D. Marty  
Received Date: 05/30/07  
Collected Date: 05/30/07  
Client Ref No: Log 6181, PO CL1391

Veterinarian: **Diane Morrison**  
Clinic:  
Phone:  
Fax:

Submitter:  
Phone:  
Fax:  
Owner: **Marine Harvest Canada**  
Phone:  
Fax:(250) 850-3275

**Animal Data**  
Species: Atlantic Salmon  
Breed:  
Sex:  
Age:  
Premise ID:

### Case History

Submitted two samples for PCR for IHN and VHS. Samples labeled 7 and 9. Collected during a routine mort dive on May 22, 2007 and kept frozen.

### Molecular Diagnostics

**PCR - IHNV** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/01/07 @ 4:47 PM

Specimen	ID	Test	Result
Tissue	A (#7)	PCR - IHNV	Negative
Tissue	B (#9)	PCR - IHNV	Negative

**PCR - VHSV** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/01/07 @ 4:47 PM

Specimen	ID	Test	Result
Tissue	A (#7)	PCR - VHSV	Negative
Tissue	B (#9)	PCR - VHSV	Negative

### Virology

**Tissue Culture** Resulted by: Hubert Wong Verified by: Liisa Nielsen on 06/29/07 @ 1:36 PM

Specimen	ID	Isolate	Result	Level
Tissue	A (#7)		No viruses isolated	
Tissue	B (#9)		No viruses isolated	



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-2114

Last Updated: 06/29/07 1:38 PM  
Pathologist: Gary D. Marty  
Received Date: 05/30/07  
Collected Date: 05/30/07  
Client Ref No: Log 6182, PO CL1392

Veterinarian: **Diane Morrison**  
Clinic:  
Phone:  
Fax:

Submitter:  
Phone:  
Fax:  
Owner: **Marine Harvest Canada**  
Phone:  
Fax:(250) 850-3275

**Animal Data**  
Species: Atlantic Salmon  
Breed:  
Sex:  
Age:  
Premise ID:

### Case History

Submitted two samples for virology for PCR of IHN and VHSV, and two cassettes for histology. Some pinpoint hemorrhaging on viscera.

### Final Diagnosis

1. Liver: intrahepatic hemorrhage, dissecting, multifocal, severe (slide 2)
- 2a. Heart: epicarditis, multifocal, lymphoplasmacytic, mild (slide 1)
- 2b. Heart: separation of endothelium from striated muscle, diffuse, moderate (slide 1)
- 3a. Mesenteric adipose tissue: peritonitis, lymphohistiocytic, fibrinous, multifocal, moderate (slide 2)
- 3b. Mesenteric adipose tissue: saponification of fat (fat necrosis), with scattered macrophages, diffuse, moderate (slide 2)

**Final Comment:** This fish might have died as a result of rupture of a major vessel in the liver. The largest dissecting focus of hemorrhage is about 8 mm long and 1 mm wide. Hemorrhage can result from trauma, and susceptibility to the effects of trauma would be increased if there was an underlying vasculitis; however, this fish had no vasculitis in the sections examined. This pattern of hemorrhage is not characteristic of the VHSV cases I have seen in Atlantic salmon.

Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. The space between the endothelial layer and the underlying myocardial cells sometimes contains wispy eosinophilic material, but often contains nothing. I have not seen this change before. The separation might be an artefact. As a differential, it might be a form of edema.

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated. The presence of fibrin is evidence that the inflammation is active, and might include a reaction to a secondary bacterial infection.

The lipid in the necrotic fat cells has been replaced by wispy basophilic material. Necrotic cells comprise less than 10% of the volume of the mesenteric adipose tissue. About half of the affected cells contain foamy macrophages. Saponification of fat is not a common lesion in Atlantic salmon. It has been associated with vitamin E deficiency, and it can be secondary to nearby inflammation. In severe cases, the adipose tissue appears chalky white.

Formalin-fixed tissues were submitted in 2 cassettes for histopathology.

Slide 1 (Althorp #15 May 22/07) - heart

Slide 2 (Althorp #15 May 22/07) - spleen, liver, heart, head kidney, trunk kidney

All organs were examined. Organs not listed below have no significant lesions.

**Quality control:** Liver autolysis: severe. Organs have no postfixation dehydration and no acid hematin deposits.

**Molecular Diagnostics**

**PCR - IHNV** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/01/07 @ 4:48 PM

Specimen	ID	Test	Result
Tissue	A (TA#12)	PCR - IHNV	Negative
Tissue	B (TA#12)	PCR - IHNV	Negative

**PCR - VHSV** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/01/07 @ 4:48 PM

Specimen	ID	Test	Result
Tissue	A (TA#12)	PCR - VHSV	Positive
Tissue	B (TA#12)	PCR - VHSV	Negative

**Virology**

**Tissue Culture** Resulted by: Hubert Wong Verified by: Liisa Nielsen on 06/29/07 @ 1:38 PM

Specimen	ID	Isolate	Result	Level
Tissue	A (TA#12)		No viruses isolated	
Tissue	B (TA#12)		No viruses isolated	



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-2115

Last Updated: 06/09/07 2:50 PM

Pathologist: Gary D. Marty

Received Date: 05/30/07

Collected Date: 05/30/07

Client Ref No: Log 6187, PO CL 1394

Veterinarian: **Diane Morrison**

Clinic:

Phone:

Fax:

Submitter:

Phone:

Fax:

Owner: **Marine Harvest Canada**

Phone:

Fax:(250) 850-3275

### Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID:

### Case History

Submitted histo of two gills, follow up, continuance to case 6161. Mortality is minimal. Looking for gill damage due to jelly fish.

### Final Diagnosis

1a. Gill, tips of filaments, base of lamellae: epithelial hyperplasia, diffuse, moderate to severe (slide G1)

1b. Gill: lamellar telangiectasis, multifocal, with organizing thrombosis, mild (slide G1)

1c. Gill: lamellar capillary thrombosis, multifocal, acute, mild (slide 2)

**Final Comment:** Hyperplasia of the epithelium lining gill lamellae is a nonspecific response to irritation. Inciting causes include parasites, bacteria, viruses, and toxins. The hyperplasia in gill G1 is unusual in that it is pronounced at the base of the lamellae, but the tips of the lamellae are lined by fairly normal, thin epithelial cells. Hyperplasia is more severe between lamellae at the distal ends of the filaments. Slide G1 contains a section of a primitive invertebrate that is 250 µm long and about 150 µm wide (coordinates on my microscope = 23 x 111). This might be a jellyfish tentacle, but I am not sure; consider submitting a tentacle preserved in 10% neutral buffered formalin for confirmatory histopathology (no charge).

Telangiectasis (rupture of the small lamellar blood vessels) in the gill most commonly results from trauma (e.g., handling). The presence of thrombi with karyorrhexis is evidence that these thrombi have been present for only a few hours.

Thrombosis in a few lamellar capillaries is evidence of increased coagulability. This can result from endothelial damage related to virus, bacterial, or parasitic infection.

### Histopathology

Two formalin-fixed parts of gill arches (with filaments and lamellae) were submitted for histopathology (follow-up from case 2007-1861). The gills were decalcified overnight in 10% EDTA and then processed routinely onto slides G1 and G2.

**Quality control:** Autolysis is none to mild in slide G1, severe in slide G2 (i.e., the gill in slide G2 is too autolyzed for diagnosis of many common gill lesions). Organs have no postfixation dehydration and no acid hematin deposits.



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-2116

Last Updated: 06/29/07 1:40 PM  
Pathologist: Gary D. Marty  
Received Date: 05/30/07  
Collected Date: 05/30/07  
Client Ref No: Log 6188, CL 1393

Veterinarian: **Diane Morrison**  
Clinic:  
Phone:  
Fax:

Submitter:  
Phone:  
Fax:  
Owner: **Marine Harvest Canada**  
Phone:  
Fax:(250) 850-3275

**Animal Data**  
Species: Atlantic Salmon  
Breed:  
Sex:  
Age:  
Premise ID:

### Case History

Five histo labeled 1, 2, 3, 5, and 6. Five virology samples for PCR for IHNV and VHSV. Fish rolling over in seine. Gill pallor. Please check hearts for anything unusual.

### Final Diagnosis

- 1a. Heart: myocardial karyomegaly, multifocal, mild (slides 1, 2, 5)
- 1b. Heart: epicarditis, lymphoplasmacytic, regionally diffuse, mild (slides 1, 2)
- 1c. Heart: endocarditis, lymphohistiocytic, with intralesional *Kudoa thyrsites* spores, multifocal, moderate (slide 2)
- 1d. Heart: endocarditis, lymphohistiocytic, multifocal, moderate (slide 5)
- 2a. Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slide 4), moderate (slide 1); multifocal, mild (slide 2)
- 2b. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slide 2)
- 2c. Liver: yellow-brown to yellow-green pigmented macrophage aggregates and sinusoidal macrophages, disseminated, mild (slides 2, 3, 4, 5)
- 3a. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, moderate (slides 1, 2, 3)
- 3b. Spleen: parenchymal golden pigment, scattered, intracellular, mild (slide 2)
- 3c. Spleen: splenic congestion, diffuse, moderate (slides 4, 5)
4. Head kidney: vascular congestion, diffuse, mild (slides 1, 4, 5)
5. Trunk kidney: renal tubular mineralization, focal, mild (slide 2)

**Final Comment:** These fish have several lesions that might have contributed to morbidity. Based on the clinical signs of gill pallor, other

diagnostic procedures to consider (if not already done) include gill histopathology and examination of blood smears for inclusions consistent with Viral Erythrocytic Necrosis.

Mild myocardial karyomegaly is somewhat common in cultured salmonids (e.g., 5.3% of the 503 Atlantic salmon hearts sampled as part of the BC Fish Health Auditing and Surveillance Program during 2006). The cause and significance is unknown, but there might be a genetic predisposition to developing the lesion. Karyomegaly in other cell types has been associated with exposure to algal toxins (e.g., hepatocytes exposed to microcystin LR in netpen liver disease). Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine.

*Kudoa thyrsites* is a myxosporean that is most common in skeletal muscle, but sometimes occurs in heart muscle. This example has characteristic stellate spores with four unequal polar capsules converging on one end. Extensive inflammation is common in severe infections (source Kent, M.L., and T.T. Poppe. 1998. Diseases of seawater netpen-reared salmonid fishes. Quadra Printers, Ltd. Nanaimo, B.C., Canada).

Lymphohistiocytic inflammation in the heart is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine.

The atrium in slide 3 seems dilated, but the tissues have no inflammation.

Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition. Basophilic cytoplasm in hepatocytes is an indication of active protein synthesis. It is normal in mature females producing protein for deposition in their eggs. In other fish it might be related to increased protein needed as part of an inflammatory response.

Pigment in the liver could be lipofuscin, hemosiderin, or both; pigment in the spleen is usually lipofuscin. Accumulation of lipofuscin 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. Conditions that lead to moderate to abundant 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.

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated. Splenic congestion is sometimes associated with bacterial or viral infections (e.g., VHSV).

Renal congestion has been associated with VHSV, bacteria, and it may occur as a sampling artefact.

Renal mineralization is common in cultured fish species; when severe, the condition is termed nephrocalcinosis. The lesion is not considered fatal, although feed conversion may be adversely affected. The pathogenesis is not fully understood, but renal mineralization has been experimentally reproduced through high carbon dioxide levels, magnesium deficiency, selenium toxicity, and a diet low in minerals (source, "Systemic Pathology of Fish", 1989, by H. Ferguson). Clinically, renal mineralization is most commonly associated with high carbon dioxide levels.

## Histopathology

Formalin-fixed tissues were submitted in 5 cassettes for histopathology (note that the slides are not in numerical order by cassette #).

Slide 1 (6188-1) - spleen, liver, heart, head kidney, trunk kidney

Slide 2 (6188-6) - spleen, liver, heart, head kidney, trunk kidney

Slide 3 (6188-3) - spleen, liver, heart (ventricle and atrium), head kidney, trunk kidney

Slide 4 (6188-5) - spleen, liver, heart (ventricle and atrium), head kidney, trunk kidney (with 3-mm -diameter Corpuscle of Stannius)

Slide 5 (6188-2) - spleen, liver, heart, head kidney

All organs were examined. Organs not listed below have no significant lesions.

**Quality control:** Liver autolysis: mild (slide 2), moderate (slides 3, 5), severe (slides 1, 4). Organs have no postfixation dehydration and no acid hematin deposits.

## Molecular Diagnostics

**PCR - IHNV** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/01/07 @ 4:48 PM

Specimen	ID	Test	Result
Tissue	A (#1)	PCR - IHNV	Negative
Tissue	B (#2)	PCR - IHNV	Negative
Tissue	C (#3)	PCR - IHNV	Negative
Tissue	D (#4)	PCR - IHNV	Negative
Tissue	E (#5)	PCR - IHNV	Negative

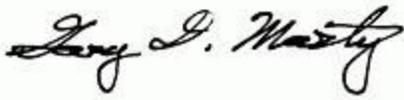
**PCR - VHSV** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/01/07 @ 4:48 PM

Specimen	ID	Test	Result
Tissue	A (#1)	PCR - VHSV	Negative
Tissue	B (#2)	PCR - VHSV	Negative
Tissue	C (#3)	PCR - VHSV	Negative
Tissue	D (#4)	PCR - VHSV	Negative
Tissue	E (#5)	PCR - VHSV	Negative

## Virology

**Tissue Culture** Resulted by: Hubert Wong Verified by: Liisa Nielsen on 06/29/07 @ 1:39 PM

Specimen	ID	Isolate	Result	Level
Tissue	A (#1)		No viruses isolated	
Tissue	B (#2)		No viruses isolated	
Tissue	C (#3)		No viruses isolated	
Tissue	D (#4)		No viruses isolated	
Tissue	E (#5)		No viruses isolated	



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-2117

Last Updated: 06/29/07 1:40 PM  
Pathologist: Gary D. Marty  
Received Date: 05/30/07  
Collected Date: 05/30/07  
Client Ref No: Log 6189, PO CL 1390

Veterinarian: **Diane Morrison**  
Clinic:  
Phone:  
Fax:

Submitter:  
Phone:  
Fax:  
Owner: **Marine Harvest Canada**  
Phone:  
Fax:(250) 850-3275

**Animal Data**  
Species: Atlantic Salmon  
Breed:  
Sex:  
Age:  
Premise ID:

### Case History

Submitted virology from Pen 4 and Pen 6 collected on May 23, 2007 and kept frozen. Fish had hemorrhage of visceral, organs, and muscles. Mortality is low. Please do PCR for IHNV and VHSV.

### Molecular Diagnostics

**PCR - IHNV** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/01/07 @ 4:48 PM

Specimen	ID	Test	Result
Tissue	A (Pen 4)	PCR - IHNV	Negative
Tissue	B (Pen 6)	PCR - IHNV	Negative

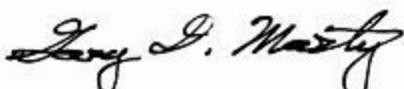
**PCR - VHSV** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/01/07 @ 4:50 PM

Specimen	ID	Test	Result
Tissue	A (Pen 4)	PCR - VHSV	Positive
Tissue	B (Pen 6)	PCR - VHSV	Positive

### Virology

**Tissue Culture** Resulted by: Hubert Wong Verified by: Liisa Nielsen on 06/29/07 @ 1:40 PM

Specimen	ID	Isolate	Result	Level
Tissue	A (Pen 4)		No viruses isolated	
Tissue	B (Pen 6)		No viruses isolated	



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-2118

Last Updated: 06/29/07 1:33 PM  
 Pathologist: Gary D. Marty  
 Received Date: 05/30/07  
 Collected Date: 05/30/07  
 Client Ref No: Log 6191, CL 1398

Veterinarian: **Diane Morrison**  
 Clinic:  
 Phone:  
 Fax:

Submitter:  
 Phone:  
 Fax:  
 Owner: **Marine Harvest Canada**  
 Phone:  
 Fax:(250) 850-3275

**Animal Data**  
 Species: Atlantic Salmon  
 Breed:  
 Sex:  
 Age:  
 Premise ID:

### Case History

Submitted 6 samples for PCR for IHNV and VHSV. Samples labeled 1, 3, 5, 7, 8, and 9.

### Molecular Diagnostics

**PCR - IHNV** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/01/07 @ 4:50 PM

Specimen	ID	Test	Result
Tissue	A (Pen 1)	PCR - IHNV	Negative
Tissue	B (#3)	PCR - IHNV	Negative
Tissue	C (#5)	PCR - IHNV	Negative
Tissue	D (#7)	PCR - IHNV	Negative
Tissue	E (Pen 8)	PCR - IHNV	Negative
Tissue	F (Pen 9)	PCR - IHNV	Negative

**PCR - VHSV** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/01/07 @ 4:50 PM

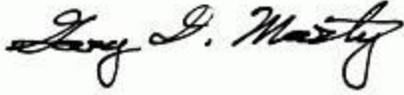
Specimen	ID	Test	Result
Tissue	A (Pen 1)	PCR - VHSV	Negative
Tissue	B (#3)	PCR - VHSV	Positive
Tissue	C (#5)	PCR - VHSV	Negative
Tissue	D (#7)	PCR - VHSV	Negative
Tissue	E (Pen 8)	PCR - VHSV	Positive
Tissue	F (Pen 9)	PCR - VHSV	Negative

### Virology

**Tissue Culture** Resulted by: Hubert Wong Verified by: Hubert Wong on 06/29/07 @ 1:33 PM

Specimen	ID	Isolate	Result	Level
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Tissue	A (Pen 1)	No viruses isolated
Tissue	B (#3)	No viruses isolated
Tissue	C (#5)	No viruses isolated
Tissue	D (#7)	No viruses isolated
Tissue	E (Pen 8)	No viruses isolated
Tissue	F (Pen 9)	No viruses isolated



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-2119

Last Updated: 06/29/07 1:39 PM  
Pathologist: Gary D. Marty  
Received Date: 05/30/07  
Collected Date: 05/30/07  
Client Ref No: Log 6197, PO CL 1397

Veterinarian: **Diane Morrison**  
Clinic:  
Phone:  
Fax:

Submitter:  
Phone:  
Fax:  
Owner: **Marine Harvest Canada**  
Phone:  
Fax:(250) 850-3275

**Animal Data**  
Species: Atlantic Salmon  
Breed:  
Sex:  
Age:  
Premise ID:

### Case History

Submitted one sample for PCR for IHN and VHS. Fish with signs of VHS.

### Molecular Diagnostics

**PCR - IHN** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/01/07 @ 4:50 PM

Specimen	ID	Test	Result
Tissue	#1	PCR - IHNV	Negative

**PCR - VHSV** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/01/07 @ 4:50 PM

Specimen	ID	Test	Result
Tissue	#1	PCR - VHSV	Negative

### Virology

**Tissue Culture** Resulted by: Hubert Wong Verified by: Liisa Nielsen on 06/29/07 @ 1:39 PM

Specimen	ID	Isolate	Result	Level
Tissue	#1		No viruses isolated	



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-2120

Last Updated: 06/29/07 1:35 PM

Pathologist: Gary D. Marty

Received Date: 05/30/07

Collected Date: 05/30/07

Client Ref No: Log 6198, PO CL 1395

Veterinarian: **Diane Morrison**

Clinic:

Phone:

Fax:

Submitter:

Phone:

Fax:

Owner: **Marine Harvest Canada**

Phone:

Fax:(250) 850-3275

### Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID:

### Case History

Histo sampled from 5 moribunds from pen 1. Fish were killed by concussion blow. Fish were lethargic, gasping and rolling over. Plankton bloom, suspect *Chrysochromulina* sp. Gills appeared irritated. No signs of disease internally. Virology for PCR for IHNV and VHSV to rule out viral etiology.

### Final Diagnosis

- 1a. Liver: hepatic necrosis, acute, multifocal, moderate (consistent with viral hemorrhagic septicemia virus, VHSV; slide 2A)
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate (slides 1A, 3A), severe (slides 4A, 5A)
- 1c. Liver: pericholangitis, lymphocytic, focal, mild (slides 2A, 3A)
- 1d. Liver: sinusoidal congestion, acute, multifocal, moderate (slide 5A)
- 2a. Spleen: peritonitis, chronic, regionally diffuse, with fibrocellular fronds, mild (slides 1A, 2A, 4A, 5A), moderate (slide 3A)
- 2b. Spleen: parenchymal golden pigment, scattered, intracellular, mild (slides 1A, 4A)
- 3a. Head kidney: interstitial vascular congestion, diffuse, mild (slide 4A)
- 3b. Head kidney: moderate numbers of intracytoplasmic eosinophilic granules in endothelial cells, diffuse (slide 4A)
4. Gill: lamellar hyperplasia and fusion, focal, mild (slide 5B)

**Final Comment:** These fish have a number of lesions that might have contributed to morbidity. Some lesions could be related to VHSV; others, to harmful algal blooms. Few of the lesions are severe enough to have killed fish alone, but the combination of lesions might have been more significant.

Hepatic necrosis can be caused by inadequate vascular perfusion or direct cytotoxicity from viral or bacterial infections. In this case, PCR results provide evidence that VHSV is the cause. Hepatic necrosis is fairly common in salmon that die in marine net pens, affecting 7.5% of the 505 Atlantic salmon and 5% of the 134 Pacific salmon examined in 2006 as part of the Province's Fish Health Auditing and Surveillance Program.

Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition. Lymphocytic inflammation around bile ductules (liver) is evidence of chronic immune stimulation. This type of inflammation can result from bacteria ascending from the intestine to the liver through the biliary system.

Sinusoidal congestion in the liver is evidence of sinusoidal damage. In BC Atlantic salmon, hepatic sinusoidal congestion is an uncommon feature of infection with viral hemorrhagic septicemia virus and *Listonella anguillarum*. Sinusoidal congestion is one of the classic lesions associated with ISAV infection, but ISAV has never been identified in British Columbia.

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated. The golden pigment in the spleen most likely is lipofuscin. Accumulation of lipofuscin 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. Conditions that lead to moderate to abundant lipofuscin have been associated with decreased growth and survival in several studies.

Renal congestion has been associated with VHSV, bacteria, and it may occur as a sampling artefact. Accumulation of eosinophilic granules in the cytoplasm of cells lining small vessels in the kidney is a distinctive finding in Atlantic salmon. These granules probably accumulate in response to systemic immune stimulation. The granules might be part of eosinophilic granular cells or endothelial cells; granule size seems too large and variable for eosinophilic granular cells. I have seen these granules associated with *Piscirickettsia salmonis*, but other cases (like this one) have no known cause.

Gill lamellar fusion with lamellar hypertrophy may be a result of physical damage from exposure to a parasite or diatoms, but this lesion contains no organisms (they might have been lost during processing).

## Histopathology

Formalin-fixed tissues were submitted in 5 cassettes for histopathology. Gills were removed from the original (A) cassettes and placed in separate (B) cassettes. Note that the slides are not in numerical order by cassette #.

Slide 1A (6198-3) - liver, trunk kidney (2 pieces), spleen, heart, and mesenteric adipose tissue

Slide 1B (6198-3) - gill

Slide 2A (6198-4) - liver, trunk kidney, head kidney, spleen, heart, intestine and mesenteric adipose tissue

Slide 2B (6198-4) - gill

Slide 3A (6198-1) - liver, trunk kidney, head kidney, spleen, heart, intestine and mesenteric adipose tissue

Slide 3B (6198-1) - gill

Slide 4A (6198-5) - liver, trunk kidney, head kidney, spleen, heart, intestine and mesenteric adipose tissue

Slide 4B (6198-5) - gill

Slide 5A (6198-2) - liver, trunk kidney, head kidney, spleen, heart, intestine and mesenteric adipose tissue

Slide 5B (6198-2) - gill

All organs were examined. Organs not listed below have no significant lesions.

**Quality control:** Liver autolysis: none (slide 5A), mild (slides 1A, 2A, 3A, 4A). Foci of erythrocytes in congested hepatic sinusoids have precipitates of acid hematin, probably as a result of these tissues becoming acidic before or during fixation. Organs have no postfixation dehydration. Gills were not decalcified, resulting in moderate artefact of the bony parts of the gill (the remainder of the gills have no more than mild artefact).

