Commission of Inquiry into the Decline of Sockeye Salmon in the Fraser River

Commission d'enquête sur le déclin des populations de saumon rouge du fleuve Fraser

Public Hearings

Audience publique

Commissioner

L'Honorable juge /
The Honourable Justice
Bruce Cohen

Commissaire

Held at:

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Federal Courthouse
701 West Georgia Street
Vancouver, B.C.

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Salle 801
Cour fédérale
701, rue West Georgia
Vancouver (C.-B.)

le mercredi 24 août 2011

APPEARANCES / COMPARUTIONS

Brock Martland Jennifer Chan Kathy L. Grant	Associate Commission Counsel Junior Commission Counsel Junior Commission Counsel
Mitchell Taylor, Q.C. Jonah Spiegelman	Government of Canada ("CAN")
Clifton Prowse, Q.C. Tara Callan	Province of British Columbia ("BCPROV")
No appearance	Pacific Salmon Commission ("PSC")
Chris Buchanan	B.C. Public Service Alliance of Canada Union of Environment Workers B.C. ("BCPSAC")
Matt Keen	Rio Tinto Alcan Inc. ("RTAI")
Alan Blair Shane Hopkins-Utter	B.C. Salmon Farmers Association ("BCSFA")
No appearance	Seafood Producers Association of B.C. ("SPABC")
Gregory McDade, Q.C. Lisa Glowacki	Aquaculture Coalition: Alexandra Morton; Raincoast Research Society; Pacific Coast Wild Salmon Society ("AQUA")
Tim Leadem, Q.C.	Conservation Coalition: Coastal Alliance for Aquaculture Reform Fraser Riverkeeper Society; Georgia Strait Alliance; Raincoast Conservation Foundation; Watershed Watch Salmon Society; Mr. Otto Langer; David Suzuki Foundation ("CONSERV")
Don Rosenbloom Katrina Pacey	Area D Salmon Gillnet Association; Area B Harvest Committee (Seine) ("GILLFSC")

APPEARANCES / COMPARUTIONS, cont'd.

No appearance	Southern Area E Gillnetters Assn. B.C. Fisheries Survival Coalition ("SGAHC")
No appearance	West Coast Trollers Area G Association; United Fishermen and Allied Workers' Union ("TWCTUFA")
No appearance	B.C. Wildlife Federation; B.C. Federation of Drift Fishers ("WFFDF")
No appearance	Maa-nulth Treaty Society; Tsawwassen First Nation; Musqueam First Nation ("MTM")
No appearance	Western Central Coast Salish First Nations: Cowichan Tribes and Chemainus First Nation Hwlitsum First Nation and Penelakut Tribe Te'mexw Treaty Association ("WCCSFN")
Brenda Gaertner Crystal Reeves	First Nations Coalition: First Nations Fisheries Council; Aboriginal Caucus of the Fraser River; Aboriginal Fisheries Secretariat; Fraser Valley Aboriginal Fisheries Society; Northern Shuswap Tribal Council; Chehalis Indian Band; Secwepemc Fisheries Commission of the Shuswap Nation Tribal Council; Upper Fraser Fisheries Conservation Alliance; Other Douglas Treaty First Nations who applied together (the Snuneymuxw, Tsartlip and Tsawout); Adams Lake Indian Band; Carrier Sekani Tribal Council; Council of Haida Nation ("FNC")
No appearance	Métis Nation British Columbia ("MNBC")

APPEARANCES / COMPARUTIONS, cont'd.

Nicole Schabus	Sto:lo Tribal Council Cheam Indian Band ("STCCIB")
No appearance	Laich-kwil-tach Treaty Society Chief Harold Sewid, Aboriginal Aquaculture Association ("LJHAH")
No appearance	Musgamagw Tsawataineuk Tribal Council ("MTTC")
Krista Robertson	Heiltsuk Tribal Council ("HTC")

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MS. CHAN: Mr. Commissioner, Jennifer Chan appearing for the Commission, and with me is Brock Martland and Kathy Grant. Today, we begin our second panel on the topic of disease. Our witnesses are Dr. Kristina Miller and Dr. Kyle Garver. If I could have the witnesses sworn or affirmed, please.

KRISTI MILLER, affirmed.

KYLE GARVER, affirmed.

THE REGISTRAR: Would you state your name, please?
DR. MILLER: Kristi Miller.
THE REGISTRAR: Thank you.
DR. GARVER: Kyle Garver.
THE REGISTRAR: Thank you. Counsel?
MS. CHAN: Mr. Commissioner, after an introduction, I'll be seeking to qualify Dr. Miller as an expert in molecular genetics, immunogenetics and functional genomics, with a specialty in salmon. If I could have Tab 16 of the Commission's list up, please?

EXAMINATION IN CHIEF ON QUALIFICATIONS BY MS. CHAN:

Q Dr. Miller, do you recognize this document as your c.v.?
DR. MILLER: Yes.
MS. CHAN: Could I have that marked as an exhibit, please?
THE REGISTRAR: Exhibit number 1510.

EXHIBIT 1510: *Curriculum vitae* of Dr. Kristi Miller

MS. CHAN:
Q Dr. Miller, you're the head of the Molecular Genetics Section of the Salmon and Freshwater Ecosystems Division of DFO's Pacific Region Science Branch; is that right?
DR. MILLER: That’s correct.
Q You're also an adjunct professor with the

Department of Forest Sciences at UBC?
DR. MILLER: Correct.
Q And you have a Ph.D. in biological sciences from Stanford University, obtained in 1992, and M.Sc. in zoology from UBC obtained in 1986, and a B.Sc. in biology from the University of California Davis in 1983?
DR. MILLER: Correct.
Q Your research interests include molecular population genetics of aquatic organisms, conservation genomics, salmon migration physiology, adaptive immunity and host responses to pathogens?
DR. MILLER: Yes.
Q And you're also the lead author of an article published in the *Journal of Science* in January 2011, entitled, "Genomic signatures predict migration in spawning failure in wild Canadian salmon," is that right?
DR. MILLER: I am.
MS. CHAN: And Mr. Commissioner, that paper is an exhibit at Exhibit 558.
Q Dr. Miller, would you consider yourself an expert in virology?
DR. MILLER: No.
MS. CHAN: And subject to any further questions, if I could ask that Dr. Miller be qualified as an expert in molecular genetics, immunogenetics and functional genomics, with a specialty in salmon?
THE COMMISSIONER: Yes, thank you, Ms. Chan.
MS. CHAN: And now to Dr. Garver. I'll be seeking to qualify Dr. Garver as an expert in molecular virology with a specialty in viruses affecting salmon. If we could have Tab 17 up, please?
Q Dr. Garver, do you recognize this document as your *CV*?
DR. GARVER: Yes, I do.
MS. CHAN: If I could have that marked as the next exhibit, please?
THE REGISTRAR: Exhibit 1511.

EXHIBIT 1511: *Curriculum vitae* of Dr. Kyle Garver

MS. CHAN:
Q Dr. Garver, you lead the Virology Research Program of the Aquatic Animal Health Section and that's in

the Salmon and Freshwater Ecosystems Division of DFO's Pacific Region Science Branch; is that right?

DR. GARVER: That's correct.

Q You hold a Ph.D. in molecular virology from Purdue University, obtained in 2000, a B.Sc. in biology from Pennsylvania State University in 1993; is that right?

DR. GARVER: That's correct.

Q And your research interests include various aquatic viruses, including viral hemorrhagic septicaemia virus, infectious hematopoietic necrosis virus, and koi herpes virus?

DR. GARVER: That's correct, I specialize mostly in fin fish.

Q And among other things, you're currently conducting research on a virus hypothesized to be associated with Dr. Miller's mortality related signature?

DR. GARVER: That's correct.

MS. CHAN: So subject to any further questions, I'd ask if Dr. Garver could be qualified as expert in molecular virology, with a speciality in viruses affecting salmon.

THE COMMISSIONER: Yes, thank you, Ms. Chan.

MS. CHAN: If we could start with Tab 18 of the Commission's documents, please, that's Exhibit 558.

EXAMINATION IN CHIEF BY MS. CHAN:

Q And Dr. Miller, do you recognize this as a science article that you published in 2011 in the *Journal of Science*?

DR. MILLER: Yes, I do.

Q Now, I understand that not all journals are regarded equally. How does the *Journal of Science* rank in comparison to others?

DR. MILLER: It ranks about the same as *Nature*. It's one of the top two leading journals in the world.

