



Government of Canada
Fisheries and Oceans

Gouvernement du Canada
Pêches et Océans

To: / John C. Davis Date: October 3, 2003
 A: Regional Director General CT#: 368384
 Subject: / **REQUEST TO IMPORT ATLANTIC SALMON EGGS FROM**
 Objet: **ICELAND**
(DECISION SOUGHT)

From / De: Laura Richards, Regional Director Science
 Via: Sue Farlinger, Regional Director HEB *By E-MAIL*
 Via: Allison Webb, A/ Director Sustainable Aquaculture *per. [Signature]*

| | |
|--|--|
| <input checked="" type="checkbox"/> For Signature / Pour Signature | <input type="checkbox"/> Information |
| <input type="checkbox"/> For Comments / Pour commentaires | <input type="checkbox"/> Material for the Minister / Documents à l'intention du ministre |

Remarks: /
Remarques :

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Tara Neale, Regional Director
Pam Goh, Regional Director's

2004

~~9727~~
~~1638~~

Drafting Officer / Rédacteur :

9727 / S. Farlinger
J.C. Davis

BRIEFING NOTE FOR THE REGIONAL DIRECTOR GENERAL**REQUEST TO IMPORT ATLANTIC SALMON EGGS FROM ICELAND**

(Decision Sought)

SUMMARY

- Two BC salmon farming companies wish to import Atlantic salmon eggs from Stofnfiskur, an Icelandic company which is not certified under the Canadian Fish Health Protection Regulations (FHPR).
- Under the *Fisheries Act*, a Section 4 approval can be granted for such an importation if risks are deemed to be acceptable.
- Failure to provide permission for egg importation may trigger a trade challenge under the World Trade Organization, because Canada's requirements are more stringent than recommended by the World Animal Health Organization.
- Additionally, DFO could also be viewed as causing a competitive disadvantage of the aquaculture industry by denying them access to alternate strains.
- An approach, using established procedure through the mechanism of a risk assessment, is proposed.

Issue:

- Two BC Aquaculture companies wish to import Atlantic salmon eggs from Iceland. The Stofnfiskur farm in Iceland, while having an excellent fish health history, is not certified under the FHPR and therefore do not meet the Policy for Importation of Atlantic Salmon. This causes difficulty for DFO in terms of processing this request.

Background

- Stolt Seafarm and Mainstream Canada (formerly Pacific National Aquaculture) have expressed significant interest in importing Atlantic salmon eggs from Stofnfiskur LTD, in Iceland.

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- Stofnfiskur is not certified under the FHPR. However, because of the interest by BC companies as well as an ACRDP project in PEI, health records from Stofnfiskur were received by the National Registry of Fish Diseases (NRFD) to determine if the company's testing records were sufficient to be granted FHPR certification status. Currently the testing does not meet the lot-based sampling requirement of the FHPR.
- Stofnfiskur is a reputable company with a long history of health testing which meets European standards. It also has an isolated water supply (fresh and sea water boreholes) which exclude introduction of pathogens from other fish stocks in the area. The company has not introduced new stock into the farm in more than 5 years.
- The company's history and fish health protection measures were taken into account in this assessment. For this reason, a *Fisheries Act* Section 4 licence for Stofnfiskur eggs was given to the PEI ACRDP project on condition that eggs and resultant fish were held in quarantine. This sets a precedent for the importation of eggs from the Stofnfiskur facility. However, Section 4 licencing has frequently been used for the introduction of stocks into New Brunswick where US farms did not meet FHPR standards.

Analysis / DFO Comment

- The NRFD considers that for the purposes of the FHPR, the company's health testing history can be considered equivalent to the first of four inspections.
- The company meets European testing standards and is currently not prepared to increase the level of testing to meet FHPR requirements.
- Discussions have been initiated by the NRFD to review the definitions on which FHPR sampling procedures are based. In the future, Canada may accept a testing level stipulated by the World Animal Health Organization (OIE) which matches the European requirements.
- Given that the company meets OIE and European health testing requirements, Canada and especially BC may be challenged on restrictive trade practices should egg importation not be permitted. The effects of such a trade challenge may be profound such that it is difficult to maintain the safeguards (FHPR, Atlantic Salmon Importation Policy) which Canada and the Region currently have in place to prevent inadvertent importation of exotic fish pathogens.

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- In addition, further challenge could come from the aquaculture industry who may view this as constraining their development and causing a competitive disadvantage by not being able to access the best available strains.
- The Atlantic Salmon Importation Policy states: All importations must comply with the FHPR. Because this requirement cannot be met, RDG approval is required to move this application forward based on the steps suggested below. In addition, agreement from the Provincial agencies is recommended for this proposal.
- Section 4 of the *Fisheries Act* which states: "Nothing in this *Act* precludes the granting by the Minister of Fisheries written permission to obtain fish for purposes of stocking or artificial breeding or for scientific purposes" is therefore a legal means by which to provide permission to import eggs, notwithstanding that Stofnfiskur is not FHPR certified.
- A risk assessment as outlined by the National Code on Introductions and Transfers would be required. Only if the risk assessment together with appropriate mitigation measures showed the risks to be low, would a request for a Section 4 approval be submitted.
- Erosion of the intent of the FHPR can readily be avoided by ensuring that future requests for Section 4 approvals also meet equally rigorous standards to prevent the importation of exotic pathogens.
- A risk assessment to establish the fish health risks associated with the importation of eggs from Stofnfiskur would be undertaken to determine whether risk levels are low, and to stipulate additional mitigation measures, e.g., 100 % lethal testing of each broodstock fish used for BC egg production, to be applied to ensure that the risk is not increased by accepting uncertified stock.

Recommendations

- We recommend that the ITC prepare a risk assessment, especially on the fish health implications, of the proposed importation as part of a phased approach for this proposal.
- Obtain concurrence from provincial agencies (possibly through Introduction and Transfers Committee-ITC) for acceptance of proposal which does not meet Atlantic Salmon Importation Policy.

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- Should the risks be acceptably low, and the provincial agencies accept the proposal, we recommend that a Section 4 approval be sought to permit the importation of Atlantic salmon eggs from Stofnfiskur.

By EMAN

Laura Richards
Regional Director Science

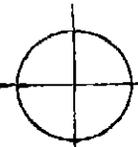


I Concur:
John C. Davis
Regional Director General

D. Kieser / A. Webb / S. Farlinger / L. Richards / J.C. Davis

CT#368384

Fish Health Service Report



Company: **Mainstream Canada**
Site: **Boot Lagoon Hatchery**
Work done: **Quarantine - Virus screening**
Date submitted: **March 30, 2004**
Case number: **4-1687**
Contact: **Howie Manchester / Caroline Cherry**
Pr#: **4060**

Final report issued: **April 13, 2004**

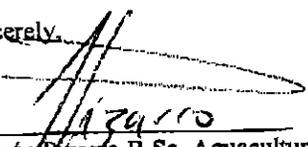
Sample description: Thirty Atlantic salmon fry were submitted for virus screening. A representative fish length is 0.17g and 2.55mm.

Virus screening: Six pools of 5 fish each were screened for the presence of virus as outlined in the Canadian Fish Health Protection Regulations Manual of Compliance (section X.A-E). The samples were inoculated onto the EPC (Epithelioma Papillosum Cyprini) and CHSE-214 (Chinook Salmon Embryo) cell lines and were allowed to incubate for 14 days at 15°C.

Results: No cytopathic effects were observed during the viral screening. These samples have been determined to be free of virus based on the scope and sensitivity of this assay.

If you have any questions regarding these results, please call us.

Sincerely,


Hernán Bazarro B.Sc. Aquaculture
Fish Health Technician


Tim Hewison B.Sc.
Fish Health Official

Microtek International (1998) Ltd.

6761 Kirkpatrick Crescent, Saanichton, B.C. Canada V8M 1Z3

Tel (250) 652-4482, Toll Free in Canada 1-800 667-5062, Fax (250) 652-4802, e-mail services@microtek-intl.com

Fish Health Service Report

Company: **Mainstream Canada**
 Site: **Boot Lagoon Hatchery**
 Work done: **Quarantine - Virus screening**
 Date submitted: **April 28, 2004**
 Case number: **4-1699**
 Contact: **Howie Manchester / Caroline Cherry**
 Pr#: **4064**

Final report issued: **May 12, 2004**

Sample description: Thirty Atlantic salmon fry were submitted for virus screening. The fish were contained in plastic bags labeled the following:

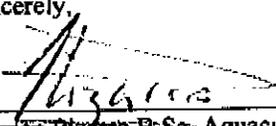
| <u>Bag label</u> | <u># fish/bag</u> | <u>Virology pool #</u> | <u>Avg. weight</u> | <u>Bag label</u> | <u># fish/bag</u> | <u>Virology pool #</u> | <u>Avg. weight</u> |
|------------------|-------------------|------------------------|--------------------|------------------|-------------------|------------------------|--------------------|
| Q2 small | 5 fish | 1 | .184g | Q2 large | 5 fish | 1 | .44 g |
| Q2 small | 5 fish | 2 | | Q2 large | 5 fish | 2 | |
| Q2 small | 5 fish | 3 | | Q2 large | 5 fish | 3 | |

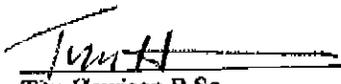
Virus screening: Six pools of 5 fish each were screened for the presence of virus as outlined in the Canadian Fish Health Protection Regulations Manual of Compliance (section X.A-E). The samples were inoculated onto the EPC (Epithelioma Papillosum Cyprini) and CHSE-214 (Chinook Salmon Embryo) cell lines and were allowed to incubate for 14 days at 15°C.

Results: No cytopathic effects were observed during the viral screening. These samples have been determined to be free of virus based on the scope and sensitivity of this assay.

If you have any questions regarding these results, please call us.

Sincerely,


 Hernán Pizarro B.Sc. Aquaculture
 Fish Health Technician


 Tim Hewison B.Sc.
 Fish Health Official

Microtek International Inc.