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/01/07 @ 4:50 PM

Specimen	ID	Test	Result
Tissue	A (#1)	PCR - IHNV	Negative
Tissue	B (#2)	PCR - IHNV	Negative
Tissue	C (#3)	PCR - IHNV	Negative
Tissue	D (#4)	PCR - IHNV	Negative
Tissue	E (#5)	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/01/07 @ 4:50 PM

Specimen	ID	Test	Result
Tissue	A (#1)	PCR - VHSV	Positive
Tissue	B (#2)	PCR - VHSV	Negative
Tissue	C (#3)	PCR - VHSV	Negative
Tissue	D (#4)	PCR - VHSV	Negative
Tissue	E (#5)	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Liisa Nielsen Verified by: Liisa Nielsen on 06/29/07 @ 1:34 PM

Specimen	ID	Isolate	Result	Level
Tissue	A (#1)		No viruses isolated	
Tissue	B (#2)		No viruses isolated	
Tissue	C (#3)		No viruses isolated	
Tissue	D (#4)		No viruses isolated	
Tissue	E (#5)		No viruses isolated	



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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END OF REPORT

## Final Report AHC Case: 07-2123

Last Updated: 06/29/07 1:39 PM

Pathologist: Gary D. Marty

Received Date: 05/30/07

Collected Date: 05/30/07

Client Ref No: Log 6199, PO CL 1396

Veterinarian: **Diane Morrison**

Clinic:

Phone:

Fax:

Submitter:

Phone:

Fax:

Owner: **Marine Harvest Canada**

Phone:

Fax:(250) 850-3275

### Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID:

### Case History

Submitted 5 histo and 5 virology samples. Similar to case 6198, fish lethargic and gasping at surface. Five moribunds sampled. Gill damage on fish #1 may have been old damage. Diffusers, tarps down. Five virology for PCR for IHNV and VHSV. Rule out viral etiology. Fish had no signs of infectious disease.

### Final Diagnosis

1a. Gill: lamellar hyperplasia and fusion, multifocal, with intralesional spiny algae consistent with *Dictyocha* sp., moderate (slides 1B, 2B, 3B, 5B)

1b. Gill: lamellar subepithelial edema, multifocal, mild (slides 1B, 4B)

2a. Liver: hepatic necrosis, acute, multifocal, consistent with viral hemorrhagic septicemia virus, VHSV, mild (slide 1A), moderate (slide 3), severe (slide 5)

2b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slide 4), severe (slides 1A, 2A, 5)

2c. Liver: hepatitis, lymphocytic, focal, mild (slide 1A)

2d. Liver: pericholangitis, lymphocytic, focal, mild (slide 5)

2e. Liver: sinusoidal congestion, acute, multifocal, moderate (slide 2A)

2f. Liver: yellow-brown to yellow-green pigmented macrophage aggregates and sinusoidal macrophages, disseminated, mild (slide 4)

3a. Spleen: peritonitis, chronic, regionally diffuse, with fibrocellular fronds, mild (slides 1A), moderate (slides 3, 5)

3b. Mesenteric adipose tissue: peritonitis, chronic, focal, with fibrocellular fronds, mild (slides 3)

3c. Intestine: peritonitis, chronic, focal, with fibrocellular fronds, mild (slides 5)

4a. Head kidney: interstitial cell hyperplasia, multifocal to diffuse, mild (slide 3)

5. Heart: endocarditis, multifocal, lymphoplasmacytic, moderate (slide 4)

**Final Comment:** These fish have a number of lesions that might have contributed to morbidity. Some lesions could be related to VHSV; others, to harmful algae. Clarifying the relation of lesions to cause might benefit from PCR and histopathology on the same fish. Few of the lesions are severe enough to have killed fish alone, but the combination of lesions might have been more significant.

Gill lamellar fusion with lamellar hypertrophy may be a result of physical damage from exposure to a parasite or diatoms. The presence of spiny processes within the lesion is consistent with *Dictyocha* sp. as the cause of the lesion. A good example in slide 1B is at coordinates 22 x 110.7 on the microscope in my office (see photo).

Separation of gill lamellar epithelium from underlying pillar cells can be a result of edema, and it also is a common postmortem artefact. In this case, accumulation of wispy proteinaceous fluid deep to the separated epithelium supports the diagnosis of edema over artefact. Lamellar edema is commonly associated with exposure to toxicants, including formalin and hydrogen peroxide overdose. In this case, it probably is related to irritation from the *Dictyocha*. Lamellar edema is reversible if the inciting cause is removed.

Hepatic necrosis can be caused by inadequate vascular perfusion or direct cytotoxicity from viral or bacterial infections. In this case, PCR results provide evidence that VHSV could be the cause, but necrosis might be a result of adverse environmental conditions. Hepatic necrosis is fairly common in salmon that die in marine net pens, affecting 7.5% of the 505 Atlantic salmon and 5% of the 134 Pacific salmon examined in 2006 as part of the Province's Fish Health Auditing and Surveillance Program.

Lymphocytic inflammation in the liver is evidence of chronic immune stimulation; differentials include a low grade bacterial or viral infection.

Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition.

Pigment in the liver could be lipofuscin, hemosiderin, or both. 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. 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.

Lymphocytic inflammation around bile ductules (liver) is evidence of chronic immune stimulation. This type of inflammation can result from bacteria ascending from the intestine to the liver through the biliary system. In slide 5, enlarged nuclei of biliary epithelial cells are an uncommon feature of pericholangitis in Atlantic salmon.

Sinusoidal congestion in the liver is evidence of sinusoidal damage. In BC Atlantic salmon, hepatic sinusoidal congestion is an uncommon feature of infection with viral hemorrhagic septicemia virus and *Listonella anguillarum*. Sinusoidal congestion is one of the classic lesions associated with ISAV infection, but ISAV has never been identified in British Columbia.

Peritonitis of the spleen, intestine, and mesenteric adipose tissue is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

Interstitial cell hyperplasia in the kidney results from increased demand for erythrocytes or white blood cells somewhere in the body (renal interstitial cells are the blood-forming or hematopoietic cells in the kidney).

Lymphoplasmacytic inflammation in the heart is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine.

## Histopathology

Formalin-fixed tissues were submitted in 5 cassettes for histopathology. Gills were removed from the original cassettes and placed in separate (B) cassettes. Note that the slides are not in numerical order by cassette #.

Slide 1A (6199-4) - liver, trunk kidney, head kidney, spleen, heart, intestine and mesenteric adipose tissue

Slide 1B (6199-4) - gill

Slide 2A (6199-3) - liver, trunk kidney, head kidney, spleen, heart, intestine and mesenteric adipose tissue

Slide 2B (6199-3) - gill

Slide 3 (6199-1) - liver, trunk kidney, head kidney, spleen, heart, intestine and mesenteric adipose tissue

Slide 3B (6199-1) - gill

Slide 4 (6199-5) - liver, trunk kidney (2 pieces), spleen, heart, intestine and mesenteric adipose tissue

Slide 4B (6199-5) - gill

Slide 5 (6199-2) - liver, trunk kidney, head kidney, spleen, heart, intestine and mesenteric adipose tissue

Slide 5B (6199-2) - gill

All organs were examined. Organs not listed below have no significant lesions.

**Quality control:** Liver autolysis: none (slides 1A, 2A, 3, 4, 5). Foci of erythrocytes in the spleen (slide 4) and liver (slide 2A) have precipitates of acid hematin, probably as a result of these tissues becoming acidic before or during fixation. Organs have no postfixation dehydration. Gills were not decalcified, resulting in moderate artefact of the bony parts of the gill (the remainder of the gills have no more than mild artefact).

### Molecular Diagnostics

**PCR - IHNV** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/01/07 @ 4:51 PM

Specimen	ID	Test	Result
Tissue	A (#1)	PCR - IHNV	Negative
Tissue	B (#2)	PCR - IHNV	Negative
Tissue	C (#3)	PCR - IHNV	Negative
Tissue	D (#4)	PCR - IHNV	Negative
Tissue	E (#5)	PCR - IHNV	Negative

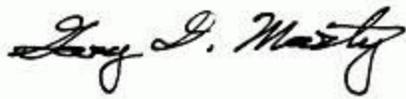
**PCR - VHSV** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/01/07 @ 4:51 PM

Specimen	ID	Test	Result
Tissue	A (#1)	PCR - VHSV	Positive
Tissue	B (#2)	PCR - VHSV	Negative
Tissue	C (#3)	PCR - VHSV	Negative
Tissue	D (#4)	PCR - VHSV	Negative
Tissue	E (#5)	PCR - VHSV	Negative

### Virology

**Tissue Culture** Resulted by: Hubert Wong Verified by: Liisa Nielsen on 06/29/07 @ 1:39 PM

Specimen	ID	Isolate	Result	Level
Tissue	A (#1)		No viruses isolated	
Tissue	B (#2)		No viruses isolated	
Tissue	C (#3)		No viruses isolated	
Tissue	D (#4)		No viruses isolated	
Tissue	E (#5)		No viruses isolated	



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-2227

Last Updated: 07/04/07 4:01 PM

Pathologist: Gary D. Marty

Received Date: 06/07/07

Collected Date: 06/07/07

Client Ref No: Log 6193, PO CL 1400

Veterinarian: **Diane Morrison**

Clinic:

Phone:

Fax:

Submitter:

Phone:

Fax:

Owner: **Marine Harvest Canada**

Phone:

Fax:(250) 850-3275

### Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID:

### Case History

Submitted 12 samples for PCR. Samples collected on May 29 and kept frozen. Virology samples for PCR for IHN and VHS labeled 6193 1 through 6193 6 and 6193 31 through 6193 36. Routine fish health check, no mortality to speak of.

### Molecular Diagnostics

**PCR - IHN** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/12/07 @ 5:44 PM

Specimen	ID	Test	Result
Tissue	6193-1	PCR - IHNV	Negative
Tissue	-2	PCR - IHNV	Negative
Tissue	-3	PCR - IHNV	Negative
Tissue	-4	PCR - IHNV	Negative
Tissue	-5	PCR - IHNV	Negative
Tissue	-6	PCR - IHNV	Negative
Tissue	-31	PCR - IHNV	Negative
Tissue	-32	PCR - IHNV	Negative
Tissue	-33	PCR - IHNV	Negative
Tissue	-34	PCR - IHNV	Negative
Tissue	-35	PCR - IHNV	Negative
Tissue	-36	PCR - IHNV	Negative

**PCR - VHSV** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/12/07 @ 11:39 AM

Specimen	ID	Test	Result
Tissue	6193-1	PCR - VHSV	Negative
Tissue	-2	PCR - VHSV	Negative
Tissue	-3	PCR - VHSV	Negative
Tissue	-4	PCR - VHSV	Negative
Tissue	-5	PCR - VHSV	Negative

Tissue	-6	PCR - VHSV	Negative
Tissue	-31	PCR - VHSV	Negative
Tissue	-32	PCR - VHSV	Negative
Tissue	-33	PCR - VHSV	Negative
Tissue	-34	PCR - VHSV	Negative
Tissue	-35	PCR - VHSV	Negative
Tissue	-36	PCR - VHSV	Negative

**Virology**

**Tissue Culture** Resulted by: Liisa Nielsen Verified by: Dr. J. Robinson on 07/04/07 @ 4:01 PM

Specimen	ID	Isolate	Result	Level
Tissue	6193-1		No viruses isolated	
Tissue	-2		No viruses isolated	
Tissue	-3		No viruses isolated	
Tissue	-4		No viruses isolated	
Tissue	-5		No viruses isolated	
Tissue	-6		No viruses isolated	
Tissue	-31		No viruses isolated	
Tissue	-32		No viruses isolated	
Tissue	-33		No viruses isolated	
Tissue	-34		No viruses isolated	
Tissue	-35		No viruses isolated	
Tissue	-36		No viruses isolated	



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-2321

Last Updated: 07/11/07 1:32 PM  
Pathologist: Gary D. Marty  
Received Date: 06/13/07  
Collected Date: 06/13/07  
Client Ref No: 6214, PO CL 16759

Veterinarian: **Diane Morrison**  
Clinic:  
Phone:  
Fax:

Submitter:  
Phone:  
Fax:  
Owner: **Marine Harvest Canada**  
Phone:  
Fax:(250) 850-3275

**Animal Data**  
Species: Atlantic Salmon  
Breed:  
Sex:  
Age:  
Premise ID:

### Case History

Submitted three samples for PCR IHN and VHS and one for histology. Collected on June 7 and frozen. Re-sample at same site as our case 6182 (2007/02114). One hiso taken.

### Final Diagnosis

1. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
2. Mesenteric adipose tissue: peritonitis, granulomatous, regionally diffuse, with occasional fine fibrocellular fronds, moderate

**Final Comment:** This fish has mild to moderate lesions that might have contributed to morbidity; however, the lesions do not seem of sufficient severity to have caused death.

Basophilic cytoplasm in hepatocytes is an indication of active protein synthesis. It is normal in mature females producing protein for deposition in their eggs. In other fish it might be related to increased protein needed as part of an inflammatory response.

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

### Histopathology

Formalin-fixed tissues were submitted in a cassette for histopathology and processed onto one slide.

Slide 1 (AT-8-6-7, 6214) - liver, heart, trunk kidney, intestinal ceca, and mesenteric fat

All organs were examined. Organs not listed below have no significant lesions.

**Quality control:** Liver autolysis: none. The trunk kidney and the margins of the liver have evidence of postfixation dehydration (e.g., nuclei stain dull blue; erythrocyte cytoplasm stains yellow instead of red; cytoplasm of other cell types stains poorly or not at all). This most commonly results when preserved tissues are removed from liquid for more than a few minutes (e.g., during shipment). Erythrocyte cytoplasm stains poorly; this often occurs when tissues are preserved in an alcohol-based fixative (e.g., Bouin's or Davidson's) and then returned to water or a

water- based fixative (e.g., 10% formalin) before being processed into paraffin. Organs have no acid hematin deposits.

### Molecular Diagnostics

**PCR - IHNV** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/18/07 @ 5:00 PM

Specimen	ID	Test	Result
Tissue	6214-1	PCR - IHNV	Negative
Tissue	6214-2	PCR - IHNV	Negative
Tissue	6214-3	PCR - IHNV	Negative

**PCR - VHSV** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/18/07 @ 12:09 PM

Specimen	ID	Test	Result
Tissue	6214-1	PCR - VHSV	Negative
Tissue	6214-2	PCR - VHSV	Negative
Tissue	6214-3	PCR - VHSV	Negative

### Virology

**Tissue Culture** Resulted by: Hubert Wong Verified by: Dr. J. Robinson on 07/11/07 @ 1:32 PM

Specimen	ID	Isolate	Result	Level
Tissue	6214-1		No viruses isolated	
Tissue	6214-2		No viruses isolated	
Tissue	6214-3		No viruses isolated	



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-2323

Last Updated: 07/11/07 1:21 PM  
Pathologist: Gary D. Marty  
Received Date: 06/13/07  
Collected Date: 06/13/07  
Client Ref No: 6202, PO CL 16755

Veterinarian: **Diane Morrison**  
Clinic:  
Phone:  
Fax:

Submitter:  
Phone:  
Fax:  
Owner: **Marine Harvest Canada**  
Phone:  
Fax:(250) 850-3275

**Animal Data**  
Species: Atlantic Salmon  
Breed:  
Sex:  
Age:  
Premise ID:

### Case History

Submitted two samples collected on May 29 and kept frozen. Both samples submitted for PCR for IHN and VHS. Mortality is low at sea site and no signs of VHS at site. Re-sample at same site as #6086 (AHC 1013).

### Molecular Diagnostics

**PCR - IHNV** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/18/07 @ 5:00 PM

Specimen	ID	Test	Result
Tissue	6202-1	PCR - IHNV	Negative
Tissue	6202-2	PCR - IHNV	Negative

**PCR - VHSV** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/18/07 @ 12:09 PM

Specimen	ID	Test	Result
Tissue	6202-1	PCR - VHSV	Negative
Tissue	6202-2	PCR - VHSV	Negative

### Virology

**Tissue Culture** Resulted by: Hubert Wong Verified by: Dr. J. Robinson on 07/11/07 @ 1:21 PM

Specimen	ID	Isolate	Result	Level
Tissue	6202-1		No viruses isolated	
Tissue	6202-2		No viruses isolated	



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-2325

Last Updated: 07/11/07 1:22 PM  
Pathologist: Gary D. Marty  
Received Date: 06/13/07  
Collected Date: 06/13/07  
Client Ref No: 6217 PO CL 1676 3

Veterinarian: **Diane Morrison**  
Clinic:  
Phone:  
Fax:

Submitter:  
Phone:  
Fax:  
Owner: **Marine Harvest Canada**  
Phone:  
Fax:(250) 850-3275

**Animal Data**  
Species: Atlantic Salmon  
Breed:  
Sex:  
Age:  
Premise ID:

### Case History

Submitted three virology samples for PCR for IHN and VHS. Histo from suspect Netpen liver disease fish. Livers small and greenish brown.

### Final Diagnosis

- 1a. Liver: hepatocellular karyomegaly and megalocytosis, diffuse, mild (slide 3), moderate (slides 1, 2)
- 1b. Liver: yellow-brown-pigmented sinusoidal macrophages, diffuse, moderate (slides 1, 2), severe (slide 3)
- 1c. Liver: hepatocellular single cell necrosis, diffuse, mild (slides 1, 2, 3)
- 1d. Liver: hepatitis, lymphoplasmacytic, multifocal, mild (slide 3), moderate (slides 1, 2)
- 1e. Liver: hydropic degeneration, diffuse, mild (slides 1, 2, 3)
2. Spleen and surrounding mesenteric adipose tissue: peritonitis, granulomatous, diffuse, mild (slides 2, 3), with intralesional vacuoles about 1 mm in diameter, severe (slide 1)
- 3a. Trunk kidney: renal tubular epithelial necrosis, multifocal, acute, moderate (slide 3)
- 3b. Trunk kidney: renal tubular luminal casts of protein and yellow-brown pigment, multifocal, mild (slide 2)

**Final Comment:** Many features of these slides are consistent with gross assessment of netpen liver disease, which is thought to be caused by the algal toxin microcystin-LR in the water. Other toxins in the water or feed (e.g., aflatoxins) are less likely differentials. All liver sections have a fairly consistent pattern: a combination of sinusoidal pigment accumulation (probably lipofuscin), single cell necrosis, hepatocellular karyomegaly, and variable amounts of lymphoplasmacytic inflammation. Hepatic megalocytosis can result from exposure to several types of toxicants, including aflatoxins, pyrrolizidine alkaloids, complex chemical mixtures from marine sediment extracts, and the algal toxin microcystin-LR. Hydropic degeneration in is probably a precursor to the more chronic lesions. 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.

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated. Vacuoles probably are a result of vaccine material lost during tissue processing.

Renal tubular epithelial necrosis (slide 3) results from acute damage to renal epithelial cells; damage is reversible if the basement membrane is spared (as in this case). Renal tubular epithelial necrosis was fairly common among fish sampled in 2006 as part of the Ministry's Fish Health Auditing and Surveillance Program: Atlantic salmon (prevalence = 7.5%; n = 495) and Pacific salmon (prevalence = 2.2%; n = 134); the cause was not determined in many cases. Differentials include viral hemorrhagic septicemia virus (VHSV) and exposure to toxins (e.g., bacterial toxins, heavy metals, or aminoglycoside antibiotics such as gentamicin). In this case, PCR results rule out VHSV if the tissues tested included tissues from the fish on slide 3.

Pigments in the renal tubules (e.g., slide 2) probably include lipofuscin. Accumulation of lipofuscin 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.

## Histopathology

Formalin-fixed tissues were submitted in 3 cassettes for histopathology.

Slide 1 (6217-1) - spleen and surrounding mesenteric adipose tissue, liver, heart, and head kidney/trunk kidney transition region

Slide 2 (6217-2) - spleen and surrounding mesenteric adipose tissue, liver, heart, and head kidney/trunk kidney transition region

Slide 3 (6217-3) - heart, liver, spleen, and trunk kidney

All organs were examined. Organs not listed below have no significant lesions.

**Quality control:** Liver autolysis: none. Organs have no postfixation dehydration and no acid hematin deposits.

## Molecular Diagnostics

**PCR - IHNV** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/18/07 @ 5:00 PM

Specimen	ID	Test	Result
Tissue	6217-1	PCR - IHNV	Negative
Tissue	6217-2	PCR - IHNV	Negative
Tissue	6217-3	PCR - IHNV	Negative

**PCR - VHSV** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/18/07 @ 12:09 PM

Specimen	ID	Test	Result
Tissue	6217-1	PCR - VHSV	Negative
Tissue	6217-2	PCR - VHSV	Negative
Tissue	6217-3	PCR - VHSV	Negative

## Virology

**Tissue Culture** Resulted by: Hubert Wong Verified by: Dr. J. Robinson on 07/11/07 @ 1:22 PM

Specimen	ID	Isolate	Result	Level
Tissue	6217-1		No viruses isolated	
Tissue	6217-2		No viruses isolated	



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-2330

Last Updated: 07/11/07 1:22 PM  
Pathologist: Gary D. Marty  
Received Date: 06/13/07  
Collected Date: 06/13/07  
Client Ref No: 6213; PO CL16762

Veterinarian: **Diane Morrison**  
Clinic:  
Phone:  
Fax:

Submitter:  
Phone:  
Fax:  
Owner: **Marine Harvest Canada**  
Phone:  
Fax:(250) 850-3275

**Animal Data**  
Species: Atlantic Salmon  
Breed:  
Sex:  
Age:  
Premise ID:

### Case History

Submitted one histo and one viral sample, collected on June 7. Sorry, no information on lesions at this time.

### Final Diagnosis

1. Stomach: peritonitis, granulomatous, diffuse, with intralesional vacuoles about 1 mm in diameter, severe
2. Spleen: peritonitis, granulomatous, diffuse, with intralesional vacuoles about 0.5 mm in diameter, moderate

**Final Comment:** Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated. Vacuoles probably are a result of vaccine material lost during tissue processing. This lesion might have been of sufficient severity to have killed the fish. Alternatively, the fish had significant lesions (e.g., mouth rot or brain lesions) in organs not submitted for histopathology.

### Histopathology

Formalin-fixed tissues were submitted in a cassette for histopathology and processed onto one slide.

Slide 1 (LB 24, 6/8/7; 6213) - liver, heart, spleen, and stomach

All organs were examined. Organs not listed in the diagnoses have no significant lesions.

**Quality control:** Liver autolysis: severe. Organs have no postfixation dehydration and no acid hematin deposits.

### Molecular Diagnostics

**PCR - IHN** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/19/07 @ 10:12 AM

Specimen	ID	Test	Result
Tissue	organs	PCR - IHN	Negative

**PCR - VHSV** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/19/07 @ 10:12 AM

Specimen	ID	Test	Result
Tissue	organs	PCR - VHSV	Negative

### Virology

**Tissue Culture** Resulted by: Hubert Wong Verified by: Dr. J. Robinson on 07/11/07 @ 1:22 PM

Specimen	ID	Isolate	Result	Level
Tissue	organs		No viruses isolated	



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-2373

Last Updated: 06/21/07 11:55 AM

Pathologist: Gary D. Marty

Received Date: 06/18/07

Collected Date: 06/18/07

Client Ref No: Bennet

Veterinarian: **Barry Milligan**

Clinic: **Grieg Seafoods BC Ltd.**

Phone: (250) 286-0838

Fax: (250) 286-1883

Submitter:

Phone:

Fax:

Owner: **Grieg Seafoods BC Ltd.**

Phone: (250) 286-0838

Fax: (250) 286-1883

### Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID:

### Case History

Submitted formalized tissue for histology. June 14, low level of BKD present. *Dictyocha* (phytoplankton) in high numbers near farm. Fining at surface, slack tide. Discoloured gills, friable livers.

