**COMMISSION OF INQUIRY INTO THE DECLINE OF SOCKEYE SALMON IN THE FRASER RIVER**

In the matter of His Excellency the Governor General in Council, on the recommendation of the Prime Minister, directing that a commission do issue under Part I of the Inquiries Act and under the Great Seal of Canada appointing the Honourable Bruce Cohen as Commissioner to conduct an inquiry into the decline of sockeye salmon in the Fraser River.

**Submissions of the Province on the recent ISAV Reports**

1. Prior to December, 2010, the Province ran a comprehensive health management program for salmon aquaculture in British Columbia out of its offices in Courtenay and Abbotsford, British Columbia.

2. The objectives of the Provincial Fish Health Program were to monitor and minimize the risks of disease in farmed fish; to ensure access to accurate and verifiable data on the disease status of cultured salmon; and to facilitate public and agency confidence that aquaculture health management in British Columbia occurred at a high standard.

*(Exhibit 1560, Province of BC Annual Report Fish Health Program, 2009, p. 5)*

3. All salmon aquaculture and commercial facilities reported site-specific information to the BC Salmon Farmers database, including mortality, causes of mortality and Fish Health Events. From that database, quarterly reports of industry's fish health status were submitted to government and posted for public viewing.

*(Exhibit 1560, Province of BC Annual Report Fish Health Program, 2009, p. 5)*

4. As regulator, the Province conducted regular and frequent inspections. For example, 116 Fish Health Audits were conducted in 2009.

*(Exhibit 1560, Province of BC Annual Report Fish Health Program, 2009, p. 13)*

5. During each Fish Health inspection, fresh silver carcasses (fish that recently died) were collected and tested by the Animal Health Branch in Abbotsford British Columbia.

*(Exhibit 1560, Province of BC Annual Report Fish Health Program, 2009, p. 14)*

6. While the Province was operating the fish health audit program, Infectious Salmon Anaemia virus "ISAV" was one of a number of pathogens that was frequently and repeatedly tested for, as Atlantic salmon are susceptible to this disease (Pacific salmon are not). As a result 4726 fish were tested between 2003 and 2010 for ISAV by reverse transcriptase polymerase chain reaction tests "RT-PCR", and all results were negative.

| Year | # Positive | # Negative |
|---|---|---|
| 2003 | 0 | 648 |
| 2004 | 0 | 675 |
| 2005 | 0 | 586 |
| 2006 | 0 | 644 |
| 2007 | 0 | 763 |
| 2008 | 0 | 588 |
| 2009 | 0 | 585 |
| 2010 | 0 | 237 |
| TOTAL | **0** | **4,726** |

*(Exhibit 1471, Publicly Available PCR Test Results for ISAV in BC Farmed Salmon, 2003-2010)*

7. In addition, the Province's fish health auditing and surveillance program tested for the disease Infectious Salmon Anaemia "ISA" through examination of mortality records, necropsies of fish by trained fish health technicians, RT-PCR, and examination of microscope slides by a board-certified veterinary pathologist.

*(Exhibit 1678, Histopathology FHAS 2006-2010)*

8. Since the transfer to the federal government, the federal government conducts the inspections; however, samples from their inspections are still sent to the British Columbia Ministry of Agriculture's Animal Health Centre in Abbotsford for testing and analysis.

9. As a result the BC government has continued to conduct ISAV RT-PCR tests on farmed fish for ISAV. In addition, the Animal Health Centre also tests samples submitted directly by the veterinarians from the salmon farms. To date 7002 tests have been conducted.

| Year | # Positive | # Negative |
|---|---|---|
| 2003 | 0 | 648 |
| 2004 | 0 | 675 |
| 2005 | 0 | 586 |
| 2006 | 0 | 644 |
| 2007 | 0 | 763 |
| 2008 | 0 | 588 |
| 2009 | 0 | 585 |
| 2010 | 0 | 907 |
| 2011 | 0 | 1,606 |
| TOTAL | **0** | **7,002** |

*(Exhibit 2079, All ISAV Tested Samples – January 2011 to Present; Exhibit QQQ, Summary of Animal Health Centre PCR test results for infectious salmon anaemia virus (ISAV) in tissues from British Columbia farmed salmon, 2003 – 2011; and*

*Exhibit 1471, Publicly Available PCR Test Results for ISAV in BC Farmed Salmon, 2003-2010)*

10. None of these tests have been positive for ISAV using the Province's usual and customary validated ISAV RT-PCR tests. This is encouraging as farmed Atlantic salmon are susceptible to ISA and ISAV.