6761 Kirkpatrick Crescent, Saanichton, B.C. Canada V8M 1Z8

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Fish Health Service Report

Company: **Mainstream Canada**
 Site: **Boot Lagoon Hatchery**
 Work done: **Quarantine**
 Date submitted: **June 1, 2004**
 Case number: **4-1708**
 Contact: **Howie Manchester**
 Pr#: **4068**

Final report issued: **June 16, 2004**

Sample description: Sixty live Atlantic salmon fry were submitted for a quarantine screening. The fish were approximately 2.47g and 5.5cm in size. The fish were contained in plastic bags labeled the following:

| <u>Bag label</u> | <u>Virology pool #</u> | <u>Bacteriology #, VEN #, <i>P. salmonis</i> #, <i>C. shasta</i> #, Marine Anemia #</u> |
|------------------|------------------------|---|
| Q2-60 - 56 | 1, 2 | 1 - 10 |
| Q2-66 - 70 | 3, 4 | 11 - 20 |
| Q2-71 - 75 | 5, 6 | 21 - 30 |
| Q2-76 - 80 | 7, 8 | 31 - 40 |
| Q2-81 - 85 | 9, 10 | 41 - 50 |
| Q2-86 - 89 | 11, 12 | 51 - 60 |

Virus screening: Twelve pools of 5 fish each were screened for the presence of virus as outlined in the Canadian Fish Health Protection Regulations Manual of Compliance (section X.A-E). The samples were inoculated onto the EPC (Epithelioma Papillosum Cyprini), CHSE-214 (Chinook Salmon Embryo), and SHK-1 (Atlantic salmon head kidney) cell lines and were allowed to incubate for 14 days at 15°C.

***Aeromonas salmonicida*:** Kidney tissues were plated onto Trypticase Soy Agar (TSA) media and TSA containing 5% blood and screened for the causative agent of furunculosis using the culture method (incubated at 20°C and 16°C respectively).

***Yersinia ruckeri*:** Kidney tissues were plated onto Trypticase Soy Agar (TSA) media and screened for the causative agent of Enteric Red Mouth (ERM) disease using the culture method (incubated at 20°C).

***Ceratomyxa shasta*:** Gut smears were prepared on slides and viewed for spores of *C. shasta*, as outlined in the Canadian Fish Health Protection Regulations Manual of Compliance (section XI.C).

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Plasmacytoid leukemia (Marine Anemia): Liver imprints were prepared on slides, then Gram's stained and viewed for the presence of numerous plasmablasts as an indication of marine anemia.

***Piscirickettsia salmonis*:** Liver tissue imprints were prepared on slides, stained with Gram's stain and viewed for *Piscirickettsia salmonis* for the causative agent of salmonid rickettsial septicemia (SRS).

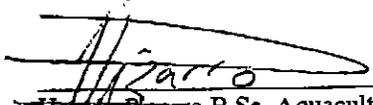
Viral Erythrocytic Necrosis (VEN): Blood smears were prepared on slides, then stained with Giemsa and viewed for the presence of inclusion bodies as outlined in the American Fisheries Society Bluebook (section VII. E.1).

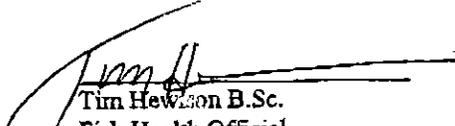
***Myxobolus cerebralis*:** Head cartilage material was prepared and slide preparations were viewed for spores of *M. cerebralis*, the causative agent of whirling disease, as outlined in the Canadian Fish Health Protection Regulations Manual of Compliance (section XI.B.3.a).

Results: No cytopathic effects were observed during the viral screening. These samples have been determined to be free of virus based on the scope and sensitivity of this assay. No *Y. ruckeri* or *A. salmonicida* were found in any of the 60 fish. No *C. shasta* or *M. cerebralis* were observed in the 60 fish. No inclusion bodies indicative of VEN were observed in any of the 60 fish. None of the 60 fish had noticeable amounts of plasmablast cells observed in the liver tissues.

If you have any questions regarding these results, please call us.

Sincerely,


Herman Pizarro B.Sc. Aquaculture
Fish Health Technician

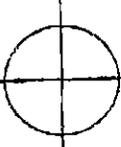

Tim Hewison B.Sc.
Fish Health Official

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Fish Health Service Report



Company: Mainstream Canada
Site: Boot Lagoon Hatchery
Work done: Schedule II, Plasmacytoid leukemia screening, *Piscirickettsia salmonis* screening, Viral Erythrocytic Necrosis screening
Date submitted: October 14, 2004
Case number: 4-1767
Contact: Howie Manchester
Pr#: 4085

Preliminary report issued: October 29, 2004

Sample description: One hundred and fifty Atlantic salmon were submitted to Microtek for a Schedule II, Marine Anemia, *P. salmonis* screening, VEN, and ISAV screening. The representative fish size was 64.3g and 17.5cm. Fifteen fish were contained in each of the plastic bags labeled the following:

| Bag # | Virology | Bacteriology and slides | Bag # | Virology | Bacteriology and slides |
|-------|----------|-------------------------|-------|----------|-------------------------|
| 600 | 1 - 3 | 1 - 15 | 605 | 16 - 18 | 76 - 90 |
| 601 | 4 - 6 | 16 - 30 | 606 | 19 - 21 | 91 - 105 |
| 602 | 7 - 9 | 31 - 45 | 607 | 22 - 24 | 106 - 120 |
| 603 | 10 - 12 | 46 - 60 | 608 | 25 - 27 | 121 - 135 |
| 604 | 13 - 15 | 61 - 75 | 609 | 28 - 30 | 136 - 150 |

Virus screening: Thirty pools of 5 fish each were screened for the presence of virus as outlined in the Canadian Fish Health Protection Regulations Manual of Compliance (section X.A-E). The samples were inoculated onto the EPC (Epithelioma Papillosum Cyprini) and CHSE-214 (Chinook Salmon Embryo) cell lines and allowed to incubate for 14 days at 15°C. The samples were also inoculated onto the SHK-1 (Atlantic Salmon Head Kidney) cell line and allowed to incubate for 14 days at 15°C. The samples will then be subsequently passed onto fresh SHK-1 cells then incubated for a further 14 days at 15°C.

Aeromonas salmonicida: Kidney tissues were plated onto Trypticase Soy Agar (TSA) media and screened for the causative agent of furunculosis using the culture method (incubated at 20°C).

Yersinia ruckeri: Kidney tissues were plated onto Trypticase Soy Agar (TSA) media and screened for the causative agent of Enteric Red Mouth (ERM) disease using the culture method (incubated at 20°C).

Ceratomyxa shasta: Gut smears were prepared on slides and viewed for spores of *C. shasta*, as outlined in the Canadian Fish Health Protection Regulations Manual of Compliance (section XI.C).

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Myxobolus cerebralis: Head cartilage material was prepared and slide preparations were viewed for spores of *M. cerebralis*, the causative agent of whirling disease, as outlined in the Canadian Fish Health Protection Regulations Manual of Compliance (section XLB.3.a).

Marine Anemia (Plasmacytoid leukemia): Liver imprints were prepared on slides, then stained with Giemsa and viewed for indications of marine anemia.

Piscirickettsia salmonis: Liver tissue imprints were prepared on slides, stained with Giemsa and viewed for *P. salmonis*.

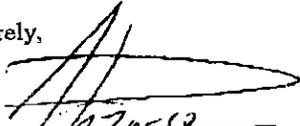
Viral Erythrocytic Necrosis (VEN): Blood smears were prepared on slides, then stained with Giemsa and viewed for the presence of inclusion bodies.

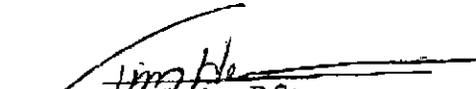
Results: No cytopathic effects were observed during the viral screening. These samples have been determined to be free of virus based on the scope and sensitivity of this assay.

No *A. salmonicida* or *Y. ruckeri* were cultured from any of the 150 fish. No *C. shasta* or *M. cerebralis* were observed in any of the fish. None of the fish had numerous immature red blood cells (plasmablasts) indicative of plasmacytoid leukemia. No *P. salmonis* or inclusion bodies indicative of VEN were observed in any of the fish.

If you have any questions regarding these results, please call us.

Sincerely,

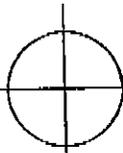

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Fish Health Technician


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Canadian Fish Health Official

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Fish Health Service Report



Company: Mainstream Canada
Site: Boot Lagoon Hatchery
Work done: Schedule II, Plasmacytoid leukemia screening, *Piscirickettsia salmonis* screening, Viral Erythrocytic Necrosis screening
Date submitted: October 14, 2004
Case number: 4-1767
Contact: Howie Manchester
Pr#: 4085

Final report issued: November 15, 2004

Sample description: One hundred and fifty Atlantic salmon were submitted to Microtek for a Schedule II, Marine Anemia, *P. salmonis* screening, VEN, and ISAV screening. The representative fish size was 64.3g and 17.5cm. Fifteen fish were contained in each of the plastic bags labeled the following:

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| 603 | 10 - 12 | 46 - 60 | 608 | 25 - 27 | 121 - 135 |
| 604 | 13 - 15 | 61 - 75 | 609 | 28 - 30 | 136 - 150 |

Virus screening: Thirty pools of 5 fish each were screened for the presence of virus as outlined in the Canadian Fish Health Protection Regulations Manual of Compliance (section X A-E). The samples were inoculated onto the EPC (Epithelioma Papillosum Cyprini) and CHSE-214 (Chinook Salmon Embryo) cell lines and allowed to incubate for 14 days at 15°C. The samples were also inoculated onto the SHK-1 (Atlantic Salmon Head Kidney) cell line and allowed to incubate for 14 days at 15°C. The samples were then subsequently passed onto fresh SHK-1 cells and incubated for a further 14 days at 15°C.