### Final Diagnosis

- 1a. Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slide 4), moderate (slides 2, 3), severe (slide 1)
- 1b. Liver: hepatic necrosis, acute, multifocal, mild (slide 4), moderate (slides 2, 3, 5A)
- 1c. Liver: sinusoidal congestion, acute, multifocal, mild (slides 1, 2, 4), moderate (slide 3)
- 2a. Spleen: peritonitis, granulomatous, regionally diffuse, with occasional fine fibrocellular fronds, severe (slides 2, 3)
- 2b. Spleen: splenitis, granulomatous, focal, moderate (slide 5A)
- 3a. Trunk kidney: nephritis, interstitial, granulomatous, multifocal, severe (slide 5A)
- 3b. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild (slide 3)

**Final Comment:** Hepatic necrosis and granulomatous inflammation are the most significant lesions in these fish. The most common organism associated with disseminated granulomas in BC salmon is *Renibacterium salmoninarum*, the cause of bacterial kidney disease. However, lack of organisms on the Gram stain mostly rules out *Renibacterium salmoninarum* as the cause of the granulomas in slide 5A. The gills have no *Dictyocha*, but organisms might have been lost during processing; also, moderate gill autolysis limited my ability to interpret the sections.

Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition.

Hepatic necrosis can be caused by inadequate vascular perfusion or direct cytotoxicity from viral or bacterial infections (e.g., viral hemorrhagic septicemia virus, *Piscirickettsia salmonis*, and *Renibacterium salmoninarum*); the cause is not determined in most cases. This case has no obvious organisms (i.e., no bacteria on Twort's Gram stain of slides 2 and 5A). Lack of proliferative lesions in the biliary system is evidence against a chronic toxic cause for the hepatic necrosis. Hepatic necrosis is somewhat common in salmon that die in marine net pens, affecting 7.5% of the 505 Atlantic salmon and 5% of the 134 Pacific salmon examined in 2006 as part of the Province's Fish Health Auditing and

## Surveillance Program.

Sinusoidal congestion in the liver is evidence of sinusoidal damage. In BC Atlantic salmon, hepatic sinusoidal congestion is an uncommon feature of infection with viral hemorrhagic septicemia virus and *Listonella anguillarum*. Sinusoidal congestion is one of the classic lesions associated with ISAV infection, but ISAV has never been identified in British Columbia.

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated. In slide 2, the lack of bacteria in the Gram stain mostly rules out *Renibacterium salmoninarum* as a differential for a 600- $\mu$ m- diameter focus of macrophages.

Renal tubular epithelial protein droplets are normal in some species, or they might be an indication of glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2006 as part of the Ministry's Fish Health Auditing and Surveillance Program: Atlantic salmon (prevalence = 22%; n = 495) and Pacific salmon (prevalence = 38%; n = 134).

## Histopathology

Formalin-fixed tissues were submitted in 6 cassettes for histopathology.

Slide 1 - spleen, liver, trunk kidney, and intestinal ceca

Slide 2 - spleen, liver, trunk kidney

Slide 3 - spleen, liver, trunk kidney

Slide 4 - spleen, liver, trunk kidney

Slide 5A - spleen, liver, trunk kidney

Slide 5B - gill

Slide 6 - gill (3 pieces)

All organs were examined. A Twort's Gram stain was done on slides 2, 5A, 5B, and 6. Organs not listed below have no significant lesions.

**Quality control:** Liver autolysis: mild (slides 1, 3), moderate (slides 2, 4, 5A); gill preservation is fair. Large foci of erythrocytes (e.g., foci of hepatic congestion in slide 1) have precipitates of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic before or during fixation. Organs have no postfixation dehydration.



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-2389

Last Updated: 06/21/07 2:10 PM

Pathologist: Gary D. Marty

Received Date: 06/19/07

Collected Date: 06/19/07

Client Ref No: SS-201

Veterinarian: **Dr. Sonja Saksida**

Clinic: **Sea to Sky Veterinary Ser**

Phone: (250) 287-2363

Fax: (250) 287-2430

Submitter:

Phone:

Fax:

Owner:

Phone:

Fax:

### Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID:

### Case History

Submitted formalized tissue for histology. Mortality increasing, fish exhibiting large amounts of serosanguineous fluid in the abdomen.

### Final Diagnosis

1a. Liver: hepatocellular karyomegaly and megalocytosis, diffuse, moderate (slides 1-1, 3-1A)

1b. Liver: yellow-brown-pigmented sinusoidal macrophages, diffuse, abundant (slides 1-1, 3-1A)

1c. Liver: hepatocellular single cell necrosis, diffuse, mild (slide 3-1A), moderate (slide 1-1)

1d. Liver: hepatitis, lymphoplasmacytic, multifocal, mild (slide 3-1A), moderate (slides 1-1)

1e. Liver: hydropic degeneration, diffuse, mild (slides 1-1, 3-1A)

2a. Mesenteric adipose tissue: atrophy, diffuse, moderate (slide 1-1)

2b. Mesenteric adipose tissue: steatitis, granulomatous, multifocal, with intralesional vacuoles about 50 µm in diameter, moderate (slide 1-1)

2c. Stomach: peritonitis, granulomatous, diffuse, with intralesional vacuoles about 1 mm in diameter, severe (slide 3-1A)

3a. Trunk kidney: renal tubular mineralization, multifocal, mild (slide 3-1A)

3b. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild (slide 3-1A)

**Final Comment:** Many features of these slides are consistent with netpen liver disease, which is thought to be caused by the algal toxin microcystin-LR in the water. Other toxins in the water or feed (e.g., aflatoxins) are less likely differentials. All liver sections have a fairly consistent pattern: a combination of sinusoidal pigment accumulation (probably lipofuscin), single cell necrosis, hepatocellular karyomegaly, and variable amounts of lymphoplasmacytic inflammation. Hepatic megalocytosis can result from exposure to several types of toxicants, including aflatoxins, pyrrolizidine alkaloids, complex chemical mixtures from marine sediment extracts, and the algal toxin microcystin-LR. Hydropic degeneration is probably a precursor to the more chronic lesions in other fish. 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

Case: 07-2389

(microcystin-LR). Conditions that lead to moderate to abundant hepatic lipofuscin have been associated with decreased growth and survival in several studies.

The primary reported clinical sign--serosanguineous ascites--is not described as a feature of netpen liver disease; the ascites might be a result of a secondary bacterial infection.

Lack of abundant adipose tissue in the mesenteries is evidence that these fish have been sick for a long time (weeks?). Granulomatous inflammation in the mesenteric adipose tissue and exocrine pancreas is consistent with a reaction to foreign material; it is common in fish that have been vaccinated. Vacuoles probably are a result of vaccine material lost during tissue processing.

Renal mineralization is common in cultured fish species; when severe (not here), the condition is termed nephrocalcinosis. The lesion is not considered fatal, although feed conversion may be adversely affected. The pathogenesis is not fully understood, but renal mineralization has been experimentally reproduced through high carbon dioxide levels, magnesium deficiency, selenium toxicity, and a diet low in minerals (source, "Systemic Pathology of Fish", 1989, by H. Ferguson). Clinically, renal mineralization is most commonly associated with high carbon dioxide levels.

Renal tubular epithelial protein droplets are normal in some species, or they might be an indication of glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2006 as part of the Ministry's Fish Health Auditing and Surveillance Program: Atlantic salmon (prevalence = 22%; n = 495) and Pacific salmon (prevalence = 38%; n = 134).

## Histopathology

Formalin-fixed tissues were submitted for histopathology. Gills were embedded into a block separate from the other tissues.

Slide 1-1 - liver, heart, trunk kidney (2 pieces, one with Corpuscles of Stannius), intestinal ceca and exocrine pancreas

Slide 1-2 - gill (this gill was decalcified overnight in EDTA before processing)

Slide 3-1A - spleen, trunk kidney, head kidney, liver, heart, brain, stomach

Slide 3-1B - gill (this small piece of gill was not noticed by the trimming technician, and it was not decalcified)

All organs were examined. Organs not listed below have no significant lesions.

**Quality control:** Tissue preservation is fair for most organs; the gills in slide 1-2 have moderate to severe autolysis; the gill in slide 1-3B has mild autolysis. Organs have no postfixation dehydration and no acid hematin accumulation. Gill decalcification is complete.



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-2430

Last Updated: 07/13/07 10:47 AM

Pathologist: Gary D. Marty

Received Date: 06/20/07

Collected Date: 06/20/07

Client Ref No: 6226 Kokish

Veterinarian: **Diane Morrison**

Clinic:

Phone:

Fax:

Submitter:

Phone:

Fax:

Owner: **Marine Harvest Canada**

Phone:

Fax:(250) 850-3275

### Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID:

### Case History

Submitted 15 bact samples, 3 pools of 5 fish for viral, 5 histo and 4 gill histo, missing one gill for 5. Fish 1 to 6 mowi, viral 1 mowi, viral 2 mix, viral 3 McConnell. VHS and IHN as well as culture.

### Final Diagnosis

1a. Trunk kidney: renal tubular epithelial necrosis, multifocal, mild, with moderate interstitial fibrosis, subacute (slide 5)

1b. Kidney: nephritis, interstitial, granulomatous, multifocal, mild (slide 5)

2. Gill: lamellar fusion, multifocal, moderate, with occasional superficial filamentous bacteria (slide 1B)

3a. Intestine and intestinal ceca: peritonitis, granulomatous, multifocal, with occasional fine fibrocellular fronds, mild (slide 3A), moderate (slide 2A)

3b. Spleen: peritonitis, granulomatous, multifocal, moderate (slide 4A), with occasional fine fibrocellular fronds and intralesional vacuoles about 150 µm in diameter, mild (slide 5), severe (slide 3A)

4. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slides 1A, 2A)

**Final Comment:** Some of these fish have significant lesions, particularly in the kidney.

Renal tubular epithelial necrosis results from acute damage to renal epithelial cells; damage is reversible if the basement membrane is spared. Fibrosis is evidence of more extensive damage and efforts at repair. Causes in fish include viral hemorrhagic septicemia virus (VHSV), exposure to toxicants (e.g., bacterial toxins, or aminoglycoside antibiotics such as gentamicin), or hypoxia followed by re-oxygenation. I have also seen this change in salmon exposed to high concentrations of chromium (Farag et al. 2006). If this site has a history of a VHSV in the past couple months, the fibrosis in this fish might be a resolving VHSV lesion.

The most common organism associated with granulomatous nephritis in salmon is *Renibacterium salmoninarum*, the cause of bacterial kidney disease. However, the lack of organisms on the Gram stain makes *Renibacterium salmoninarum* less likely in this case. Differentials include a vaccine reaction or a chronic infection with *Yersinia ruckeri*.

Gill lamellar fusion, without lamellar hypertrophy, has been associated with exposure to heavy metals and with hypoxia (thought to be from fish

gasping for oxygen at the water-air interface). Thin rod-shaped to filamentous bacteria are common on the gills of debilitated juvenile salmonids. Although bacterial culture or PCR is required for a definitive diagnosis, the common species in the gill include *Flavobacterium columnare* (the cause of columnaris disease), *F. psychrophilum* (the cause of coldwater disease), and *F. branchiophilum* (the cause of bacterial gill disease). Infections are usually associated with crowding or poor water quality. Small numbers of organisms on histology sections are often associated with significant clinical disease (bacterial numbers might decrease during processing). Severity of infection needs to be determined from examination of gill wet mounts from live (anesthetized) fish.

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated. Vacuoles probably are a result of vaccine material lost during tissue processing.

Basophilic cytoplasm in hepatocytes is an indication of active protein synthesis. It is normal in mature females producing protein for deposition in their eggs. In other fish it might be related to increased protein needed as part of an inflammatory response.

#### Literature cited:

Farag, A. M., T. May, G. D. Marty, M. Easton, D. D. Harper, E. E. Little, and L. Cleveland. 2006. The effect of chronic chromium exposure on the health of juvenile Chinook salmon (*Oncorhynchus tshawytscha*). *Aquatic Toxicology* 76:246-257.

## Histopathology

Formalin-fixed tissues were submitted in cassettes wrapped in moist paper towel for histopathology. The gills were decalcified overnight in EDTA and then processed routinely into paraffin.

Slide 1A - liver, trunk kidney, head kidney, heart, skin, skeletal muscle, intestine, intestinal ceca, and mesenteric adipose tissue

Slide 1B - gill (2 pieces)

Slide 2A - liver, trunk kidney, head kidney, heart, skin, skeletal muscle, intestine, intestinal ceca, and mesenteric adipose tissue

Slide 2B - gill (2 pieces)

Slide 3A - liver, trunk kidney, head kidney, heart, spleen, skin, skeletal muscle, intestine, intestinal ceca, and mesenteric adipose tissue

Slide 3B - gill (2 pieces)

Slide 4A - liver, trunk kidney, head kidney, heart, skeletal muscle, intestine, intestinal ceca, and mesenteric adipose tissue

Slide 4B - gill

Slide 5 - liver, trunk kidney (2 pieces), heart, intestine, intestinal ceca, and mesenteric adipose tissue; a Twort's Gram stain was done on a step section from this block.

All organs were examined. Organs not listed below have no significant lesions.

**Quality control:** Tissue preservation is good for most organs. Some tissues (especially intestine) in slide 1A have small numbers of irregular vacuoles, consistent with freezing artefact. The margins of many organs have evidence of postfixation dehydration (e.g., nuclei stain dull blue; erythrocyte cytoplasm stains yellow instead of red; cytoplasm of other cell types stains poorly or not at all). This most commonly results when preserved tissues are removed from liquid for more than a few minutes (e.g., during shipment). [Our necropsy technician noted that the tissues were very dry and hard upon arrival.] Erythrocyte cytoplasm in slides 1, 2, and 5 stains poorly; this often occurs when tissues are preserved in an alcohol-based fixative (e.g., Bouin's or Davidson's) and then returned to water or a water-based fixative (e.g., 10% formalin) before being processed into paraffin. [I do not see the problem of lost erythrocyte staining when formalin-fixed tissues are transferred to water or physiologic saline before processing. Erythrocytes in slides 3 and 4 stain normally.] Large foci of erythrocytes (e.g., some liver vessels in slide 3A) have precipitates of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic before or during fixation. Gill decalcification is complete.

## Molecular Diagnostics

**PCR - IHNV** Resulted by: Julie Bidulka Verified by: Julie Bidulka on 06/25/07 @ 8:43 AM

Specimen	ID	Test	Result
Tissue	A-6226-1	PCR - IHNV	Negative
Tissue	B-6226-2	PCR - IHNV	Negative
Tissue	C-6226-3	PCR - IHNV	Negative

**PCR - VHSV** Resulted by: Julie Bidulka Verified by: Julie Bidulka on 06/25/07 @ 8:43 AM

Specimen	ID	Test	Result
Tissue	A-6226-1	PCR - VHSV	Negative
Tissue	B-6226-2	PCR - VHSV	Negative
Tissue	C-6226-3	PCR - VHSV	Negative

## Virology

**Tissue Culture** Resulted by: Liisa Nielsen Verified by: Dr. J. Robinson on 07/13/07 @ 10:47 AM

Specimen	ID	Isolate	Result	Level
Tissue	A-6226-1		No viruses isolated	
Tissue	B-6226-2		No viruses isolated	
Tissue	C-6226-3		No viruses isolated	



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-2553

Last Updated: 07/23/07 3:14 PM  
Pathologist: Gary D. Marty  
Received Date: 06/27/07  
Collected Date: 06/27/07  
Client Ref No: Log 6227 PO BB15112

Veterinarian: **Brad Boyce**  
Clinic:  
Phone:  
Fax:

Submitter:  
Phone:  
Fax:  
Owner: **Marine Harvest Canada**  
Phone:  
Fax:(250) 850-3275

**Animal Data**  
Species: Atlantic Salmon  
Breed:  
Sex:  
Age:  
Premise ID:

### Case History

Submitted one bag of tissue for PCR for IHN and VHS.

### Molecular Diagnostics

**PCR - IHN** Resulted by: A Scouras Verified by: Dr. J. Robinson on 07/04/07 @ 4:00 PM

Specimen	ID	Test	Result
Tissue	6227-1	PCR - IHN	Negative

**PCR - VHSV** Resulted by: A Scouras Verified by: Dr. J. Robinson on 07/04/07 @ 4:00 PM

Specimen	ID	Test	Result
Tissue	6227-1	PCR - VHSV	Negative

### Virology

**Tissue Culture** Resulted by: Liisa Nielsen Verified by: Dr. J. Robinson on 07/23/07 @ 3:14 PM

Specimen	ID	Isolate	Result	Level
Tissue	6227-1		No viruses isolated	



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-2554

Last Updated: 07/23/07 3:14 PM  
Pathologist: Gary D. Marty  
Received Date: 06/27/07  
Collected Date: 06/27/07  
Client Ref No: Log 6243, PO BB15112

Veterinarian: **Brad Boyce**  
Clinic:  
Phone:  
Fax:

Submitter:  
Phone:  
Fax:  
Owner: **Marine Harvest Canada**  
Phone:  
Fax:(250) 850-3275

**Animal Data**  
Species: Atlantic Salmon  
Breed:  
Sex:  
Age:  
Premise ID:

### Case History

Submitted five bags of tissue for PCR for IHN and VHS.

### Molecular Diagnostics

**PCR - IHN** Resulted by: A Scouras Verified by: Dr. J. Robinson on 07/04/07 @ 4:05 PM

Specimen	ID	Test	Result
Tissue	A-6243-1	PCR - IHN	Negative
Tissue	B-6243-2	PCR - IHN	Negative
Tissue	C-6243-3	PCR - IHN	Negative
Tissue	D-6243-4	PCR - IHN	Negative
Tissue	E-6243-5	PCR - IHN	Negative

**PCR - VHSV** Resulted by: A Scouras Verified by: Dr. J. Robinson on 07/04/07 @ 4:05 PM

Specimen	ID	Test	Result
Tissue	A-6243-1	PCR - VHSV	Negative
Tissue	B-6243-2	PCR - VHSV	Negative
Tissue	C-6243-3	PCR - VHSV	Negative
Tissue	D-6243-4	PCR - VHSV	Negative
Tissue	E-6243-5	PCR - VHSV	Negative

### Virology

**Tissue Culture** Resulted by: Liisa Nielsen Verified by: Dr. J. Robinson on 07/23/07 @ 3:14 PM

Specimen	ID	Isolate	Result	Level
Tissue	A-6243-1		No viruses isolated	
Tissue	B-6243-2		No viruses isolated	

Tissue	C-6243-3
Tissue	D-6243-4
Tissue	E-6243-5

No viruses isolated
No viruses isolated
No viruses isolated



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-2591

Last Updated: 07/06/07 11:20 AM

Pathologist: Gary D. Marty

Received Date: 06/29/07

Collected Date: 06/29/07

Client Ref No: Log 6233 PO#BB15113

Veterinarian: **Marine Harvest Canada**

Clinic:

Phone:

Fax:

Submitter:

Phone:

Fax:

Owner: **Marine Harvest Canada**

Phone:

Fax:(250) 850-3275

### Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID:

### Case History

Log #6233. PO #BB15113. Submitted 6 histo samples. Saltwater entry 06. Fresh mortis sampled for histo (2 cassettes per fish). High numbers of jellyfish and caprellids in water.

### Final Diagnosis

- 1a. Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slides 8A, 8(2)A, 12A), moderate (slide 2A), severe (slide 11A)
- 1b. Liver: pericholangitis, lymphocytic, multifocal, mild (slides 2A, 8A, 12A)
- 1c. Liver: biliary preductular cell hyperplasia, diffuse, mild (slide 2A)
- 1d. Liver: hepatocellular single cell necrosis (apoptosis), disseminated, acute, mild (slides 2A, 8(2)A, 11A)
- 1e. Liver: yellow-brown to yellow-green pigmented macrophage aggregates and sinusoidal macrophages, disseminated, mild (slide 2A)
- 1f. Liver: hepatic necrosis, acute, focal, mild (slide 8A); multifocal, mild (slide 11A)
2. Head kidney: intracytoplasmic eosinophilic granules in endothelial cells, diffuse, moderate numbers (slide 12A), abundant (slide 2A)
- 3a. Spleen: parenchymal golden pigment, scattered, intracellular, mild (slide 2A)
- 3b. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slides 5A, 8A, 8(2)A, )
4. Intestine: peritonitis, chronic, focal, with fibrocellular fronds, mild (slide 8A)
5. Skeletal muscle: flocculated sarcoplasm of white myofibres, acute, mild (slide 5A)
- 6a. Gill: lamellar telangiectasis, multifocal, with organizing thrombosis, mild (slide 8B)
- 6b. Gill: lamellar telangiectasis, multifocal, with acute thrombosis, mild (slides 8(2)B, 11B)
- 6c. Gill: branchial granuloma, focal, mild (slide 8B)

**Final Comment:** These fish have several lesions, but none are sufficient alone to explain their death. In the BC Fish Health Auditing and Surveillance Program, about half of the "cause of death" diagnoses are made based on brain histopathology. If additional samples are collected from this group of fish, consider including brain to increase the chance of detecting significant lesions. Details of each lesion follow:

Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition. Lymphocytic inflammation around bile ductules (liver) is evidence of chronic immune stimulation. This type of inflammation can result from bacteria ascending from the intestine to the liver through the biliary system.

Biliary preductular cell hyperplasia 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). Biliary preductular cell hyperplasia is fairly common in salmon that die in marine net pens, affecting 15% of the 505 Atlantic salmon and 4.5% of the 134 Pacific salmon examined in 2006 as part of the Province's Fish Health Auditing and Surveillance Program; the sample size of Atlantic salmon in 2006 was sufficient to identify a trend towards greater prevalence in the fall and winter (20-26%) than in the spring and fall (4-12%).

Causes of hepatocellular single cell necrosis have not been well defined in fish. Possible differentials include exposure to toxins (endogenous or exogenous), or a viral infection (VHSV). A similar change, apoptosis, occurs with remodelling of the liver in rapidly growing fish that suddenly go off feed about 24 hours before death. Apoptosis is the normal way in which hepatocyte numbers are decreased (i.e., the hepatocytes are not needed when growing fish stop feeding because few to no nutrients are being absorbed into the blood and entering the liver for processing).

Pigment in the liver could be lipofuscin, hemosiderin, or both. Pigment in the spleen is usually just lipofuscin. Accumulation of lipofuscin 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), but the affected liver lacks the more diagnostic feature of netpen liver disease: hepatic megalocytosis. 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.

Hepatic necrosis can be caused by inadequate vascular perfusion or direct cytotoxicity from viral or bacterial infections (e.g., viral hemorrhagic septicemia virus, *Piscirickettsia salmonis*, and *Renibacterium salmoninarum*); the cause is not determined in most cases. This case has no obvious organisms. Lack of proliferative lesions in the biliary system is evidence against a chronic toxic cause for the hepatic necrosis. Hepatic necrosis is somewhat common in salmon that die in marine net pens, affecting 7.5% of the 505 Atlantic salmon and 5% of the 134 Pacific salmon examined in 2006 as part of the Province's Fish Health Auditing and Surveillance Program.

Accumulation of eosinophilic granules in the cytoplasm of cells lining small vessels in the kidney is a distinctive finding in Atlantic salmon. These granules probably accumulate in response to systemic immune stimulation. The granules might be part of eosinophilic granular cells or endothelial cells; granule size seems too large and variable for eosinophilic granular cells. I have seen these granules associated with *Piscirickettsia salmonis*, but other cases (like this one) have no known cause.

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

Flocculated sarcoplasm of skeletal muscle is a degenerative change that can result from trauma, bacterial, or parasitic infections. It is common in fish that are handled within a few hours of death. These sections have no evidence of parasitic infections.

Telangiectasis (rupture of the small lamellar blood vessels) in the gill most commonly results from trauma (e.g., handling). The presence of organizing thrombi (i.e., blood clots being re-canalized by new vessels) is evidence that these thrombi have been present for at least a few days.

Granulomas develop in response to persistent foreign material. Differentials include vaccine adjuvant and a bacterial infection. The lesion in slide 8B contains no obvious organisms.

Gill lamellar fusion with lamellar hyperplasia may be a result of physical damage from exposure to a parasite or diatoms, but this lesion contains no organisms (they might have been lost during processing).

## Histopathology

Formalin-fixed tissues were submitted in 12 cassettes for histopathology. The gills were decalcified overnight in EDTA and then all tissues

were processed routinely into paraffin. Slide #s are the same as case numbers.

