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.

REPLY SUBMISSIONS OF THE PROVINCE ON THE ISSUE OF RECENT TESTING OF ISAV IN BRITISH COLUMBIA

Reply to points raised by the Aquaculture Coalition regarding the sufficiency of the Province's Primers and Probes

The Province's ISA primers and probes

1. The Province's Animal Health Centre is certified by the American Association of Veterinary Diagnosticians "AAVLD". The Animal Health Centre has a proven track record of being able to efficiently and correctly test for various veterinary diseases. Staff from the Animal Health Centre conducted diagnostic support for the CFIA during the avian influenza outbreak and there were no problems with the reliability of the Animal Health Centre's results.

(Dr. Wright, December 19, 2011, p. 19, ll. 7- 38)

2. The requirements of the AAVLD are stringent. Dr. Wright outlined the requirements in his evidence on December 19th.

19 Q And Dr. Wright, the provincial veterinary
20 diagnostic laboratory is certified by the American
21 Association of Veterinary Laboratory
22 Diagnosticians. Are you familiar with this
23 certification process?
24 DR. WRIGHT: Yes, I am.
25 Q Could you summarize the process in relation to the
26 reliability of results from an AAVLD or the
27 American Association of Veterinary Laboratory
28 Diagnosticians --
29 DR. WRIGHT: Okay.
30 Q -- certified laboratory?
31 DR. WRIGHT: Sure. If I may, I'll just call them
32 AAVLD, and within the last seven or so years they
33 have revamped their accreditation program, and
34 what they have done as their base document,
35 they've actually accepted the OIE quality standard
36 for testing laboratories and they've modified it
37 somewhat, because that standard was actually
38 written for laboratories that actually test for
39 infectious diseases, so they've modified it
40 slightly to incorporate other types of testing,
41 you know, toxicology, this type of thing. That
42 standard, the OIE standard, is actually an
43 interpretation of ISO 17025 specifically for
44 veterinary laboratories involved in testing.
45 So in essence, it's the equivalent to a 17025
46 without the requirement to have a scope listing
47 every test for every pathogen for every host

19
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1 species that you're testing. It's broader in
2 terms of scope, it's more general, but in terms of
3 the quality standard, it's essentially 17025.
4 Q So then you would think that the AAVLD standards
5 are good standards?
6 DR. WRIGHT: I have no problem with that.

(Dr. Wright, December 19, 2011, p. 18, l 47 – p.19, l. 6)

3. To be certified with the AAVLD diagnostic tests must be validated to confirm their tests are measuring what they are supposed to be measuring and the assay is up to date and catches recently discovered strains of viruses. The Province has fulfilled this requirement with respect to its ISAV RT-PCR test.

(Exhibit C, section 5.4.3 American Association of Veterinary Laboratory Diagnosticians, Requirements for an Accredited Veterinary Medical Diagnostic Laboratory, Validation of test methods of Exhibit 1675, Affidavit #2 of Dr. Gary D. Marty)

4. The Aquaculture Coalition on page 4, section 4, para 2 of their argument states "the evidence is clear that Dr. Marty was conducting PCR tests with no confirmed validity" and the Province's "primer had never been through the validation process" and quotes page 107 lines 21-23 for this proposition. The Province outrightly disputes these statements. This section of the transcript cited by the Aquaculture Coalition for this proposition states:

> 21 Q But the B.C. lab, the assay that they use, isn't
> 22 validated either, is it?
> 23 DR. WRIGHT: No. But we're encouraging anybody – and

(Dr. Wright, December 19, 2011, p. 107, ll. 21-23)

5. The quote provided was only one small portion of the series of questions posed by Ms. Robertson that was objected to by the Province. Dr. Wright did not have first-hand knowledge and clarified this point later a few lines down in the same question that he did not know whether the Province had validated its primers or not. Given the proposition was wholly misleading as the proposition was contrary to the evidence (Exhibits 2041 or Provincial Tab 10 was put to Ms. Robertson during the exchange on the objection however Exhibits 2049 and 2082 also

stand for the same proposition), Ms. Robertson properly withdrew the question. The transcripts of the whole exchange is as follows:

21 Q But the B.C. lab, the assay that they use, isn't
22 validated either, is it?
23 DR. WRIGHT: No. But we're encouraging anybody – and
24 I'm speaking from the OIE perspective - that they
25 should, whether they can populate that -- I mean,
26 this is a template and it could very well be that
27 they have that validation data in bits and pieces
28 that need to be fed into that template so you can
29 actually see the flow. **Whether they've done it or**
30 not, I don't know. But we certainly encourage
31 people to do it.
32 Q But you're not --
33 DR. WRIGHT: So it is one way and that when anybody
34 comes in with questions whether it's a trading
35 partner audit or whether it's a quality audit,
36 that you have all of your evidence in one place
37 and every year, because it's a quality document,
38 it should be reviewed and updated because it's an
39 ongoing process. And you'll be able to add more
40 validation data to it on a year-to-year basis or
41 more frequently if you want.
42 So it becomes a living document but at least
43 for the analytical bits and pieces and the
44 diagnostic bits and pieces, if there's a new
45 strain that comes up, the expectation is you will
46 enter that data to show that you can detect that
47 strain. But you have to start somewhere and

1 especially with new tests, I mean, there are many
2 tests out there in the world, whether terrestrial
3 or aquatic, where you will not find this dossier
4 because many of them have been grandfathered in.
5 They've been used for the last six or seven years.
6 Q So does this mean --
7 DR. WRIGHT: But if they're ever challenged they should
8 be able to come up with those criteria and have
9 them fulfilled in that type of pathway.

10 Q So are you concerned that at the moment, the lab
11 that DFO relies on to test its auditing samples
12 from the salmon farms, hasn't been validated in
13 that manner?
14 MS. CALLAN: I'm just going to step in. This is Tara
15 Callan appearing on behalf of Her Majesty The
16 Queen in Right of the Province of British
17 Columbia. As far as I understand, I think this
18 question is misleading in the sense that there is
19 no evidence that it's not validated. On the
20 contrary, it has been validated.
21 MS. ROBERTSON: Could you, Ms. Callan, point to the
22 evidence where it has been validated?
23 MS. CALLAN: Well, there was the document that talked
24 about the primers and the validation that
25 occurred. I believe it's provincial tab 10. And
26 also, there are no provincial witnesses on the
27 panel, but suggesting that it's not validated
28 without the proper evidentiary basis in the
29 Province's submission, is incorrect.
30 MS. ROBERTSON: What I heard yesterday is, in fact, Dr.
31 Kibenge and Dr. Nylund both are -- or, pardon me,
32 last week, both indicated that they'd never heard
33 of the test. So I'm going to just move on because
34 I'm running out of time here and I have one
35 question left.

(Dr. Wright, December 19, 2011, p. 107, l. 21 – p. 108, l.35)

6. Exhibit 2049 clearly and unequivocally sets out that the Province's assay is sensitive to 30 copies when nested, and that the validation was completed by Rachel Richardson (who is a different person from then SFU Masters student Lisa Wegener who developed the primers). There was no criticism levelled on Ms. Richardson's level of education.

> This assay is sensitive to 30 copies when nested. Validation completed by Rachel Richardson.

(Exhibit 2049, ISAV – AHC (Real-Time Assay), using Wegener Primers and Probe; and

Exhibit 2082, Email re Will ISAV PCR Tests Run by the BC Animal Health Centre Detect All Strain of ISAV)

7. Dr. Marty provided an affidavit in response to the Aquaculture Coalition's objection to entering document QQQ as an exhibit, and at para 4 of the Affidavit confirms Exhibits 2049 and 2082 outline the Province's primers and probes were fully validated and used to conduct ISAV tests by the Animal Health Centre.

(Affidavit of Dr. Marty #3, para 4)

8. Additionally at page 4, section 4 para 2 the Aquaculture Coalition criticizes the Province's primers and probes stating the primer had never been through "a peer-reviewed publication". This criticism is unwarranted, as viruses develop as time goes on, and relying on published tests would result in only capturing older viruses, which would result in missing new strains.

9. To stay on top of the newly emerging strains of viruses the procedure followed by the Province is the correct approach, and further this is how both Dr. Kibenge and Ms. Gagne at the DFO Moncton lab developed their assays. This is the OIE's recommended procedure. Dr. Kibenge and Ms. Gagne testified to this accordingly as follows:

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?