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***Yersinia ruckeri*:** Kidney tissues were plated onto Trypticase Soy Agar (TSA) media and screened for the causative agent of Enteric Red Mouth (ERM) disease using the culture method (incubated at 20°C).

***Ceratomyxa shasta*:** Gut smears were prepared on slides and viewed for spores of *C. shasta*, as outlined in the Canadian Fish Health Protection Regulations Manual of Compliance (section XI.C).

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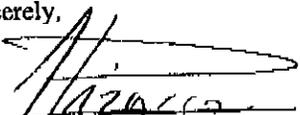
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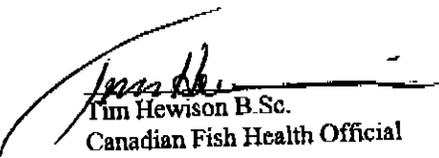
Results: No cytopathic effects were observed during the viral screening. These samples have been determined to be free of virus based on the scope and sensitivity of this assay.

No *A. salmonicida* or *Y. ruckeri* were cultured from any of the 150 fish. No *C. shasta* or *M. cerebralis* were observed in any of the fish. None of the fish had numerous immature red blood cells (plasmablasts) indicative of plasmacytoid leukemia. No *P. salmonis* or inclusion bodies indicative of VEN were observed in any of the fish.

If you have any questions regarding these results, please call us.

Sincerely,


Herman Pizarro B.Sc. Aquaculture
Fish Health Technician


Tim Hewison B.Sc.
Canadian Fish Health Official

Microtek International Inc.

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CERTIFICATE NO.:
006/04

FHPR FISH HEALTH CERTIFICATE

(Eggs Only)

Name of facility/source: **Stofniskur Ltd.**
 Address: **P.O. Box 24, 222 Hafnarfjörður, ICELAND**
 Telephone No.: **(+354) 564 6300** Fax No.: **(+354) 564 6301**

I, **Dr. Gísli Jónsson**, approved by the Secretary of the Department of the Interior, last recertified on May 31st 2004 as a Certifying Official for Iceland, as required by the Title 50, CFR pursuant to the provisions of part 16.13, do hereby certify that the source indicated above was inspected by the methods approved by the Minister of Fisheries and Oceans Canada and that the following pathogen status was determined as required by the Canadian Fish Health Protection Regulations C.R.C.

| Pathogen | Detected | Not Detected | Not Tested |
|---|--------------------------|-------------------------------------|-------------------------------------|
| Viral Hemorrhagic Septicemia Virus (VHS) | <input type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> |
| Infectious Hematopoietic Necrosis Virus (IHN) | <input type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> |
| Infectious Pancreatic Necrosis Virus (IPN) | <input type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> |
| Other filterable replicating agent | <input type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> |
| <i>Aeromonas salmonicida</i> | <input type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> |
| <i>Yersinia ruckeri</i> | <input type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> |
| <i>Mycobolus cerebralis</i> | <input type="checkbox"/> | <input type="checkbox"/> | <input checked="" type="checkbox"/> |
| <i>Caratomyxa shasta</i> | <input type="checkbox"/> | <input type="checkbox"/> | <input checked="" type="checkbox"/> |
| Bacterial Kidney Disease (BKD) | <input type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> |

Date of the last four inspections:

November 8th 2004 (D/M/Y) June 3rd 2004 (D/M/Y) May 10th 2004 (D/M/Y) May 3rd 2004 (D/M/Y)

December 14th 2004 (Date of Issue) *Gísli Jónsson* (Signature) Keflavík, Reykjavík (Place) (+354) 585 5100 (Telephone No.)

This certificate expires on the date the pathogen status changes or August 5th 2005, whichever is the earlier. (Date)

EXPORTER'S DECLARATION:

I, Víðfus Jónsson owner manager of the above noted facility which was last inspected on Dec. 14th declare that, to my knowledge, no disease agent listed in Schedule II of the Fish Health Protection Regulations (FHPR) that would alter the above described pathogen status have been detected, in this facility, according to the procedures outlined in the FHPR Manual of Compliance since the last FHPR inspection, that no introduction of fish or fish eggs from any source that would alter the above pathogen status has been made into the facility, that the shipment described below will be derived solely from this facility, and that eggs in the shipment will be surface disinfected prior to leaving the source.

I can also declare that these eggs will be surface disinfected and that they derive solely from the above inspected source.

This shipment consists of:

160 litres Live Eggs Species: Atlantic salmon (*Salmo salar* L.)

Number

Dec. 14th *Víðfus Jónsson* (+354) 564 6300

Date Signature of Owner, Manager or Consignor Telephone No

IMPORTING INFORMATION:

Departing city and country: Keflavík, ICELAND Carrier: Air Canada no. AC 855

Bill of lading No.: 108-1562-3543 Date: December 16th 2004

Anticipated port of arrival in Canada (City and Province): Vancouver December 17th 2004
Date

Name and address of importer: **Mainstream Canada**
Boot Lagoon Hatchery
A Division of EWOS
CANADA

Date Signature of Importer Telephone No.

Fish Health Service Report

Company: **Mainstream Canada**
 Site: **Boot Lagoon Hatchery**
 Work done: **Virus screening**
 Date submitted: **April 14, 2005**
 Case number: **5-1911**
 Contact: **Howie Manchester**
 Pr#: **12030**

Final report issued: **May 5, 2005**

Sample description: Thirty live Atlantic salmon fry were submitted to Microtek for virus screening. A representative fish size was 0.70g and 4.3cm. The fish were contained in plastic bags labeled the following:

| <u>Bag #</u> | <u>Virology pool #</u> |
|--------------|------------------------|
| Q1-26-35 | 1, 2 |
| Q1-46-56 | 3, 4 |
| Q1-36-45 | 5, 6 |

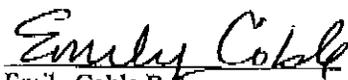
Virus screening: Six pools of 5 fish each were screened for the presence of virus as outlined in the Canadian Fish Health Protection Regulations Manual of Compliance (section X.A-E). The samples were inoculated onto the EPC (Epithelioma Papillosum Cyprini) and CHSE-214 (Chinook Salmon Embryo) cell lines and allowed to incubate for 14 days at 15°C.

The samples were also inoculated onto the SHK-1 (Atlantic Salmon Head Kidney) cell line and allowed to incubate for 21 days at 15°C.

Results: No cytopathic effects were observed during the viral assay. This sample has been determined to be free of virus based on the scope and sensitivity of this assay.

If you have any questions regarding these results, please call us.

Sincerely,


 Emily Coble B.Sc.
 Fish Health Technician


 Tim Hewison B.Sc.
 Canadian Fish Health Official

Microtek International Inc.

6761 Kirkpatrick Crescent, Saanichton, B.C. Canada V8M 1Z8

Tel (250) 652-4482, Toll Free in Canada 1-800 667-5062, Fax (250) 652-4802, e-mail services@microtek-intl.com

Fish Health Service Report

Company: **Mainstream Canada**
 Site: **Boot Lagoon Hatchery**
 Work done: **Schedule II / Quarantine screening**
 Date submitted: **April 26, 2005**
 Case number: **5-1912**
 Contact: **Howie Manchester**
 Pr#: **12031**

Final report issued: **May 17, 2005**

Sample description: Sixty live Atlantic salmon fry were submitted to Microtek for Quarantine screening including infectious salmon anemia virus (ISA), viral erythrocytic necrosis (VEN), marine anemia and salmonid rickettsial septicemia (SRS) screening. The fish size ranged from 0.8g, 4.5cm to 2.0g, 5.5cm. The fish were contained in plastic bags labeled the following:

| Bag # | No. fish/bag | Virology pool # | Bacteriology, Marine Anemia, SRS # | VEN # |
|----------|--------------|-----------------|------------------------------------|---------------|
| Q1-26-30 | 10 | 1, 2 | 1 - 10 | 1, 2, 4, 7-10 |
| Q1-31-35 | 10 | 3, 4 | 11 - 20 | 11 - 20 |
| Q1-36-40 | 10 | 5, 6 | 21 - 30 | 21 - 30 |
| Q1-41-45 | 10 | 7, 8 | 31 - 40 | 31 - 40 |
| Q1-46-51 | 11 | 9, 10 | 41 - 50 | 3, 41 - 50 |
| Q1-56-57 | 5 | 11 | 51 - 55 | 51 - 55 |
| Q1-56-57 | 5 | 12 | 56 - 60 | 56 - 60 |

Note: Blood collection was unsuccessful (due to the small size of fish) when attempted from three fish in bag Q1-26-30. The extra fish from bag Q1-46-51 was used for a blood smear.

Virus screening: Twelve pools of 5 fish each were screened for the presence of virus as outlined in the Canadian Fish Health Protection Regulations Manual of Compliance (section X.A-E). The samples were inoculated onto the EPC (Epithelioma Papillosum Cyprini) and CHSE-214 (Chinook Salmon Embryo) cell lines and allowed to incubate for 14 days at 15°C. The samples were also inoculated onto the SHK-1 (Atlantic Salmon Head Kidney) cell line and allowed to incubate for 21 days at 15°C.

Aeromonas salmonicida: Kidney tissues were plated onto Trypticase Soy Agar (TSA) and TSA containing 5% sheep blood (BA) media and screened for the causative agent of furunculosis using the culture method (the plates were incubated at 20°C and 16°C respectively).

Yersinia ruckeri: Kidney tissues were plated onto Trypticase Soy Agar (TSA) media and screened for the causative agent of Enteric Red Mouth (ERM) disease using the culture method (the plates were incubated at 20°C).

Microtek International Inc.

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Ceratomyxa shasta: Gut smears were prepared on slides and viewed for spores of *C. shasta*, as outlined in the Canadian Fish Health Protection Regulations Manual of Compliance (section XI.C).

Myxobolus cerebralis: Head cartilage material was prepared and slide preparations were viewed for spores of *M. cerebralis*, the causative agent of whirling disease, as outlined in the Canadian Fish Health Protection Regulations Manual of Compliance (section XI.B.3.a).