Q And Dr. Scott Hinch is a co-author, I see, on the third line, there, in the list of authors. He testified earlier on some of the biotelemetry aspects of the paper. And I'll be asking you about some of the conclusions that you've reached, but before I do, I just want to, for the purposes of our discussion today, canvass with you my

understanding of some of the technology and methods used and see if you agree with my understanding. So first of all, the microarray of technology that you used, first of all, you used microarray technology for this paper?
DR. MILLER: Yes, correct.
Q And this is a tool that allows you to take a tissue sample, looks at tens of thousands of genes in that sample all at once to see which genes are turned on and which genes are turned off; is that right?
DR. MILLER: Yes.
Q Now, by looking at these genes and which ones are turned on and turned off, you may gain information about the physiological condition of the tissue being tested?
DR. MILLER: Yes.
Q And that condition may express as a pattern, sometimes referred to as genomic profile or a genomic signature?
DR. MILLER: Yes.
Q Okay. So that's the microarray technology. If we could see if you agree with my understanding of the method here. Now, members of your team, so there's the authors listed on the front of that paper, there, captured and tagged fish in the ocean, in the river, at the spawning grounds, and then took samples of the gill tissue from that fish; is that right?
DR. MILLER: Yes, non-destructive samples of the gill tissue.
Q So the fish didn't die?
DR. MILLER: No, and there's been quite a few studies that Scott Hinch and Tony Farrell's group had done previous to this study to show that there was a very minimal impact on survivorship of taking tissue samples, gill tissue samples from these fish.
Q And then you took that gill tissue and you tested it using the genomic microarray that we just discussed; is that right?
DR. MILLER: Yes.
Q And you also used biotelemetry, that Dr. Hinch described to us when he was here, to see which fish made it to the spawning grounds and which ones successfully spawned?
DR. MILLER: Yes, and importantly, we also ran genetic

stock ID on all of those fish so that we knew where they were going when they were migrating and we could look at stock-specific differences.

Q So when you compared the migration and spawning information from the biotelemetry to the microarray information from the gill tissue, what did you find?

DR. MILLER: Well, we basically were able to contrast the genomics of the fish that made it to the spawning ground successfully, or in the case of the study at the spawning grounds, the fish that were successfully spawned with those that were unsuccessful, either in terms of their migration or their spawning. And in doing so, we found that in all three of our independent tagging studies, that the same genomic signature was associated with poor success no matter whether the fish were tagged in the marine environment about 200 kilometres before they enter the river, whether they were tagged in the lower river, or whether they were tagged at the spawning grounds. The same signal was emanating from the data.

Q So this signal, and I also heard you say the word, "genomic signature," is this the same as the mortality-related signature that we've seen?

DR. MILLER: Yes. Yes, that is what we have termed the mortality-related signature. And in the marine environment, when fish carry that signature, they had 13.5 times lower probability of making it to the spawning grounds and that was also seen, although it was not as high a difference in probability in the lower river and, again, at the spawning grounds.

Q Now, this paper, if I understand correctly, looked at 2006 returning adults; is that right?

DR. MILLER: Yes, it did.

Q Beyond this paper, have you looked at the mortality-related signature, which I'll call MRS, in other years of returning fish, or in other tissues, or --

DR. MILLER: We have. It's unusual to be able to have this tagging program and that only happens when there's a lot of fish coming back. We are actually conducting a study that basically is a mirror of this, only with even more fish from fish that were tagged in 2010. But we have conducted quite a large number of studies using

destructively sampled tissues. We have profiled liver tissue, brain tissue, gill tissue in other studies, as well, and muscle tissue, white muscle tissue, and we've also looked at hypothalamus, as well. Our studies date back all the way to 2003 returning adults. Most of these studies don't have the contrast between successful and unsuccessful fish because we don't have radio tracking and with these other tissues, we can't sample a liver tissue or brain tissue not destructively so you can't directly relate any kind of fate with the signatures that you see, but, yes, we have looked at other tissues and one of the findings that we made after we had done this particular study was that we were observing this same signature, this mortality-related signature in other tissues, as well.

Q So you have this additional information on the mortality-related signature in other tissues and you said also for other returning years and other lifecycles?

DR. MILLER: Yes, in 2005, we profiled gill, liver and brain tissue in all the same fish, and we observed the mortality-related signature in each of those tissues, but interestingly, very different prevalence rates in different tissues and individuals didn't necessarily contain that signature in all tissues. In fact, it was more common for them to contain the signature in only one or two tissues.

Q With this additional information, and we've heard that the DFO and PSC keeps records of sockeye migration success and sockeye spawning success, have you been able to compare your tests looking at the MRS prevalence to the success of migration and spawning from those data?

DR. MILLER: We have the fish, certainly, to do that, not to do direct comparisons. We don't have the fish that are from radio-tracking programs, where we can compare success versus lack of success, but we have samples. We have adult fish that have been collected all the way back to 2003 and, basically, virtually every year since then. And so what we have not done, microarrays are very expensive to run, they are about $400 an individual to run so we are moving towards a new technology that is faster and cheaper and where we

can run through thousands of fish and simply score them, do they carry the MRS signature, or not, and that's a technology that we're employing now in our lab. And we will be running through, basically, our whole archive of about 2,500 samples that have been collected since 2003 and so we will have a better feel for that because we're interested now in the prevalence of the signature across multiple tissues now that we have discovered that it isn't simply in gill tissue.

Q Okay. So it sounds like you're working on the technology to scan for the MRS prevalence in a more efficient way, but looking at the MRS scanning technique that you've used in the past, the genomic microarray, have you seen a correlation between the MRS prevalence in the samples that you've already looked at to returning fish, for example?

DR. MILLER: You mean to pre-spawning or en-route mortality?

Q To en-route mortality or pre-spawn mortality, or even the numbers returning from the ocean, have you seen any correlations between MRS prevalence and sockeye survival?

DR. MILLER: Well, we've done a lot of work on 2005, and that would have been the brood year for the 2009 returns. And those fish carried, if you added up the prevalence of that signature in each of the different tissues, or the presence of that signature in each of the different tissues, that was gill, liver, brain, that we surveyed, the overall prevalence would have been 75 percent of the fish contained that signature in at least one tissue. And that was really pretty high compared to other years that we had looked at, but there wasn't any other years that we had looked at that same suite of tissues, and that's why this faster technology's going to be very valuable, because I really do believe that it isn't simply that it's present in one tissue, but how many tissues is it present in?

Q So just to check that I heard you correctly, the brood year of 2005 fish, you found over 75 percent prevalence of the MRS signature in at least one tissue?

DR. MILLER: Correct, a fairly low prevalence rate in gill tissue, however, compared to 2006.

MS. CHAN: I wonder if we could turn to the Commission's Tab 22, please?

Q Dr. Miller, do you recognize this document? I understand it's something that you might have created?

DR. MILLER: Yes.

Q And can you give us any of the contextual background for this document? Where was it made, when was it presented, to whom?

DR. MILLER: I believe this is the document that was prepared for the Pacific Salmon Commission meeting, which was in 2010, I believe, in June of 2010, and so we were asked, a number of scientists were asked by the Commission to present their unique hypotheses and the data that they had in support of those hypotheses being an impact on the salmon returns.

MS. CHAN: If I could have this marked as the next exhibit, please?

THE REGISTRAR: Exhibit 1512.

EXHIBIT 1512: Hypothesis prepared for Pacific Salmon Commission meeting, June 2010

MS. CHAN:

Q Now, if we go down to the bottom of the first page, and it's the second bullet from the top, it reads:

> In 2008, 60% of smolts left the Fraser River with the unhealthy signature in brain, 40% in liver, with 82% of fish affected in at least one tissue. There was a 30% reduction in brain prevalence of unhealthy signature fish from summer to fall in the ocean, and a 50% reduction in liver.

I'm just going to jump to the last bullet, there, on the bottom:

> If these decreases in prevalence were due to mortality, and if we assumed that 120 million smolts left the river in 2008 (there may have been more), we could account for the loss of more than 27 million salmon in 2008 associated with the unhealthy signature alone.

Now, the smolts that left in 2008, when would they have returned?
DR. MILLER: In 2010.
Q So just picking up on your comment earlier about the 2005 brood year fish coming back in 2009, with 75 percent MRS positives, in these fish --
DR. MILLER: That's adults.
Q Okay. So that's as adults?
DR. MILLER: That's in returning adults.
Q Okay. So this one, here, is looking at smolts?
DR. MILLER: This is smolts. This is smolts leaving the river.
Q Okay. 82 percent of smolts having the MRS positive signature, is that a high percentage?
DR. MILLER: In 2008 was the first year that we had done any smolt studies and so we didn't have a benchmark for smolts. 2008 was really the year that our program on smolts starts and so we only had a very small number of 2007 fish, which I'm sure we'll come into later. What we have observed with this signature is that the highest prevalence that we observe in any of the tissues occurs before fish leave the river. And so we always see, and we are accumulating more years of data on this, but we have observed that we can see very high prevalences of this signature, but very variable among different years, of fish leaving the river. The fish that return, we still see the signature in some portion of the adults that return. In the two years where we have any data, the proportion of adults affected is much less than what we see of smolts leaving the river with.
Q Okay. So but 82 percent of smolts leaving the river with the MRS positive, would that have predicted mortality, or would that be considered normal?
DR. MILLER: 82 percent of the fish leaving the river with at least one tissue affected, we don't have a way to directly assess mortality and this is why we're just looking at shifts in the prevalence of this signature. If you contrast what we observed in 2007, which is really the only other piece of data we had on this signature at the time, it was after this study, one thing that is really highly different between them is that most of these smolts that carried this signature, only 20 percent of these fish carried the signature in

both tissues, okay? And in 2007, virtually all of the fish carried the signature in both tissues. So I think it's a difference in the intensity of the signature, in that it's carried across multiple tissues. And it was virtually, again, sample sizes, and we can get into 2007, were very small, there were very few fish available to us, but a fish leaving the river, 10 out 10 for both brain and liver contained the signature.