Slide 2A - liver, trunk kidney, head kidney, spleen, heart, intestine, and mesenteric adipose tissue

Slide 2B - gill

Slide 5A - liver, trunk kidney, head kidney, spleen, heart, skeletal muscle, intestine, and mesenteric adipose tissue

Slide 5B - gill

Slide 8A - liver, trunk kidney, head kidney, spleen, heart, skeletal muscle, intestine, and mesenteric adipose tissue

Slide 8B - gill

Slide 8(2)A - liver, trunk kidney, head kidney, spleen, heart, skeletal muscle, intestine, and mesenteric adipose tissue

Slide 8(2)B - gill

Slide 11A - liver, trunk kidney, head kidney, spleen, heart, skeletal muscle, intestine, and mesenteric adipose tissue

Slide 11B - gill

Slide 12A - liver, trunk kidney, head kidney, spleen, heart, skeletal muscle, intestine, and mesenteric adipose tissue

Slide 12B - gill

All organs were examined. Organs not listed elsewhere have no significant lesions.

**Quality control:** Liver autolysis: mild (slides 2A, 8A, 8(2)A, 11A, 12A), severe (slide 5A); gill autolysis: mild (slide 2B, 8(2)B, 11B, 12B), moderate (slide 8B), severe (slide 5B). Most organs have no acid hematin deposits, but the spleen in slide 11A has a few precipitates of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic before or during fixation. Organs have no postfixation dehydration. Gill decalcification is complete.



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-2593

Last Updated: 07/25/07 5:18 PM

Pathologist: Gary D. Marty

Received Date: 06/29/07

Collected Date: 06/29/07

Client Ref No: Log 6242, PO#BB15113

Veterinarian: **Brad Boyce**

Clinic:

Phone:

Fax:

Submitter:

Phone:

Fax:

Owner: **Marine Harvest Canada**

Phone:

Fax:(250) 850-3275

### Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID:

### Case History

Viral PCR for IHN and VHS. Bags #1 to 6 (pools of 5), Bags #7 to 9 (pools of 3 fresh mortis). Saltwater entry 06.

### Molecular Diagnostics

**PCR - IHN** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 07/09/07 @ 9:50 AM

Specimen	ID	Test	Result
Tissue	A-6242-1	PCR - IHN	Negative
Tissue	B-6242-2	PCR - IHN	Negative
Tissue	C-6242-3	PCR - IHN	Negative
Tissue	D-6242-4	PCR - IHN	Negative
Tissue	E-6242-5	PCR - IHN	Negative
Tissue	F-6242-6	PCR - IHN	Negative
Tissue	G-6242-7	PCR - IHN	Negative
Tissue	H-6242-8	PCR - IHN	Negative
Tissue	I-6242-9	PCR - IHN	Negative

**PCR - VHSV** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 07/06/07 @ 3:30 PM

Specimen	ID	Test	Result
Tissue	A-6242-1	PCR - VHSV	Negative
Tissue	B-6242-2	PCR - VHSV	Negative
Tissue	C-6242-3	PCR - VHSV	Negative
Tissue	D-6242-4	PCR - VHSV	Negative
Tissue	E-6242-5	PCR - VHSV	Negative
Tissue	F-6242-6	PCR - VHSV	Negative
Tissue	G-6242-7	PCR - VHSV	Negative
Tissue	H-6242-8	PCR - VHSV	Negative
Tissue	I-6242-9	PCR - VHSV	Negative

**Tissue Culture** Resulted by: Liisa Nielsen Verified by: Dr. J. Robinson on 07/25/07 @ 5:18 PM

Specimen	ID	Isolate	Result	Level
Tissue	A-6242-1		No viruses isolated	
Tissue	B-6242-2		No viruses isolated	
Tissue	C-6242-3		No viruses isolated	
Tissue	D-6242-4		No viruses isolated	
Tissue	E-6242-5		No viruses isolated	
Tissue	F-6242-6		No viruses isolated	
Tissue	G-6242-7		No viruses isolated	
Tissue	H-6242-8		No viruses isolated	
Tissue	I-6242-9		No viruses isolated	



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-2711

Last Updated: 07/16/07 12:22 PM

Pathologist: Stephen Raverty, DVM

Received Date: 07/11/07

Collected Date: 07/11/07

Client Ref No:

Veterinarian: **Mainstream Canada-T**

Clinic:

Phone:

Fax:

Submitter:

Phone:

Fax:

Owner: **Mainstream Canada-T**

Phone:

Fax:(250) 725-1250

### Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID:

### Case History

4 VHS and IHN for PCR. 2 VHS and IHN pools for PCR. 4 histology. 2 histology pools. Many non-performers dropping off. Have samples from various pens, non performs as well as healthy, fish were culled. Currently finishing Tribissen treatment for mouth rot.

### Final Diagnosis

#### MORPHOLOGIC DIAGNOSES:

1A:

- 1). Liver: Granuloma, mild, multifocal, chronic
- 2). Brain: Hemorrhage, subdural, moderate, diffuse, acute
- 3). Adipose tissue: Peritonitis, mild, nodular to diffuse, granulomatous (vaccine induced)
- 4). Gills: Respiratory epithelia, hypertrophy, moderate, focally extensive with blunting of secondary lamellae

There are no overt lesions within the posterior kidney, peripheral vasculature, small intestine, peripheral nerves, spleen, anterior kidney, or heart.

1B:

- 1). Liver: Atrophy, marked, diffuse with pronounced karyocytomegaly, occasional vesiculated nuclei with prominent nucleoli, ductular hyperplasia, expansile clear vacuoles, rare Langhan's type multinucleated cells, scattered fibrin deposition and fibroplasia
- 2). Adipose tissue: Peritonitis, moderate, nodular to diffuse, granulomatous (vaccine induced)
- 3). Spleen capsule: Serositis, moderate, focally extensive, granulomatous, chronic
- 4). Spleen: Congestion, moderate, diffuse

There are no significant lesions within the liver, peripheral vasculature, peripheral nerves, brain, heart, small intestine, or posterior kidney.

1C:

There are no significant lesions within the gills or liver.

2A and 2B:

- 1). Liver: Karyocytomegally, moderate, multifocal, with biliary ductular hyperplasia, lobular collapse and rare intranuclear pseudo-inclusions
- 2). Brain: Hemorrhage, subdural, mild, multifocal, acute
- 3). Adipose tissue: Peritonitis, moderate, focal, granulomatous (presumptive vaccine induced)

There are no significant lesions within the posterior kidney, spleen, small intestine, peripheral nerves, heart, gills, or peripheral vasculature.

3A:

- 1). Adipose tissue: Peritonitis, mild, multifocal, granulomatous (compatible with vaccine induced peritonitis)

There are no significant lesions within the liver, spleen, kidney, peripheral vasculature, peripheral nerves, small intestine or brain.

3B:

- 1). Liver: Karyocytomegally, moderate, multifocal, with biliary ductular hyperplasia, lobular collapse and rare intranuclear pseudoinclusions
- 2). Heart: Endocarditis, mild, multifocal, random, proliferative, subacute
- 3). Stomach: Edema, submucosal, moderate, diffuse, acute
- 4). Kidney, posterior: Tubuloectasia, moderate, diffuse acute with scattered tubuloproteinosis

There are no significant lesions within the liver, spleen, peripheral vasculature, peripheral nerves, small intestine or brain.

**COMMENTS:**

The liver lesions are suggestive with net pen liver disease and would have been sufficiently severe to account for antemortem morbidity and presumably the loss of these animals. With the treatment for mouthrot, the possibility of some adverse reaction to the medication cannot be entirely discounted. If possible, follow up submission of liver from 5-10 moribund animals upon completion of the treatment may be considered. In multiple sections, there was low to intermediate grade granulomatous peritonitis which is most likely due to vaccination, although infectious and other inflammatory processes may not be entirely discounted. In select animals there is scattered reactive change suggestive of a low grade antigenemia, which is likely a sequela to impaired hepatobiliary function.

**Histopathology**

Refer to Morphologic Diagnoses

**Molecular Diagnostics**

**PCR - IHNV** Resulted by: A Scouras Verified by: Dr. J. Robinson on 07/16/07 @ 12:22 PM

Specimen	ID	Test	Result
Tissue	DX 104,108	PCR - IHNV	Negative
Tissue	DX(NP) 104,108	PCR - IHNV	Negative
Tissue	DX 109	PCR - IHNV	Negative
Tissue	DX(NP) 109	PCR - IHNV	Negative
Tissue	DX 110	PCR - IHNV	Negative
Tissue	DX(NP) 110	PCR - IHNV	Negative

Specimen	ID	Test	Result
Tissue	DX 104,108	PCR - VHSV	Negative
Tissue	DX(NP) 104,108	PCR - VHSV	Negative
Tissue	DX 109	PCR - VHSV	Negative
Tissue	DX(NP) 109	PCR - VHSV	Negative
Tissue	DX 110	PCR - VHSV	Negative
Tissue	DX(NP) 110	PCR - VHSV	Negative



Stephen Raverty, DVM  
Stephen.Raverty@gov.bc.ca

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**END OF REPORT**

## Final Report AHC Case: 07-2940

Last Updated: 08/27/07 12:03 PM

Pathologist: Gary D. Marty

Received Date: 07/30/07

Collected Date: 07/30/07

Client Ref No: PO BB 15116

Veterinarian: **Diane Morrison**

Clinic: **Marine Harvest Canada**

Phone: (250) 850-3276

Fax: (250) 850-3275

Submitter:

Phone:

Fax:

Owner: **Marine Harvest Canada**

Phone:

Fax:(250) 850-3275

### Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID:

### Case History

Submitted fish tissue. 6268 please do tissue culture and PCR for IHN and VHS. 6260 Please do histology and tissue culture/PCR for IHN and VHS. 6258, 6275, 6276 please do histology. Tissue culture/PCR for VHS and IHN. 6276 8 cassettes for histology (4x2) 5 vials for PCR IHN/VHS. Note: fish were going through a mild hetaro bloom, moribunds were killed with clove oil.

### Final Diagnosis

1a. Liver: hydropic degeneration, diffuse, moderate (slide 8-2)

1b. Liver: hepatocellular karyomegaly, diffuse, moderate (netpen liver disease? slide 8-2)

1c. Liver: hepatocellular single cell necrosis, diffuse, with parenchymal collapse, moderate (slide 8-2)

1d. Liver: lipofuscin-pigmented sinusoidal macrophages, diffuse, mild (slide 8-2)

1e. Liver: hepatitis, lymphoplasmacytic, multifocal, moderate (slide 8-2)

1f. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate (slide 5-1)

1g. Liver: biliary ductular and preductular cell hyperplasia, diffuse, mild (slide 5-1)

1h. Liver: hepatitis, granulomatous, with multinucleate giant cells, focal, mild (slide 6-2); multifocal, coalescing, moderate (slide 6-1)

1i. Liver: peritonitis, chronic, focal, with fibrocellular fronds, mild (slide 6-1), moderate (slide 6-4)

1j. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slide 6-3)

1k Liver: hepatocellular eosinophilic cytoplasmic inclusions, multifocal, severe (slide 0-4)

1l. Liver: cholangitis, focal, acute, with edema and epithelial necrosis, moderate (slide 0-4)

2a. Heart: epicarditis, multifocal, lymphoplasmacytic, mild (slides 5-1, 6-1)

2b. Heart: endocarditis, multifocal, lymphohistiocytic, mild (slide 6-1)

- 3a. Spleen, intestinal ceca, and mesenteric adipose tissue: peritonitis, granulomatous, multifocal, with intralesional vacuoles about 50 µm in diameter, moderate (slides 6-1, 6-4), severe (slide 6-3)
- 3b. Spleen, intestinal ceca (or, in slide 8-1, intestine), and mesenteric adipose tissue: peritonitis, granulomatous, multifocal, moderate (slides 6-2, 8-1)
- 3c. Spleen: leukocytic karyorrhexis, disseminated, moderate (slide 8-1)
- 4a. Trunk kidney: renal tubular epithelial necrosis and regeneration, focal, acute, mild (slide 6-2); multifocal, acute, mild (slide 8-2)
- 4b. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild (slides 6-2, 6-3), moderate (slide 6-1)
- 4c. Trunk kidney: glomerulonephritis, membranous, segmental, mild (slide 6-4), moderate (slide 6-2)
- 4d. Trunk kidney: renal tubular mineralization, multifocal, mild (slide 8-2)
5. Head kidney and trunk kidney: nephritis, granulomatous, multifocal, mild (slide 6-2)
6. Gill: lamellar subepithelial edema, focal, mild (slide 8-1G)
7. Mesenteric adipose tissue: atrophy, diffuse, severe (slide 8-2)
8. Blood vessel (anterior cardinal vein): eosinophilic granular cells in vascular wall, abundant (normal or vasculitis? slides 0-1, 0-4, 0-5, 0-7)
9. Brain: meningeal hemorrhage, focal, mild (slides 0-1, 0-5)

**Final Comment:** Features of the liver in slide 8-2 are characteristic of net pen liver disease. Netpen liver disease can be reproduced in the laboratory with water exposure to the algal toxin microcystin-LR, but the source of the toxin in the field is unknown. Other toxins in the water or feed (e.g., aflatoxins) are less likely differentials. Changes in the affected liver (slide 8-2) include single cell necrosis, hepatocellular karyomegaly, sinusoidal lipofuscin accumulation, and variable amounts of lymphoplasmacytic inflammation. Widespread parenchymal collapse is evidence that the process has been going on for a long time (months?). Lack of lipid stores in the mesenteric adipose tissue of this fish is further evidence of chronic impairment. Hepatic megalocytosis can result from exposure to several types of toxicants, including aflatoxins, pyrrolizidine alkaloids, complex chemical mixtures from marine sediment extracts, and the algal toxin microcystin-LR. Hydropic degeneration is a precursor to single cell necrosis. 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; it is a common feature of netpen liver disease. Conditions that lead to moderate to abundant hepatic lipofuscin have been associated with decreased growth and survival in several studies. The other fish have several significant lesions, but none have evidence of netpen liver disease. None of the fish have *Heterosigma* spp. in the gills.

Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition.

Biliary ductular and preductular cell hyperplasia 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). Biliary ductular hyperplasia is not common, but preductular cell hyperplasia is fairly common in salmon that die in marine net pens, affecting 15% of the 505 Atlantic salmon and 4.5% of the 134 Pacific salmon examined in 2006 as part of the Province's Fish Health Auditing and Surveillance Program; the sample size of Atlantic salmon in 2006 was sufficient to identify a trend towards greater prevalence in the fall and winter (20-26%) than in the spring and summer (4-12%).

Granulomatous hepatitis could be part of a vaccine reaction or response to chronic bacterial infection (e.g., *Yersinia ruckeri*). Lack of organisms on the Twort's Gram stain (slide 6-1) makes bacterial kidney disease an unlikely differential.

Basophilic cytoplasm in hepatocytes is an indication of active protein synthesis. It is normal in mature females producing protein for deposition in their eggs. In other fish it might be related to increased protein needed as part of an inflammatory response.

Homogeneous cytoplasmic inclusions in hepatocytes (e.g., slide 0-4) are a distinct change. Some of the inclusions are up to 25 µm in diameter, and margins of the inclusions are well-demarcated. The inclusions might be phagocytosed cellular debris or plasma protein, or accumulation of protein synthesized in hepatocytes. Features of the inclusions are not consistent with viral inclusions. Transmission electron microscopy might help determine the nature of this change, but TEM is not available at the Animal Health Centre. I have seen this change in fish that have been exposed to toxins, but this change is not described in common fish pathology books. Cholangitis in this fish might have the same underlying cause as the hepatocellular droplets.

Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. Lymphohistiocytic endocarditis in the heart is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine.

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated. Vacuoles probably are a result of vaccine material lost during tissue processing.

The presence of degenerating nuclei (karyorrhexis) in the spleen (slide 8-1) is evidence of increased cell turnover, possibly as part on an active inflammatory response.

Renal tubular epithelial necrosis results from acute damage to renal epithelial cells; damage is reversible if the basement membrane is spared (as in this case). Renal tubular epithelial necrosis was fairly common among fish sampled in 2006 as part of the Ministry's Fish Health Auditing and Surveillance Program: Atlantic salmon (prevalence = 7.5%; n = 495) and Pacific salmon (prevalence = 2.2%; n = 134); the cause was not determined in many cases. Differentials include viral hemorrhagic septicemia virus (VHSV) and exposure to toxins (e.g., bacterial toxins, heavy metals, or aminoglycoside antibiotics such as gentamicin).

Renal tubular epithelial protein droplets are normal in some species, or they might be an indication of glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2006 as part of the Ministry's Fish Health Auditing and Surveillance Program: Atlantic salmon (prevalence = 22%; n = 495) and Pacific salmon (prevalence = 38%; n = 134).

Differentials for granulomatous nephritis in the head kidney (slide 6-2) include bacterial kidney disease, vaccine reaction, or other chronic bacterial infection (e.g., *Yersinia ruckeri*).

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 syndrome (in Atlantic salmon), nephrocalcinosis, and infections with a number of bacteria and parasitic species. The segmental pattern (i.e., only part of the glomerulus affected) in the kidney on slide 6-2 is unusual.

Renal mineralization is common in cultured fish species. The pathogenesis is not fully understood, but renal mineralization has been experimentally reproduced through high carbon dioxide levels, magnesium deficiency, selenium toxicity, and a diet low in minerals (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Clinically, renal mineralization is most commonly associated with high carbon dioxide levels.

Separation of gill lamellar epithelium from underlying pillar cells can be a result of edema, and it also is a common postmortem artefact. In this case, accumulation of proteinaceous fluid deep to the separated epithelium supports the diagnosis of edema over artefact. Lamellar edema is commonly associated with exposure to toxicants, including harmful algae, formalin, and hydrogen peroxide overdose. Lamellar edema is reversible if the inciting cause is removed.

The walls of the anterior cardinal vein (drains from the brain/head to the sinus venosus and then to the heart) contain abundant eosinophilic granular cells (EGCs, up to 6 cell-layers thick). EGCs appear in a number of studies with salmonid diseases; they might be a nonspecific indicator of inflammation and/or stress. Alternatively, they might be normal.

The most common cause of meningeal hemorrhage is trauma.

## Histopathology

Formalin-fixed tissues were submitted for histopathology. The gills mid-sagittal sections were decalcified overnight in 10% EDTA before being processed routinely into paraffin.

Slide 5-1 (6275-1) - spleen, liver, heart, head kidney, trunk kidney

Slide 5-1G - gill

Slide 6-1 - spleen, liver, heart, head kidney, trunk kidney, brain, intestinal ceca, and mesenteric adipose tissue; a Twort's Gram stain was done on a step section from this block.

Slide 6-1G - gill

Slide 6-2 - spleen, liver, heart, head kidney, trunk kidney, brain, intestinal ceca, and mesenteric adipose tissue

Slide 6-2G - gill

Slide 6-3 - spleen, liver, heart, head kidney, trunk kidney, brain, intestinal ceca, and mesenteric adipose tissue

Slide 6-3G - gill

Slide 6-4 - spleen, liver, heart, head kidney, trunk kidney, brain, stomach, intestinal ceca, and mesenteric adipose tissue

Slide 6-4G - gill

Slide 8-1 (PA-1 7-13-07) - spleen, liver, heart, head kidney, trunk kidney, brain, stomach, intestinal ceca, and mesenteric adipose tissue

Slide 8-1G (PA-1 7-13-07) - gill

Slide 8-2 (PA-2 Jul 7/07) - spleen, liver, heart, head kidney, trunk kidney, brain, skeletal muscle, intestinal ceca, and mesenteric adipose tissue

Slide 8-1G (PA-2 Jul 7/07) - gill

Slides 0-1, 0-2, and 0-3 - midsagittal sections of head and body of a smolt-sized fish

Slide 0-4 - two midsagittal sections of a smolt-sized fish

Slides 0-5, 0-6 - left and right midsagittal sections of a smolt-sized fish (on half per slide)

Slides 0-7, 0-8 - left and right midsagittal sections of a smolt-sized fish (one half per slide)

All organs were examined. Organs not listed below have no significant lesions.

**Quality control:** Liver autolysis: none (slides 5- 1, 6-1, 6-2, 6-3, 6-4, 8-1, 8-2), mild (slide 0-4), moderate (slides 0-2, 0-5, 0-7). Gill autolysis: none (slides 6-1G, 6-2G, 6-3G, 6-4G, 0-1, 0-4, 0-6, 0-7), mild (slides 5-1-G, 8-1G, 8-2G). Gill decalcification is complete. Organs have no postfixation dehydration and no acid hematin deposits.

**Measures of physiologic condition** (smolt-sized fish only, "0" slides):

Hepatocellular glycogen - none (slide 0-4), small amounts (slides 0-5, 0-7), moderate amounts (slide 0-3)

Lipid in mesenteric adipose tissue - abundant (slides 0-2, 0-4, 0-5, 0-7)

Gastrointestinal food - moderate amounts (slides 0-2, 0-4, 0-5, 0-7)

These patterns in the measures of physiologic condition are consistent with healthy growing fish that were eating at the time they were sampled. Some of the fish (e.g., the fish in slide 0-4) might have eaten less than normal for a few days before they were sampled.

## Molecular Diagnostics

**PCR - IHNV** Resulted by: Ken Sojonky Verified by: Dr. J. Robinson on 08/01/07 @ 4:20 PM

Specimen	ID	Test	Result
Tissue	A)6260-4	PCR - IHNV	Negative
Tissue	B)6260-5	PCR - IHNV	Negative
Tissue	C)6260-6	PCR - IHNV	Negative
Tissue	D)6260-7	PCR - IHNV	Negative

Tissue	E)6260-8	PCR - IHNV	Negative
Tissue	F)6268	PCR - IHNV	Negative
Tissue	G)6273	PCR - IHNV	Negative
Tissue	H)6275 #1-3	PCR - IHNV	Negative
Tissue	I)6276 #1-5	PCR - IHNV	Negative

**PCR - VHSV** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 08/01/07 @ 4:20 PM

Specimen	ID	Test	Result
Tissue	A)6260-4	PCR - VHSV	Negative
Tissue	B)6260-5	PCR - VHSV	Negative
Tissue	C)6260-6	PCR - VHSV	Negative
Tissue	D)6260-7	PCR - VHSV	Negative
Tissue	E)6260-8	PCR - VHSV	Negative
Tissue	F)6268	PCR - VHSV	Negative
Tissue	G)6273	PCR - VHSV	Negative
Tissue	H)6275 #1-3	PCR - VHSV	Negative
Tissue	I)6276 #1-5	PCR - VHSV	Negative

### Virology

**Tissue Culture** Resulted by: Liisa Nielsen Verified by: Liisa Nielsen on 08/27/07 @ 12:03 PM

Specimen	ID	Isolate	Result	Level
Tissue	A)6260-4		No viruses isolated	
Tissue	B)6260-5		No viruses isolated	
Tissue	C)6260-6		No viruses isolated	
Tissue	D)6260-7		No viruses isolated	
Tissue	E)6260-8		No viruses isolated	
Tissue	F)6268		No viruses isolated	
Tissue	G)6273		No viruses isolated	
Tissue	H)6275 #1-3		No viruses isolated	
Tissue	I)6276 #1-5		No viruses isolated	



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-3106

Last Updated: 08/30/07 9:45 AM  
Pathologist: Gary D. Marty  
Received Date: 08/08/07  
Collected Date: 08/08/07  
Client Ref No: Log 6279 PO CL16767

Veterinarian: **Diane Morrison**  
Clinic:  
Phone:  
Fax:

Submitter:  
Phone:  
Fax:  
Owner: **Marine Harvest Canada**  
Phone:  
Fax:(250) 850-3275

**Animal Data**  
Species: Atlantic Salmon  
Breed:  
Sex:  
Age:  
Premise ID:

### Case History

Submitted fresh tissue collected on July 24 and frozen. Please run tissue culture and PCR for IHN and VHS.

### Molecular Diagnostics

**PCR - IHN** Resulted by: A Scouras Verified by: Dr. J. Robinson on 08/13/07 @ 4:43 PM

Specimen	ID	Test	Result
Tissue	6279	PCR - IHN	Negative

**PCR - VHSV** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 08/13/07 @ 2:35 PM

Specimen	ID	Test	Result
Tissue	6279	PCR - VHSV	Negative

### Virology

**Tissue Culture** Resulted by: Liisa Nielsen Verified by: Liisa Nielsen on 08/30/07 @ 9:45 AM

Specimen	ID	Isolate	Result	Level
Tissue	6279		No viruses isolated	



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-3108

Last Updated: 08/30/07 9:46 AM

Pathologist: Gary D. Marty

Received Date: 08/08/07

Collected Date: 08/08/07

Client Ref No: Log 6285 PO CL16768

Veterinarian: **Diane Morrison**

Clinic:

Phone:

Fax:

Submitter:

Phone:

Fax:

Owner: **Marine Harvest Canada**

Phone:

Fax:(250) 850-3275

### Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID:

### Case History

Submitted 4 samples collected on August 1 and frozen. Please run tissue culture and PCR for IHN and VHS.