Marine Anemia (Plasmacytoid leukemia): Liver imprints were prepared on slides, then stained with Giemsa and viewed for indications of marine anemia.

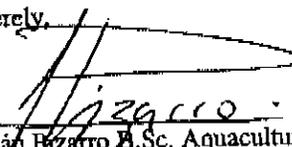
Piscirickettsia salmonis: Liver tissue imprints were prepared on slides, then stained with Giemsa and viewed for *Piscirickettsia salmonis*.

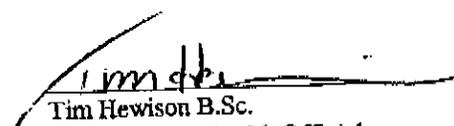
Viral Erythrocytic Necrosis (VEN): Blood smears were prepared on slides, then stained with Giemsa and viewed for the presence of inclusion bodies.

Results: No cytopathic effects were observed during the viral assay. This sample has been determined to be free of virus based on the scope and sensitivity of this assay. No *A. salmonicida* or *Y. ruckeri* were cultured from any of the 60 fish. No *C. shasta* or *M. cerebralis* were observed in any of the fish. None of the fish had numerous immature red blood cells (plasmablasts) indicative of plasmacytoid leukemia. No *P. salmonis* were observed in any of the fish. No inclusion bodies indicative of VEN were observed in any of the 58 fish tested.

If you have any questions regarding these results, please call us.

Sincerely,


Hernán Pizarro B.Sc. Aquaculture
Fish Health Technician

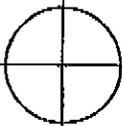

Tim Hewison B.Sc.
Canadian Fish Health Official

Microtek International Inc.

6761 Kirkpatrick Crescent, Saanichton, B.C. Canada V8M 1Z8

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Fish Health Service Report



Company: Mainstream Canada
Site: Boot Lagoon Hatchery
Work done: Schedule II / Quarantine screening
Date submitted: September 2, 2005
Case number: 5-1971
Contact: Howie Manchester
Pr#: 12042

Final report issued: September 23, 2005

Sample description: One hundred and fifty Atlantic salmon were submitted to Microtek for Quarantine screening including infectious salmon anemia virus (ISA), viral erythrocytic necrosis (VEN), marine anemia and salmonid rickettsial septicemia (SRS) screening. The fish had a representative fish size of 25.5g, 12.5cm. The fish were contained in plastic bags labeled the following:

| Bag # | No. fish/bag | Virology pool # | Bacteriology, Marine Anemia, SRS #, VEN # |
|---------|--------------|-----------------|---|
| 501 | 15 | 1 - 3 | 1 - 15 |
| 502 | 15 | 4 - 6 | 16 - 30 |
| 503 | 15 | 7 - 9 | 31 - 45 |
| 504 | 15 | 10 - 12 | 46 - 60 |
| 612 | 15 | 13 - 15 | 61 - 75 |
| 614 | 15 | 16 - 18 | 76 - 90 |
| 616 | 15 | 19 - 21 | 91 - 105 |
| 617 | 15 | 22 - 24 | 106 - 120 |
| 611 | 15 | 25 - 27 | 121 - 135 |
| 104/107 | 15 | 28 - 30 | 136 - 150 |

Virus screening: Thirty pools of 5 fish each were screened for the presence of virus as outlined in the Canadian Fish Health Protection Regulations Manual of Compliance (section X.A-E). The samples were inoculated onto the EPC (Epithelioma Papillosum Cyprini) and CHSE-214 (Chinook Salmon Embryo) cell lines and allowed to incubate for 14 days at 15°C. The samples were also inoculated onto the SHK-1 (Atlantic Salmon Head Kidney) cell line and allowed to incubate for 21 days at 15°C.

Aeromonas salmonicida: Kidney tissues were plated onto Trypticase Soy Agar (TSA) and TSA containing 5% sheep blood (BA) media and screened for the causative agent of furunculosis using the culture method (the plates were incubated at 20°C and 16°C respectively).

Microtek International Inc.

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Tel (250) 652-4482, Toll Free in Canada 1-800 667-5062, Fax (250) 652-4802, e-mail services@microtek-intl.com

Yersinia ruckeri: Kidney tissues were plated onto Trypticase Soy Agar (TSA) media and screened for the causative agent of Enteric Red Mouth (ERM) disease using the culture method (the plates were incubated at 20°C).

Ceratomyxa shasta: Gut smears were prepared on slides and viewed for spores of *C. shasta*, as outlined in the Canadian Fish Health Protection Regulations Manual of Compliance (section XI.C).

Myxobolus cerebralis: Head cartilage material was prepared and slide preparations were viewed for spores of *M. cerebralis*, the causative agent of whirling disease, as outlined in the Canadian Fish Health Protection Regulations Manual of Compliance (section XI.B.3.a).

Marine Anemia (Plasmacytoid leukemia): Liver imprints were prepared on slides, then stained with Giemsa and viewed for indications of marine anemia.

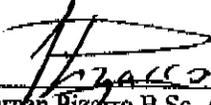
Piscirickettsia salmonis: Liver tissue imprints were prepared on slides, then stained with Giemsa and viewed for *Piscirickettsia salmonis*.

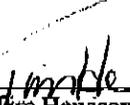
Viral Erythrocytic Necrosis (VEN): Blood smears were prepared on slides, then stained with Giemsa and viewed for the presence of inclusion bodies.

Results: No cytopathic effects were observed during the viral assay. This sample has been determined to be free of virus based on the scope and sensitivity of this assay. No *A. salmonicida* or *Y. ruckeri* were cultured from any of the 60 fish. No *C. shasta* or *M. cerebralis* were observed in any of the fish. None of the fish had numerous immature red blood cells (plasmablasts) indicative of plasmacytoid leukemia. No *P. salmonis* were observed in any of the fish. No inclusion bodies indicative of VEN were observed in any of the 60 fish tested.

If you have any questions regarding these results, please call us.

Sincerely,


Herpan Pizarro B.Sc. Aquaculture
Fish Health Technician


Tim Hewison B.Sc.
Canadian Fish Health Official

Microtek International Inc.

6761 Kirkpatrick Crescent, Saanichton, B.C. Canada V8M 1Z8

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ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road
Abbotsford BC V0G 2M9
Telephone: (804) 866-8003
Facsimile: (804) 866-3010
Toll-Free: 1-800-661-9903

Case Report

| | | | | | |
|---------------------------------|---------------------|-----------------------------------|------------------|-------------------------------------|--|
| Submission #: 2004-01975 | | Date Received: 01-Jun-2004 | | Report Dat: 02-Jul-2004 | |
| Submitter: 8447 | Stolt Sea Farm Inc. | Report To | Copies To | <input checked="" type="checkbox"/> | |
| Owner: 8447 | Stolt Sea Farm Inc. | | | <input type="checkbox"/> | |
| Farm: | | | | <input type="checkbox"/> | |
| Vet Clinic: | | | | | |
| Attending Vet: | | | | | |
| Specimen: Whole Animal | | Flock Herd Size: | | | |
| Species: Atlantic Salmon | | Age: | | | |
| Breed: | | Sex: | | | |
| Feed: | | Feed Supplier: | | | |
| Vaccination: | | | | | |
| Treatment: | | | | | |
| Diagnosis: | | | | | |

S.A. Flaverty, D.V.M.,
Veterinary Pathologist

AUG 10 2004 13:26 FR ANIMAL HEALTH CENTER 604 556 3010 TO STOLT SEA - LEE P.02/03

Submission #: 2004-01975**Case Report****History/Symptoms**

Submitted 4 Atlantic salmon. Fourth quarantine sample from Dalrymple. Please test as per the e-mail sent to J. Robinson and S. Raverty.

Bacteriology

Please note: Sample was not received in Bacteriology Laboratory until Aug 9th.
Results pending.

Virology

Fish viruses negative by culture.

* Results faxed July 2/04.

Molecular Diagnostics/PCR

ISA Virus negative by PCR.

Mxyobolus cerebralis (Whirling Disease) negative by PCR.

Piscirickettsia salmonis negative by PCR.

Aeromonas salmonicida negative by PCR.

Yersinia ruckerii (ERM) negative by PCR.

Note: *Aeromonas salmonicida* and *Yersinia ruckerii* (ERM) are also being cultured. No charge PCR for these two tests. *Ceratomyxa shasta* PCR in progress. IPN and VHS in tissue culture, ongoing, negative to date.

* Results faxed July 14th and Aug. 10/04.

/bb

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Telephone: 604 566-3003
Facsimile: 604 566-3010
Toll-Free: 1 800 661-0903

MOLECULAR DIAGNOSTICS

Specimen No.: 04-1975
Date Received: 2004/07/01
Owner: Stolt Sea Farm-Dalrymple
Submitter: Same
Pathologist: SAR
Species: Salmonid
CHG: x-3
NC:

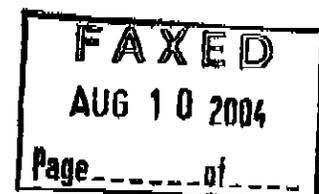
Results: **ISA Virus Negative by PCR**
Myxobolus cerebralis (Whirling Disease) Negative by PCR
Piscirickettsia salmonis Negative by PCR
Aeromonas salmonicida Negative by PCR
Yersinia ruckerii (ERM) Negative by PCR

Note: **Aeromonas salmonicida and Yersinia ruckerii (ERM) are also being cultured. No charge PCR for these two tests. Certomyxa shasta PCR in progress**

IPN & VHS in tissue culture, ongoing, negative to date.

Date: August 9, 2004

John H. Robinson DVM, Ph.D.
Head, Virology & Molecular Diagnostics
Animal Health Centre
BCMAF&F
Abbotsford, B.C.