Q In 2007?

DR. MILLER: In 2007.

Q I think we do have some of your 2007 information.

MS. CHAN: If we could turn to, I believe it's Tab 24 of the Commission's list, please?

Q Dr. Miller, is this a presentation that you've given? Here, I'm reading the title, "Genomic studies suggest that a novel disease is affecting sockeye and may be an important contributor to the Fraser River sockeye situation"?

DR. MILLER: Yes.

Q And who did you give this presentation to?

DR. MILLER: This was an inter-departmental meeting, intra-departmental meeting that was really aimed to provide more information from whatever was presented at the Pacific Salmon Commission meeting so it was an update meeting for the Department.

MS. CHAN: Okay. If I could have this marked as the next exhibit, please?

THE REGISTRAR: Exhibit 1513:

EXHIBIT 1513: Presentation entitled, "Genomic studies suggest that a novel disease is affecting sockeye and may be an important contributor to the Fraser River sockeye situation"

MS. CHAN:

Q And I want to bring you to slide number 6, and I think we can use that to follow up on the 2007 data that you were just describing. Is this slide the one that you were referring to, or that you were discussing the 2007 data?

DR. MILLER: This is a slide that refers to the contrast between 2007 and 2008. These fish were only fish that were sampled in the marine environment, in the end of June, around the same week in both years, yes.

Q And what does it mean when it says there that:

> 90% prevalence of MRS fish late June in the ocean in 2007

And:

> 40% prevalence of MRS fish late June in the ocean in 2008

DR. MILLER: We work with the bioinformatics group at the University of British Columbia, led by Paul Pavlidis, and we've discovered that we can identify this signature quite readily as using principled component analysis. And in general, it comes up as explaining the largest source of genomic variation among individual fish and so this shows the ranking for principle component analysis and here, the MRS signature are the individuals that rank negatively with principle component 1. And so what this shows is that of the 10 2007 fish that were sampled, nine out of 10 of those in the ocean at the end of June contained this signature. If you compare that with 2008, with fish that are sampled around the same time, it's a much lower percentage, it's somewhere around 40 percent.

Q What does that tell us about predicting returns for 2009 and 2010?

DR. MILLER: Well, that's something that we're still studying, right? So understanding the predictability with the smolt signature, because we don't have the ability to directly contrast, you know, the successful and unsuccessful individuals, we are, right now, trying to gather information about prevalence, and that kind of information needs to be gathered over a number of years of study where we know what the outcomes were. And so this highlights the first study where we show that this signature does exist in smolts, that it is in quite different levels of prevalence in these two years, with the caveat that it's a very small sample size for 2007. And so I would say I don't know that we understand completely how predictable this is at this point in smolts. This is where our research is going. But I can say that, you know, if we look at Chilko

fish, where we have a lot of data about Chilko because they're a very large stock and it's very easy to pick those up in the ocean environment, we do see very dramatic differences in the prevalence of this signature in the summer in the ocean. And where we're looking to go is to establish whether or not it's simply the prevalence of the signature in the ocean, or whether it's the shift in prevalence that we observe over time that's more important in terms of being a predictor.

Q And in terms of the fish going out into the ocean and the shifting of prevalence, have you done any investigations about the ocean environmental, or other environmental conditions and looked to see whether or not those affect the MRS prevalence?

DR. MILLER: I'm working with colleagues at DFO, with Mark Trudel and Dick Beamish, and more recently, with the PARR program, getting samples earlier in the year, and we do collect data on these individual fish on other parameters, like whether they were feeding, or not, and what the ocean conditions were like when they were captured, but we need to get enough years of data to start to pull those together, and I have a post-doc who will be modelling these relationships because I do feel that there's a very high probability, if this is important in the early marine environment, that it probably has to be seen in the context of the overall environmental conditions that are present there.

Q And understanding that your work is ongoing in this area, have you had any indication to tell you whether or not the MRS prevalence is more of a determinant factor relative to ocean conditions or whether or not environmental factors play a greater or larger role?

DR. MILLER: My speculation is that what will be the best predictor will be the shift in prevalence that we observe between fish that leave the freshwater environment and fish sampled in the ocean, and that that shift may reflect the differences in the ocean environment in different years and how survivable it is for fish. If fish enter the river in poor condition and then -- or into the ocean, I'm sorry, into the ocean in poor condition, and then into an ocean that is additionally stressed, like my colleague suggests

was the case in 2007, that that may have a more profound effect on their survivorship than if they enter the ocean in good condition, and the ocean is in good conditions, like we observed in 2008.

Q Now, we've discussed that you've looked for this MRS in other fish and other tissues. Have you looked for the MRS in farm fish or in hatcheries?

DR. MILLER: We have begun working on -- we have coho and chinook salmon, as well, collected within our program, and we are working on coho salmon and we have quite an extensive hatchery collection from them.

Q Sorry, so that's the hatchery, do you have fish farm fish to test for the MRS, as well?

DR. MILLER: We have some chinook salmon from Creative Salmon for another project and we are working with the industry and will be getting samples very shortly.

Q Just to clarify, you said you have chinook salmon from Creative Salmon --

DR. MILLER: Creative Salmon, yes.

Q -- for another project. Is that looking for the MRS prevalence?

DR. MILLER: No, that project is about a jaundice syndrome that has created problems with mortality over winter in cultured chinook salmon, and we are using genomics to try to determine whether or not that syndrome is more likely to be environmentally induced or due to a pathogen.

Q Okay. Focussing on the MRS prevalence, have any Atlantic fish farms provided you with samples to test for the prevalence of this MRS?

A Not at this time.

Q Have you asked for samples from fish farms, from Atlantic salmon fish farms?

A When the paper came out in Science, I was approached by Mary Ellen Walling about what this meant and the potential of testing their fish, and we had a discussion about that and she was going to follow up and talked to the vets about it, and I believe, at the time, the vets weren't comfortable with testing for a signature. And more recently, we have been in conversation because we have identified now a candidate virus associated with this signature and we have approached the industry again about testing now for this virus and they've agreed to do so.

Q Just so that the record is clear, could you tell us who Mary Ellen Walling is, please?
DR. MILLER: She's the head of the B.C. Salmon Growers Association.
Q Have you asked anyone at DFO to assist you in obtaining samples from Atlantic salmon farms for the purpose of testing for the MRS?
DR. MILLER: Yes, I brought this up within our department, with our fish health group, I guess it was in July of this year, once we had obtained the virus sequence for the parvovirus, and we've done a fair amount of screening of wild fish to know that this is a virus that is highly prevalent in sockeye salmon that we observe in the same tissues that we observe this signature in, and we are doing some large-scale surveys, both of sockeye salmon and of hatchery and wild chinook and coho salmon and so I felt that it was time that we also look at a broader range and look at the aquaculture and, specifically, Atlantic salmon, as well. We do know that this signature and the virus are found in chinook salmon, but I have not had any samples of Atlantic salmon. There was some discussion about this and whether or not this was the time to test because we haven't demonstrated in a laboratory that this virus can cause disease, that it can cause mortality, and that is work that is ongoing that Kyle and I are working on now. And so there were questions as to whether or not we should be testing now or wait till we had all of that information, and I know that there were some emails that came out because of that meeting.

Since that meeting, I met again with Mark Saunders and Andy Thompson, as well as, I believe, Stewart Johnson was there, as well, and Andy suggested that he simply approach the industry about this, you know, and see if they would be willing to collaborate with us to test their fish, and they've agreed to do so.

Q So as it stands, though, and just to clarify, you have asked for Atlantic salmon samples from fish farms to test for the MRS signature, and you have not received them and you have not tested Atlantic salmon fish farms for the presence of the MRS? Leaving aside the tests for parvovirus, you have not looked at these fish for MRS?

DR. MILLER: No, we haven't.
Q Okay. Now, going back to your Science paper, when you find a genomic profile like MRS, does that tell you with certainty what caused that profile?
DR. MILLER: No.
Q Looking at which genes are turned on and which genes are turned off, does that give you an inference or lead to a hypothesis as to the cause?
DR. MILLER: It absolutely does. I mean, the whole point of this program of using genomics is a way of assessing whole organismal physiology, and there are a plethora of controlled laboratory studies that have shown a genomic response to a variety of different environmental stressors, toxicants, diseases, et cetera. And so we use that information as a backdrop so that when we obtain a genomic signature, we can then look to see what are the similarities between the signature that we are observing with other controlled studies. And it is from that kind of analysis, which we call a functional analysis, that we proposed that this signature, the most likely explanation for this signature is that it is virally mediated, that it's a response to a viral infection. And in the particular case of this signature, the more data that we obtain, the more validation we get for that hypothesis. In the beginning, it was based on what we observed in the paper, in the Science paper, based on the genes that were being stimulated, the biological processes that those genes were involved in, which involved a number of immune system processes that were specific to fighting viruses or intracellular pathogens. 65 percent of the processes that were affected were processes that were known to be affected by viruses.