## Molecular Diagnostics

**PCR - IHNV** Resulted by: A Scouras Verified by: Dr. J. Robinson on 08/13/07 @ 4:43 PM

Specimen	ID	Test	Result
Tissue	6285-1	PCR - IHNV	Negative
Tissue	-2	PCR - IHNV	Negative
Tissue	-3	PCR - IHNV	Negative
Tissue	-4	PCR - IHNV	Negative

**PCR - VHSV** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 08/13/07 @ 2:37 PM

Specimen	ID	Test	Result
Tissue	6285-1	PCR - VHSV	Negative
Tissue	-2	PCR - VHSV	Negative
Tissue	-3	PCR - VHSV	Negative
Tissue	-4	PCR - VHSV	Negative

## Virology

**Tissue Culture** Resulted by: Liisa Nielsen Verified by: Liisa Nielsen on 08/30/07 @ 9:46 AM

Specimen	ID	Isolate	Result	Level

Tissue	6285-1
Tissue	-2
Tissue	-3
Tissue	-4

No viruses isolated



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-3271

Last Updated: 09/17/07 5:32 PM  
Pathologist: Stephen Raverty, DVM  
Received Date: 08/21/07  
Collected Date: 08/21/07  
Client Ref No: Log 6308

Veterinarian: **Diane Morrison**  
Clinic:  
Phone:  
Fax:

Submitter:  
Phone:  
Fax:  
Owner: **Marine Harvest Canada**  
Phone:  
Fax:(250) 850-3275

**Animal Data**  
Species: Atlantic Salmon  
Breed:  
Sex:  
Age:  
Premise ID:

### Case History

Submitted two samples collected on August 15 and frozen. Please run PCR for IHN and VHS.

Log 6308.

### Molecular Diagnostics

**PCR - IHN** Resulted by: A Scouras Verified by: Julie Bidulka on 08/24/07 @ 10:12 AM

Specimen	ID	Test	Result
Tissue	A-Coho Viral	PCR - IHN	Negative
Tissue	B-Coho Viral	PCR - IHN	Negative

**PCR - VHSV** Resulted by: A Scouras Verified by: Julie Bidulka on 08/24/07 @ 10:11 AM

Specimen	ID	Test	Result
Tissue	A-Coho Viral	PCR - VHSV	Negative
Tissue	B-Coho Viral	PCR - VHSV	Negative

### Virology

**Tissue Culture** Resulted by: Hubert Wong Verified by: Dr. J. Robinson on 09/17/07 @ 5:32 PM

Specimen	ID	Isolate	Result	Level
Tissue	A-Coho Viral		No viruses isolated	
Tissue	B-Coho Viral		No viruses isolated	



Stephen Raverty, DVM  
Stephen.Raverty@gov.bc.ca

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**END OF REPORT**

## Final Report AHC Case: 07-3272

Last Updated: 09/17/07 5:32 PM  
Pathologist: Stephen Raverty, DVM  
Received Date: 08/21/07  
Collected Date: 08/21/07  
Client Ref No: Log 6296

Veterinarian: **Diane Morrison**  
Clinic:  
Phone:  
Fax:

Submitter:  
Phone:  
Fax:  
Owner: **Marine Harvest Canada**  
Phone:  
Fax:(250) 850-3275

**Animal Data**  
Species: Atlantic Salmon  
Breed:  
Sex:  
Age:  
Premise ID:

### Case History

Submitted one pooled sample of 5 fish for PCR for IHN and VHS. Sample collected on August 8, 2007 and frozen.

Log 6296

### Molecular Diagnostics

**PCR - IHNV** Resulted by: A Scouras Verified by: Julie Bidulka on 08/24/07 @ 10:12 AM

Specimen	ID	Test	Result
Tissue		PCR - IHNV	Negative

**PCR - VHSV** Resulted by: A Scouras Verified by: Julie Bidulka on 08/24/07 @ 10:13 AM

Specimen	ID	Test	Result
Tissue		PCR - VHSV	Negative

### Virology

**Tissue Culture** Resulted by: Hubert Wong Verified by: Dr. J. Robinson on 09/17/07 @ 5:32 PM

Specimen	ID	Isolate	Result	Level
Tissue			No viruses isolated	



v

Stephen Raverty, DVM  
Stephen.Raverty@gov.bc.ca

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**END OF REPORT**

## Final Report AHC Case: 07-3273

Last Updated: 09/17/07 5:32 PM  
Pathologist: Stephen Raverty, DVM  
Received Date: 08/21/07  
Collected Date: 08/21/07  
Client Ref No: Log 6299

Veterinarian: **Diane Morrison**  
Clinic:  
Phone:  
Fax:

Submitter:  
Phone:  
Fax:  
Owner: **Marine Harvest Canada**  
Phone:  
Fax:(250) 850-3275

**Animal Data**  
Species: Atlantic Salmon  
Breed:  
Sex:  
Age:  
Premise ID:

### Case History

Sampled fresh and dead fish with hemorrhage on intestine and swim bladder, spleen, and liver. Please run PCR for IHN and VHS. Histo taken from above fish. All samples collected on August 14, 2007. Viral samples frozen.

### Final Diagnosis

1. Spleen and intestinal ceca: peritonitis, granulomatous, multifocal, moderate
- 2a. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 2b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 3a. Brain: hemorrhage, multifocal, mild
- 3b. Brain: capillary (vascular) congestion, diffuse, mild to moderate
4. Heart: myocardial karyomegaly, multifocal, mild

**Final Comment:** This fish has several lesions that might provide clues to its death; however, the lesions are not consistent with death due to VHSV or IHN.

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

### Histopathology

Histopathology is reported by Dr. Gary Marty. Formalin-fixed tissues were submitted a cassette and prepared for histopathology on one slide: spleen, brain, liver, heart, trunk kidney (2 pieces), intestinal ceca, and mesenteric adipose tissue

All organs were examined. Organs not listed elsewhere have no significant lesions.

**Quality control:** Liver autolysis: moderate. Large foci of erythrocytes (e.g., spleen) have precipitates of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic before or during fixation. Organs have no postfixation dehydration.

### Molecular Diagnostics

**PCR - IHNV** Resulted by: A Scouras Verified by: Julie Bidulka on 08/24/07 @ 10:13 AM

Specimen	ID	Test	Result
Tissue		PCR - IHNV	Negative

**PCR - VHSV** Resulted by: A Scouras Verified by: Julie Bidulka on 08/24/07 @ 10:13 AM

Specimen	ID	Test	Result
Tissue		PCR - VHSV	Negative

### Virology

**Tissue Culture** Resulted by: Hubert Wong Verified by: Dr. J. Robinson on 09/17/07 @ 5:32 PM

Specimen	ID	Isolate	Result	Level
Tissue			No viruses isolated	



Stephen Raverty, DVM  
Stephen.Raverty@gov.bc.ca

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**END OF REPORT**

## Final Report AHC Case: 07-3442

Last Updated: 09/26/07 5:12 PM

Pathologist: Gary D. Marty

Received Date: 09/04/07

Collected Date: 09/04/07

Client Ref No: Log 6316

Veterinarian: **Diane Morrison**

Clinic:

Phone:

Fax:

Submitter:

Phone:

Fax:

Owner: **Marine Harvest Canada**

Phone:

Fax:(250) 850-3275

### Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID:

### Case History

Submitted one sample for PCR for IHN and VHS, collected on August 23 and kept frozen.

Atlantic, regular, saltwater, high mortality.

### Molecular Diagnostics

**PCR - IHN** Resulted by: A Scouras Verified by: Dr. J. Robinson on 09/06/07 @ 4:35 PM

Specimen	ID	Test	Result
Tissue	6316-1	PCR - IHN	Negative

**PCR - VHSV** Resulted by: A Scouras Verified by: Dr. J. Robinson on 09/06/07 @ 4:35 PM

Specimen	ID	Test	Result
Tissue	6316-1	PCR - VHSV	Negative

### Virology

**Tissue Culture** Resulted by: Hubert Wong Verified by: Dr. J. Robinson on 09/26/07 @ 5:12 PM

Specimen	ID	Isolate	Result	Level
Tissue	6316-1		No viruses isolated	



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-3443

Last Updated: 09/26/07 5:12 PM

Pathologist: Gary D. Marty

Received Date: 09/04/07

Collected Date: 09/04/07

Client Ref No: Log 6319

Veterinarian: **Diane Morrison**

Clinic:

Phone:

Fax:

Submitter:

Phone:

Fax:

Owner: **Marine Harvest Canada**

Phone:

Fax:(250) 850-3275

### Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID:

### Case History

Submitted two samples for PCR. #1 sample (3 fish pool), and #2 sample (2 fish pool) for PCR of IHN and VHS. Routine sample of site experiencing low DO. One fish with hemorrhage of viscera.

### Molecular Diagnostics

**PCR - IHNV** Resulted by: A Scouras Verified by: Dr. J. Robinson on 09/06/07 @ 4:35 PM

Specimen	ID	Test	Result
Tissue	A-6319-1,2,3	PCR - IHNV	Negative
Tissue	B-6319-4,5	PCR - IHNV	Negative

**PCR - VHSV** Resulted by: A Scouras Verified by: Dr. J. Robinson on 09/06/07 @ 4:35 PM

Specimen	ID	Test	Result
Tissue	A-6319-1,2,3	PCR - VHSV	Negative
Tissue	B-6319-4,5	PCR - VHSV	Negative

### Virology

**Tissue Culture** Resulted by: Hubert Wong Verified by: Dr. J. Robinson on 09/26/07 @ 5:12 PM

Specimen	ID	Isolate	Result	Level
Tissue	A-6319-1,2,3		No viruses isolated	
Tissue	B-6319-4,5		No viruses isolated	



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D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-3488

Last Updated: 10/03/07 4:34 PM

Pathologist: Gary D. Marty

Received Date: 09/06/07

Collected Date: 09/06/07

Client Ref No: Log 6340

Veterinarian: **Diane Morrison**

Clinic:

Phone:

Fax:

Submitter:

Phone:

Fax:

Owner: **Marine Harvest Canada**

Phone:

Fax:(250) 850-3275

### Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID:

### Case History

Submitted three samples for virology and three histo cassettes.

Slight increase in mortality at site. Three fresh mortis had petechial hemorrhage of liver, inflamed intestine, no feed in gut. Samples collected on Aug 31 and kept frozen. Please run PCR for IHN and VHS.

## Final Diagnosis

- 1a. Liver: sinusoidal congestion, with intracytoplasmic spherical amphophilic inclusions, acute, multifocal, mild (slides 1, 2), moderate (slide 3)
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate (slide 1)
- 1c. Liver: cholangitis, pleocellular, focal, mild (slides 1, 2)
2. Spleen, mesenteric adipose tissue, and liver (slide 1 only): peritonitis, chronic, multifocal, with fibrocellular fronds, mild (slides 2, 3), moderate (slide 1)
- 3a. Heart: endocarditis, multifocal, lymphohistiocytic, mild (slides 1, 3)
- 3b. Heart: epicarditis, multifocal, lymphohistiocytic, mild (slides 2, 3)
4. Brain: capillary (vascular) congestion, diffuse, mild (slides 1, 2)
5. Head kidney and Trunk kidney: moderate numbers of intracytoplasmic eosinophilic granules in endothelial cells, diffuse, moderate (slide 1)
6. Head kidney: interstitial vascular congestion, diffuse, mild (slide 2)

**Final Comment:** These fish have several lesions that provide evidence of systemic damage, consistent with clinical findings. The changes tend to be fairly nonspecific, and could be a result of toxin exposure, or infection with various bacteria or viruses.

Sinusoidal congestion in the liver is evidence of sinusoidal damage. In BC Atlantic salmon, hepatic sinusoidal congestion is evidence of sinusoidal damage. I have seen it associated with viral hemorrhagic septicemia virus and *Listonella anguillarum* infection. The amphophilic cytoplasmic inclusions in hepatocytes are large, up twice the size of hepatocyte nuclei. The inclusions might be remnants of ingested erythrocytes (this type of inclusion has not been described with any salmon virus).

Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition.

Lymphocytic inflammation in and around bile ductules (liver) is evidence of chronic immune stimulation. This type of inflammation can result from bacteria ascending from the intestine to the liver through the biliary system.

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

Lymphohistiocytic inflammation in the heart is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine.

Congestion of brain capillaries is evidence of circulating vasodilators; differentials include viral, bacterial, and parasitic infections. The neuropil normally contains a rich network of capillaries, but in any given section, the majority of capillaries contain no erythrocytes. By comparison, when cerebral capillaries are congested (as in this case), a greater proportion of capillaries in the section contain erythrocytes.

Accumulation of eosinophilic granules in the cytoplasm of cells lining small vessels in the kidney is a distinctive finding in Atlantic salmon. These granules probably accumulate in response to systemic immune stimulation. The granules might be part of eosinophilic granular cells or endothelial cells; granule size seems too large and variable for eosinophilic granular cells. I have seen these granules associated with *Piscirickettsia salmonis*, but other cases (like this one) have no known cause. They have also been described in Atlantic salmon naturally infected with chronic pancreas disease in Norway (Salmonid alphavirus subtype 3, SAV3; McLoughlin and Graham 2007), but SAV3 has not been identified in BC salmon.

Renal vascular congestion has been associated with VHSV and bacteria. It probably has a pathogenesis similar to hepatic sinusoidal congestion.

#### Literature Cited:

McLoughlin, M.F. and D.A. Graham. 2007. Alphavirus infections in salmonids - a review. *Journal of Fish Diseases*. 30(9):511-531.

## Histopathology

Formalin-fixed tissues were submitted in 3 full cassettes for histopathology.

Slide 1 (6340-1) - brain, spleen, liver, heart, head kidney, trunk kidney, intestinal ceca, and mesenteric adipose tissue

Slide 2 (6340-2) - brain, spleen, liver, heart, head kidney, trunk kidney, intestinal ceca, and mesenteric adipose tissue

Slide 3 (6340-3) - spleen, liver, heart, head kidney, trunk kidney, intestinal ceca, and mesenteric adipose tissue

All organs were examined. Organs not listed elsewhere have no significant lesions.

**Quality control:** Liver autolysis: mild (slides 1, 2, 3). Organs have no postfixation dehydration and no acid hematin deposits.

## Molecular Diagnostics

**PCR - IHNV** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 09/11/07 @ 2:23 PM

Specimen	ID	Test	Result
Tissue	A-6340-1	PCR - IHNV	Negative

Case: 07-3488

Tissue	B-6340-2	PCR - IHNV	Negative
Tissue	C-6340-3	PCR - IHNV	Negative

**PCR - VHSV** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 09/11/07 @ 2:23 PM

Specimen	ID	Test	Result
Tissue	A-6340-1	PCR - VHSV	Negative
Tissue	B-6340-2	PCR - VHSV	Negative
Tissue	C-6340-3	PCR - VHSV	Negative

### Virology

**Tissue Culture** Resulted by: Liisa Nielsen Verified by: Dr. J. Robinson on 10/03/07 @ 4:34 PM

Specimen	ID	Isolate	Result	Level
Tissue	A-6340-1		No viruses isolated	
Tissue	B-6340-2		No viruses isolated	
Tissue	C-6340-3		No viruses isolated	



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-3489

Last Updated: 10/03/07 4:35 PM

Pathologist: Gary D. Marty

Received Date: 09/06/07

Collected Date: 09/06/07

Client Ref No: Log 6341

Veterinarian: **Diane Morrison**

Clinic:

Phone:

Fax:

Submitter:

Phone:

Fax:

Owner: **Marine Harvest Canada**

Phone:

Fax:(250) 850-3275

### Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID:

### Case History

Submitted four pooled samples for PCR for IHN and VHS. Samples labeled as 1-5, 6-10, 11-15, 16-20. Fish may be moved. Routine health check prior to move. Samples collected Aug 30 and kept frozen. Histo taken from four fish labeled 1, 2, 3, and 4.

### Final Diagnosis

1. Liver: hepatitis, perivascular, lymphocytic, focal, mild (slides 1, 2)
2. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild (slide 3)

**Final Comment:** These fish have only minor lesions that are common in cultured juvenile salmonids.

Lymphocytic inflammation around a vessel in the liver is evidence of chronic immune stimulation, e.g., from a bacterial infection.

Renal tubular epithelial protein droplets are normal in some species, or they might be an indication of glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2006 as part of the Ministry's Fish Health Auditing and Surveillance Program: Atlantic salmon (prevalence = 22%; n = 495) and Pacific salmon (prevalence = 38%; n = 134).

### Histopathology

**Histopathology:** Formalin-fixed tissues were submitted in 4 cassettes for histopathology.

Slide 1 (UH-1 8/30/07) - skin, spleen, liver, heart, trunk kidney (2 pieces), intestinal ceca, and mesenteric adipose tissue

Slide 2 (UH-2 8/30/07) - brain, spleen, liver, heart, head kidney, trunk kidney, stomach, intestinal ceca, and mesenteric adipose tissue

Slide 3 (UH-3 8/30/07) - skin, skeletal muscle, spleen, liver, heart, head kidney, trunk kidney, stomach, intestinal ceca, and mesenteric adipose tissue

All organs were examined. Organs not listed elsewhere have no significant lesions.

**Quality control:** Liver autolysis: none (all 4 slides). Organs have no postfixation dehydration and no acid hematin deposits.

**Measures of physiologic condition:**

Hepatocellular glycogen: small amounts (slide 4), moderate amounts (slides 1, 3), abundant (slide 2)

Mesenteric adipose tissue: abundant (all 4 slides)

These measures of physiologic condition are consistent with healthy fish; fish #4 did not eat as much recently as the other fish.

**Molecular Diagnostics**

**PCR - IHNV** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 09/11/07 @ 2:23 PM

Specimen	ID	Test	Result
Tissue	A-6341 1-5	PCR - IHNV	Negative
Tissue	B-6341 6-10	PCR - IHNV	Negative
Tissue	C-6341 11-15	PCR - IHNV	Negative
Tissue	D-6341 16-20	PCR - IHNV	Negative

**PCR - VHSV** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 09/11/07 @ 2:23 PM

Specimen	ID	Test	Result
Tissue	A-6341 1-5	PCR - VHSV	Negative
Tissue	B-6341 6-10	PCR - VHSV	Negative
Tissue	C-6341 11-15	PCR - VHSV	Negative
Tissue	D-6341 16-20	PCR - VHSV	Negative

**Virology**

**Tissue Culture** Resulted by: Liisa Nielsen Verified by: Dr. J. Robinson on 10/03/07 @ 4:35 PM

Specimen	ID	Isolate	Result	Level
Tissue	A-6341 1-5		No viruses isolated	
Tissue	B-6341 6-10		No viruses isolated	
Tissue	C-6341 11-15		No viruses isolated	
Tissue	D-6341 16-20		No viruses isolated	



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-3520

Last Updated: 09/11/07 11:10 AM

Pathologist: Gary D. Marty

Received Date: 09/07/07

Collected Date: 09/07/07

Client Ref No: Log #6343

Veterinarian: **Marine Harvest Canada**

Clinic:

Phone:

Fax:

Submitter:

Phone:

Fax:

Owner: **Marine Harvest Canada**

Phone:

Fax:(250) 850-3275

### Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID:

### Case History

Log No. 6343. Four histo cassettes of gill. Species: Atlantic. Sex: Regular. Saltwater entry: 2007 S1. Saltwater. *Chaetoceros* bloom on site coming to an end. Gill wet mounts revealed very little in the way of cells present. Sample size: 4 histo cassettes of gills. Gill histo collected to assess any damages, cassettes labelled CH1, CH2, CH3 and CH4. Date: Sept. 6/07

### Final Diagnosis

These fish have lesions consistent with the clinical history of exposure to *Chaetoceros*. The diatoms *Chaetoceros concavicornis*, *C. convolutus*, and a *Corethron* sp. have been associated with mortality of salmon reared in seawater netpens at numbers as low as 5/mL (Taylor and Harrison 2002). In some cases, the space between the base of gill filaments often contain moderate numbers of unstained structures that are consistent with the diatom *Chaetoceros*. Chains of cell bodies are about 25 µm in diameter, and individual spines/setae are about 3.5 µm in diameter. In this case, only one fish has organisms in the section, but others have similar lesions.

Mortality due to *Chaetoceros* can occur within a few days of exposure, as a result of physical damage to the gills and the resultant inflammatory response. Affected fish are also more vulnerable to *Vibrio* infections. Larger fish tend to be more susceptible than small fish. Characteristic gill lesions include mucous cell hyperplasia, lamellar epithelial cell hyperplasia and necrosis, and variable numbers of neutrophils. [Source: Kent, M.L., and T.T. Poppe. 1998. Diseases of seawater netpen-reared salmonid fishes. Quadra Printers, Ltd. Nanaimo, B.C., Canada.]

### Literature cited:

Taylor, F.J.R., and P.J. Harrison. 2002. Harmful algal blooms in western Canadian coastal waters. In Report #23 of the North Pacific Marine Science Organization, "Harmful algal blooms in the PICES region of the North Pacific."

### Histopathology

Four formalin-fixed gill arches, each in a separate cassette were submitted for histopathology. Cassettes were labeled 9/5/07, and progressively from CH-1 through CH-4 (slide numbers correspond to CH #s). The gills were decalcified in 10% EDTA over the weekend and then processed routinely into paraffin.

**Quality control:** Tissue preservation is excellent. Tissues have no postfixation dehydration and no deposits of acid hematin.



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-3522

Last Updated: 09/11/07 11:08 AM

Pathologist: Gary D. Marty

Received Date: 09/07/07

Collected Date: 09/07/07

Client Ref No: Log #6342

Veterinarian: **Marine Harvest Canada**

Clinic:

Phone:

Fax:

Submitter:

Phone:

Fax:

Owner: **Marine Harvest Canada**

Phone:

Fax:(250) 850-3275

### Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID:

### Case History

Log No. 6342. Four histo cassettes of gill. Species: Atlantic. Sex: Regular. Saltwater entry: 2007 S1. Netpen ID: 8. Saltwater. Collected Sept 5/07. Sample size: 4 histo cassettes of gills. Site has been in midst of *Chaetoceros* bloom for 17 days. Cell counts are coming down. Gill samples taken to assess any damage, if any. Cassettes labelled LB1, LB2, LB3, LB4.

### Final Diagnosis

1. Gill: lamellar epithelial hyperplasia and fusion, multifocal, moderate (slide 1), with intralesional diatom spines/setae, moderate (slides 2, 3, 4)
2. Gill: branchitis, fibrinous, with neutrophils and multinucleate giant cells, multifocal, with intralesional diatom setae, severe (slide 4)

**Final Comment:** These fish have lesions consistent with the clinical history of exposure to *Chaetoceros*. The diatoms *Chaetoceros concavicornis*, *C. convolutus*, and a *Corethron sp.* have been associated with mortality of salmon reared in seawater netpens at numbers as low as 5/mL (Taylor and Harrison 2002). In some cases, the space between the base of gill filaments often contain moderate numbers of unstained structures that are consistent with the diatom *Chaetoceros*. Chains of cell bodies are about 25 µm in diameter, and individual spines/setae are about 3.5 µm in diameter. In this case, three gills have organisms in the section, and the other gill has similar lesions.

Mortality due to *Chaetoceros* can occur within a few days of exposure, as a result of physical damage to the gills and the resultant inflammatory response. Affected fish are also more vulnerable to *Vibrio* infections. Larger fish tend to be more susceptible than small fish. Characteristic gill lesions include mucous cell hyperplasia, lamellar epithelial cell hyperplasia and necrosis, and variable numbers of neutrophils. [Source: Kent, M.L., and T.T. Poppe. 1998. Diseases of seawater netpen-reared salmonid fishes. Quadra Printers, Ltd. Nanaimo, B.C., Canada.]

### Literature cited:

Taylor, F.J.R., and P.J. Harrison. 2002. Harmful algal blooms in western Canadian coastal waters. In Report #23 of the North Pacific Marine Science Organization, "Harmful algal blooms in the PICES region of the North Pacific."