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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)

10. Additionally the Aquaculture Coalition argued "Dr. Kibenge testified that in his opinion this test would not be sensitive to finding ISA". This is not an accurate representation of what Dr. Kibenge stated. The testimony quoted for this proposition is:

45 Q Dr. Kibenge?
46 DR. KIBENGE: Yeah, I'm not familiar with this test,

47 but I notice here that the target is
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1 (indiscernible) PB1 gene, which is probably one of
2 the largest genes in the virus and my thinking is
3 that probably the copy numbers for this gene may
4 not be as high as we see in segments 7 and 8. And
5 just on that basis, I would expect this not to be
6 as sensitive as segments 7 and 8.

(Dr. Kibenge, December 15, 2011, p. 111, l. 45 – p.112, l. 6)

11. It is clear that Dr. Kibenge was unfamiliar with the test which is understandable given he had not reviewed it in advance. It was clear Dr. Kibenge was speaking without specifically reviewing all of the necessary information to give an informed fully considered answer. Had Dr. Kibenge had the necessary information (such as the validation data in Exhibit 2049) his opinion likely would have been different. However, since the Aquaculture Coalition did not put Exhibit 2049 which contained the validation data to Dr. Kibenge which sets out that this particular test is sensitive to 30 copies (which is very sensitive), we do not know what his opinion would be if he fully reviewed the materials.

12. The Aquaculture Coalition also levels the inflammatory statement at page 4, section 4, para 3:

> "...the failure of Dr. Marty to advise the Commission when he testified that the PCR process done by the province was a "self invented" one, should be subject to substantial criticism at the very least. In our respectful submission this "non disclosure" is tantamount to deliberate deception, given the significance that Dr. Marty placed on these PCR tests in attempting to refute and dismiss Dr. Morton's interpretation of the 1,100 + instance of ISA-like lesions found in the provincial audits".

The Province says that this criticism is wholly inappropriate given Dr. Kibenge and Ms. Gagne both agreed that the method undertaken by the Province outlined

in Exhibit 2082 was an appropriate way to develop an assay. Further if this was really a concern, why did the Aquaculture Coalition not ask Dr. Marty about this when he was on the stand in August? The issue of ISAV and the RT-PCR tests was clearly a prominent issue. If asked, Dr. Marty would have clearly outlined how the primers and probes were developed as well as the rationale for doing so. Because the Aquaculture Coalition had the opportunity to ask the question, but chose to not do so, it is wholly unfair to level this criticism.

13. Further, the criticism of the Province's ISAV primers by the Aquaculture Coalition is hypocritical and seems to be a result of these tests producing negative results, even though these results were consistent with histopathology by a board certified veterinary pathologist and analysis of farm mortality records by a fish health veterinarian (Exhibit 2080). In contrast, Dr. Miller's positive ISAV results are preliminary, unproven and use unvalidated methods (which were conducted without a positive control), yet the Aquaculture Coalition says "Dr. Miller's genetic approach is novel and advanced, but that is not grounds to reject it." Further Dr. Miller's sequencing data includes a stop codon which would make the protein being sequenced unfunctional.

(Dr. Nylund, December 15, 2011, p. 100, ll. 17-33; and
(Exhibit 2080, Molecular Diagnostics Sequence Identification Summary, Case 2011-0855)

14. Given the weak, erroneous and inconsistent arguments, the Aquaculture Coalition's position is without merit, and ought to be rejected.

Dr. Miller's Research

15. Dr. Miller's work is preliminary, and is ongoing. What Dr. Miller is finding this week, may differ from what she may find next week because her work is simply developing in real time.

16. However, at this time, in at least two cases Dr. Miller's negative control samples tested positive for ISAV (Exhibit 2061, worksheet "Ct data", cell G273; and Exhibit 2060, worksheet "Plate 1", cell I103). In any test a positive test result from a negative control sample indicates that the test results are unreliable and the test must be repeated.

(Exhibit 2060, Test Results of 96 Samples with All Five Primer Sets; and Exhibit 2061, Test Results from the 7900)

17. The Aquaculture Coalition infers at page 5 that Dr. Miller's research and in particular Brad Davis's paper at Exhibit 2052 stands for the proposition that "ISA is causing negative health symptoms in infected Pacific salmon". It is important to note there are serious deficiencies in this preliminary non peer reviewed document, including that 6 of the 18 pieces of data was ignored or changed.

> sequence variation in locations where the primers and/or probe attach. Consequently, I identified the 'negative' (putative **false negatives**) samples which appeared more similar to *positive* than *negative* samples and removed them from the data set and repeated the analysis (e.g. Step 2-4 above).