In addition to that, when we started seeing this signature in other tissues, again, the fact that this signature was present in other tissues, but was not necessarily present in all tissues within an organism at once really fits well with a pathogen kind of model. It doesn't fit very well with a general stressor or other kinds of things that one could evoke to explain a signature of this nature, and it does not fit well with a toxicant kind of exposure where toxicants would exert an effect primarily on the liver tissue

because that's a detoxification tissue, and then it might, you know, have weaker effects on other tissues. In this particular case, we could see strong effects in a liver tissue and no effect on a brain, we could see strong effects on a brain tissue and a gill tissue and no effect on a liver. This is much more a pattern that is associated oftentimes with pathogenic agents.

Q Okay. And I believe we do have your hypothesis about the virus in the Science paper, it's on page 216 of the document we have on screen, and I'll read it to you. I'm sure you're very familiar with it. The last sentence, there, says:

> Our hypothesis is that the genomic signal associated with elevated mortality is in response to a virus affecting fish before river entry and that persists to the spawning areas.

And you've described, as I understand it, some of the gene signalling that you observed and that led you to that hypothesis, but I just want to understand, when you say "hypothesis," does your Science article conclude that a virus is causing the fish to die en route, or at the spawning grounds?

DR. MILLER: No, it does not. And I think that I should clarify that in my view, the most important finding in this Science paper is that the fish are already conditionally challenged before they're entering the river. A lot of the work that my colleagues have done, Scott Hinch and Tony Farrell, has also seen this using what I would call directed physiological indicators for stress, for osmo-regulation, for maturation, and for energy, and they have seen, in other years that involved radio tracking, that there was a correlation oftentimes with stress and with osmo-regulation in terms of successful migration. But from those studies, they could never really propose a mechanism for why some of the fish were sometimes much more ready for freshwater, why there was a portion of fish that were so ready for freshwater that they probably would be uncomfortable in the marine environment, and why there were so many stress indicators in those

fish. We understood genomics to try to provide a much deeper level of understanding of the mechanisms that might create the kinds of patterns that they were observing. This study absolutely was a really good follow-up to what they found and, in fact, we found that these same fish had the same difficulties with osmo-regulation in that they showed a pattern of osmo-regulatory preparedness when they were 200 kilometres in the river that looked like a freshwater fish. They were probably very uncomfortable in the marine environment.

All that is to say that what we have seen here provides a deeper mechanistic understanding to what I believe that they have observed in previous years using other kinds of markers that only allow them to know that fish were stressed and had differences in osmo-regulatory preparation, but didn't provide any kind of mechanistic explanation.

At the time of this paper, that this was caused by a virus was a hypothesis, we did not have a specific virus.

Q So at the time of this paper, for example, if someone were to cite this Science paper as proof that a virus was killing sockeye salmon, would that be correct?

DR. MILLER: That would not have been the way I would have cited this paper. To me, this paper was proof that river conditions alone are not probably the only indicators or only exacerbating factors in terms of salmon mortalities. I mean, we have had mortalities of salmon in the river. Up to 99 percent of some stocks in some years have died before they spawned and, really, there is very little understanding for why that occurs. A lot of the research is focussed on the river environment, around the temperatures in the river, around the pathogens that they pick up when they enter the river. This is the first study that says, "Look, this could be a pathogen that they carry in with them into the river, not simply something that's picked up in the river, that might also be undermining their performance." It's probably not the only thing undermining their performance, but what this study showed was that fish were already compromised before they enter

the river, and I believe that that is what we are going to find, as well, with smolts.

Q Okay. So moving along with the viral hypothesis, I understand -- Dr. Garver, this is where you fit in -- now, have you done work on trying to identify this virus that's related to the MRS? And, in particular, I'm interested in your work with Dr. Tang of the BCCDC, if you could tell us about that, please?

DR. GARVER: Yes, when Kristi first approached me regarding a hypothesis about a virus potentially being associated with the MRS, I suggested several different diagnostic methods that we could try to get at the answer of if there is indeed a virus in these tissues that she's characterizing as unhealthy or having the MRS. And so to do that, one approach was a traditional virological approach, and this is kind of a broad method in which you put the sample onto cell culture. And so this is in vitro, you grow fish cells, you put the sample on the tissue and you observe for virus infectivity in those tissues. So we tried various different cell lines. We weren't fortunate enough to culture any virus, but, again, it's a broad technique and a lot of viruses are unculturable.

Another method that we're trying, and my research program is quite interested in developing novel detection methods for viruses, and so one area we're pursuing is a technology similar to the microarray technology that Kristi is using for gene expression, but we're looking at a microarray that is able to survey for thousands of viruses at one time. So basically, it's a slide that has thousands of viral sequences on that slide, and you basically apply your sample to that slide and if there's a virus in your sample that is complementary to one of those on the slide, you will get a fluorescent and, hence, an indication that you have a particular type of virus. And so this is called -- the person that developed it was Joe DeRisi, down in the University of San Francisco. It's called the ViroChip. We're into several generations of it now. As new sequences come in, it's updated. And so what we did is when Kristi approached me, we took the MRS tissues versus tissues that were not exhibiting an MRS sequence and we applied these to the slide. And

at the time we did the analysis, I should also point out that we're also validating this chip to work on fish viruses. It's mostly used in human virus. So predominantly on the slide, the sequences that are there are human viruses. That's not to say that they don't have fish viruses.

Q I just want to check that I understood you correctly there. So you're working with Dr. Tang from the BCCDC; is that correct?

DR. GARVER: That is correct, he has the slide.

Q And you've referred to this slide, is this also called the ViroChip?

DR. GARVER: The ViroChip.

Q And how did Dr. Tang learn how to use the ViroChip?

DR. GARVER: Dr. Tang did a post-doctoral fellow research position with Joe DeRisi, the developer of the chip.

Q So that means he's worked with the inventor of this chip; is that right?

DR. GARVER: That's correct.

Q Would you say he's fairly experienced in using it?

DR. GARVER: Yes.

Q And just so I understand the technology that you just explained, this ViroChip is a tool to test for the presence of viruses?

DR. GARVER: That's correct.

Q And it's a microarray, as you said, and it contains bits of genetic material representing all known viruses; is that right?

DR. GARVER: That is correct.

Q And the idea is you take a sample, for example, from a fish, process it, put it on the ViroChip and if I understand you correctly, if your sample contains a bit of viral genetic sequence matching what's on the microarray, the ViroChip, you will see a positive signal; is that right?

DR. GARVER: That is exactly right.

Q Okay. So has this ViroChip been used to discover new viruses before?

DR. GARVER: It has. Actually, it first really made its highlight and its use with the SARS virus.

Q Okay.

MS. CHAN: If I could have Canada's Tab number 7 brought up, please? This is Canada's Tab number 7? Is this the correct one, for diseases?

I believe they have a separate list for today. Thank you, Mr. Lunn.
Q Do you recognize this paper on the screen?
DR. GARVER: Yes, I do.
Q It's titled, "Using a Pan-Viral Microarray Assay (Virochip) to Screen Clinical Samples for Viral Pathogens." And I see there that Joseph DeRisi is one of the authors, and you just told us that he was an inventor of this ViroChip?
DR. GARVER: Yes, he was.
Q Does this paper set out the protocol to be followed when using the ViroChip?
DR. GARVER: I believe so. Yes, it does.
Q Would it be the protocol that you would have followed?
DR. GARVER: Yes, we would use something very similar in Patrick's lab.
Q Okay. And when you then used this chip and this protocol, what did you find when you compared the MRS positive fish and MRS negative fish?
DR. GARVER: At the time when we did the analysis, we didn't see any conclusive viral signal coming from -- in other words, there was no significant difference between the MRS sample and the non-MRS sample so we were unable to differentiate conclusively if there was a specific virus between the difference between the two samples.
Q So there was no difference?
DR. GARVER: There was no difference.
Q Did it indicate the presence of any novel viruses?
DR. GARVER: No, we weren't able to find any viral signals that cropped up in the MRS, however, I should note, this technology, there are some limitations to it, one being it is a hybridization so you need a lot of sample and if you suspect there's a virus in your sample, you need a lot of virus to be able to bind to produce a signal. So if you don't have ample quantities in your sample of that virus, you will not detect it. Another significant feature of this is it's based on all the known viral sequences in a public database. And the problem with that is if there's new viruses that are significantly different than those that are appearing on the chip, you won't get hybridization. So there's two limitations, you need a lot of virus to find binding, but you also need something that's at least genetically

similar to what's on the viruses. So if it's quite a bit different than what's on the viruses, it won't bind and give you a fluorescent signal.

Q Okay. So a negative signal, does that necessarily mean that the virus isn't there?

DR. GARVER: No.

Q Okay. If I could --

DR. MILLER: Can I just add something?

Q Oh, yes. Oh, your microphone, please.

DR. MILLER: In general, this chip has been used with cultured viruses and one way to get a lot of viral concentration is through culturing viruses on cells. It's application for use using a tissue sample where you're trying to get enough virus out of a tissue sample, it hasn't been used anywhere near as much. There's other issues associated with using a tissue sample in that when you have a tissue sample, you also have the background of the genome of whatever animal you obtained that tissue sample from. And so there were some questions about how one might deal with that in terms of the way that the data are treated and the data are normalized, et cetera, and so really, we were exploring not only, you know, could we pick up any kind of signal from this from a tissue sample, because that's what we had, but also, you know, are there methods that could be used to better tease out, you know, the background that the salmon genome would have on the slide.