11. In contrast to Atlantic Salmon, all tested Pacific salmon have been shown to be resistant to ISA and ISAV. Dr. McWilliams testified to this point and stated:

> 45 Q And you've worked on ISAV, then?
> 46 DR. MacWILLIAMS: Yes, I did that during my Master's
> 47 thesis work. And infectious anaemia virus is --
>
> 22
> PANEL NO. 55
> In chief by Mr. Martland
> August 22, 2011
> 1 has just been shown to cause natural infections in
> 2 marine farmed Atlantic salmon. Under experimental
> 3 conditions they have -- certain labs, including
> 4 mine, have been able to experimentally infect
> 5 using a high dose of a very pathogenic strain of
> 6 the virus and cause disease in other species. In
> 7 my case it was rainbow trout or Oncorhynchus
> 8 genus.
> 9 And but work done on Pacific salmon has shown
> 10 that Pacific salmon are relatively resistant to
> 11 the disease. You can infect them with a high dose
> 12 of a strain in very unnatural conditions in a
> 13 laboratory, and you can -- but most Pacific salmon
> 14 species, they weren't able to cause disease. They
> 15 were able to just have application of the virus,
> 16 but the fish did not actually get sick.
> 17 So it is important to note that Atlantic
> 18 salmon are the only species that have ever shown
> 19 natural infection in a wild environment.

*(Dr. MacWilliams, August 23, 2011, p. 21, l. 45 – p. 22, l. 19)*

12. This is consistent with all of the expert witness evidence to date (Dr. F. Kibenge, Dr. Nylund, Dr. Miller, Dr. Gagne, Dr. M. Kibenge) as none of them have identified any signs of disease in any of the sockeye salmon tested.

13. Regardless of the fact that sockeye salmon have never presented naturally with ISA, after the Province became aware of Dr. F. Kibenge's results, it reran all of its ISAV RT-PCR tests for the 2011 year (January through October 25, 2011) using at least two different RT-PCR tests recommended in the OIE Manual for ISA primers and probes. All repeated tests were negative for ISAV.

*(Exhibit 2079, All ISAV Tested Samples – January 2011 to Present and Exhibit 2000, OIE Validation and Certification of Diagnostic Assays)*

14. Of these results, the Province noted that in two cases at least one of the duplicate wells reacted with primer set Conventional OIE-Ref. 20, M1 gene (segment 8). Each one of these tests was repeated and the results were not reproducible.

*(Exhibit 2079, All ISAV Tested Samples – January 2011 to Present)*

15. Out of an abundance of caution, however, the Province sequenced the bands that reacted with the Conventional OIE-Ref 20 M1 gene (segment 8) to determine what was being picked up.

16. In each case where sequencing was conducted the results showed the band was not a match to ISAV. The closest matches were to mouse (*Mus musculus*). Accordingly the Province did not detect ISAV in any of its samples.

*(Exhibit 2079, All ISAV Tested Samples – January 2011 to Present and*

*Exhibit 2080, Molecular Diagnostics Sequence Identification Summary, Case 2011-0855)*

17. In another case (2011-4562), samples in a single test reacted with the Province's primers and probes (i.e. Real-time AHC M1 gene, Segment 8). RT-PCR testing on these samples was repeated in triplicate with the same primers and probes, and all were negative for ISAV. Additional PCR testing was done in triplicate with four other tests recommended by the OIE Manual for ISA, and all were negative for ISAV.

*(Exhibit 2079, All ISAV Tested Samples – January 2011 to Present)*

## THE PROVINCE'S PRIMERS AND PROBES ARE DESIGNED PROPERLY AND MEASURE WHAT THEY ARE SUPPOSED TO MEASURE

18. The Aquaculture Coalition criticized the Province's primers arguing that the primers were created in house. However it did not produce any evidence that the primers used by the Province were insufficient. To the contrary, Dr. Kibenge and Ms. Gagne agreed that the procedure followed by the Province was the correct way to develop and ensure that primers and probes target all newly discovered genotypes of ISAV.