AUG 10 2004 09:52 FR ANIMAL HEALTH CENTER 604 556 3010 TO STOLT SEA - LEE P.01/01

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1767 Angus Campbell Road
Abbotsford BC V3G 2M3
Telephone: 604 556-3003
Facsimile: 604 556-3010
Toll-Free: 1 800 661-9903**MOLECULAR DIAGNOSTICS**

Specimen No.: 04-1975
Date Received: 2004/07/01
Owner: Stolt Sea Farm-Dalrymple
Submitter: Same
Pathologist: SAR
Species: Salmonid
CHG: x-3
NC:

Results: ISA Virus Negative by PCR
Myxobolus cerebralis (Whirling Disease) Negative by PCR
Piscirickettsia salmonis Negative by PCR
Aeromonas salmonicida Negative by PCR
Yersinia ruckerii (ERM) Negative by PCR

Note: *Aeromonas salmonicida* and *Yersinia ruckerii* (ERM) are also being cultured. No charge PCR for these two tests. *Ceratomyxa shasta* PCR in progress

IPN & VHS in tissue culture, ongoing, negative to date.

Date: August 9, 2004

John H. Robinson DVM, Ph.D.
Head, Virology & Molecular Diagnostics
Animal Health Centre
BCMAF&F
Abbotsford, B.C.



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1767 Angus Campbell Road
Abbotsford BC V3G 2M9
Telephone: (804) 556-3000
Facsimile: (804) 856-3010
Toll-Free: 1-800-861-9903

Bacteriology Results

| | |
|---|---|
| Submission #: 2004-01975 | Owner: Stolt sea Farm Inc. |
| Date Received: 01-Jun-2004 | Submitter: Stolt Sea Farm Inc. |
| Date Completed: 02-Jul-2004 | Fax: 250-286-1062 Phone: 250-286-3532 |
| Pathologist: S.A. Raverty, D.V.M., | Farm: |
| | Location: CAMPBELL RIVER |
| Species: Atlantic salmon | Vet Clinic: |
| Breed: | Fax: Phone: |
| Age: | Attending Vet: |

Preliminary Date: **Completed Date: 11-Aug-2004**

whole body, multiple organ - Fish

Comments

Please note: Sample was not received in Bacteriology Laboratory until Aug 9th.
 No Bacteria isolated after 48 hrs.
 No Yersinia ruckeri isolated
 No Aeromonas salmonicida isolated
 Completed by H. Gannon

AUG 04 2004 08:45 FR ANIMAL HEALTH CENTER 604 556 3010 TO STOLT SEA - LEE P.01/01



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Facsimile: (604) 556-3010
Toll-Free: 1-800-661-0909

Virology Laboratory Results

| | |
|---|---|
| Submission #: 2004-02295 | Owner: Dalrymple Quarantine, PO #50717 |
| Date Received: 07-JUL-2004 | Submitter: Stolt Sea Farm Inc. |
| Date Completed: 14-JUL-2004 | Fax: 250-286-1062 Phone: 250-286-3532 |
| Pathologist: S.A. Raverty, D.V.M., | Farm: |
| Species: Fish, Not spe | Location: CAMPBELL RIVER |
| Breed: | Vet Clinic: |
| Age: | Fax: Phone: |
| | Attending Vet: |

| | | |
|-----------------------------------|--------------------------|------------------------------------|
| Date Received: 07-JUL-2004 | Preliminary Date: | Completed Date: 03-AUG-2004 |
|-----------------------------------|--------------------------|------------------------------------|

| Sample# | Description | VTG | Ext. Ref | Titre Test |
|---------|-------------|-----|----------|------------|
|---------|-------------|-----|----------|------------|

Comments

Fish Viruses Negative
Tissue culture = negative

AUG 03 2004 09:55 FR ANIMAL HEALTH CENTER 604 556 3010 TO STOLT SEA - LEE P.01/02



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1767 Angus Campbell Road
Abbotsford BC V2G 2M3
Telephone: (804) 556-3003
Facsimile: (804) 556-9010
Toll-Free: 1-800-861-9005

Case Report

Submission #: 2004-02295 Date Received 07-Jul-2004 Report Dat 14-Jul-2004

| | | Report To | Copies To |
|-----------------|---------------------------------|-------------------------------------|-----------|
| Submitter: 8447 | Stolt Sea Farm Inc. | <input checked="" type="checkbox"/> | |
| Owner: 12250 | Dalrymple Quarantine, PO #50717 | <input type="checkbox"/> | |
| Farm: | | <input type="checkbox"/> | |
| Vet Clinic: | | | |
| Attending Vet: | | | |

| | |
|------------------------------|------------------|
| Specimen: Whole Animal | Flock Herd Size: |
| Species: Fish, Not Specified | Age: |
| Breed: | Sex: |

| | |
|-------|----------------|
| Feed: | Feed Supplier: |
|-------|----------------|

Vaccination:

Treatment:

Diagnosis:

S.A. Raverty, D.V.M.,
Veterinary Pathologist

AUG 03 2004 09:55 FR ANIMAL HEALTH CENTER 604 556 3010 TO STOLT SEA - LEE P.02/02

Submission #: 2004-02295

Case Report**History/Symptoms**

Dalrymple quarantine testing of Stofnfiskurs eggs.

| Disease | Method | Comments | Commence testing | by whom |
|-----------------------|---------------|------------|------------------|----------------|
| IPN | cell culture | pools of 5 | immediately | AHC |
| VHS | cell culture | pools of 5 | immediately | AHC |
| IPN | cell culture | pools of 5 | immediately | AHC |
| Whirling Disease | PCR | | 120 days | AHC |
| Geratomyxa shasta | PCR | | 120 days | AHC |
| Aeromonas salmonicida | Bac-t culture | | 4cm or 120 days | AHC |
| ERM | Bac-t culture | | 4cm or 120 days | AHC |
| (VENEIBS) | blood smears | | 2nd sample | AHC-S Raverty) |
| (Marine anemia) | histology | | 4cm or 120 days | AHC-S Raverty) |
| ISA | PCR | pools of 5 | immediately | AHC |
| Rickettsial dis | PCR | pools of 5 | immediately | AHC |

All testing to be done in accordance with the FHPR manual of compliance or the O.I.E. Diagnostic Manual for Aquatic Animal Diseases, 2002.

Bacteriology

No Bacteria isolated (including *Aeromonas salmonicida* and *Yersinia ruckeri*) from Fish after extended incubation at 15 and 22 degrees Celsius.
Completed by E. Whitton.

* Results faxed July 12/04.

Molecular Diagnostics/PCR

Samples A, B, C - *Piscirickettsia salmonis* negative by PCR.
Samples A, B, C - ISA Virus negative by PCR.
Samples A, B, C - *Myxobolus cerebralis* negative by PCR.

* Results faxed July 14/04.

Final Comments

Histology samples have been forwarded to an outside reference lab and results to follow.

/bb

JUL 14 2004 11:40 FR ANIMAL HEALTH CENTER 604 556 3010 TO STOLT SEA - LEE P.02/02

**ANIMAL HEALTH CENTRE**
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1767 Angus Campbell Road
Abbotsford BC V3G 2M3
Telephone: 604 656-3003
Facsimile: 604 656-3010
Toll-Free: 1 800 661-0903**MOLECULAR DIAGNOSTICS**

SUBMISSION NO: 04-2295
DATE RECEIVED: 2004/07/07
OWNER: Dalrymple Quarantine PO #50717
SUBMITTER: Stolt Sea Farm
PATHOLOGIST: SAR
SPECIES: Salmonid
CHG: x-9
NC:

Results: Samples A, B, C *Piscirickettsia salmonis* Negative by PCR
Samples A, B, C ISA Virus Negative by PCR
Samples A, B, C *Myxobolus cerebralis* Negative by PCR**DATE:** July 12/ 2004**John H. Robinson DVM, Ph.D.**
Animal Health Centre
B.C. Ministry of Agriculture, Food, & Fisheries
Abbotsford

JUL 12 2004 09:52 FR ANIMAL HEALTH CENTER 604 556 3010 TO STOLT SEA - LEE P.01/01



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Facsimile: (804) 556-3010
Toll-Free: 1-800-681-9903

Bacteriology Results

| | |
|--|---|
| Submission #: 2004-02285 | Owner: Dalrymple Quarantine, PO #50717 |
| Date Received: 07-Jul-2004 | Submitter: Stolt Sea Farm Inc. |
| Date Completed: | Fax: 250-286-1062 Phone: 250-286-3532 |
| Pathologist: S.A. Raverty, D.V.M. | Farm: |
| Species: Fish, Not Spc | Location: CAMPBELL RIVER |
| Breed: | Vet Clinic: |
| Age: | Fax: Phone: |
| Attending Vet: | |
| Preliminary Date: | Completed Date: 12-Jul-2004 |

Whole body, multiple organ - Fish

Comments

No Bacteria isolated (including *Aeromonas salmonicida* and *Yersinia ruckeri*) from Fish after extended incubation at 15 and 22 degrees Celsius.
Completed by E. Whinton



Stolt Sea Farm Inc.

A subsidiary of
Stolt-Nielsen S.A.

1761 Redwood Street
Campbell River, B.C.
V9W 3K7

Telephone: (250) 286-3532
Facsimile: (250) 286-1062

June 16, 2004

June 21/04
left message
re missing Sched II
test results

Dorothee Kieser
By Fax: 250-756-7053
Fisheries and Oceans
Pacific Biological Station
3190 Hammond Bay Road
Nanaimo, BC V9t 6N7

Dear Dorothee,

Subject: Results of the Third Testing on the Stofnfiskur Strain Held in the Dalrymple Quarantine Unit.

Attached are the results from the sample sent on May 3, 2004 to the Animal Health Centre in Abbotsford.

The fourth sample was sent June 1, 2004. I will forward the results when they are available.