### Histopathology

Four formalin-fixed gill arches, each in a separate cassette were submitted for histopathology. Cassettes were labeled 9/5/07, and progressively from LB-1 through LB-4 (slide numbers correspond to LB #s). The gills were decalcified in 10% EDTA over the weekend and then processed routinely into paraffin.

**Quality control:** Tissue preservation is excellent. Tissues have no postfixation dehydration and no deposits of acid hematin.

A handwritten signature in black ink that reads "Gary D. Marty". The signature is written in a cursive style with a large, prominent "G" and "M".

Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

These results relate only to the animals or items tested.

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**END OF REPORT**

## Final Report AHC Case: 07-3561

Last Updated: 10/03/07 4:35 PM

Pathologist: Gary D. Marty

Received Date: 09/11/07

Collected Date: 09/11/07

Client Ref No: Log 6344

Veterinarian: **Diane Morrison**

Clinic:

Phone:

Fax:

Submitter:

Phone:

Fax:

Owner: **Marine Harvest Canada**

Phone:

Fax:(250) 850-3275

### Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID:

### Case History

Submitted 3 histo and 3 virology samples submitted as follow up to sudden overnight die off. Fresh dead fish shipped down on ice. Histo and virology samples taken. Please run PCR for IHNV and VHSV on all samples. Atlantic, 2006, S1, Saltwater.

### Final Diagnosis

- 1a. Liver: biliary preductular cell hyperplasia, diffuse, moderate (slide 1)
- 1b. Liver: sinusoidal congestion, acute, multifocal, moderate (slide 2)
- 1c. Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slide 1)
2. Heart: myocardial karyomegaly, multifocal, moderate (slide 2)
- 3a. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slide 1)
- 3b. Spleen: peritonitis, focal, granulomatous, mild (slide 2)
- 3c. Spleen: peritonitis, granulomatous, regionally diffuse, with intralesional vacuoles about 50 µm in diameter, moderate (slide 3)
4. Head kidney and trunk kidney: interstitial vascular congestion, diffuse, mild (slide 1), moderate (slide 2)

**Final Comment:** None of the significant lesions in these fish are consistent in all 3 fish. Biliary preductular cell hyperplasia in fish #1 is evidence of exposure to toxins, but other fish lacked this lesion. Vascular congestion in the liver and kidney of fish #2 is consistent with acute septicemia as the cause of death, but congestion in other fish is no more than mild. Specific comments follow:

Biliary preductular cell hyperplasia 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). Biliary preductular cell hyperplasia is fairly common in salmon that die in marine net pens, affecting 15% of the 505 Atlantic salmon and 4.5% of the 134 Pacific salmon examined in 2006 as part of the Province's Fish Health Auditing and Surveillance Program; the sample size of Atlantic salmon in 2006 was sufficient to identify a trend towards greater prevalence in the fall and winter (20-26%) than in the spring and summer (4-12%).

Congestion in hepatic sinusoids and renal capillaries is nonspecific evidence of sinusoidal damage. In BC Atlantic salmon, sinusoidal congestion is an uncommon feature of infection with viral hemorrhagic septicemia virus and *Listonella anguillarum*, but other bacteria and viruses cannot be ruled out. Vascular congestion is one of the classic lesions associated with ISAV infection, but ISAV has never been

identified in British Columbia.

Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition.

Mild myocardial karyomegaly is somewhat common in cultured salmonids (e.g., 5.3% of the 503 Atlantic salmon hearts sampled as part of the BC Fish Health Auditing and Surveillance Program during 2006). The cause and significance is unknown, but there might be a genetic predisposition to developing the lesion. Karyomegaly in other cell types has been associated with exposure to algal toxins (e.g., hepatocytes exposed to microcystin LR in netpen liver disease).

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated. Vacuoles probably are a result of vaccine material lost during tissue processing.

## Histopathology

Formalin-fixed tissues were submitted in 3 cassettes for histopathology.

Slide 1 (6344-1) - spleen, liver, heart, trunk kidney, head kidney

Slide 2 (6344-2) - spleen, liver, heart, trunk kidney, head kidney

Slide 3 (6344-3) - spleen, liver, heart, trunk kidney, head kidney

All organs were examined. Organs not listed elsewhere have no significant lesions.

**Quality control:** Liver autolysis: severe (slides 1, 2, and 3). Trunk kidney autolysis: moderate (slide 1, 2, and 3). Organs have no postfixation dehydration and no acid hematin deposits.

## Molecular Diagnostics

**PCR - IHNV** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 09/13/07 @ 4:35 PM

Specimen	ID	Test	Result
Tissue	A)6344-1	PCR - IHNV	Negative
Tissue	B)6344-2	PCR - IHNV	Negative
Tissue	C)6344-3	PCR - IHNV	Negative

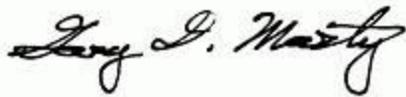
**PCR - VHSV** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 09/13/07 @ 4:37 PM

Specimen	ID	Test	Result
Tissue	A)6344-1	PCR - VHSV	Negative
Tissue	B)6344-2	PCR - VHSV	Negative
Tissue	C)6344-3	PCR - VHSV	Negative

## Virology

**Tissue Culture** Resulted by: Liisa Nielsen Verified by: Dr. J. Robinson on 10/03/07 @ 4:35 PM

Specimen	ID	Isolate	Result	Level
Tissue	A)6344-1		No viruses isolated	
Tissue	B)6344-2		No viruses isolated	
Tissue	C)6344-3		No viruses isolated	



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-3585

Last Updated: 09/18/07 9:42 AM

Pathologist: Gary D. Marty

Received Date: 09/13/07

Collected Date: 09/13/07

Client Ref No:

Veterinarian: **Barry Milligan**

Clinic:

Phone:

Fax:

Submitter:

Phone:

Fax:

Owner: **Grieg Seafoods BC Ltd.**

Phone: (250) 286-0838

Fax:(250) 286-1883

### Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID:

### Case History

Submitted 4 cassettes for histology. Two fish per cassette.

Plankton bloom. High mortality.

## Final Diagnosis

1. Gill: interlamellar bacteria (all sections), with cylindrical algae (*Skeletonema costatum* ; slides 1.1, 2.1, 3.1, and 4.1) and spiny algae (*Dictyocha sp.*, slide 3.1)

2a. Liver: hepatic necrosis, acute, multifocal, mild (slide 1, one liver), moderate (slide 1, one liver; slide 3, both livers; slide 4, one liver)

2b. Liver: sinusoidal congestion, acute, multifocal, mild (slide 1, one liver), moderate (slide 1, one liver)

3. Liver, spleen, intestinal ceca and mesenteric fat: peritonitis, chronic, multifocal, with fibrocellular fronds, moderate (slides 1, 2, 3, 4)

4a. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild (slides 1, 2, 3, 4)

4b. Trunk kidney: renal tubular casts of protein and yellow-brown pigment, with tubular dilation, focal, mild (slide 4)

**Final Comment:** These fish probably died of complications related to exposure to harmful algal blooms, although infectious disease cannot be ruled out unless bacteriology and virology are done.

The spaces between the tips of gill lamellae of adjacent filaments contain several foci of plump bacteria and cylindrical algae. Most of the algae have morphologic features consistent with *Skeletonema costatum* (i.e., strutted processes linking cells, ~40 µm long and 5 µm in diameter; coordinates on my microscope on slide 1 = 23 × 117.5). At least one organism in slide 3 has spiny processes consistent with *Dictyocha speculum* (coordinates on my microscope = 24 × 111). The gills are severely autolyzed, and they have no evidence of any proliferative response. Much of the bacterial growth probably occurred postmortem.

Hepatic necrosis can be caused by inadequate vascular perfusion or direct cytotoxicity from viral or bacterial infections (e.g., viral hemorrhagic septicemia virus, *Piscirickettsia salmonis*, and *Renibacterium salmoninarum*); the cause is not determined in most cases. This case has no obvious organisms in the liver, and necrosis was probably secondary to gill lesions. Hepatic necrosis is somewhat common in salmon that die in marine net pens, affecting 7.5% of the 505 Atlantic salmon and 5% of the 134 Pacific salmon examined in 2006 as part of the Province's Case: 07-3585

Sinusoidal congestion in the liver is nonspecific evidence of sinusoidal damage. In BC Atlantic salmon, hepatic sinusoidal congestion is an uncommon feature of infection with viral hemorrhagic septicemia virus and *Listonella anguillarum*. In this case, it probably is related to hepatic necrosis. Sinusoidal congestion is one of the classic lesions associated with ISAV infection, but ISAV has never been identified in British Columbia.

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

Renal tubular epithelial protein droplets are normal in some species, or they might be an indication of glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2006 as part of the Ministry's Fish Health Auditing and Surveillance Program: Atlantic salmon (prevalence = 22%; n = 495) and Pacific salmon (prevalence = 38%; n = 134).

Pigment in the renal tubules (slide 4) probably includes lipofuscin. Accumulation of lipofuscin 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.

## Histopathology

Formalin-fixed tissues were submitted in 4 cassettes for histopathology. Gills were removed from the original cassettes and placed in separate (.1) cassettes. The gills were decalcified overnight in EDTA and then processed routinely into paraffin.

Slide 1 - gill, spleen, liver, head kidney, trunk kidney, intestinal ceca, and mesenteric adipose tissue

Slide 1.1 - gill

Slide 2 - spleen, liver, head kidney, trunk kidney, intestinal ceca, and mesenteric adipose tissue

Slide 2.1 - gill

Slide 3 - spleen, liver, head kidney, trunk kidney, intestinal ceca, and mesenteric adipose tissue

Slide 3.1 - gill

Slide 4 - spleen, liver, head kidney, trunk kidney (including 2-mm -diameter Corpuscles of Stannius), intestinal ceca, and mesenteric adipose tissue

Slide 4.1 - gill

All organs were examined. Organs not listed elsewhere have no significant lesions.

**Quality control:** Liver autolysis: severe (slides 1, 2, 3, 4); gill autolysis: severe (all sections). Gill decalcification is complete. Organs have no postfixation dehydration and no acid hematin deposits.



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-3717

Last Updated: 10/16/07 4:40 PM

Pathologist: Gary D. Marty

Received Date: 09/25/07

Collected Date: 09/25/07

Client Ref No: Log 6371

Veterinarian: **Diane Morrison**

Clinic: **Marine Harvest Canada**

Phone: (250) 850-3276

Fax: (250) 850-3275

Submitter:

Phone:

Fax:

Owner: **Marine Harvest Canada**

Phone:

Fax:(250) 850-3275

### Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID:

### Case History

Submitted one pooled sample for PCR for IHNV and VHSV. Sample collected on September 20 from three fish showing hemorrhage throughout viscera and kept frozen. Mortality is low at sea site. First virology sample from this population at this site. Also submitted one histo cassette.

## Final Diagnosis

1. Intestinal ceca: peritonitis, granulomatous, regionally diffuse, with scattered macrophages filled with Gram - positive material (tissues are PCR-positive for *Renibacterium salmoninarum*), severe
2. Spleen: peritonitis, chronic, diffuse, with fibrocellular fronds, mild
3. Heart: endocarditis, multifocal, lymphohistiocytic, moderate
- 4a. Liver: hepatitis, granulomatous, multifocal, moderate
- 4b. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
5. Trunk kidney: nephritis, interstitial, granulomatous, multifocal, moderate

### Final Comment:

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated. Because the pattern of inflammation and Gram stain is different from most cases of BKD that I see, I ordered a PCR test (no extra charge to client); the test confirms that tissues from this fish contain *Renibacterium salmoninarum* DNA. The granulomatous inflammation surrounding the intestinal ceca contains small numbers of macrophages that have abundant Gram -positive material in the cytoplasm. On 100x oil immersion, the material is slightly polymorphic; although the diameter is the same as *Renibacterium salmoninarum*, much of the material is more spherical than rod-shaped, and it stains poorly. The cases of *Renibacterium salmoninarum* that I usually see have a positive correlation between number of macrophages filled with Gram-positive bacilli and number of bacilli within each macrophage. This case does not fit the usual pattern; i.e., this case has too few macrophages filled with too much Gram - positive material. The inflammation includes a multinucleate giant cell, but the section of this cell contains no Gram -positive material.

Lymphohistiocytic inflammation in the heart is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. The foci are negative for bacteria on the Gram stain.

The single focus of granulomatous inflammation in the kidney that contains a multinucleate giant cell with some poorly staining material that probably came from a vaccine; however, the presence of infectious disease cannot be entirely ruled out. The most common organism associated with granulomatous nephritis in salmon is *Renibacterium salmoninarum*, the cause of bacterial kidney disease. However, chronic infections with *Yersinia ruckeri* have also been associated with granulomatous inflammation. The focus was not included on the Gram - stained section.

Foci of granulomatous inflammation in the liver probably have the same pathogenesis as in the kidney. Basophilic cytoplasm in hepatocytes is an indication of active protein synthesis. It is normal in mature females producing protein for deposition in their eggs. In other fish it might be related to increased protein needed as part of an inflammatory response.

## Histopathology

Formalin-fixed tissues were submitted in a cassette for histopathology: spleen, liver, heart, head-trunk kidney transition area, intestinal ceca, and mesenteric adipose tissue. All organs were examined. A section was also stained with Twort's gram stain. Organs not listed elsewhere have no significant lesions.

**Quality control:** Liver autolysis: mild; intestinal autolysis: none. Organs have no postfixation dehydration and no acid hematin deposits.

## Molecular Diagnostics

**PCR-Renibacterium salmoni** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 10/03/07 @ 4:31 PM

Specimen	ID	Test	Result
Tissue	6371-1	PCR-Renibacterium salmoninaru	Positive

**PCR - IHNV** Resulted by: A Scouras Verified by: Dr. J. Robinson on 09/28/07 @ 3:59 PM

Specimen	ID	Test	Result
Tissue	6371-1	PCR - IHNV	Negative

**PCR - VHSV** Resulted by: A Scouras Verified by: Dr. J. Robinson on 09/28/07 @ 3:59 PM

Specimen	ID	Test	Result
Tissue	6371-1	PCR - VHSV	Negative

## Virology

**Tissue Culture** Resulted by: Liisa Nielsen Verified by: Dr. J. Robinson on 10/16/07 @ 4:40 PM

Specimen	ID	Isolate	Result	Level
Tissue	6371-1		No viruses isolated	



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-3719

Last Updated: 10/16/07 4:40 PM

Pathologist: Gary D. Marty

Received Date: 09/25/07

Collected Date: 09/25/07

Client Ref No: Log 6365

Veterinarian: **Diane Morrison**

Clinic:

Phone:

Fax:

Submitter:

Phone:

Fax:

Owner: **Marine Harvest Canada**

Phone:

Fax:(250) 850-3275

### Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID:

### Case History

Submitted three bags for virology. Routine check of broodstock prior to brood season. Please run PCR for IHNV and VHSV.

### Molecular Diagnostics

**PCR - IHNV** Resulted by: A Scouras Verified by: Dr. J. Robinson on 10/01/07 @ 4:14 PM

Specimen	ID	Test	Result
Tissue	6365-1	PCR - IHNV	Negative
Tissue	6365-2	PCR - IHNV	Negative
Tissue	6365-3	PCR - IHNV	Negative

**PCR - VHSV** Resulted by: A Scouras Verified by: Dr. J. Robinson on 10/01/07 @ 4:15 PM

Specimen	ID	Test	Result
Tissue	6365-1	PCR - VHSV	Negative
Tissue	6365-2	PCR - VHSV	Negative
Tissue	6365-3	PCR - VHSV	Negative

### Virology

**Tissue Culture** Resulted by: Liisa Nielsen Verified by: Dr. J. Robinson on 10/16/07 @ 4:40 PM

Specimen	ID	Isolate	Result	Level
Tissue	6365-1		No viruses isolated	
Tissue	6365-2		No viruses isolated	



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-3923

Last Updated: 11/02/07 2:29 PM

Pathologist: Gary D. Marty

Received Date: 10/11/07

Collected Date: 10/11/07

Client Ref No: Log 6383

Veterinarian: **Diane Morrison**

Clinic: **Marine Harvest Canada**

Phone: (250) 850-3276

Fax: (250) 850-3275

Submitter: **Marine Harvest**

Phone:

Fax:

Owner: **Marine Harvest Canada**

Phone:

Fax:(250) 850-3275

### Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID:

### Case History

Submitted two fresh tissue samples for PCR for IHN and VHS on samples A and B.

Slight increase in mortality at site. Routine sample to rule out viruses. NVL on 5 fish. Samples collected October 5, 2007 and frozen.

## Molecular Diagnostics

**PCR - IHN** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 10/16/07 @ 2:21 PM

Specimen	ID	Test	Result
Tissue	A	PCR - IHN	Negative
Tissue	B	PCR - IHN	Negative

**PCR - VHSV** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 10/15/07 @ 4:35 PM

Specimen	ID	Test	Result
Tissue	A	PCR - VHSV	Negative
Tissue	B	PCR - VHSV	Negative

## Virology

**Tissue Culture** Resulted by: Liisa Nielsen Verified by: Dr. J. Robinson on 11/02/07 @ 2:29 PM

Specimen	ID	Isolate	Result	Level
Tissue	A		No viruses isolated	
Tissue	B		No viruses isolated	



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-3924

Last Updated: 10/15/07 4:19 PM

Pathologist: Gary D. Marty

Received Date: 10/11/07

Collected Date: 10/11/07

Client Ref No: Log 6385

Veterinarian: **Diane Morrison**

Clinic: **Marine Harvest Canada**

Phone: (250) 850-3276

Fax: (250) 850-3275

Submitter: **Diane Morrison**

Phone:

Fax:

Owner: **Marine Harvest Canada**

Phone:

Fax:(250) 850-3275

### Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID:

### Case History

Submitted one container and two bags of tissue for histology. Heart abnormalities observed in production fish. Sampled fish were on feed; had good fat stores, and no other visible lesions. One container holds a number of sampled hearts. Full histo from 2 fish are contained in two separate bags labeled 10-1 and 7-1.

### Final Diagnosis

- 1a. Heart: cardiac dysplasia (situs inversus? slides 1, 2, 7-1)
- 1b. Heart: bulbus arteriosus dilation, (lumen 7 mm in diameter, slide 4B)
- 1c. Heart: epicarditis, histiocytic, multifocal, mild (slides 1, 10-3)
- 1d. Heart: myocardial karyomegaly, multifocal, mild (slides 1, 4B, 7-1)
2. Liver, trunk kidney, and brain: capillary congestion, multifocal to diffuse, mild (slide 7-2), moderate (slide 10-2)
3. Spleen: peritonitis, granulomatous, regionally diffuse, with intralesional vacuoles about 50 µm in diameter, mild (slide 10-2), moderate (slide 7-2)
4. Intestinal ceca and mesenteries: peritonitis, granulomatous, multifocal, mild (slide 10-2)

### Final Comment:

Mid-sagittal sections of the heart are normally triangular, with the bulbus arteriosus on the anterior margin and the atrioventricular valve on the dorso-anterior margin. The hearts in slides 1, 2, and 7-1 have both the bulbus arteriosus and the atrioventricular valve on the anterior margin. Histology is a poor method for detecting abnormalities in gross anatomy, but the atrium in these slides seems to be directed anteriorly instead of posteriorly; therefore, the hearts in these slides might be cases of the abnormality termed "situs inversus", which has been described in up to 10% of some Atlantic salmon cohorts (source: Kent, M.L., and T.T. Poppe. 1998. Diseases of seawater netpen-reared salmonid fishes. Quadra Printers, Ltd. Nanaimo, B.C., Canada). Dilation of the bulbus arteriosus is one of the features described for situs inversus. The hearts on slides 3 and 10-3 might have similar changes, but the sections are not cut to include diagnostic angles through critical structures.

Macrophages and small numbers of lymphocytes line a few foci of the epicardial surface of the heart on slides 1 and 10- 3. These infiltrates

provide evidence of chronic immune stimulation; differentials include a low grade bacterial infection or a reaction to a vaccine.

Mild myocardial karyomegaly is somewhat common in cultured salmonids (e.g., 5.3% of the 503 Atlantic salmon hearts sampled as part of the BC Fish Health Auditing and Surveillance Program during 2006). The cause and significance is unknown, but there might be a genetic predisposition to developing the lesion. Karyomegaly in other cell types has been associated with exposure to algal toxins (e.g., hepatocytes exposed to microcystin LR in netpen liver disease).

Congestion of capillaries in several organs is evidence of circulating vasodilators; differentials include viral, bacterial, and parasitic infections.

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated. Vacuoles probably are a result of vaccine material lost during tissue processing.

## Histopathology

Formalin-fixed tissues were submitted for histopathology. One jar contained 4 hearts, which were distributed onto slides 1, 2, 3, and 4A/4B. Two separate bags (AL7.1 and AL10.1) each contained multiple tissues from one fish each.

Slide 7.1 - heart

Slide 7.2 - skeletal muscle, spleen, liver, head kidney, trunk kidney, intestinal ceca, and mesenteric adipose tissue

Slide 7.3 - gill

Slide 10.1 - gill

Slide 10.2 - skeletal muscle, spleen, liver, head kidney, trunk kidney, intestinal ceca, and mesenteric adipose tissue

Slide 10.3 - heart

All organs were examined. Organs not listed elsewhere have no significant lesions.

**Quality control:** Heart autolysis: none (all heart sections); liver autolysis: mild (slides 7-2, 10-2); gill autolysis: moderate (slides 7-3, 10-1). Large foci of erythrocytes (e.g., liver in slide 7-2) have precipitates of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic before or during fixation. Organs have no postfixation dehydration.



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-4000

Last Updated: 10/22/07 1:29 PM

Pathologist: Gary D. Marty

Received Date: 10/18/07

Collected Date: 10/18/07

Client Ref No: Log 6393

Veterinarian: **Diane Morrison**

Clinic: **Marine Harvest Canada**

Phone: (250) 850-3276

Fax: (250) 850-3275

Submitter: **Marine Harvest Canada**

Phone:

Fax:

Owner: **Marine Harvest Canada**

Phone:

Fax:(250) 850-3275

### Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID:

### Case History

Submitted formalized tissue.

Heart abnormalities as discussed with Dr. Marty. Please show samples to Dr. Marty prior to trimming.

### Final Diagnosis

1. Heart: cardiac dysplasia, with extranumerary blind- ended bulbus arteriosus (two extra, slide 1; one extra, slide 2; one extra, slide 3)
2. Heart: epicarditis, lymphocytic, multifocal, mild (slide 2)

**Final Comment:** The pale foci on the these hearts have features consistent with the bulbus arteriosus; they are composed of an outer band of elastic connective tissue and an inner cavity filled by blood that communicates with the ventricular lumen. They lack the normal central channels of the bulbus arteriosus, but are otherwise very similar. Coronary arteries at the periphery of these lesions are normal. Although several types of heart abnormalities have been described in Atlantic salmon, I have not seen this one described. Based on the clinical history of normal growth, these lesions might be congenital and of little significance for fish health. The blind pouch might have been more susceptible to thrombosis than a normal heart. Some of the structures identified as bulbus arteriosus in the previous case (2007-3924) might have been extranumerary bulbus arteriosus.

Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine.

### Histopathology

Three cassettes of formalin-fixed heart were submitted for histopathology wrapped in a moist paper towel and enclosed in a zip-seal plastic bag. Tissues from each cassette were processed onto a slide with the same number.

Slide 1 (6393-1) - the atrium and bulbus arteriosus had been opened by the submitter; I cut a single sagittal section through the ventricle, to include two pale foci (aneurysms?) on the ventral margin; each pale area is about 3 mm in diameter.

Slide 2 (6393-2) - the atrium and bulbus arteriosus were intact when received; I cut a sagittal section through the ventricle, to include a single pale focus (aneurysm?) on the dorsal margin. I also cut a transverse section through the pale focus.

Slide 3 (6393-3) - the submitter had cut a section through the heart that included bulbus arteriosus, ventricle, and a dilated focus that is about 10 mm in diameter. The atrium was not included. I did not cut this piece further; the slide contains a section of the face cut by the submitter.

**Quality control:** Tissue preservation is excellent. Tissues have no postfixation dehydration and no deposits of acid hematin.