> 30 Q. So in order to stay current, to develop a proper
> 31 assay, it's necessary to keep it updated.
> 32 MS. GAGNE: Yes.
> 33 Q. And then once you -- and one way to do that is by
> 34 regularly using GenBank and appropriate software
> 35 to develop one that targets all known strains or
> 36 variance?
> 37 MS. GAGNE: I would say mostly by reviewing the
> assay
> 38 you're using with additional sequences as they
> 39 become available.
> 40 Q. Would you agree that that's the proper way to keep
> 41 current, Dr. Kibenge?
> 42 DR. KIBENGE: That is correct and actually in fact
> 43 that's what the OIE manual recommends.
> 44 Q. So then once you do that procedure, then you must
> 45 conduct validation tests to ensure that what
> 46 you're picking up is actually ISAV; is that
> 47 correct?
>
> 4
> PANEL NO. 66
> Cross-exam by Ms. Callan (BCPROV)(cont'd)
> December 16, 2011
> 1 DR. KIBENGE: Yes.
> 2 Q. Now, this is what your labs do?
> 3 MS. GAGNE: Yes.
> 4 Q Dr. Kibenge as well?
> 5 DR. KIBENGE: Yes.
> 6 MS. CALLAN: Now, if we could turn to provincial Tab
> 7 10, Mr. Lunn. Yes, please.

8 MS. PANCHUK: Tab 12 is now marked as 2086.
9
10 EXHIBIT 2086: (See Exhibit 2041)
11
12 MS. CALLAN:
13 Q. Would you agree based on a review of the document
14 that this is what the province does as well?
15 MS. GAGNE: That's what the document says.

*(Dr. Gagne and Dr. Kibenge, December 16, 2011, p. 3, l. 30 – p.4, l. 15)*

19. Ms. Gagne further testified that the Province's ISAV primers were a good primer set. She also testified that the Province's ISAV primers would have caught whatever Dr. Miller and Dr. Molly Kibenge was measuring if it were in the fish they sampled.

38 Q. So you'd agree, then, that the provincial primer
39 set is a good primer set?
40 MS. GAGNE: It looks like it, and I can add that based
41 on Dr. Miller sequencing information provided
42 during this inquiry, it's showing on the parts of
43 the sequences that she has obtained that these
44 primers should detect ISA.

*(Gagne, December 16, 2011, p.4, ll. 38-44)*

20. Accordingly ISAV would have been picked up if it were causing disease in the fish samples that the Province tested. The fact that it wasn't picked up means that ISAV was not present.

## Dr. MILLERS RESULTS

21. Dr. Miller testified that she had been conducting various tests as a follow up on her genomic signature work. One of the viruses she was testing for was ISAV.

22. Dr. Miller as opposed to all of the other experts conducted her testing using a "Fluidigm" methodology where she mixed the sample with a low concentration of approximately 20 primer sets at once, ran the cocktail for 14 cycles without a probe, and then ran the sample again with a specific probe for 40 cycles using a greater concentration of each primer set in each well. Therefore, for the 40 cycles, each well included only a single primer set.

*(Exhibit 2076 SOP for Fluidigm Real-Time PCR TaqMan Assay, Dec 6 2011)*

23. This methodology is not standard for virologists and Dr. Nylund was critical of this testing methodology stating at page 94 of the Dec. 15 transcript:

> 35 Q. Dr. Nylund, would you agree that this isn't
> 36 standard methodology for virus research?
> 37 DR. NYLUND: Yeah, like Dr. Miller said, this is not
> 38 standard for -- I mean, I've never been acquainted
> 39 with this method before and it's a bit worrying
> 40 the way they're doing it, but as I said, it could
> 41 lead to false positives.

*(Dr. Nylund, December 15, 2011, p. 94, ll. 35-41)*

24. Further raising concerns regarding the validity of the results, Dr. Garver could not confirm many if any of Dr. Miller's results despite clear attempts to do so.