DR. GARVER: And I can add one other point. We have been validating this chip since and we have conducted it on tissues, as well as amplified isolates, as Kristi's alluding to, and it does work with both, particularly for fish that have exhibited disease signs due to a viral signal. And so particularly, the main diseases that I work on, IHN, VHS, it's worked considerably well for those.

MS. CHAN: Okay. If we could return to Tab 22, please. I think that is now -- oh, and first, perhaps, if I could mark this paper as the next exhibit?

THE REGISTRAR: Exhibit number 1514.

EXHIBIT 1514: Video article entitled, "Using a Pan-Viral Microarray Assay (Virochip) to Screen Clinical Samples for Viral Pathogens"

MS. CHAN: And returning to Tab 22 of the Commission's list, please? I believe this is now Exhibit 1512. If we could go to the second page, please, near the bottom?

Q I'm just going to read to you the second bullet from the bottom, there. It says, under the heading, "Additional Evidence of Potential Pathogen Involvement," and this is returning to you -- Dr. Miller, your presentation at the PSC in June of 2010:

> A VIRAL PATHOGEN?: In collaboration with B.C. Centre for Disease Control, we ran both healthy and unhealthy RNA on a Viral Array (used to identify viral strains in humans and agricultural animals), and found the unhealthy tissue gave 6x higher intensity binding to the array than healthy tissue. There was a 3-fold over-representation of Retroviral family DNA.

So when I read that, it seems to me to differ from the conclusion that Dr. Garver just described, saying that there was no difference between the MRS positive and the MRS negative fish. Can you explain that?

DR. MILLER: Okay. Yes, sure. In the methods that Dr. Tang uses on these arrays, again, they usually are using cell culture. And one of the issues with dealing with a tissue culture is that you have a much higher background binding because you have a lot of other DNA in the mixture. And I spent considerable time with post-doc there and discussed. They don't normally -- anytime anyone runs a microarray, you always have to background correct for what's the background fluorescent signal on the array because you only really want to demarcate what's the signal on each of the spots on the array. And in every slide, you're going to have a different level of background, and so unless you correct for that, you really don't know which of those spots are binding significantly above background. And so I took the slide results into my lab and treated it like we would treat any of our other microarray slides and background corrected it. And when I did that, another typical measure used in microarrays is to

only count spots that have at least two standard deviations greater binding from the background intensity. Okay, so you're trying to differentiate what's nothing in terms of binding and what's actually truly binding to something. And we found when we did that analysis that we had three different tissues that we ran for what we called at the time, unhealthy, which is the same thing as MRS and samples that we classified as being healthy or non-MRS samples. And we found that over all three tissues, the level of binding to the specific probes on the array was six times greater for the MRS positive or unhealthy positive than the negatives.

Now, this is not a kind of measure that Dr. Tang generally uses and but this was our own observation. The other observation was if you look -- there's a very different representation of the different families of viruses on these arrays, depending on how common those viruses are. There is, you know, a plethora of herpes viruses and retroviruses on those arrays. There's very few of some of the smaller viral families. And so one of the caveats of doing what I did in terms of looking at higher-intensity binding is if you had two samples and one of them was a herpes virus and another one was a very uncommon or, you know, a family that's not well represented on the array, you know, you might incorrectly assume that the one with the herpes virus is the one with the virus and the other one isn't, which is a caveat of using just this higher-intensity binding. But what we found when we looked within family, so when you took into account how many different spots were present represented in each family, the only family that had a higher representation in the unhealthy or MRS tissue, compared to the other tissue, was the retro-viral family DNA.

Now, I have to say one caveat to this is that retroviruses can insert their DNA into the host genome. Salmon carry all kinds of endogenous retroviruses. Those are called endogenous, which means that at some point that might have been, you know, hundreds of years ago, even, retroviruses have inserted their sequence into the DNA of the host and it remains there. And so you do get a background binding of endogenous retroviruses on

this array and we do find with the MRS signature that we get a spike in the production of the RNA that comes from those endogenous retroviruses, which is one potential signal that there is a retroviral infection because, in general, endogenous retroviruses can be stimulated by exogenous retroviruses so those are self-replicating retroviruses. So that, I believe, is what led to this over-representation of the retroviral family.

Q At this point in time, when you wrote this presentation, was it your hypothesis that a retrovirus was the cause of the MRS?

DR. MILLER: That was a sub. I mean, our key hypothesis was that it was viral and after that, that it was possible that it was retroviral, and there were a lot of elements within the genes that were being stimulated that were known to be stimulated and co-opted by retroviruses and so we were quite interested in the potential for retroviruses, recognizing that they are one of the hardest families to try to work with.

I should also say that -- and I know you don't want to get too far into the virus that we did eventually identify in this tissue --

Q We will get there.

DR. MILLER: -- but the one comment about that when it comes to this array is it only has about 30 percent homology and in order to get binding of a virus, a good binding to this array, you need at least 50 percent homology at a nucleotide level. So the virus that we have identified is highly divergent and would not have bound very effectively to this array.

Q So that's just to say the negative result is not definitive in your words; is that accurate, then?

DR. MILLER: Well, neither Kyle or I assumed that this test would -- if you didn't get a positive result, it didn't say there wasn't a virus, but we hoped that it would be helpful.

Q Okay. So with the retrovirus as a sub-hypothesis of the viral hypothesis, as you were saying, are there some retroviruses that are known to cause cancer?

DR. MILLER: Well, many retroviruses are oncogenic and associated with cancer. I mean, the well known ones are leukemia, but there's a swim bladder

virus that Atlantic salmon carry that is also oncogenic so yes, they tend to be associated with cancers.

MS. CHAN: If we could go to Tab 20, please, of the Commission's documents? This is Exhibit 613G.

Q Dr. Miller, this is already an exhibit. Do you recognize this as a presentation that you gave?

DR. MILLER: Yes, this was a presentation at the first DFO meeting that we had, an intra-departmental meeting where we were asked to look at each of our research programs and that we wanted to start a discussion in DFO about what various hypotheses people had that might pertain to the salmon declines, and this was a presentation that I gave at that time.

Q When you say intra-departmental, who was in attendance and --

DR. MILLER: It was only DFO staff, DFO scientists, largely, but there were some managers in attendance, as well.

Q Did you create this presentation based on that, with the expectation that it would be broadly distributed?

DR. MILLER: Actually, at the time, I was presenting it as a presentation to stimulate discussion within DFO about some of this work. I should say that at this time, we had begun to suspect that our signature could relate to a retrovirus. We had also been looking closely about what we knew about retroviruses in salmon and had found the literature that Mike Kent and others had put forward on the plasmacytoid leukemia or the salmon leukemia virus, and so we had a considerable amount of interest in that particular disease. And we were hoping that through giving this talk and putting forth to the Department the various pieces of evidence that we had, that there would be some expertise in the Department to move forward with how do we determine whether or not that particular disease is important.

Q Okay. Reading the title here, it says:

> Epidemic of a novel, cancer-causing viral disease may be associated with wild salmon declines in B.C.

And I just want to move to page 7 of the

presentation, please, and here we have some pictures. It says:

> Large dark attached tumour mass

And I believe if we go to the next page, page 8, just reading the top:

> Optic lob is has large tumour mass and is hemorrhagic (tumours are attached, blood is a different consistency)

And I wanted to follow up with you, have you done any additional work, or obtained any additional information on these tumours?

DR. MILLER: I would like to provide the backdrop in that one of the observations associated with plasmacytoid leukemia was that they observed optic tumours and so when we were talking to various colleagues and trying to figure out what other kinds of information could we glean from our fish to try to match, to determine whether or not this particular disease might be causative of the signatures that we have, one obvious place to look was to look for these optic tumours. And the unfortunate thing when we started delving into this was that nobody had tissue samples associated with this particular disease. There were no tissue samples left within DFO and nobody was collecting them, and nobody was studying them in British Columbia. And so we had gone and looked, we had archives of hundreds or thousands, actually, of brains in both smolts and adults, and we went and looked at the optic lobes of those brains to see if we saw any indications of potential tumours. And lo and behold, we actually did see that quite a large proportion of those brains, on the outside of the optic lobe carried very heavy vascularization, so blood vessels, and in some brains, it would be very white-looking and you wouldn't see this heavy vascularization, and in other brains, you would see this heavy vascularization. When you opened up the optic lobe, in a good portion of those brains, you would see what looked like these pink mass growths, and you could see them connected to the blood vessels inside. Blood, loose blood is quite a different

consistency, it's quite dark, it's not attached, you can just pick it up. Some of this was just, you know, a few cell layers deep of heavily vascularized, very pink tissue, compared to a very white background. And so we talked at the time, we brought the Fish Health staff up to see if anyone had observed the tumours associated with plasmacytoid leukemia and no one knew what they looked like. And I asked if anyone had seen anything like this. So we consulted the staff and they suggested we do histology to determine whether or not these are tumours.

Q Okay. And with that background, are these tumours?

DR. MILLER: The time I gave this talk was right in the middle, when we were doing all of this, and about a month-and-a-half after I gave this talk, we had the results from histology and the histology results, which were read by Gary Marty, suggested that these were haemorrhages.

Q Not tumours?

DR. MILLER: Not tumours.

Q So we're looking at this one exhibit that refers to tumours and with this same clarification that you've just given, that these are not tumours, would that apply equally to other documents regarding your research that referred to tumours?