(Exhibit 2052, Identification of the ISAv7 Genomic Expression Profile in the 07-10 44K Liver Microarray Data, p. 2)

> Next we tried re-analyzing the data again, this time including the putative **false negative** samples, but manually changing their status to being positive samples and repeating the analysis. If these samples are truly **false negatives** then re-analyzing the data should produce a similar or stronger signal to the one found when the samples were removed.

(Exhibit 2052, Identification of the ISAv7 Genomic Expression Profile in the 07-10 44K Liver Microarray Data, p. 4)

18. In such circumstances, where data is clearly being manipulated and results are being changed from negative to positive, nothing of value can be taken from this paper.

Comparison between Dr. Miller's results and Ms. Morton's charts

19. Page 4 of the Aquaculture Coalition's argument sets out "It should be noted that the percentage of unexplained ISA-like symptoms which were highlighted by Dr. Morton in her testimony, and set out in Exhibit 1976 (p. 28) are remarkably similar to the percentages found by Dr. Miller in her results".

20. Dr. Miller only examined a small number of farmed Chinook salmon. She did not examine any farmed Atlantic Salmon in her study. Dr. Kibenge also testified that he does not consider the SSC or the HEM lesion to be considered to be classic lesions for ISA or ISAV in Pacific salmon.

35 MS. CALLAN:
36 Q Now, Dr. Kibenge, would you agree that the lesions
37 SSC and HEM (sic) that were discussed in that
38 report are not evidence of ISA in Pacific salmon
39 and are non-specific symptoms otherwise?
40 DR. KIBENGE: Well, the lesions of ISA have only been
41 documented in Atlantic salmon, so as far as I
42 know, the Pacific salmon are not known to develop

43 ISA, so those would be not lesions of ISA in
44 Pacific salmon.
45 Q And you'd agree that if an Atlantic salmon was
46 shown to be having the SSC or the Heem [sic] lesion, a
47 PCR test would then indicate, if it were a

7
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1 negative, that ISA wasn't present in that fish.
2 DR. KIBENGE: That is correct, but I'll qualify that
3 that depends on the specificity of that test.

(Dr. Kibenge, December 16, 2011, p. 6, l. 35 – p.7, l. 3; and Exhibit 2086, Confidential Report – Opinion – Infectious Salmon Anaemia (ISA) diagnostics on farmed salmon in British Columbia, July 14, 201, p. 6 ll. 9-10)

Heart Skeletal and Muscle Inflammation "HSMI"

21. On page 7 of the Aquaculture Coalition's argument they state:

> "Dr. Miller's findings of HSMI in the creative salmon fish farm in Clayoquot Sound should raise some substantial concern, and should trigger a DFO response."

Dr. Miller did not provide evidence that she found HSMI, and in fact said she did not see HSMI disease, but rather a piscine reovirus[1]. Specifically and in her words, Dr. Miller testified:

27 Q You're beginning to see positives for HSMI in
28 sockeye?
29 DR. MILLER: Not for HSMI, the disease, we see

[1] The transcript appears to incorrectly use the term "pasendrial virus" instead of "piscine reovirus"

30 pasendrial virus in our wild migrating sockeye
31 salmon.

(Dr. Miller, December 15, 201, p. 113, ll. 27-31)

22. The Aquaculture Coalition further sets out:

> "[Dr. Miller's] research comparing 2007 to 2008 smolts also showed a "high positive rate" for the causative virus for HSMI."

This again is an incorrect interpretation of Dr. Miller's evidence. She said that piscine reovirus is only possibly causative for HSMI:

5 And when we sampled them in the marine
6 environment, they had quite a high positive rate
7 for the pasendrial virus that is possibly
8 causative of HSMI....

(Dr. Miller, December 15, 201, p. 114, ll. 5-8)

This is a substantial difference, and is misleading for the Aquaculture Coalition to state this in such certain language.

23. Exhibit 1482 is a document that the Aquaculture Coalition put into evidence which addresses HSMI. This article says that HSMI is only assumed to be caused by a reovirus. Dr. Marty affirmed in his affidavit at Exhibit 1675 that some scientists believe that HSMI is caused by a salmon alphavirus. However, causation has not been proven under controlled experimental conditions.