Sincerely yours,

Lee Beile
Fish Health Manager

cc Keng Pee Ang

Lee Beile

From: SRaverty [SRaverty@telus.net]
Sent: Sunday, May 09, 2004 9:58 PM
To: Lee Beile
Subject: fish health check

Hi Lee,
No indication of any viral inclusions or abnormalities within the examined smears. Hope you had an enjoyable weekend.
Stephen



ANIMAL HEALTH CENTRE

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Ministry of
Agriculture, Food and Fisheries
1787 Angus Campbell Road
Abbotsford BC V3G 2M3
Telephone: (604) 336-3003
Facsimile: (604) 658-3010
Toll-Free: 1-800-661-9803

Submission Summary Report

| | | | | | |
|--|--|-------------------------------------|--|---------------------|--|
| Submission #: 2004-01641 | | Date Received: 04-May-2004 | | Report Date: | |
| Submitter: 8447 Stolt Sea Farm Inc. | | <input checked="" type="checkbox"/> | | Report To | |
| Owner: 8447 Stolt Sea Farm Inc. | | <input type="checkbox"/> | | Copies To | |
| Farm: | | | | | |
| Pathologist: J.H. Robinson, D.V.M. | | Phone: | | Fax: | |
| Vet Clinic: | | | | | |
| Attending Vet: | | | | | |
| Specimen: Whole Animal | | Flock Herd Size: | | | |
| Species: Fish, Not Specified | | Age: | | | |
| Breed: | | Sex: | | | |
| Feed: | | Feed Supplier: | | | |
| Vaccination: | | | | | |
| Treatment: | | | | | |

History / Symptoms:

Submitted one cooler - 2 bags x15 alevins for IHNV, VHSV, IPNV, and ISAV (PCR).
Rickettsial disease (PCR).

PO# - 50395.

MAY 08 2004 16:52 FR ANIMAL HEALTH CENTER 604 556 3010 TO 812502861062

P. 02/02

**ANIMAL HEALTH CENTRE**
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1767 Angus Campbell Road
Abbotsford BC V8G 2M3
Telephone: 804 556-3003
Facsimile: 804 556-3010
Toll-Free: 1 800 661-0903**MOLECULAR DIAGNOSTICS**

Specimen No.: 03-1641
Date Received: 2004/05/04
Owner: Stolt Sea Farm Inc.
Submitter: Same
Pathologist: JHR
Species: Salmonid
CHG: x-6
NC:

Results: Samples composite 1, 2, 3 ISA Virus Negative by PCR
Samples composite 1, 2, 3 *Piscirickettsia salmonis*
Negative by PCR

Date: May 8, 2004

John H. Robinson DVM, Ph.D.
Head, Virology & Molecular Diagnostics
Animal Health Centre
BCMAF&F
Abbotsford, B.C.



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Telephone: (804) 556-3000
Facsimile: (804) 556-3010
TollFree: 1-800-961-0903

Virology Laboratory Results

| | | | |
|-----------------|-----------------------|----------------|----------------------------------|
| Submission #: | 2004-01641 | Owner: | Stolt Sea Farm Inc. |
| Date Received: | 04-May-2004 | Submitter: | Stolt Sea Farm Inc. |
| Date Completed: | | Fax: | 250-286-1062 Phone: 250-286-3532 |
| Pathologist: | J.H. Robinson, D.V.M. | Farm: | |
| Species: | Fish, Not spe | Location: | CAMPBELL RIVER |
| Breed: | | Vet Clinic: | |
| Age: | | Fax: | Phone: |
| | | Attending Vet: | |

| | | | | |
|----------------|-------------|-------------------|-----------------|-------------|
| Date Received: | 04-May-2004 | Preliminary Date: | Completed Date: | 02-Jun-2004 |
|----------------|-------------|-------------------|-----------------|-------------|

| Sample# | Description | VTG | Ext. Ref | Titre Test |
|---------|-------------|-----|----------|------------|
|---------|-------------|-----|----------|------------|

Comments

3 samples inoculated onto tissue culture - all negative.

Stolt Sea Farm Inc.

Stolt Sea Farm Inc.
1761 Redwood Street,
Campbell River, B.C.
Canada V9W 3K7
Phone (250) 286-3532 Fax (250) 286-1062

FAX MESSAGE

TO:

COMPANY:

FAX NUMBER:

Dorothee Keiser

DFD

1250 756-7053

FROM:

DATE:

NUMBER OF PAGES
(including cover sheet)

Lee Beile

Aug 6, 04

12

SUBJECT:

Multiple horizontal lines for subject text input.

Stolt Sea Farm Inc.

A subsidiary of
Stolt-Nielsen S.A.

1781 Redwood Street
Campbell River, B.C.
V9W 3K7

Telephone: (250) 286-3532
Facsimile: (250) 286-1062

August 6, 2004

Dorothee Kieser
By Fax: 250-756-7053
Fisheries and Oceans
Pacific Biological Station
3190 Hammond Bay Road
Nanaimo, BC V9t 6N7

Dear Dorothee,

Subject: Preliminary Results of the Fourth Testing on the Stofnfiskur Strain Held in the Dalrymple Quarantine Unit.

Attached are the preliminary results from the sample sent on June 01, 2004 to the Animal Health Centre in Abbotsford.

Although it was requested, the samples were not initially tested for *Ceratomyxa Shasta*, *Aeromonas salmonicida* or *Yersinia ruckeri*. They are presently testing for the latter two by PCR. The results for *Ceratomyxa Shasta* are pending the arrival of a positive control, which I am told should arrive next week.

The fifth sample was sent July 6, 2004. This sample, per our discussion will not be tested for VEN/EIBS or marine anemia. A sample testing for these will be sent to Animal Health upon Dr. Steve Raverty's return to work in two weeks.. I will forward the results when they are available.

Sincerely yours,

Lee Beile
Fish Health Manager

cc Keng Pee Ang

Lee Beile

From: Lee Beile
Sent: Monday, May 31, 2004 3:59 PM
To: John Robinson (E-mail)
Cc: 'SRaverty@telus.net'
Subject: Dalrymple testing

I have sent off the fourth quarantine sample to the Animal Health Centre this afternoon. Please test as per the 120 day or 4 cm requirement.

I have sent 30 fish for you John and 30 fish for Dr Raverty for the blood and histo samples. Hopefully you will have sufficient fish for all the samples.

Kind Regards, Lee

Daily sample quarantine testing of Stofniskurs eggs

| Disease | Method | Comments | Commence testing | by whom |
|-----------------------|---------------|------------|------------------|-----------------------------|
| IPN | cell culture | pools of 5 | immediately | Animal Health |
| VHS | cell culture | pools of 5 | immediately | Animal Health |
| IPN | cell culture | pools of 5 | immediately | Animal Health |
| Whirling Disease | PCR | | 120 days | Animal Health |
| Ceratomyxa shasta | PCR | | 120 days | Animal Health |
| Aeromonas salmonicida | Bact culture | | 4 cm or 120 days | Animal Health |
| ERM | Bac-t culture | | 4 cm or 120 days | Animal Health |
| VEN/EBS | blood smears | | 2nd sample | Animal Health/Steve Raverty |
| Marine anemia | histology | | 4 cm or 120 days | Animal Health/Steve Raverty |
| ISA | PCR | pools of 5 | immediately | Animal Health |
| Rickettsial disease | PCR | pools of 5 | immediately | Animal Health |

All testing to be done in accordance with the FHPR manual of compliance or the O.I.E. Diagnostic Manual for Aquatic Animal Diseases, 2002.



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Toll-Free: 1 800 861-9903

MOLECULAR DIAGNOSTICS

SUBMISSION NO: 04-1975
DATE RECEIVED: 2004/06/01
OWNER: Stolt Sea Farm
SUBMITTER: Same
PATHOLOGIST: SAR
SPECIES: Salmonid
CHG:
NC: X

Results: *ISA Virus Negative by PCR*
Myxobolus cerebralis Negative by PCR
Pliscirickettsia Negative by PCR

DATE: July 12/ 2004

John H. Robinson DVM, Ph.D.
Animal Health Centre
B.C. Ministry of Agriculture, Food, & Fisheries
Abbotsford



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Facsimile: (804) 666-3010
Toll-Free: 1-800-661-0003

Virology Laboratory Results

| | | | |
|-----------------|-----------------------|----------------|----------------------------------|
| Submission #: | 2004-01875 | Owner: | Stolt Sea Farm Inc. |
| Date Received: | 01-Jun-2004 | Submitter: | Stolt Sea Farm Inc. |
| Date Completed: | | Fax: | 250-286-1062 Phone: 250-286-3632 |
| Pathologist: | S.A. Raverty, D.V.M., | Farm: | |
| Species: | Atlantic salmon | Location: | CAMPBELL RIVER |
| Breed: | | Vet Clinic: | |
| Age: | | Fax: | Phone: |
| | | Attending Vet: | |

| | | | | | |
|----------------|-------------|-------------------|--|-----------------|-------------|
| Date Received: | 01-Jun-2004 | Preliminary Date: | | Completed Date: | 02-Jul-2004 |
|----------------|-------------|-------------------|--|-----------------|-------------|

| Sample# Description | VTG | Ext. Ref | Titre Test |
|---------------------|-----|----------|------------|
|---------------------|-----|----------|------------|

Comments

Fish viruses negative by culture.

Submission #: 2004-01975

Case Report

History/Symptoms

Submitted 4 Atlantic salmon. Fourth quarantine sample from Dalrymple. Please test as per the e-mail sent to J. Robinson and S. Raverty.

Virology

Fish viruses negative by culture.

* Results faxed July 2/04.

Molecular Diagnostics/PCR

ISA Virus negative by PCR.

Mxyobolus cerebralis negative by PCR.

Piscirickettsia negative by PCR.

* Results faxed July 14/04.

/bb

CASE NUMBER: 04-F-048, 120 day or 4 cm testing
DATE: June 9, 2004

MORHOLOGIC DIAGNOSES:

COMMENTS:

| | | | |
|----------|--------------|--|------------|
| VEN/EIBS | blood smears | | 2nd sample |
|----------|--------------|--|------------|

FINAL REPORT

Stolt Sea Farm Inc.