DR. MILLER: Yes. This is the main document, and we probably should have used the term "lesion," not "tumours" here because we hadn't established that they were tumours at this time. I think the reason that that jump was made was because they were tumours in association with plasmacytoid leukemia. The other thing that wasn't revealed to me until a much later time was that Mike Kent never actually looked at brains in his studies of plasmacytoid leukemia. The tumours that they found were observed in the back of the eye and not in the optic lobe. That was not clear from the literature, they simply called them "optic tumours." And if you listened to Dr. Kent's testimony just a couple of days ago, he backed up a little bit about that even being tumours. He suggested that the lesions that he saw on the back of those eyes might have actually been inflammation and not tumours. We were going by the information that we had associated with this

disease. Now it seems that there's a lot of backtracking on that information by the experts on that disease.

Q Okay. If we turn to page 11 of this presentation, just reading the title at the top, it says:

> Strong Linkages of Genomic and Brain Tumour Data With Plasmacytoid Leukemia caused by the Salmon Leukemia Virus.

Now, you've just clarified that these weren't brain tumours. Did you find any linkages between these what you thought were tumours and the mortality-related signature?

DR. MILLER: Okay. The answer is no, but you have to understand that at the time that we were dissecting these brains for looking in the optic lobes, in order to do microarrays, we have to take RNA from an entire brain and so all of our studies that delineate this signature would have used up all of the brains. And so when we went to look for evidence of plasmacytoid leukemia in these brains, we had to sample brand new brains. So we followed this up with a study that where we had scored individual brains for whether or not they contained these lesions, which turned out to be, according to Gary Marty, according to a sample size of about 12 fish, that turned out to be haemorrhages and from that, we determined that our signature was not correlated with the presence of these lesions.

Q Are you still looking to plasmacytoid leukemia or salmon leukemia virus as a possible cause of the MRS?

DR. MILLER: I have not discounted it, but it is not something that's going to be easy to get to because there are not people who are studying it and there are no samples available of fish that are positive for plasmacytoid leukemia. And now, if you look at what the experts had to say in the last couple of days, they're even kind of backtracking on whether or not it is a single disease or whether the histological signature might be associated with a variety of different pathogens. So it's still of interest to me, mostly because of the history in terms of when it was first observed, that sockeye salmon was shown

to be highly susceptible to it. I have not discounted it, but I am at a bit of a loss as to where to move forward with it.

MS. CHAN: If we could have Tab 26 of the Commission's documents, please?

Q There are two documents that are at Tab 26, one is an email and one is what looks like a memorandum to the Minister, or at least a draft with some comment bubbles on the side, and Dr. Garver, we have this document from you. Do you recognize this as a memorandum with your comments on the side?

MS. CHAN: Perhaps if we bring up the email that attaches this document.

Q Do you recognize this as an email from yourself to Dr. Miller?

DR. GARVER: I do, yes.

MS. CHAN: And if we could have this marked as the next exhibit, please?

THE REGISTRAR: Exhibit 1515.

EXHIBIT 1515: Email dated 2009-Oct-08 from Kyle Garver to Kristi Miller-Saunders entitled, Re: Ministers memo - DRAFT

MS. CHAN: And if we could go to the document that's attaching it, or that's attached to it. I'll just read some of these thought bubbles. If we could have this document marked as the next exhibit after, so it would be 1516, please?

THE REGISTRAR: That's correct, 1516.

EXHIBIT 1516: Memorandum for the Minister, "Epidemic of a Novel, Cancer-causing Viral Disease may be Associated with Wild Salmon Declines in B.C."

MS. CHAN:

Q So the title, "Epidemic of a Novel, Cancer-causing Viral Disease may be Associated with Wild Salmon Declines in B.C.," that seems to be the same title as the previous presentation we just saw. And just looking at some of the thought bubbles, Dr. Garver, you're saying an alternative title suggestion, and the second one down:

Decline in tumour prevalence does not

necessarily mean fish with tumours died, it could simply tumours regressed.

And the third thought bubble:

Is there strong evidence to directly link tumour decline and mortality?

What was the context of this and what was the message that you were trying to convey in your email in this attached comments to Dr. Miller?

DR. GARVER: My main concern with this document at the time, and Kristi alluded to this, the genomic profiling is not a definitive diagnostic. So in other words, to be able to link it to a specific virus, in other words, differentiate between Virus A versus B, you really need to know what those signatures of those viruses are to differentiate. And in fish health, as far as genomic profiles, as far as obtaining those signatures of different viruses, say we have Virus A and Virus B, we need to determine that signature. And in fish health, that just hasn't been done. So there is no biomarker or signatures for specific viruses. There is a few, but to say that it's a retroviral agent, I just was not comfortable in that the data suggested that so I tried to rephrase it to "viral disease."

Q Now, I'm looking at the time, and just to move on to the current hypothesis, which, Dr. Miller, you've mentioned a parvovirus, so when did you find this parvovirus and how did you find it?

DR. MILLER: With Dr. Garver's help, we attempted to isolate viral particles from tissues that contain the MRS. We did this through using sucrose gradients, which is a technique that's often used to isolate viruses and then we extracted DNA and RNA from those and we sent them off to a genome centre in Quebec to be sequenced. And we used 454 sequencing, which is a very rapid sequencing technology that allows you to get hundreds of thousands of reads very cheaply and quite quickly.

We obtained the results from that in early 2011 and we identified the parvovirus using bioinformatic approaches in late February of 2011. Parvoviruses are a small DNA virus and so we did about 260,000 reads from DNA that was isolated

from MRS-positive livers of smolts and adults. We found the parvovirus sequence in both the positive smolts and adults and we found it 76 times. And we obtained a sequence that was about half the size of the genome so we have about 2,200 bases of the sequence. And it's probability value of being a parvovirus sequence is E to the minus 63. It's a very, very powerful positive for a parvovirus. We have since aligned it with the conserved regions of a number of parvoviruses and shown that it contains all of the -- all of the conserved regions are conserved in this virus, as well.

Q You mentioned you have part of the DNA sequence. If it's a DNA sequence, is it a retrovirus, then?

DR. MILLER: No, it's a parvovirus. It's in the parvovirus.

Q Not in the retrovirus?

DR. MILLER: Not in a retrovirus.

Q You mentioned finding it in MRS positive samples. Have you found it in MRS negative samples?

DR. MILLER: Well, so we didn't run the sequencing on MRS negative samples, but we developed molecular markers for this virus and have screened individuals that we have run on microarrays in the past, and it is associated with the presence of the MRS in liver tissue. That's the one tissue we validated so far. That's the tissue we observed this in originally.

Q Have you ever found the parvovirus in tissues that were MRS negative?

DR. MILLER: We did not find it in any of the livers that were MRS negative.

Q Any of the other tissues that were MRS negative?

DR. MILLER: We are in the throes of doing that. One of the difficulties that we had was that if you look at the Science paper, we used non-destructively sampled gill tissues and all of the genomic work is based on RNA, which is different from DNA, and in order to get enough RNA to run on microarrays, you have to use an entire sample. And so we did not have tissue remaining to extract DNA to look for the virus in those particular samples. So that is something that we're doing over samples from other studies that we have identified the MRS in.

Q And Dr. Garver, I understand that you're involved in this work, as well. Now, for a parvovirus,

parvovirus, is that seen in other animals?
DR. GARVER: It has been observed.
Q Microphone, please.
DR. GARVER: Yes, the parvovirus has been observed in other animals.
Q When it's observed in other animals, is it of a size that if you have the right kind of microscope, say, a scanning electronic microscope, is it visible?
DR. GARVER: It is visible, yes.
Q Have you looked for parvovirus particles in these MRS positive tissues?
DR. GARVER: We have not done that yet, no.
Q Do you intend to?
DR. GARVER: That is one of the diagnostics that we plan to do.
Q Are you working on testing infectivity of the parvovirus?
DR. GARVER: Yes, so when you have a disease agent or a etiological agent that might be causing a disease, one of the first things you need to do is identify if it is transmissible, if it's infectious. And so when Kristi came up with this sequence, identified this sequence, we then proposed to take those infected tissues and see if they are infectious to naive hosts. And so what that means is you take the infected tissue and inject it or subject it to a naive host, in this case, sockeye salmon that are thought to be free of the parvovirus, and then we look for transmission of that agent to see if it is indeed infectious. And more in particular, then you follow that infection study and look for disease signs and you do that using pathology and histology.
Q And have you found that it's infectious?
DR. GARVER: That's a good question. We just started that challenge yesterday, actually.
Q Okay. So still in progress. Now, if there's a parvovirus in these fish, does it necessarily mean that there's going to be a disease?
DR. GARVER: No, not necessarily. As in probably the past two days, I'm sure you've heard, not all pathogens equate to disease. It's a complex, multi-factoral interaction among the host/pathogen environment to actually get what we call the sweet spot of disease.
Q Is it possible that the parvovirus is not