*(Exhibit 2043, Garver Results, by Experiment)*

25. Dr. Miller also testified that she did not have positive controls in her lab, which would have made it impossible for her to validate her methods. Validation of the test methods is critical in viral diagnostic work.

26. There is also a stop codon being identified in the Plarre 7 tests so whatever Dr. Miller is targeting cannot be ISAV. Dr. Nylund testified to this as follows:

> 11 Q.Okay. And Dr. Nylund, one last question. Earlier
> 12 on in Commission counsel's evidence, you mentioned
> 13 an issue about stop codons. I was very interested
> 14 in hearing it and I was hoping you could answer
> 15 what your concerns were with respect to the stop
> 16 codon issue.
> 17 DR. NYLUND: Yeah, well, if you look at that
> 18 presentation by Miller, she has an alignment of
> 19 the ISA-7 showing three fixed differences.
> 20 Actually, if you look at that alignment, and I
> 21 meant alignment because I have a lot of sequences
> 22 in my lab that hasn't been published yet, there
> 23 are seven differences in the space between the two
> 24 primers and those seven differences cannot be
> 25 found in Canadian or European ISA virus. But
> 26 unfortunately, those differences also introduces a
> 27 stop codon into this sequence, which means that
> 28 it's not a functional sequence, it can't be coding
> 29 for an ISA virus or another virus protein because
> 30 you don't have stop codons in there. A stop codon
> 31 means that it's the end of the sequence, coding
> 32 sequence and this is not the end of the coding
> 33 sequence for an ISA virus.
> 34 Q. Thank you for answering that question
> 35 DR. NYLUND: So that means that I find it hard to
> 36 believe that this could be a functional sequence.
> 37 I think this could be due to unspecific annealing
> 38 of the primers that are picking up something else
> 39 than actually virus.

*(Dr. Nylund, December 15, 2011, p.100, ll. 11-39)*

CHINOOK FARMED TEST RESULTS

27. Dr. Miller also testified that she tested some samples of farmed Chinook salmon provided for a study into why certain Chinook salmon were jaundiced, and Dr. Miller testified that about 30% of her samples were testing positive for ISAV.

28. This was interesting because the Province had also been given samples from the same fish and conducted histopathology. Dr. Marty confirmed that the healthy fish that were sampled had only minor lesions, whereas the jaundiced fish all had lesions of sufficient severity to explain their death. Interestingly in the samples Dr. Miller tested the healthy fish and the jaundiced fish had the same prevalence for what Dr. Miller thought was ISAV. This indicates if Dr. Miller's testing is correct, what she is detecting did not cause the jaundice syndrome.

*(Exhibit 2078, Evidence that Jaundice Syndrome in Farmed Chinook Salmon is Not Associated with Positive PCR Test Results for ISAV*
*Exhibit 2077 Marty, Histopathology of Chinook Salmon Sampled as Part of a Study into the Cause of Jaundice Syndrome, undated [Excel])*

29. The Province also ran ISAV RT-PCR tests on other diseased fish from the same farm. When the Province did its RT-PCR testing, all of the tests except for Conventional OIE-Ref 20 M1 gene were routine negative results (i.e. no reaction with the probe whatsoever). When Conventional OIE-Ref 20 M1 gene (segment 8) was run, a duplicate well reacted with primer set Conventional OIE-Ref. 20, M1 gene (segment 8), but it was sequenced by the Province and was not a match for ISAV. (This is the case discussed in paras 14-16 of the Province's submissions.)

*(Exhibit 2079, All ISAV Tested Samples – January 2011 to Present; and*

*Exhibit 2080, Molecular Diagnostics Sequence Identification Summary, Case 2011-0855)*

30. The sequencing results showed that the band was not a match for ISAV but was actually closer to mouse (*Mus musculus*). Accordingly, whatever Dr. Miller is finding is probably not ISAV, but regardless further testing using standard methodology should be continued to confirm this definitively.

## DR. MOLLY KIBENGE'S PAPER

31. Dr. Molly Kibenge conducted some research in 2004 on ISAV in wild salmon, and got unusual ISAV testing results which could not be validated by the DFO Moncton laboratory, despite numerous attempts to do so.