(Exhibit 1675, Affidavit #2 of Dr. Gary D. Marty, p. 22)

24. Regardless, reoviruses in Pacific salmon are neither novel nor lethal. Thirty years ago, Dr. James Winton reported the discovery of a reovirus in chum

salmon and included results from controlled laboratory exposure of kokanee salmon (a type of sockeye salmon), chum salmon, and Chinook salmon to the virus. Dr. Winton published a paper on this topic in the Journal of Fish Pathology and stated at page 160:

> No significant mortality occurred in the chum, chinook, or kokanee salmon fry injected with the virus and no gross external signs were seen in fry held for 42 days.

(Isolation of a New Reovirus from Chum Salmon Oncorhynchus-Keta in Japan, Winton J.R., Kannan C.N. et al. Journal of Fish Pathology Volume 15 issue 3-4 pages 155-162 1981)

25. Further while the chum and Chinook salmon tested in Winton's study initially had lesions, they recovered from the reovirus after three weeks. In contrast, the sockeye salmon had no lesions. At page 161 of the Winton article, he set out:

> Histological examination provided evidence of pathology in chum and Chinook fry. Areas of focal necrosis were observed in the liver of infected fish. In chum salmon fry, these lesions began as small areas of necrosis at day 8. By day 14, the lesions were acute and numerous. At day 21 and after, the lesions began to heal and normal liver cellular architecture began to return. A similar histological picture was seen in Chinook fry; however, the extent and severity of the lesions were less. **No pathology was observed in the liver of the infected Kokanee salmon.** Other organs of all three species were unremarkable.

(Isolation of a New Reovirus from Chum Salmon Oncorhynchus-Keta in Japan, Winton J.R., Kannan C.N. et al. Journal of Fish Pathology Volume 15 issue 3-4 pages 155-162 1981)

26. The Aquaculture Coalition seems to make much of Dr. Miller's finding writing in their argument "the presence of HSMI in a fish farm [on the West Coast of Vancouver Island] which has been showing evidence of a hitherto unexplained disease, and its confirmation in the 2007 smolts may be evidence of a direct link from the fish farm disease to the 2009 sockeye collapse." However, Dr. Miller only had 10 Sockeye smolts available to her in 2007, and the farm in question that was testing was on the West Coast of Vancouver Island so this argument appears weak and somewhat fanciful since the farm is nowhere near the Fraser River Sockeye salmon migration route.

(Dr. Miller August 24, 2011 p. 9 l. 37- p. 10 l. 11)

27. Accordingly, the issue of whether or not HSMI has been found in the creative salmon fish tested by Dr. Miller is not a significant issue for the Commission to consider.

Reply to Dr. Kibenge's submissions

28. Dr. Kibenge noted at page 2 that the primers in question have been optimized for Atlantic Salmon and not Pacific Salmon. Further Dr. Kibenge notes that viruses can change over time and can take on different characteristics in different fish. Dr. Kibenge's testimony on this point is quite compelling and bears being repeated:

> 41 Q Okay. Now, these RC-PCR (sic) tests are optimized
> 42 for Atlantic salmon. Can you describe problems
> 43 that may arise when using the same Atlantic salmon
> 44 PCR tests and applying them to other species such
> 45 as sockeye or chinook?
> 46 DR. KIBENGE: Yes. Actually, both the conventional RT
> 47PCR and the real-time RT-PCR were developed for
>
> 2

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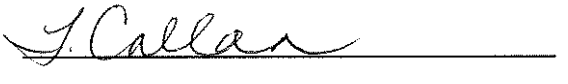
1 detecting the virus in -- from Atlantic salmon, so
2 the actual tests are designed to detect the
3 presence of the virus in -- from the fish in which
4 they developed disease, and I think they're fairly
5 consistent in detecting the virus in those species
6 -- in that species, Atlantic salmon.
7 When you apply the same test to the wild
8 fish, we run into problems because, first of all,
9 we don't know what is the best tissue to test, in
10 which case the tissue that will have the most
11 amount of virus. We also don't know how long that
12 virus will be in that particular tissue.
13 But the other thing is that we really don't
14 know the exact variation of this virus within
15 those species, so I would say that these tests are
16 not designed to particularly detect infection in
17 wild fish.

(Dr. Kibenge, December 16, 2011, p. 1, l. 41 – p. 2, l.17)

29. The Province agrees with Dr. Kibenge on this point, and thinks this may be further be leading to the problems exemplified by the various virology results on the wild Pacific salmon. It would further explain why the farmed Atlantic salmon tests are targeting the right viral sequences, and the wild Pacific Salmon tests seem to not consistently do so.

All of which is respectfully submitted,

January 3rd, 2012



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