associated with any disease in these fish?
DR. GARVER: It's possible, yes.
Q That's something under investigation?
DR. GARVER: Yeah, that's exactly what we're looking for, to see if there is disease that is associated with the parvovirus.
Q And Dr. Miller, you alluded to this earlier, are you looking for the parvovirus in Atlantic salmon fish farms?
DR. MILLER: Yes, we will be as soon as we get the samples, yes.
Q And are those on their way to you?
DR. MILLER: This all came about about a week before I was due to testify, and that was when we had the agreement that the four major salmon farming companies would work with us on a sampling program, and I believe after the aquaculture hearings, I will be getting together with the vets and Kyle and we will be designing a sample program for the industry because I want to make sure that we cover the life history stages where we've seen this virus in wild fish.
Q All right. Now, I see that I'm nearing the end of my time. As my last issue to put to you, it's something that's been raised as an issue recently, and that is whether or not anyone at DFO, any of your superiors, have ever told you not to speak to the public, not to speak to other scientists, or not to share your research? Has that ever happened?
DR. MILLER: Well, yes, I'm not to speak to the public because of the ongoing inquiry. I am free to speak with colleagues and other scientists, and I have been able to attend some scientific meetings.
Q Have you ever been told not to attend a scientific meeting?
DR. MILLER: Yes.
Q And when was that?
DR. MILLER: Well, it was really a think tank, an SFU think tank, but it wasn't me exclusively. DFO decided that nobody, no scientist from DFO was to attend that meeting.
Q Have you ever been told not to publish your research?
DR. MILLER: No, absolutely not. You know, this is one of my worries with this whole process and the way that this has played out in the media, you know,

the integrity of science in DFO is absolutely withheld. As scientists, you know, we do our research, we come up with our conclusions, we write our papers and there's nothing to stop us from publishing our research anywhere that we would like to publish our research. We do provide a reprint of what we are going to be submitting for publication, but there has never, to my knowledge, been anyone who's been prevented from publishing their research.

Q And you said that at DFO, science integrity has been withheld, and what did you mean by that?

DR. MILLER: I mean the integrity of the science is strong, that there's nobody telling anybody what they can and can't publish or what they can or can't say in a publication.

Q Have you ever been told not to research a particular issue?

DR. MILLER: Probably, the answer would be yes, and not pertaining to this, but you know, we, as employees of the federal government, need to make sure that our programs fall within the mandate of DFO and that we are doing research that fulfills that mandate. I can't think of a specific example, but there certainly could be examples of areas of research that DFO did not deem to be within their mandate. So that's certainly a possibility. I can't think of a specific example.

MS. CHAN: Mr. Commissioner, those are my questions and perhaps this would be a good time for the break.

THE COMMISSIONER: Yes, thank you very much, Ms. Chan. It's 11:20.

THE REGISTRAR: The hearing will now recess for 15 minutes.

(PROCEEDINGS ADJOURNED FOR MORNING RECESS)
(PROCEEDINGS RECONVENED)

THE REGISTRAR: The hearing is now resumed.

MR. MARTLAND: Mr. Taylor is looking over so maybe I will rise just to say Canada is the next participant examining this panel. It's 65 minutes.

MR. TAYLOR: Mitchell Taylor for the participant, Government of Canada. Mr. Commissioner, with me is Jonah Spiegelman. And as I mentioned the other day, a law student, Jeff Miller, is with us as

well.

CROSS-EXAMINATION BY MR. TAYLOR:

Q I'm going to start by asking both of you some questions that are picking up on some things that Ms. Chan asked you and then I'll proceed to ask some questions of Dr. Miller and then Dr. Garver. Now, Dr. Miller, you said at one point in answering a question from Ms. Chan that the signature was found in at least one tissue of a lot of fish but it's important to see it in more than one tissue. Do you recall that? And can you expand on your point about more than one -- seeing it in more than one tissue?

DR. MILLER: Sure. Yes, I did make that statement and it is my view, if this is validated to be a viral infection, which is something we now have a candidate virus and it's something that we are working on, it's probably the intensity of infection that really matters here, not that a salmon is simply a carrier in a single tissue. And Kyle may be able to comment further on this. When there is an active infection, that infection can spread through a large number of tissues. But you can have, in a less active infection, a positive for a virus that, in a single tissue or maybe even in one or two tissues, that's not highly active at the time.

And so in my view, if we do validate that this is caused by a virus and is caused by the parvovirus, in particular. Probably the level of infection and the level of infection in multiple different tissues and the copy number of the virus in multiple different tissues would probably be the best indicator of potential negative impacts on those fish. That is certainly something that came from our comparison of 2007 and 2008, out-migrating smolts. The really big difference between them was at the time that those smolts were leaving the river in the brain and the liver, virtually all of the fish in 2007, very small sample size, but they all had the signature in both tissues. And in 2008, very few of the fish carried it in both of those tissues.

Q All right. Now, you were also asked about getting fish from fish farms to do testing for your work

and you explained to Ms. Chan that that has recently been set about to happen. As I understand it, there's a process now where you and veterinarians for the fish farms are developing a protocol for getting the fish and then screening and testing the fish; is that right?

DR. MILLER: Yes, I haven't actually spoken specifically to any of the veterinarians yet. I have had email contact with Mary Ellen Walling and she has spoken to various vets but that is the procedure that we will work with the vets and design a sampling program.

Q And as you explained earlier, but just to remind us all, Mary Ellen Walling is the executive director or similar title for the Salmon Farmers Association, is she?

DR. MILLER: Yes, that's my understanding.

Q And why is it important as a scientist to get a protocol for your screening and testing in place, as opposed to just doing it?

DR. MILLER: Well, it's important if was want to know -- you know, ideally, one would do this over multiple years and determine whether or not Atlantic salmon and other species can carry this virus over multiple years. I think in the beginning we're really just going to look at a single year of samples but we need to get a broad range of samples from similar life history stages, as what we've seen in wild fish. We see in wild fish a lot of fish coming out of the rivers with this virus and it's the virus that we're looking for in the industry in the beginning, not the signature. And we see, you know, that there are shifts in prevalence during their time of ocean residence. So I would like to be able to get samples of Atlantic salmon coming out of the rivers before they're put on the open net pen farms and also at various different stages of development of those Atlantic salmon on the net pens. And specifically getting samples of salmon during times when wild salmon might be migrating by salmon farms.

Q Okay. So as I hear you, and trying to sum up, so tell me if I've got it right or wrong, I think you're saying that as a scientist you want to be clear what it is you're getting and you want to ensure that what's done is going to be

scientifically sound and consistent year-to-year?
DR. MILLER: Correct. I mean simply taking 20 fish from a single salmon farm would not be adequate to say whether or not that virus could be carried by Atlantic salmon.
Q You can't just ad hoc it, so to speak?
DR. MILLER: I don't believe you can. And I think that right now, this is a research question. I have to be clear that we are interested in whether or not this viral sequence is present in Atlantic salmon. That doesn't necessarily equate to saying that this viral -- this virus causes disease in Atlantic salmon. That would be step two after we determined if it was actually present.
Q All right. Is it your opinion as a scientist that it is not scientifically sound to ad hoc it or do one-offs or otherwise just go at it without a clear protocol and plan in place?
DR. MILLER: I'm a very broad thinker and so when I design a program, it usually ends up being very broad and, yes, I do think that we have to go at it with a protocol to make sure that we can definitely say at least in the one year that we'll start this work that there is or is not presence of this parvovirus sequence in Atlantic salmon.
Q All right. Dr. Garver --
DR. GARVER: Mitch, I'd just like to add something on top of this topic, as I also run up the diagnostic portion of Virology Lab for the Aquatic Animal Health program. And there is a clear protocol to establish freedom from disease at sites. And I believe Dr. Stephen has alluded to that in the past two days. There's a strict regimen of how to follow number of fish. As Kristi alluded to, you want to look at your life stage, where the disease is most prevalent, you need to know the prevalence of the disease, you need to know what tissues that disease is most prevalent in, you also need to approach it with a validated diagnostic test. So you have to be sure that the disease you're looking for is going to be detected with your method of detection that you're using.
Q All right. Thank you. And speaking even more broadly than that, and as a scientist, do you agree with what Dr. Miller was just saying about having protocols and scientifically sound methodology in place, as opposed to one-offs and

ad hoc and just getting whatever you get without consistency and a clear plan?

DR. GARVER: Yes, most definitely.

MR. TAYLOR: Now, if I could ask Mr. Lunn to bring up Exhibit 613G or Tab 20 of the Commission, whichever is easiest? Yes, thank you.

Q Dr. Miller, you earlier identified that as a paper that you presented to an internal DFO Science meeting. And I'm not sure if you said when but do you recall when that meeting was and this presentation was made?

DR. MILLER: September 2009. The date on this document says 2008 and that's a bad habit of mine that I take previous slides from previous talks and I overwrite them and I did not change the date on this slide, which should have been 2009.

Q All right. And do I take it also that the 27, the actual day, that's not necessarily correct?

DR. MILLER: The date on this, I simply missed changing that date when I wrote this talk.

Q Now, you see the title at the top there, and this is 2009 you wrote this, but knowing now what you have in mind, would you use a different title on that paper with the knowledge you have now?

DR. MILLER: Sure. You know, in science, it's important to understand the scientific process. As scientists, what we do is we gather information either from published studies from other people or from our own data and we develop hypotheses about those and then we develop methods in which to test those hypotheses to either support or refute the hypotheses that we generate. At the time that this talk was given, we were asked to put forward hypotheses based on the data that we had at the time.