32. Of the most concern was that according to her test results 100% of the 64 Cultus Lake Sockeye Salmon tested had reacted with the primers.

33. However it is important to examine the results more closely. When the Cultus Lake Sockeye samples were sequenced it became clear that ISAV was not being picked up in Dr. M. Kibenge's testing. Dr. F. Kibenge agreed to this point, and testified this was significant because it shows that the internal sequences being measured were not the same as ISAV and therefore it wasn't a match for ISAV.

9 Q. Okay. If we could turn to page 11 of the paper?
10 Now, would you agree that this paper discusses the
11 Cultus Lake sockeye samples and that it indicates
12 at the bottom of the first paragraph that the
13 nucleotide sequence of these inserts had identity
14 to ISAV only in the primer sequence?
15 DR. KIBENGE: Yes.
16 Q Now, what's the significance of that?
17 DR. KIBENGE: Well, you can look at it in several ways,
18 but in my view, for the primers to anneal, they
19 have to be homologous to the target. So clearly

20 they annealed to a target in these samples and the
21 sequence was amplified. The internal sequences
22 that we amplified were probably not identical to
23 those that had been deposited in the GenBank.
24 That's why only the primer sequences were
25 identical to the ISA virus.
26 The ISA virus stated here would be
27 corresponding to all those sequences that are
28 available in the GenBank at that time.
29 Q. So it wasn't a match, then, for ISAV?
30 DR. KIBENGE: It wasn't.

*(Dr. Kibenge, December 16, 2011, p.7, ll. 9-30)*

34. Ms. Gagne further commented on this point that the amplified sequence had nothing to do with fish, and was actually a match for mouse. She thought it could be non-specific amplification (although Dr. F. Kibenge disagreed with this notion just prior to Ms. Gagne giving evidence on this point.)

32 Q. Ms. Gagné, you've heard me ask those fairly
33 detailed questions. Do you have any comment or
34 evidence on that question?
35 MS. GAGNE: I have seen in the disclosed documents the
36 sequence of these non-specific, and the match has
37 nothing to do with any fish. The match is random
38 mouse, human, and I have seen that with FA3/RA3
39 primers we were using at the time. We dropped
40 using them because we found that they were
41 matching non-specifically to the salmon RNA and
42 producing non-specific amplification in the same
43 size as the positive product, but upon sequencing
44 it's clear that it's a non-specific product.
45 Q. And what do you draw from that?
46 MS. GAGNE: This actually, for me, if I read that
47 without any knowledge, I just see that you have
81
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accidentally obtained a product 1 of the wrong --
2 it's a non-specific amplification, and this is not
3 uncommon.

*(Gagne, December 16, 2011, p. 80, l. 32 – p. 81, l.3)*

35. This is significant, because this result mirrors the test results of the Province on the errant band on the Conventional OIE-Ref 20 m1 gene test.

36. Accordingly, the Province submits, either non specific amplification on some of the tests mirrors a segment of the mouse genome, or some of the segment 8 assays (such as the Conventional OIE-Ref 20 m1 gene (segment 8)) are not specific enough to ISAV, and is what is causing the errant results. This means ISAV is likely not being found.

## CONCLUSION

37. It appears if the various scientists conduct appropriate follow up work, they will likely come to a consensus on the assays and what is actually being measured with the various tests which came back as presumptively positive.

38. This work should be encouraged, and the Province will do its part to work together with the various DFO, CFIA, and nongovernmental virologists.

39. The Province submits that sampling of wild fish for ISAV should continue to ensure that if ISAV is present in BC waters, it is definitively found with appropriate sampling techniques. This way if the virus is present, it can be identified appropriately, and there will be no question about the integrity of the samples or the quality of the RNA.

40. The Province also submits that additional sampling of wild fish should include a broader range of diagnostic tests, including but not limited to bacteriology, virology, parasitology, and histopathology. This is needed to understand the relation of any individual findings with disease and population change.

All of which is respectfully submitted,

December 29, 2011

D.C. Prowse Q.C., Boris Tyzuk Q.C., and Tara Callan, Counsel for Her Majesty the Queen in Right of the Province of British Columbia