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-4001

Last Updated: 11/03/07 10:25 AM  
Pathologist: Gary D. Marty  
Received Date: 10/18/07  
Collected Date: 10/18/07  
Client Ref No: Log 6379

Veterinarian: **Diane Morrison**  
Clinic: **Marine Harvest Canada**  
Phone: (250) 850-3276  
Fax: (250) 850-3275

Submitter: **Marine Harvest Canada**  
Phone:  
Fax:  
Owner: **Marine Harvest Canada**  
Phone:  
Fax:(250) 850-3275

**Animal Data**  
Species: Atlantic Salmon  
Breed:  
Sex:  
Age:  
Premise ID:

### Case History

Submitted one bacti plate for identification and antibiotic sensitivity.

### Bacteriology

**Aerobic Culture - Prod** Resulted by: Erin Zabek Verified by: Erin Zabek on 10/29/07 @ 2:46 PM

Specimen	ID	Isolate	Result	Level
Isolate		Serratia sp.	Positive	

**Fish** Resulted by: Erin Zabek Verified by: Erin Zabek on 10/29/07 @ 2:46 PM

Organism	ID	e	ffc	sor	s3	sxt	ot
Serratia sp.		r	s	s	r	s	s

\*\* Antibiotic sensitivity legend: e = Erythromycin, ffc = Florfenicol, sor = Romet 30, s3 = Tri-Sulfas, sxt = Sulfamethoxazole/Trimethopri ot = Oxytetracycline



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-4140

Last Updated: 11/21/07 5:06 PM

Pathologist: Gary D. Marty

Received Date: 10/29/07

Collected Date: 10/29/07

Client Ref No: Log 6413

Veterinarian: **Diane Morrison**

Clinic: **Marine Harvest Canada**

Phone: (250) 850-3276

Fax: (250) 850-3275

Submitter: **Marine Harvest Canada**

Phone:

Fax:

Owner: **Marine Harvest Canada**

Phone:

Fax:(250) 850-3275

### Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID:

### Case History

Submitted 4 histo cassettes and 5 virology samples for PCR for IHN and VHS. Histo #1 and #2 are from dead fish. Histo #3 and #4 are from moribund fish. Virology samples are 2 fish pools. Virology sample #1 is of 2 moribunds, virology sample #2 through #5 are fresh dead. All fish were anemic, had ascites, and hemorrhage of fat, p.c, and peritoneum. Some fish had petechiation in muscle and spleen was enlarged and friable.

Urgent results please. Samples in Virology. Log 6413.

### Final Diagnosis

- 1a. Liver: hydropic degeneration, diffuse, mild (slide 3), moderate (slide 4), severe (slides 1, 2)
- 1b. Liver: hepatocellular karyomegaly, diffuse, mild (slide 2), moderate (slides 1, 3, 4)
- 1c. Liver: hepatocellular single cell necrosis, diffuse, mild (slides 2), moderate (slides 1, 3, 4)
- 1d. Liver: lipofuscin-pigmented sinusoidal macrophages, diffuse, abundant (slides 1,2, 3, 4)
- 1e. Liver: hepatitis, lymphoplasmacytic, multifocal, mild (slides 2, 3), moderate (slides 1, 4)
- 1f. Liver: peritonitis, lymphoplasmacytic, diffuse, mild (slide 2), moderate (slide 1)
- 1g. Liver: mesothelial cell hypertrophy, diffuse, moderate (slides 1, 2, 3, 4)
- 1h. Liver: hepatopathy, diffuse, subacute, severe (consistent with netpen liver disease, all slides)
- 2a. Trunk kidney: renal tubular epithelial necrosis, multifocal, acute, mild (slide 2), moderate (slides 1, 3)
- 2b. Trunk kidney: renal tubular epithelial yellow- brown pigment (probably lipofuscin), multifocal, mild (slides 2, 4)
3. Intestine: mesenteric capillary congestion, diffuse, mild (slide 3), moderate to severe (slide 2).

4. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slides 1, 4)

5a. Heart: mural thrombosis, focal, mild (slide 2)

5b. Heart: epicarditis, regionally diffuse, lymphohistiocytic, mild (slide 2)

6. Head kidney: intracytoplasmic eosinophilic granules in cells lining capillaries, diffuse, mild (slide 3)

**Final Comment:** Features of these slides are consistent with netpen liver disease. Netpen liver disease can be reproduced in the laboratory with water exposure to the algal toxin microcystin-LR, but the source of the toxin in the field is unknown. Other toxins in the water or feed (e.g., aflatoxins) are likely differentials. Changes in the affected liver include single cell necrosis, hepatocellular karyomegaly, sinusoidal lipofuscin accumulation, and variable amounts of lymphoplasmacytic inflammation. Most of the remaining hepatocytes have basophilic cytoplasm indicative of regenerative hepatocytes: probably in response to hepatocellular necrosis. Widespread hydropic degeneration and single cell necrosis is evidence that the process may be relatively acute (1 or 2 weeks). The presence of abundant lipid stores in the mesenteric adipose tissue is further evidence that the disease is in its early phase (netpen liver disease sometimes affects fish over a period of months). Hepatic megalocytosis can result from exposure to several types of toxins, including aflatoxins, pyrrolizidine alkaloids, complex chemical mixtures from marine sediment extracts, and the algal toxin microcystin-LR. Hydropic degeneration is a precursor to single cell necrosis. 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; it is a common feature of netpen liver disease. Conditions that lead to moderate to abundant hepatic lipofuscin have been associated with decreased growth and survival in several studies. Mesothelial cell hypertrophy in the hepatic capsule is evidence of ascites.

Renal tubular epithelial necrosis results from acute damage to renal epithelial cells; damage is reversible if the basement membrane is spared (as in this case). Tubules in slide 3 have few necrotic cells but many tubular epithelial cells contain nuclei that have marginated chromatin (a type of nuclear degeneration). In slide 3, one tubule is lined by cells with thin basophilic epithelium; this tubule probably had epithelial necrosis following by stretching of the few remaining cells to keep the basement membrane covered. Renal tubular epithelial necrosis was fairly common among fish sampled in 2006 as part of the Ministry's Fish Health Auditing and Surveillance Program: Atlantic salmon (prevalence = 7.5%; n = 495) and Pacific salmon (prevalence = 2.2%; n = 134); the cause was not determined in many cases. Differentials include viral hemorrhagic septicemia virus (VHSV) and exposure to toxins (e.g., bacterial toxins, heavy metals, or aminoglycoside antibiotics such as gentamicin). Renal tubular necrosis is not a feature commonly associated with netpen liver disease, but I don't know if the controlled studies of netpen liver disease included histopathology of kidney.

Pigments in the renal tubules probably include lipofuscin. Accumulation of lipofuscin 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 this case, the same toxin that caused netpen liver disease might have also adversely impacted the kidney.

Distension of capillaries in the mesenteric adipose tissue is often part of the inflammatory response to many infectious diseases; hemorrhage sometimes occurs in severe cases. In British Columbia, mesenteric congestion and hemorrhage is most commonly associated with VHSV and bacterial infections. In this case, however, it might be a result of exposure to the same toxins that caused the netpen liver disease.

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

Thrombosis in the heart is evidence of increased coagulability. This can result from endothelial damage related to virus, bacterial, or parasitic infection, or exposure to toxins.

Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine.

Accumulation of eosinophilic granules in the cytoplasm of cells lining small vessels in the kidney is a distinctive finding in Atlantic salmon. These granules probably accumulate in response to systemic immune stimulation. The granules might be part of eosinophilic granular cells or endothelial cells; granule size seems too large and variable for eosinophilic granular cells. I have seen these granules associated with *Piscirickettsia* salmonis infection and with severe cerebral *Renibacterium salmoninarum* (in at least one case, the affected fish had no granulomatous inflammation in the kidney), but other cases (like this one) have no known cause. Renal eosinophilic granules have also been described in Atlantic salmon naturally infected with chronic pancreas disease in Norway (Salmonid alphavirus subtype 3, SAV3; McLoughlin and Graham 2007), but SAV3 has not been identified in BC salmon.

#### Literature Cited:

McLoughlin, M.F. and D.A. Graham. 2007. Alphavirus infections in salmonids - a review. *Journal of Fish Diseases*. 30(9):511-531.

Case: 07-4140

## Histopathology

Formalin -fixed tissues were submitted in 4 cassettes for histopathology.

Slide 1 (6413-1) - spleen, liver, heart, trunk kidney, head kidney

Slide 2 (6413-2) - spleen and mesenteric adipose tissue, liver, heart, trunk kidney, head kidney

Slide 3 (6413-3) - spleen, liver, heart, trunk kidney, head kidney, intestinal ceca, and mesenteric adipose tissue

Slide 4 (6413-4) - spleen, liver, heart, trunk kidney, head kidney, intestinal ceca, and mesenteric adipose tissue

All organs were examined. Organs not listed elsewhere have no significant lesions.

**Quality control:** Liver autolysis: none (slides 3, 4), mild (slides 1, 2). Organs have no postfixation dehydration and no acid hematin deposits.

**Measure of physiologic condition:**

Mesenteric adipose tissue: abundant (slides 2, 3, 4), or not included for analysis (slide 1)

Abundant mesenteric adipose tissue is evidence that the fish were feeding fairly normally until they began suffering from toxic liver disease.

## Molecular Diagnostics

**PCR - IHNV** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 10/29/07 @ 2:24 PM

Specimen	ID	Test	Result
Tissue	6413-#1	PCR - IHNV	Negative
Tissue	6413-#2	PCR - IHNV	Negative
Tissue	6413-#3	PCR - IHNV	Negative
Tissue	6413-#4	PCR - IHNV	Negative
Tissue	6413-#5	PCR - IHNV	Negative

**PCR - IPNV** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 10/29/07 @ 2:24 PM

Specimen	ID	Test	Result
Tissue	6413-#1	PCR - IPNV	Negative
Tissue	6413-#2	PCR - IPNV	Negative
Tissue	6413-#3	PCR - IPNV	Negative
Tissue	6413-#4	PCR - IPNV	Negative
Tissue	6413-#5	PCR - IPNV	Negative

**PCR - ISA** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 10/29/07 @ 2:25 PM

Specimen	ID	Test	Result
Tissue	6413-#1	PCR - ISA	Negative
Tissue	6413-#2	PCR - ISA	Negative
Tissue	6413-#3	PCR - ISA	Negative
Tissue	6413-#4	PCR - ISA	Negative
Tissue	6413-#5	PCR - ISA	Negative

Specimen	ID	Test	Result
Tissue	6413-#1	PCR - Piscirickettsia salmonis	Negative
Tissue	6413-#2	PCR - Piscirickettsia salmonis	Negative
Tissue	6413-#3	PCR - Piscirickettsia salmonis	Negative
Tissue	6413-#4	PCR - Piscirickettsia salmonis	Negative
Tissue	6413-#5	PCR - Piscirickettsia salmonis	Negative

Specimen	ID	Test	Result
Tissue	6413-#1	PCR - VHSV	Negative
Tissue	6413-#2	PCR - VHSV	Negative
Tissue	6413-#3	PCR - VHSV	Negative
Tissue	6413-#4	PCR - VHSV	Negative
Tissue	6413-#5	PCR - VHSV	Negative

**Virology**

Specimen	ID	Isolate	Result	Level
Tissue	6413-#1		No viruses isolated	
Tissue	6413-#2		No viruses isolated	
Tissue	6413-#3		No viruses isolated	
Tissue	6413-#4		No viruses isolated	
Tissue	6413-#5		No viruses isolated	



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-4141

Last Updated: 11/21/07 5:06 PM

Pathologist: Gary D. Marty

Received Date: 10/29/07

Collected Date: 10/29/07

Client Ref No: Log 6405

Veterinarian: **Diane Morrison**

Clinic: **Marine Harvest Canada**

Phone: (250) 850-3276

Fax: (250) 850-3275

Submitter: **Marine Harvest Canada**

Phone:

Fax:

Owner: **Marine Harvest Canada**

Phone:

Fax:(250) 850-3275

### Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID:

### Case History

Two virology samples labeled 6405-1, and 6405-2. Mortality is slightly increasing, mouth rot present in population. Five fish sampled, fish had not been eating; half of the examined fish also have visceral hemorrhaging. Samples collected on October 24, 2007 and kept frozen. 6405-1 is 2 fish pool; 6405-2 is a 3 fish pool. Please run PCR for IHN and VHS.

Log 6405. Samples in Virology.

## Molecular Diagnostics

**PCR - IHNV** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 10/30/07 @ 4:12 PM

Specimen	ID	Test	Result
Tissue	6405-#1	PCR - IHNV	Negative
Tissue	6405-#2	PCR - IHNV	Negative

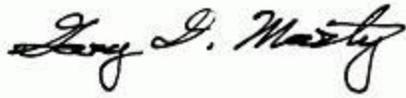
**PCR - VHSV** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 10/30/07 @ 4:12 PM

Specimen	ID	Test	Result
Tissue	6405-#1	PCR - VHSV	Negative
Tissue	6405-#2	PCR - VHSV	Negative

## Virology

**Tissue Culture** Resulted by: Cheryl Cecconi Verified by: Dr. J. Robinson on 11/21/07 @ 5:06 PM

Specimen	ID	Isolate	Result	Level
Tissue	6405-#1		No viruses isolated	
Tissue	6405-#2		No viruses isolated	



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-4142

Last Updated: 11/21/07 5:06 PM

Pathologist: Gary D. Marty

Received Date: 10/29/07

Collected Date: 10/29/07

Client Ref No: Log 6406

Veterinarian: **Diane Morrison**

Clinic: **Marine Harvest Canada**

Phone: (250) 850-3276

Fax: (250) 850-3275

Submitter: **Marine Harvest Canada**

Phone:

Fax:

Owner: **Marine Harvest Canada**

Phone:

Fax:(250) 850-3275

### Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID:

### Case History

Submitted one virology sample. Please run PCR for IHN and VHS. Mortality is low, but sampled fish had extensive hemorrhage of viscera and muscle. Sample collected on October 24, 2007 and kept frozen.

Log 6406. Samples in Virology.

## Molecular Diagnostics

**PCR - IHN** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 10/30/07 @ 4:12 PM

Specimen	ID	Test	Result
Tissue	6406	PCR - IHN	Negative

**PCR - VHSV** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 10/30/07 @ 4:12 PM

Specimen	ID	Test	Result
Tissue	6406	PCR - VHSV	Negative

## Virology

**Tissue Culture** Resulted by: Cheryl Cecconi Verified by: Dr. J. Robinson on 11/21/07 @ 5:06 PM

Specimen	ID	Isolate	Result	Level
Tissue	6406		No viruses isolated	



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-4212

Last Updated: 11/23/07 4:50 PM

Pathologist: Gary D. Marty

Received Date: 11/01/07

Collected Date: 11/01/07

Client Ref No: Log 6415

Veterinarian: **Diane Morrison**

Clinic: **Marine Harvest Canada**

Phone: (250) 850-3276

Fax: (250) 850-3275

Submitter: **Marine Harvest**

Phone:

Fax:

Owner: **Marine Harvest Canada**

Phone:

Fax:(250) 850-3275

### Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID:

### Case History

Submitted virology samples 9 samples of duplicates. Bag A contain spleen and head kidney and are for tissue culture. Bags labeled with B contain kidney, spleen, gill, and liver are for PCR for IHN and VHS. 18 samples for virology and PCR for IHN and VHS. Atlantic, Regular, 2006, Saltwater.

### Molecular Diagnostics

**PCR - IHNV** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 11/02/07 @ 2:05 PM

Specimen	ID	Test	Result
Tissue	Organ B#1	PCR - IHNV	Negative
Tissue	Organ B#2	PCR - IHNV	Negative
Tissue	Organ B#3	PCR - IHNV	Negative
Tissue	Organ B#4	PCR - IHNV	Negative
Tissue	Organ B#5	PCR - IHNV	Negative
Tissue	Organ B#6	PCR - IHNV	Negative
Tissue	Organ B#7	PCR - IHNV	Negative
Tissue	Organ B#8	PCR - IHNV	Negative
Tissue	Organ B#9	PCR - IHNV	Negative

**PCR - VHSV** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 11/02/07 @ 2:05 PM

Specimen	ID	Test	Result
Tissue	Organ B#1	PCR - VHSV	Negative
Tissue	Organ B#2	PCR - VHSV	Negative
Tissue	Organ B#3	PCR - VHSV	Negative
Tissue	Organ B#4	PCR - VHSV	Negative
Tissue	Organ B#5	PCR - VHSV	Negative
Tissue	Organ B#6	PCR - VHSV	Negative
Tissue	Organ B#7	PCR - VHSV	Negative

Tissue	Organ B#8	PCR - VHSV	Negative
Tissue	Organ B#9	PCR - VHSV	Negative

**Virology**

**Tissue Culture** Resulted by: Liisa Nielsen Verified by: Dr. J. Robinson on 11/23/07 @ 4:50 PM

Specimen	ID	Isolate	Result	Level
Tissue	A-1A		No viruses isolated	
Tissue	B-2A		No viruses isolated	
Tissue	C-3A		No viruses isolated	
Tissue	D-4A		No viruses isolated	
Tissue	E-5A		No viruses isolated	
Tissue	F-6A		No viruses isolated	
Tissue	G-7A		No viruses isolated	
Tissue	H-8A		No viruses isolated	
Tissue	I-9A		No viruses isolated	



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-4213

Last Updated: 11/23/07 4:50 PM

Pathologist: Gary D. Marty

Received Date: 11/01/07

Collected Date: 11/01/07

Client Ref No: Log 6416

Veterinarian: **Diane Morrison**

Clinic: **Marine Harvest Canada**

Phone: (250) 850-3276

Fax: (250) 850-3275

Submitter: **Marine Harvest**

Phone:

Fax:

Owner: **Marine Harvest Canada**

Phone:

Fax:(250) 850-3275

### Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID:

### Case History

Submitted 3 bags labeled #1A, 2A, 3A for tissue culture; 3 bags labeled #1B, 2B, 3B for PCR for IHN and VHS. Bags A spleen and kidney; bags B p.c, gill, spleen, and kidney.

Atlantic, Regular, 2006 S1, Saltwater.

## Molecular Diagnostics

**PCR - IHNV** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 11/02/07 @ 5:08 PM

Specimen	ID	Test	Result
Tissue	Organ B#1	PCR - IHNV	Negative
Tissue	Organ B#2	PCR - IHNV	Negative
Tissue	Organ B#3	PCR - IHNV	Negative

**PCR - VHSV** Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 11/02/07 @ 5:08 PM

Specimen	ID	Test	Result
Tissue	Organ B#1	PCR - VHSV	Negative
Tissue	Organ B#2	PCR - VHSV	Negative
Tissue	Organ B#3	PCR - VHSV	Negative

## Virology

**Tissue Culture** Resulted by: Liisa Nielsen Verified by: Dr. J. Robinson on 11/23/07 @ 4:50 PM

Specimen	ID	Isolate	Result	Level
Tissue	A-1A		No viruses isolated	

Tissue	B-2B
Tissue	C-3A

No viruses isolated
No viruses isolated



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-4466

Last Updated: 12/19/07 5:09 PM

Pathologist: Gary D. Marty

Received Date: 11/27/07

Collected Date: 11/27/07

Client Ref No: Log 6460

Veterinarian: **Diane Morrison**

Clinic: **Marine Harvest Canada**

Phone: (250) 850-3276

Fax: (250) 850-3275

Submitter: **Marine Harvest Canada**

Phone:

Fax:

Owner: **Marine Harvest Canada**

Phone:

Fax:(250) 850-3275

### Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID:

### Case History

Submitted one sample for virology labeled "ALT 8" collected November 22, 2007 and kept frozen. Fish with hemorrhage of pyloric caeca, swim bladder, liver, and peritoneum. No bacterial growth on blood agar or TSA. Please run PCR for IHN and VHS.

Atlantic, regular, 2007 S1, Saltwater.

## Molecular Diagnostics

**PCR - IHN** Resulted by: Ken Sojony Verified by: Dr. J. Robinson on 11/30/07 @ 9:45 AM

Specimen	ID	Test	Result
Tissue	ALT 8	PCR - IHN	Negative

**PCR - VHSV** Resulted by: Ken Sojony Verified by: Dr. J. Robinson on 11/30/07 @ 9:48 AM

Specimen	ID	Test	Result
Tissue	ALT 8	PCR - VHSV	Negative

## Virology

**Tissue Culture** Resulted by: Liisa Nielsen Verified by: Dr. J. Robinson on 12/19/07 @ 5:09 PM

Specimen	ID	Isolate	Result	Level
Tissue	ALT 8		No viruses isolated	



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-4467

Last Updated: 12/19/07 5:09 PM

Pathologist: Gary D. Marty

Received Date: 11/27/07

Collected Date: 11/27/07

Client Ref No: Log 6468

Veterinarian: **Diane Morrison**

Clinic: **Marine Harvest Canada**

Phone: (250) 850-3276

Fax: (250) 850-3275

Submitter: **Marine Harvest**

Phone:

Fax:

Owner: **Marine Harvest Canada**

Phone:

Fax:(250) 850-3275

### Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID:

### Case History

Submitted two samples labeled 6468 #1-3 and 6468 #4-5 for virology. Samples collected November 21 and kept frozen.

Atlantic, regular, 2007 S0, Saltwater.

## Molecular Diagnostics

**PCR - IHNV** Resulted by: A Scouras Verified by: Dr. J. Robinson on 11/30/07 @ 9:52 AM

Specimen	ID	Test	Result
Tissue	6468 1-3	PCR - IHNV	Negative
Tissue	6468 4-5	PCR - IHNV	Negative

**PCR - VHSV** Resulted by: A Scouras Verified by: Dr. J. Robinson on 11/30/07 @ 9:52 AM

Specimen	ID	Test	Result
Tissue	6468 1-3	PCR - VHSV	Negative
Tissue	6468 4-5	PCR - VHSV	Negative

## Virology

**Tissue Culture** Resulted by: Liisa Nielsen Verified by: Dr. J. Robinson on 12/19/07 @ 5:09 PM

Specimen	ID	Isolate	Result	Level
Tissue	6468 1-3		No viruses isolated	
Tissue	6468 4-5		No viruses isolated	



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

These results relate only to the animals or items tested.

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**END OF REPORT**

## Final Report AHC Case: 07-4530

Last Updated: 12/10/07 12:13 PM

Pathologist: Gary D. Marty

Received Date: 12/04/07

Collected Date: 12/04/07

Client Ref No:

Veterinarian: **Barry Milligan**

Clinic: **Grieg Seafoods BC Ltd.**

Phone: (250) 286-0838

Fax: (250) 286-1883

Submitter: **Jeanine Sumner**

Phone:

Fax:

Owner: **Grieg Seafoods BC Ltd.**

Phone: (250) 286-0838

Fax:(250) 286-1883

### Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID: Barnes

### Case History

Six histo cassettes. Please prepare for histo exam by Dr. G. Marty.

Additional history received from Dr. Milligan by e-mail (10 Dec. 2007): clinical diagnoses include *P. salmonis* (visually and by PCR) at this site. Bacteriology / virology have been clear to date (including ongoing samples). A portion of this stock had been vaccinated with a trial Kudoa vaccine with significant adverse reactions which led to halting the trial and significant mortality following vaccination complicated with *Saprolegnia* and various opportunistic bacteria.