A subsidiary of
Stolt-Nielsen S.A.

1761 Redwood Street
Campbell River, B.C.
V9W 3K7

Telephone: (250) 286-3532
Facsimile: (250) 286-1062

Dr. Ang, Keng Pee Ph.D.

Technical Manager

May 3, 2004

Dorothee Kieser
By Fax: 250-756-7053
Fisheries and Oceans
Pacific Biological Station
3190 Hammond Bay Road
Nanaimo, BC V9t 6N7

Stolt Sea Farm Inc.



A subsidiary of 1761 Redwood Street
Stolt-Nielsen S.A. Campbell River, B.C.
Canada V9W 3K7
E Mail: keng.pee.ang@stoltseafarm.com

Business Phone: (250) 286-3532
Business Fax: (250) 286-1062
Direct Dial Line: (250) 850-3558
Cell Phone: (250) ~~850-7208~~

850-9888

Dear Dorothee,

Subject: Results of the Second Testing on the Stofnfiskur Strain Held in the Dalrymple Quarantine Unit.

Attached are the results from the sample sent on March 29, 2004 to the Animal Health Centre in Abbotsford. Dr Raverty was unable to evaluate the fish for EIBS/VEN because of their size (see attached email).

The third sample was sent today, May 3, 2004. I will forward the results when they are available.

Sincerely yours,

Lee Beile
Fish Health Manager

cc Keng Pee Ang

Lee Beile

From: SRaverty [SRaverty@telus.net]
Sent: Wednesday, April 28, 2004 7:30 PM
To: Lee Beile
Subject: fish health check

Hi Lee,
Thirty fish were euthanized and after 15 animals were processed by tail bleed and exsanguination, adequate slides were identified for only 5 animals, and in each of these smears, there were insufficient numbers of red blood cells to assess. The fish were not further evaluated. Re-evaluation at a later stage of development should facilitate more adequate slide preparation.
Stephen

APR 29 2004 17:25 FR ANIMAL HEALTH CENTER 604 556 3010 TO 812502861062

P.01/02



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Submission Summary Report

| | | | | | |
|---------------------------------|-----------------------|-------------------------------------|--|---------------------------------|--|
| Submission #: 2004-01250 | | Date Received: 30-Mar-2004 | | Report Date: 02-Apr-2004 | |
| | | Report To | | Copies To | |
| Submitter: 8447 | Stolt Sea Farm Inc. | <input checked="" type="checkbox"/> | | | |
| Owner: 8447 | Stolt Sea Farm Inc. | <input type="checkbox"/> | | | |
| Farm: | | | | | |
| Pathologist: | S.A. Raverty, D.V.M., | | | | |
| Vet Clinic: | | Phone: | | Fax: | |
| Attending Vet: | | <input type="checkbox"/> | | | |
| Specimen: Tissue - Fresh | | Flock Herd Size: | | | |
| Species: Atlantic Salmon | | Age: | | | |
| Breed: | | Sex: | | | |
| Feed: | | Feed Supplier: | | | |
| Vaccination: | | | | | |
| Treatment: | | | | | |

History / Symptoms:

Submitted fish tissue.

Dalrymple quarantine sample 2. Included are two bags, tanks 1, 2, and 3 and tanks 4, 5, and 6. 30 fish are for Dr. J. Robinson for viral culture for IHN, VHS, IPN, and PCR for ISA, and Rickettsial disease. The remaining 30 are for Dr. S. Raverty for IEBS/Ven screening by blood smears.

PO# 49774.



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MOLECULAR DIAGNOSTICS

Specimen No.: 04-1250
Date Received: 20004/03/30
Owner: Stolt Sea Farm Inc.
Submitter: Same
Pathologist: SAR
Species: Salmonid
CHG: x-2
NC:

Results: IPN Virus Negative by PCR
ISA Virus Negative by PCR

Date: April 1, 2004

John H. Robinson DVM, Ph.D.
Head, Virology & Molecular Diagnostics
Animal Health Centre
BCMAF&F
Abbotsford, B.C.

FAXED
APR 29 2004
Page _____ of _____



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Facsimile: (604) 868-3010
Toll-Free: 1-800-661-0009

Virology Laboratory Results

| | |
|------------------------------------|---------------------------------------|
| Submission #: 2004-01250 | Owner: Stolt Sea Farm Inc. |
| Date Received: 30-Mar-2004 | Submitter: Stolt sea Farm inc. |
| Date Completed: 02-Apr-2004 | Fax: 250-286-1062 Phone: 250-286-3532 |
| Pathologist: S.A. Raverty, D.V.M., | Farm: |
| Species: Atlantic Salm | Location: CAMPBELL RIVER |
| Breed: | Vet Clinic: |
| Age: | Fax: Phone: |
| | Attending Vet: |

Date Received: 30-Mar-2004 Preliminary Date: Completed Date: 28-Apr-2004

Sample# Description

VTG Ext. Ref

Titre Test

Comments

2 samples inoculated onto tissue culture - both negative.

APR 14 2004 10:14 FR ANIMAL HEALTH CENTER 604 556 3010 TO 812502861062

P. 01/02



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Toll-Free: 1-800-061-8903

Submission Summary Report

| | | |
|--|-----------------------------------|---------------------------------|
| Submission #: 2004-00874 | Date Received: 02-Mar-2004 | Report Date: 10-Mar-2004 |
| Submitter: 8447 Stolt Sea Farm Inc. <input checked="" type="checkbox"/> | | Report To |
| Owner: 8447 Stolt Sea Farm Inc. <input type="checkbox"/> | | Copies To |
| Farm: | | |
| Pathologist: J.H. Robinson, D.V.M. | | |
| Vet Clinic: | | Phone: |
| Attending Vet: | | Fax: |
| | | <input type="checkbox"/> |
| Specimen: Tissue - Fresh | Flock Herd Size: | |
| Species: Fish, Not Specified | Age: | |
| Breed: | Sex: | |
| Feed: | Feed Supplier: | |
| Vaccination: | | |
| Treatment: | | |

History / Symptom:

Submitted 3 bags labeled 1/2, 3/4, and 5/6. This represents the six tanks in the quarantine building. Because of their size, I have included more than the 30 fish required to be sampled. So, to confirm, at this point you need to test via cell culture for IHN, IPN, and VHS. In addition, via PCR for ISA and Rickettsial disease. For cell culture 6 pools of 5 fish are acceptable. I am unsure of pooling for PCR by O/E standards. Any questions, please call.



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Virology Laboratory Results

| | |
|---|---|
| Submission #: 2004-00874 | Owner: Stolt Sea Farm Inc. |
| Date Received: 02-Mar-2004 | Submitter: Stolt Sea Farm Inc. |
| Date Completed: 10-Mar-2004 | Fax: 250-286-1062 Phone: 250-286-3832 |
| Pathologist: J.H. Robinson, D.V.M. | Farm: |
| species: Fish, Not Spe | Location: CAMPBELL RIVER |
| Breed: | Vet Clinic: |
| Age: | Fax: Phone: |
| | Attending Vet: |

| | | |
|-----------------------------------|--------------------------|------------------------------------|
| Date Received: 02-Mar-2004 | Preliminary Date: | Completed Date: 24-Mar-2004 |
|-----------------------------------|--------------------------|------------------------------------|

| Sample# | Description | VTG | Ext. Ref | Titre Test |
|---------|-------------|-----|----------|------------|
|---------|-------------|-----|----------|------------|

Comments

6 pools were inoculated onto tissue culture - all 6 negative.

MAR 16 2004 14:19 FR ANIMAL HEALTH CENTER 604 556 3010 TO STOLT SEA - LEE P.01/03



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Toll-Free: 1-800-661-9802

Case Report

| | | | | | |
|-------------------------------------|---------------------|-------------------------------------|--|--------------------------------|--|
| Submission #: 2004-00874 | | Date Received: 02-Mar-2004 | | Report Dat: 10-Mar-2004 | |
| | | | | Report To Copies To | |
| Submitter: 8447 | Stolt Sea Farm Inc. | <input checked="" type="checkbox"/> | | | |
| Owner: 8447 | Stolt Sea Farm Inc. | <input type="checkbox"/> | | | |
| Farm: | | <input type="checkbox"/> | | | |
| Vet Clinic: | | | | | |
| Attending Vet: | | | | | |
| Specimen: Tissue - Fresh | | Flock Herd Size: | | | |
| Species: Fish, Not Specified | | Age: | | | |
| Breed: | | Sex: | | | |
| Feed: | | Feed Supplier: | | | |
| Vaccination: | | | | | |
| Treatment: | | | | | |
| Diagnosis: | | | | | |

J.H. Robinson, D.V.M.
Veterinary Virologist

MAR 16 2004 14:20 FR ANIMAL HEALTH CENTER 604 556 3010 TO STOLT SEA - LEE P.02/03

Submission #: 2004-00874**Case Report**

History/Symptoms

Submitted 3 bags labeled 1/2, 3/4, and 5/6. This represents the six tanks in the quarantine building. Because of their size, I have included more than the 30 fish required to be sampled. So, to confirm, at this point you need to test via cell culture for IHN, IPN, and VHS. In addition, via PCR for ISA and Rickettsial disease. For cell culture 6 pools of 6 fish are acceptable. I am unsure of pooling for PCR by O/E standards. Any questions, please call.

Molecular Diagnostics/PCR

Six (6) pools (5 fish - pool): *Piscirickettsia salmonis* negative by PCR.
Six (6) pools (5 fish - pool): ISA Virus negative by PCR.

* Results faxed Mar. 10/04.