### Final Diagnosis

1. Head kidney: hematopoietic cells, focal (500 µm in diameter), with focal necrosis and intralesional spherical organisms consistent with *Piscirickettsia salmonis* (slide 4)

2a. Liver: vasculitis and hepatic necrosis, acute, multifocal, severe (focus of necrosis is 6 mm in diameter) with abundant intralesional spherical organisms consistent with *Piscirickettsia salmonis* (slide 5)

2b. Liver: hepatic necrosis, acute, multifocal, moderate (slide 3)

2c. Liver: yellow-brown pigmented macrophage aggregates and sinusoidal macrophages, disseminated, mild (slides 1A and 6; lipofuscin confirmed in slide 6)

2d. Liver: pericholangitis, lymphocytic, multifocal, mild (slide 2A)

2e. Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slides 3, 4), moderate (slide 2A)

2f. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slides 2A, 3), moderate (slide 6)

2g. Liver: biliary preductular cell hyperplasia, diffuse, mild (slide 2A)

2h. Liver: sinusoidal congestion, acute, multifocal, mild (slide 4)

3a. Intestine and surrounding mesenteries: peritonitis, granulomatous, regionally diffuse, with occasional fine fibrocellular fronds, moderate

(slide 2A), severe (slide 1A)

- 3b. Spleen: peritonitis, granulomatous, regionally diffuse, with occasional fine fibrocellular fronds, moderate (slide 6), severe (slide 3)
- 3c. Spleen: parenchymal histiocytosis, with intracellular spherical organisms consistent with *Piscirickettsia salmonis*, moderate (slide 5)
- 4a. Trunk kidney: interstitial cell hyperplasia, diffuse, moderate (slide 3), with intralosomal spherical organisms consistent with *Piscirickettsia salmonis* (slide 5)
- 4b. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild (slides 1A, 2A, 3, 5)
- 4c. Trunk kidney: interstitial congestion, diffuse, mild (slide 4)
- 4d. Trunk kidney: renal tubular degeneration, necrosis, and regeneration, with chronic-active inflammation, and intratubular and interstitial yellow-green pigment (mostly lipofuscin, with one focus of iron), diffuse, severe (slide 6)
- 4e. Trunk kidney: intratubular cellular debris and Gram-negative rods, focal, mild (slide 6)
- 4f. Trunk kidney: glomerulonephritis, membranous, diffuse, moderate (slide 6)
- 5a. Brain: capillary (vascular) congestion, diffuse, mild (slide 4), moderate (slide 2A)
- 5b. Brain: meningitis, chronic, regionally diffuse, mild (slide 1A)
- 6. Mesenteric adipose tissue: steatitis, histiocytic, with intracellular spherical organisms consistent with *Piscirickettsia salmonis*, moderate (slide 5)

**Final Comment:** These fish have evidence of death due to at least two causes: *Piscirickettsia salmonis* infection and hyperreaction to a vaccine. Other lesions might be a result of VHSV or a bacterial infection. Specific comments follow:

A small piece of head kidney admixed with brain tissue in slide #4 has a 100- $\mu$ m-diameter focus of eosinophilia with small spherical organisms with morphology consistent with *Piscirickettsia salmonis*. The fish has no other evidence of infection with *Piscirickettsia salmonis*, including the intact head kidney; therefore, I suspect that this tissue is an artefact-- perhaps from block #5 (the small piece of tissue on slide #4 might have fallen out of cassette #5 and entered cassette #4. The fish on slide #5 has abundant *Piscirickettsia salmonis* in the liver and moderate numbers in the kidney, spleen, and mesenteric adipose tissue; *P. salmonis* probably killed fish #5.

Some of the meninges in slide 1A are up to 80  $\mu$ m in diameter, and they seem a little thicker and denser than normal). This might be a result of a tangential plane of section (i.e., not a real change); alternatively, it might be a result of chronic irritation, as might happen with a parasite or bacterial infection. The slide contains no obvious organisms.

Hepatic vasculitis commonly leads to hepatic necrosis in fish infected with *Piscirickettsia salmonis*. The associated lesions probably killed fish #5. In general, hepatocellular necrosis can be caused by inadequate vascular perfusion (e.g., as occurs with harmful algal blooms or hypoxia) or direct cytotoxicity from viral or bacterial infections (e.g., viral hemorrhagic septicemia virus or *Piscirickettsia salmonis*); the cause is not determined in most cases. Hepatocellular necrosis in slide 3 has no obvious organisms. Lack of proliferative lesions in the biliary system (slide 3) is evidence against a chronic toxic cause for the hepatic necrosis. Hepatic necrosis is somewhat common in salmon that die in marine net pens, affecting 7.5% of the 505 Atlantic salmon and 5% of the 134 Pacific salmon examined in 2006 as part of the Province's Fish Health Auditing and Surveillance Program.

Pigment in the liver probably is mostly lipofuscin (confirmed in fish #6); fish #6 has no hemosiderin (confirmed with Perl's iron stain). 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. It is a fairly good marker of exposure to a serious algal bloom within the past warm season. Conditions that lead to moderate to abundant hepatic lipofuscin have been associated with decreased growth and survival in several studies. The combination of lipofuscin ("ageing pigment") with peritonitis (common in younger fish after being vaccinated) is unusual.

Lymphocytic inflammation around bile ductules (liver) is evidence of chronic immune stimulation. This type of inflammation can result from bacteria ascending from the intestine to the liver through the biliary system.

Basophilic cytoplasm in hepatocytes is an indication of active protein synthesis. It is normal in mature females producing protein for deposition in their eggs. In other fish it might be related to increased protein needed as part of an inflammatory response.

Biliary preductular cell hyperplasia 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). Biliary preductular cell hyperplasia is fairly common in salmon that die in marine net pens ("silvers"), affecting 15% of the 505 Atlantic salmon and 4.5% of the 134 Pacific salmon examined in 2006 as part of the Province's Fish Health Auditing and Surveillance Program; the sample size of Atlantic salmon in 2006 was sufficient to identify a trend towards greater prevalence in the fall and winter (20-26%) than in the spring and summer (4-12%).

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated. Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition.

Interstitial cell hyperplasia in the kidney results from increased demand for erythrocytes or white blood cells somewhere in the body (renal interstitial cells are the blood-forming or hematopoietic cells in the kidney). Interstitial cell hyperplasia more than mild is rare in Atlantic salmon sampled as part of the Provincial Fish Health Auditing and Surveillance Program (i.e., only 2 of 1098 kidneys examined to date since 2006). Erythrophagocytosis is another unusual feature of this case. Differentials in this case include hyperreaction to a vaccine, or a bacterial infection.

Renal tubular epithelial protein droplets are normal in some species, or they might be an indication of glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2006 as part of the Ministry's Fish Health Auditing and Surveillance Program: Atlantic salmon (prevalence = 22%; n = 495) and Pacific salmon (prevalence = 38%; n = 134).

Changes in the trunk kidney in slide #6 are among the most severe I have ever seen in a cultured Atlantic salmon not infected with *Renibacterium salmoninarum* or *Piscirickettsia salmonis*. The underlying lesion seems to be severe tubular necrosis. Inflammation (neutrophils and macrophages) and fibrosis are evidence that the necrosis has also destroyed segments of the basement membrane, making full regeneration impossible. Lipofuscin accumulation is evidence of the chronicity of the lesion. The lesions probably are a result of ongoing exposure to toxins; differentials include toxins in the water or feed, or toxins produced by a bacterial infection. This might be part of the adverse reaction to the *Kudoa* trial vaccine, but the reaction is different than what I usually see with other vaccines. VHSV is an unlikely differential. One tubule contains Gram -negative bacterial rods (coordinates on my microscope = 43.7 x 113); these might be a *Vibrio* species. Neutrophilic inflammation scattered throughout the kidney has no bacteria (Gram stain).

Membranous glomerulonephritis is rare in Atlantic salmon; in the Provincial Auditing and Surveillance Program, only 1 of 1083 trunk kidney examined since 2006 had moderate interstitial cell hyperplasia (none were severe). In fish, renal interstitial cell hyperplasia 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 syndrome (in Atlantic salmon), nephrocalcinosis, and infections with a number of bacteria and parasitic species. In fish #6, it might be part of the vaccine reaction (thickened glomerular membranes are PAS-positive).

Capillary congestion in the liver, kidney, and brain (slide #4) is commonly associated with circulating toxins. Inflammatory cells, bacteria, or viral infections are common sources of these toxins. Differentials include VHSV, filamentous bacteria (e.g., mouthrot) or, less likely, *Listonella anguillarum*. Renal and hepatic congestion are classic signs of infectious salmon anemia (ISA), but ISA has never been isolated from fish in BC.

## Histopathology

Formalin-fixed tissues were submitted in 6 cassettes for histopathology. For cassettes 1 and 2, gills were removed from the original (A) cassettes and placed in separate (B) cassettes.

Slide 1A- liver, head kidney with trunk kidney on margin, brain, intestinal ceca and mesenteric adipose tissue

Slide 1B- gill

Slide 2A- brain, trunk kidney, liver, intestinal ceca and mesenteric adipose tissue

Slide 2B- gill

Slide 3- liver, spleen, trunk kidney, brain

Slide 4- brain, trunk kidney, liver, spleen

Slide 5- mesenteric adipose tissue, brain, spleen, trunk kidney, liver

Slide 6- brain, trunk kidney with corpuscle of Stannius, liver, mesenteric adipose tissue, spleen; sections from the same block were stained

with Schmorl's lipofuscin, Perl's iron, Twort's Gram, and PAS

All organs were examined. Organs not listed elsewhere have no significant lesions.

**Quality control:** Liver autolysis: mild (slides 2A, 3), moderate (slides 5, 6), severe (slides 1A, 4). Organs have no postfixation dehydration and no acid hematin deposits.



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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## Final Report AHC Case: 07-4532

Last Updated: 12/08/07 10:37 AM

Pathologist: Gary D. Marty

Received Date: 12/04/07

Collected Date: 12/04/07

Client Ref No: 7-2369

Veterinarian:

Clinic:

Phone:

Fax:

Submitter: **Microtek International**

Phone:

Fax:

Owner: **Microtek International In**

Phone:

Fax:(250) 652-4802

### Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID:

### Case History

Samples enclosed for histology testing.

Samples were originally fixed in formalin then transfer to tap water for transport. Each cassette contains multiple organ tissue samples from an individual Atlantic smolt. There are 4 samples from Pen #8 and 3 samples from Pen #3. Please refer to case 7-2369 on all correspondence and invoicing. Should you require any further information, please call 250-652-4482 ext 201.

## Final Diagnosis

1. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slides 3-1, 3-2, 3-3, 8-1, 8-3, 8-4), moderate (slide 8-2)
2. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild (slides 3-1, 3-2, 3-3, 8-1, 8-2, 8-3, 8-4)
- 3a. Intestinal ceca and surrounding mesenteries: peritonitis, granulomatous, regionally diffuse, with occasional fine fibrocellular fronds, mild (slide 8-3), moderate (slide 3-1), severe (slide 8-4; slide 3-3, includes neutrophils)
- 3b. Spleen: peritonitis, granulomatous, regionally diffuse, with intralesional vacuoles 50 - 400 µm in diameter, moderate (slide 8-1, 8-3), severe (slide 8-2)
4. Heart, bulbus arteriosus: endocarditis, diffuse, lymphohistiocytic, moderate (slides 3-3, 8-1, 8-3), or not included in the section (slides 3-1, 3-2, 8-2, 8-4)
5. Nematode parasite (probably an ascarid), focal, mild (slide 3-3)

**Final Comment:** Chronic inflammation consistent with a vaccine reaction is the most consistent and severe lesion in these fish. Comments on specific lesions follow:

Basophilic cytoplasm in hepatocytes is an indication of active protein synthesis. It is normal in mature females producing protein for deposition in their eggs. In other fish it might be related to increased protein needed as part of an inflammatory response.

Renal tubular epithelial protein droplets are normal in some species, or they might be an indication of glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2006 as part of the Ministry's Fish Health Auditing and Surveillance Program: Atlantic salmon (prevalence = 22%; n = 495) and Pacific salmon (prevalence = 38%; n = 134).

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated. In most cases reaction to vaccines is an incidental finding, but the fish in slide 3-3 might have died from complications related to peritonitis. Inflammation in slide 3-3 includes abundant, maturing, highly vascular, granulation tissue (~10 x the diameter of the intestinal ceca), and small foci of neutrophils. Vacuoles in slide 8-1 probably are what is left after vaccine material was lost during processing of the tissues to the slide. A vaccine reaction might also be the cause of the lymphohistiocytic inflammation in the bulbus arteriosus, where the inflammation is evidence of chronic immune stimulation; differentials include a low grade bacterial infection.

Slide 3-3 contains a cross section of a nematode parasite with several characteristic features of ascarids: prominent lateral alae, prominent lateral cords, ovary with two thick-shelled eggs (each about 66 x 36 µm). Nematode parasites are very rare in cultured salmonids. This fish might have eaten something other than processed feed during its development. Alternatively, the parasite is a "float-on" from another case; ascarids normally live in the lumen of the intestine, but the parasite in slide 3-3 is not associated with any organ.

## Histopathology

Formalin-fixed tissues were submitted in 7 cassettes for histopathology.

Slide 3-1 (#3 Brent) - liver, trunk kidney, heart, intestinal ceca and mesenteric adipose tissue

Slide 3-2 (#3 Brent) - heart, liver, spleen(?), trunk kidney, intestinal ceca, and mesenteric adipose tissue

Slide 3-3 (#3 Brent) - trunk kidney, heart, liver, trunk kidney, intestinal ceca, and mesenteric adipose tissue

Slide 8-1 (#8 Brent) - spleen, heart, trunk kidney, liver, intestinal ceca, and mesenteric adipose tissue

Slide 8-2 (#8 Brent) - spleen, trunk kidney, heart, liver

Slide 8-3 (#8 Brent) - heart, spleen, trunk kidney, liver, intestinal ceca, and mesenteric adipose tissue

Slide 8-4 (#8 Brent) - spleen, heart, liver, trunk kidney, stomach, intestinal ceca, and mesenteric adipose tissue

All organs were examined. Organs not listed elsewhere have no significant lesions.

**Quality control:** Liver autolysis: moderate (slide 8-2), severe (slides 3-1, 3-2, 3-3, 8-1, 8-3, 8-4). Tissue quality is not ideal. In all slides, erythrocytes have lysed cytoplasm (e.g., liver vessels in slide 8-2), and kidney tubules are separated by clear space; these artefacts can result if tissues are placed into water before they are fixed by the formalin (tissues need to be in formalin (at a ratio of 10 parts formalin to 1 part tissue) for 24 hours before being transferred to water. Organs have no postfixation dehydration and no acid hematin deposits.



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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## Final Report AHC Case: 07-4668

Last Updated: 12/19/07 2:51 PM

Pathologist: Gary D. Marty

Received Date: 12/17/07

Collected Date: 12/17/07

Client Ref No: 121307

Veterinarian:

Clinic:

Phone:

Fax:

Submitter: **Mainstream Canada**

Phone:

Fax:

Owner: **Mainstream Canada-T**

Phone:

Fax:(250) 725-1250

### Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID:

### Case History

Submitted formalized tissue (2 cassettes) for histology.

Higher mortality rates observed in otherwise healthy fish which are off feed. Only internal observations are larger than normal livers which show some swelling.

Venture Pt, Pen 2

## Final Diagnosis

- 1a. Liver: hepatic necrosis, acute, multifocal, moderate (probable Viral Hemorrhagic Septicemia Virus, VHSV; slide 2)
- 1b. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slide 2)
- 1c. Liver: biliary preductular cell hyperplasia, diffuse, mild (slide 2)
- 1d. Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slides 1, 2)
- 1e. Liver: pericholangitis, lymphocytic, multifocal, mild (slide 2)
2. Spleen and surrounding fatty mesenteries: peritonitis, lymphoplasmacytic, granulomatous, regionally diffuse, with fine fibrocellular fronds, moderate (slide 1), severe (slide 2)
3. Corpuscle of Stannius: edema, diffuse, moderate (slide 2)
4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild (slide 2)

**Final Comment:** These fish probably are infected with viral hemorrhagic septicemia virus (VHSV); I recommend additional tests for confirmation (virus culture or PCR on fresh liver, kidney, or spleen). Comments on specific lesions follow:

Hepatocellular necrosis can be caused by inadequate vascular perfusion (e.g., as occurs with harmful algal blooms or hypoxia) or direct cytotoxicity from viral or bacterial infections (e.g., viral hemorrhagic septicemia virus, *Renibacterium salmoninarum*, or *Piscirickettsia salmonis*).

This case has no obvious organisms; therefore, VHSV is the most likely diagnosis. VHSV in farmed salmon often follows cohabitation with infected Pacific herring.

Basophilic cytoplasm in hepatocytes is an indication of active protein synthesis. It is normal in mature females producing protein for deposition in their eggs. In other fish it might be related to increased protein needed as part of an inflammatory response.

Biliary preductular cell hyperplasia 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). Biliary preductular cell hyperplasia is fairly common in salmon that die in marine net pens ("silvers"), affecting 15% of the 505 Atlantic salmon and 4.5% of the 134 Pacific salmon examined in 2006 as part of the Province's Fish Health Auditing and Surveillance Program; the sample size of Atlantic salmon in 2006 was sufficient to identify a trend towards greater prevalence in the fall and winter (20-26%) than in the spring and summer (4-12%).

Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition.

Lymphocytic inflammation around bile ductules (liver) is evidence of chronic immune stimulation. This type of inflammation can result from bacteria ascending from the intestine to the liver through the biliary system.

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

Edema of the Corpuscle of Stannius probably is also related the infection with VHSV.

Renal tubular epithelial protein droplets are normal in some species, or they might be an indication of glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2006 as part of the Ministry's Fish Health Auditing and Surveillance Program: Atlantic salmon (prevalence = 22%; n = 495) and Pacific salmon (prevalence = 38%; n = 134).

## Histopathology

Formalin-fixed tissues were submitted in 2 cassettes for histopathology.

Slide 1 (#2 14/12/07) - liver, spleen, trunk kidney, intestinal ceca, and mesenteric adipose tissue

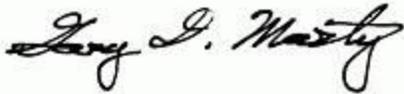
Slide 2 (Pen 2 2/12) - liver, trunk kidney with Corpuscles of Stannius, spleen, intestinal ceca, and mesenteric adipose tissue

All organs were examined. Organs not listed elsewhere have no significant lesions.

**Quality control:** Liver autolysis: mild (slide 2), severe (slide 1). Large foci of erythrocytes (e.g., spleen in slide 2) have precipitates of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic before or during fixation. Organs have no postfixation dehydration.

### Staff Comments:

19 December 2007, 14:52; I called Mainstream at 250-286-0022 and talked with Tim Wiper; I told him that the lesions were consistent with VHSV and recommended further confirmatory tests.3:04 PM 19/12/2007



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

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**END OF REPORT**

## Final Report AHC Case: 07-4697

Last Updated: 12/22/07 11:10 AM

Pathologist: Gary D. Marty

Received Date: 12/19/07

Collected Date: 12/19/07

Client Ref No: Log 6498

Veterinarian: **Diane Morrison**

Clinic: **Marine Harvest Canada**

Phone: (250) 850-3276

Fax: (250) 850-3275

Submitter: **Marine Harvest**

Phone:

Fax:

Owner: **Marine Harvest Canada**

Phone:

Fax:(250) 850-3275

### Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID:

### Case History

Submitted 4 fixed tissue samples for histology.

Four fish sampled from population rolling over in uncrowded seine. Fish #1 with hemorrhage of p.c. Fish #2 bloated. Fish #3 normal. Fish #4 moribund.

Atlantic, Regular, 2006 S1, Saltwater.

## Final Diagnosis

1. Intestinal mesenteries: capillary congestion, diffuse, moderate (slide 1A)
- 2a. Liver: hepatocellular cytoplasmic lipid, diffuse, small amounts (slides 1A, 2A), moderate amounts (slide 3A)
- 2b. Liver: biliary preductular cell hyperplasia, diffuse, mild (slides 1A, 4A)
- 2c. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slides 1A, 2A, 4A)
- 2d. Liver: yellow-brown to yellow-green pigmented macrophage aggregates and sinusoidal macrophages, disseminated, mild (slide 1A), moderate (4A)
- 2e. Liver: pericholangitis, lymphocytic, multifocal, mild (slide 3A)
3. Trunk kidney: interstitial cell hyperplasia, diffuse, moderate (slide 1A)
- 4a. Heart: myocardial karyomegaly, multifocal, mild (slides 1A, 2A)
- 4b. Heart: endocarditis, focal, lymphohistiocytic, mild (slide 4A)
- 5a. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slides 1A, 2A), moderate (slide 3A, 4A)
- 5b. Spleen: parenchymal golden pigment, scattered, intracellular, mild (slides 2A, 4A), moderate (slide 3A)

Case: 07-4697

6. Gill: branchitis, lymphohistiocytic, focal, mild (slides 2B, 4B)

**Final Comment:** These fish have several lesions, many of which are fairly common in farmed Atlantic salmon, but only a few provide clues to the cause of death. If problems continue at this farm, consider sampling brain and sampling fresh dead fish (silvers). Comments on specific lesions follow:

Distension of capillaries in the mesenteric adipose tissue is often part of the inflammatory response to many infectious diseases; hemorrhage sometimes occurs in severe cases. In British Columbia, mesenteric congestion and hemorrhage is most commonly associated with VHSV and bacterial infections. Consider bacteriology and virology, if not already done.

The liver has several changes that often occur together. First, some degree of lipid accumulation in the cytoplasm of hepatocytes might be normal. Abnormal accumulation of hepatocellular lipid (lipidosis) occurs when fish are not feeding and in cases of inadequate nutrition.

Second, biliary preductular cell hyperplasia 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). Biliary preductular cell hyperplasia is fairly common in salmon that die in marine net pens ("silvers"), affecting 15% of the 505 Atlantic salmon and 4.5% of the 134 Pacific salmon examined in 2006 as part of the Province's Fish Health Auditing and Surveillance Program; the sample size of Atlantic salmon in 2006 was sufficient to identify a trend towards greater prevalence in the fall and winter (20-26%) than in the spring and summer (4-12%).

Third, basophilic cytoplasm in hepatocytes is an indication of active protein synthesis. It is normal in mature females producing protein for deposition in their eggs. In other fish it might be related to increased protein needed as part of an inflammatory response.

And fourth, pigment in the liver and spleen probably is lipofuscin; pigment in the liver might also include hemosiderin. 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; it is also more common in older fish. Moderate lipofuscin deposits in the liver are highly unusual for farmed Atlantic salmon. Of the 1132 Atlantic salmon that I have examined as part of the FHASP since January 2006, only 13 (= 1.1%) have had moderate hepatic lipofuscin, and 4 of those fish were from a single farm visit in the 4<sup>th</sup> quarter of 2007. I wonder, did your farm have severe problems with algal blooms this past summer? 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 might result from increased turnover of red blood cells.

Lymphocytic inflammation around bile ductules (liver) is evidence of chronic immune stimulation. This type of inflammation can result from bacteria ascending from the intestine to the liver through the biliary system.

Interstitial cell hyperplasia in the kidney results from increased demand for erythrocytes or white blood cells somewhere in the body (renal interstitial cells are the blood-forming or hematopoietic cells in the kidney).

Mild myocardial karyomegaly is somewhat common in cultured salmonids (e.g., 5.3% of the 503 Atlantic salmon hearts sampled as part of the BC Fish Health Auditing and Surveillance Program during 2006). The cause and significance is unknown, but there might be a genetic predisposition to developing the lesion. Karyomegaly in other cell types has been associated with exposure to algal toxins (e.g., hepatocytes exposed to microcystin LR in netpen liver disease).

Lymphohistiocytic inflammation in the heart is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine.

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

Foci of lymphohistiocytic branchitis might be a response to a local bacterial infection; they probably are not the cause of clinical signs.

## Histopathology

Formalin-fixed tissues were submitted in 4 cassettes for histopathology. For each cassette, gills were removed from the original (A) cassettes and placed in separate (B) cassettes.

Slide 1A (6498-1)  $\delta$  liver, intestine, heart, head kidney, trunk kidney, spleen, mesenteric adipose tissue

Slide 1B (6498-1) - gill

Slide 2A (6498-2) - liver, heart, head kidney, trunk kidney, spleen, intestinal ceca, mesenteric adipose tissue

Slide 2B (6498-2) - gill

Slide 3A (6498-3) - liver, intestine, heart, head kidney, trunk kidney, spleen, mesenteric adipose tissue

Slide 3B (6498-3) - gill

Slide 4A (6498-4) - liver, intestine, heart, head kidney, trunk kidney, spleen, mesenteric adipose tissue

Slide 4B (6498-4) - gill

All organs were examined. Organs not listed elsewhere have no significant lesions.

**Quality control:** Liver autolysis: none (slides 1A, 2A, 3A, 4A). Large foci of erythrocytes (e.g., spleen in slide 1A) have precipitates of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic before or during fixation (as can happen with thick bloody pieces of tissue). Organs have no postfixation dehydration.



Gary D. Marty  
D.V.M., Ph.D., Diplomate A.C.V.P.

These results relate only to the animals or items tested.

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**END OF REPORT**