/bb

MAR 16 2004 14:20 FR ANIMAL HEALTH CENTER 604 556 3010 TO STOLT SEA - LEE P.03/03

**ANIMAL HEALTH CENTRE**
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Toll-Free: 1 800 681-8903**MOLECULAR DIAGNOSTICS**

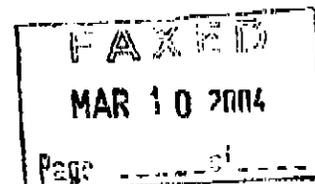
Specimen No.: 04-874
Date Received: 20004/03/02
Owner: Stolt Sea Farm Inc.
Submitter: Same
Pathologist: JHR
Species: Salmonid
CHG: x-6
NC:

Results: Six (6) pools (5 fish -pool) *Piscirickettsia salmonis*
Negative by PCR

Six (6) pools (5 fish- pool) ISA Virus Negative by PCR

Date: March 8, 2004

John H. Robinson DVM, Ph.D.
Head, Virology & Molecular Diagnostics
Animal Health Centre
BCMAF&F
Abbotsford, B.C.



JUL 29 2004 10:48 FR ANIMAL HEALTH CENTER 604 556 3010 TO 812502861062

P. 02/02

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Toll-Free: 1 800 881-9903**MOLECULAR DIAGNOSTICS**

Specimen No.: 04-2458
Date Received: 2004/07/20
Owner: Dalrymple quarantine PO # 51915
Submitter: Stolt Sea Farm
Pathologist: JHR
Species: Salmonid
CHG: x-18
NC:

Results: Specimens T 1-6: ISA Virus Negative by PCR
Piscirickettsia salmonis Negative by PCR
Myxobolus cerebralis Negative by PCR

Note: TC in progress; *Ceratomyxa shasta* PCR in progress

Date: July 28, 2004

John H. Robinson DVM, Ph.D.
Head, Virology & Molecular Diagnostics
Animal Health Centre
BCMAF&F
Abbotsford, B.C.

JUL 30 2004 12:20 FR ANIMAL HEALTH CENTER 604 556 3010 TO STOLT SEA - LEE P.02/03

Submission #: 2004-02458

Case Report

History/Symptoms

Submitted 10 fish from 6 tanks (60 fry).

Please see attached sheet for testing instructions.

Molecular Diagnostic/PCR

Specimens T 1-6:

ISA Virus negative by PCR.

Piscirickettsia salmonis negative by PCR.

Myxobolus cerebralis negative by PCR.

* Results faxed July 29/04.

/bb

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Case Report

Submission 2005-01009 **Date** 24-Mar-2005 **Report** 28-Apr-2005

Report **Copies**

Submitter: 8447 Stolt Sea Farm Inc.
Owner 8447 Stolt Sea Farm Inc.
Farm:
Vet Clinic:
Attending

Specimen: Whole Animal **Count** 60 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination**Treatmen****Diagnosis**

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

History/Symptoms

Dalrymple Quarantine Fish. Second sample. Request cell culture of 60 fish (pools of 5 fish) on CHSE and EPC.

LAB. NOTE:

On Mar. 24/05 fish were anesthetized by Dr. Raverty, separated into 5 fish pools, and frozen in the -10 degrees C freezer next to the necropsy room. The samples were removed from the freezer and submitted to virology for cell culture Mar. 30/05 by Dr. Gary Marty.

Virology

12 samples were inoculated onto tissue culture - all were negative.

- 3 fish cell lines were used ; EPC (Epithelioma Papulosum Cyprini), CHSE-214 (Chinook Salmon Embryo) and SSN-1 (Striped Snakehead).
- incubation time on tissue cultures was 25 days.

* Results faxed Apr. 28th.

Molecular Diagnostics/PCR

IHN Virus negative by PCR.
VHS Virus negative by PCR.

* Results faxed Apr. 28/05.

/bb

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Toll-Free: 1-800-661-9903

Case Report

Submission 2005-00644 **Date** 24-Feb-2005 **Report** 22-Mar-2005

Report **Copies**

Submitter: 8447 Stolt Sea Farm Inc.
Owner 13605 Dalrymple Quarantine PO 56230
Dalrymple Quarantine, PO #56230

Farm:
Vet Clinic:
Attending

Specimen: Tissue - Fresh **Count** **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

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

History/Symptoms

Submitted many tissue samples for cell culture pools of 5 (1st sample) for IPN, VHS, and IHN. Also for PCR pools of 5 (1st sample) for ISA, and Rickettsial disease. I sent enough to do 30 cell cultures and 30 for PCR.

1. 30 moribund salmonids every four weeks during the quarantine period (approximately 6 months).
2. 60 moribund salmonids no more than two weeks prior to the end of the quarantine period.
3. 150 moribund salmonids three weeks prior to their removal from the isolation facility at (9-12) months.

PO #56230

Submitted by Brad Boyce.

Virology

6 samples inoculated onto tissue culture - all negative.

* Results faxed Mar. 22/05.

Molecular Diagnostics/PCR

Sample pools 1-6: *Piscirickettsia salmonis* negative by PCR.

Sample pools 1-6: ISA Virus negative by PCR.

* Results faxed Mar. 22/05.

/bb

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Facsimile: (604) 556-3010
Toll-Free: 1-800-661-9903

Case Report

Submission 2005-00092 **Date** 12-Jan-2005 **Report** 09-Feb-2005

Report **Copies**

Submitter: 8447 Stolt Sea Farm Inc.

Owner 8447 Stolt Sea Farm Inc.

Farm:

Vet Clinic:

Attending

Specimen: Whole Animal

Count 150

Flock Herd Size:

Species: Atlantic Salmon

Age

Breed:

Sex:

Feed:

Feed

Vaccination

Treatmen

Diagnosis

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

History/Symptoms

PO # 55378. Submitted 150 live Atlantic Salmon for health screen as per Dr. G. Marty.

Gross Pathology

150 Atlantic salmon were subjected to necropsy on January 13, 2005. The average fork length of the 150 fish sampled was 15.7 cm (range = 8.8 - 19.6 cm). We collected samples from 131 live anesthetized fish and 19 recently dead fish. The following protocol was used for sample collection:

1. Anesthetize fish (for fish that were still alive), measure fork length, place fish on paper with fish number written on the paper towel.
2. Cut caudal peduncle, obtain blood, and make a blood smear on pre-labelled slide.
3. Disinfect fish, cut transverse section through dorsal skin at cranial margin of dorsal fin, swab kidney with sterile plastic loop, and streak on TSA plates (1/4 plate per fish).
4. Remove left operculum, excise 1 or 2 gill arches and place in Whirlpak bag for virology PCR.
5. Open body cavity - excise spleen for virology PCR.
6. Harvest gall bladder (expel bile) for *Ceratomyxa shasta* PCR.
7. Excise sample of intestinal ceca for *Ceratomyxa shasta* PCR, and another sample of intestinal ceca for virology PCR.
8. Sample distal intestine for histopathology (in pre-labelled vial of 10% formalin).
9. Remove head (at level of operculum); transect ~midsagittal (thinnest ½-head in pre-labelled vial of 10% formalin for histopathology, thickest ½-head in Whirlpak bag for *Myxobolus cerebralis* PCR).
10. Transverse section through body and kidney for histopathology (in pre-labelled vial of 10% formalin); remove remaining head and trunk kidney for virology PCR.
11. Final check that all samples have been taken.
12. Discard remains.

Two fish had significant gross lesions:

1. Fish #93 had a pale mass (proliferative?), about 5 x 3 x 3 mm, at base of right pectoral fin that was preserved in 10% formalin for histopathology.
2. Fish #132 did not have a left eye (it had probably been enucleated).

Tissues were separated into three groups and analyzed as follows for PCR and viral culture:

1. PCR for ISA and *Piscirickettsia*; viral culture for VHS, IHN, and IPN - gill, kidney (all head kidney and trunk kidney that was not used for histopathology), spleen, and intestinal ceca.
2. *Ceratomyxa shasta* PCR - gall bladder and intestinal ceca.
3. *Myxobolus cerebralis* PCR - ½-head.

All fish were negative for significant bacterial, viral, and parasitic pathogens.

Blood smears: Blood smears from 150 fish were stained with Giemsa, cover-slipped, and examined under oil immersion with a 100× objective lens. Twenty-fields of the feathered edge of each smear were examined for inclusions of Viral Erythrocytic Necrosis virus (VEN) and Erythrocytic Inclusion Body Syndrome (EIBS). Blood smears from all 150 fish were negative for viral inclusions.

Bacteriology

Fish #1 - 70: no growth of bacteria.
Fish #72 - 150: no growth of bacteria.
No *Yersinia ruckeri* or *Aeromonas salmonicida* isolated.

See attached sheet.

Histopathology

Samples of head, distal intestine, and body wedge (including trunk kidney) were fixed in 10% neutral buffered formalin. The head and body wedge were decalcified for 2 to 2.5 hours in Protocol B decalcifier (from Fisher Scientific) and then processed routinely to an H&E stained slide. Sections of each organ were examined from each of 150 fish (note: the intestine only was missing from fish # 9). All fish were negative for evidence of marine anemia. The white mass at the base of the pectoral fin of fish #93 was a mat of fungal hyphae (*Saprolegnia* or related species).

Virology

30 pooled samples inoculated onto tissue culture - all negative.

Molecular Diagnostics/PCR

Sample pools 1-5, 6-10, 11-15, 1-20, 21-25, 26-30, 31-35, 36-40, 41-45, 46-50, 51-55, 56-60, 61-65, 66-70, 71-75, 76-80, 81-85, 86-90, 91-95, 96-100, 101-105, 106-110, 111-115, 116-120, 121-125, 126-130, 131-135, 136-140, 141-145, 146-150:

Ceratomyxa shasta negative by PCR.
Myxobolus cerebralis negative by PCR.
ISA Virus negative by PCR.
Piscirickettsia salmonis negative by PCR.

Diagnosis

1. Skin: superficial fungal hyphae, focal, moderate (fish #93)
2. Enucleated left eye (fish #132)

Final Comments

These fish had no evidence of infectious organisms that would be of concern for their stocking into the waters of British Columbia. Lesions in two of the fish (enucleated eye and superficial fungal hyphae) routinely occur at low prevalence in cultured salmonids in British Columbia.

/bb