At the time I gave this talk, the data that was first and foremost on my mind because we had just been doing all these brain dissections in August and then trying to elicit interest in people who had been working on plasmacytoid leukemia to work with us on this, this was the topic that was utmost in my mind. We were seeing these what we thought were growths in the optic lobe that were decreasing in prevalence in smolts during their time in the ocean. So the highest prevalence that we saw in smolts was coming out of the river.

We saw a decrease in prevalence in the first few months in the ocean. We also saw a decrease in prevalence in adults of these brain lesions or what were later determined to be aneurysms in adult salmon returning to the river to spawn. This was utmost in my mind, as was the MRS signature, and this was the hypothesis that I put together to elicit discussion about these findings and this particular disease at our intradepartmental meeting. But yes, knowing what I know now, of course, I might call use a different title.

MR. TAYLOR: Okay. Thank you. Now, if we might have, if possible together, Exhibits 1515 and 1516 on the screen.

Q And this is a question for you, Dr. Garver. While it's coming up, this is the draft briefing note from back in that same period of time, which has in front of it the email you now see from you and then the briefing note. And you're familiar with this. It was up a few moments ago and you're generally familiar with it, as I understand it, Dr. Garver. This is the 2009 period of time that you're writing the email and writing on the briefing note. In what you were doing here and the balloons that you can only see a part of to the right. There we go. Is this you as a virologist injecting a pound of caution and suggesting some words that would avoid overstating what the available information would support?

DR. GARVER: Yes, both as a virologist and a scientist, I weigh the amount of evidence that's there and make a conclusion based on that.

Q Okay. Thank you. Next, and this is still picking up on a couple of points that Ms. Chan was asking you about. There was reference in a question and answer earlier to a think tank from sometime ago and DFO scientists not going. And you recall that evidence, I'm sure. Do you recall when that was and when the rationale for DFO scientists not going to that think tank was, Dr. Miller?

DR. MILLER: I believe that that was late in 2009 but I don't know the exact date. At the time, DFO was trying to get their mind around what the Commission would want the scientists within the Department to do in terms of how much we should speak publicly about our work versus leave those

discussions to be something that we'll present in the Inquiry. And I believe that they were thinking that there might be some sort of ruling from the Commission about that but that is something that you'd have to ask the upper managers. And I think that to be precautionary, they decided that they would limit the exposure of scientists to any meetings that were likely to attract public attention and media. And that SFU think tank was a meeting that they felt could have some public interest and some media in attendance. So they made the decision that no scientists in DFO was to attend that meeting.

Q All right. Thank you. In terms of speaking with the public now and of recent times, and this is a question of both of you, Dr. Miller said earlier that that's not to happen. Do you have an understanding of why that's so, why the DFO scientists right now are not to speak with the public or give public interviews?

DR. MILLER: Well, I mean what we have been told is that we're not to speak about our findings until we testify here in the Cohen Inquiry. I don't know at what point that ban in speaking to the public will be lifted. I don't believe it is lifted yet.

Q Do you have an understanding of the rationale for that?

DR. MILLER: It's only the rationale I've been told. As scientists, we're not very privy to the conversation that goes on in Ottawa about these sorts of things. We're sort of only told the result.

Q Yeah. Perhaps a better question on my part, what's your understanding of the rationale then?

DR. MILLER: Again, the understanding is that the evidence supporting or refuting various hypotheses should be heard first in the Cohen Inquiry before it becomes something of public debate.

Q All right. Thank you. Dr. Garver, do you have anything to add to that?

DR. GARVER: Yes, I basically was under the understanding that we were respecting the Cohen Commission process and presenting evidence here first.

Q All right. Thank you. Just a couple of more questions, Dr. Miller. Dr. Garver, you may have

something to add on this as well. When did you identify the parvovirus and how did you find it?

DR. MILLER: We identified it from the sequences that we obtained in late February. And what was the second part of your question?

Q How was it found? I think you've spoken to some of that before.

DR. MILLER: In late February of 2011, it was found by basically we had about 260,000 reads for each of DNA and RNA. You put those together in what's called "contigs". Each of the individual reads can be quite small. They can be anywhere from 200 to 500 bases so not a lot of sequence information. Those are developed in looking for sequences that overlap, that basically multiple sequences that contain portions of the same sequence. And they're built into something called "contigs", which are basically a contiguous sequence of representing basically a larger portion of a general sequence.

Those were what we call "blasted", or sent to various sequencing databases. There's viral databases. There are sequencing databases for all protein sequences that have been sequenced in all organisms, et cetera. So we basically did alignments using these public databases and identified in every single one of those databases that the parvovirus was the only significant hit to that particular sequence.

Q Okay. It's sometimes called a novel virus and I think novel virus has been used with the retrovirus that was earlier talked about as well. What is meant by "novel virus"?

DR. MILLER: Novel does not necessarily mean new. Novel means that it is previously un-described/unknown.

Q All right. Sort of like a planet that we don't know about. It's always been there but it takes someone to find it; is that the idea?

DR. MILLER: Yes, we certainly don't have any data at the present time on whether this is something that is new in terms of that salmon have only recently picked it up or it's something that's been there for a long period of time. That will require some epidemiological work.

Q And do you have any understanding so far as to whether this is native or something that's been

introduced?
DR. MILLER: That kind of understanding will come once we start looking more broadly at where else this viral sequence exists. At the present time, we cannot say.
Q Okay. Now, I'm going to ask you questions to let you flush out what you've been saying so far on some points. Firstly, am I correct that your 2011 paper was dealing with or addressing 2006 adult returners?
DR. MILLER: Yes. I mean in our genomic program, when we first started this program, the real interest in terms of sockeye salmon, and this came from the Pacific Salmon Commission, was the fact that these salmon were dying premature in the river and there was no way to predict what level of mortality different stocks would experience in the river. And the problem with this when it comes to management is that they open fisheries on these fish based on what they assume will be the returns to each of the different river systems.

Historically, there was only about 15 to 20 percent of fish that would go missing en route to spawning grounds and in the last 15 years or so, it's fluctuated somewhere between 40 percent to 95 percent. And it's fluctuated even between different stocks. And so our program was really built on top of Scott Hinch's program and Tony Farrell's programs that were already looking at physiology and radio-tracking to try to understand what might be going on as a way to help provide both a mechanistic understanding for why there are these very high levels of premature mortality but also to hopefully help to provide a tool that managers could use to predict what kind of levels of mortality to expect in those fish.
Q Okay. And then it was after you got the 2006 data that you then started looking at smolts about 2008, as I understand it, and they're not part of that paper, of course. Can you flush out the work that you're doing with smolts and any conclusions that you've reached with regard to smolts and/or how those conclusions are the same or differ from what you've put in your paper as to the adult returners?
DR. MILLER: Yeah, so there's basically two points in the life cycle of salmon that have begun to be of

a lot of interest in the scientific community. And these are the times when salmon are transitioning between freshwater to saltwater as smolts and back to saltwater as adults. During these periods of time, we know that there are very high levels of variation in the level of mortality that are experienced and we know that our ability to predict how much mortality will be experienced during those times is quite limited.

So there's a lot of focus on early marine ecology by my colleagues, Dick Beamish, Marc Trudel, as well as individuals in the U.S. I developed the program on smolts basically to emulate what we were already doing in adult salmon and obtained a grant from Genome British Columbia, funding from them, as well as Pacific Salmon Commission, DFO and NSERC, along with my colleagues at UBC and other colleagues at DFO.

To begin to use genomics as a way to flush out what can the physiology of the fish tell us about what types of things might be undermining their performance? As you've heard previously by other speakers talking about fish health, and I should be clear that my program is not simply about disease. The kind of approach that we're using can flush out things like do the genomics suggest that fish are feeding or not? What is their nutritional status? What is their growth status? Are they prepared for freshwater or saltwater transitions? There's a wide variety of kinds of information that we can get using genomics approaches.

And so the idea of this program really was to go out into the ocean with smolts and track their migration in the ocean and ask the question, what's the range of physiological variance in those smolts as they're leaving the rivers in various years? And which physiological signatures might be associated with poor performance? We're very lucky in working with adults that we are able to use approaches like radio-tracking where we can say something about what the fate is of the fish that we're actually studying. In smolts, radio-tracking technology has not yet been developed well enough to be able to do that. And so our questions really are, what's the range in physiology of those smolts? Which of those

physiological signatures that we uncover do we expect may be indicative of environmental stress or disease or something of that nature? What do we hypothesize might be the mechanism associated with those signatures?

And then the idea of it was this is really a discovery program and we really don't know what's happening to the salmon, why so many of them are dying in some years. And so the thought was that if we could start to use their own physiology to give us indications of what kind of stresses they might be under. This particular signature came out again in the adult study and it could have meant anything. In terms of when we were studying adults, we weren't looking for disease in particular; we were looking for anything that associated with success or lack of success to make it to the spawning grounds.

This signature, however, has come out in this program as being the single most powerful genomic signature in everything that we have looked at. This signature is stronger than the genes that are differentiated along the migration route back to the spawning grounds. We see more genes that are affected by this particular signature than are affected when a salmon moves from Queen Charlotte Islands all the way back to the spawning grounds. The number of genes that have to change, and you have to understand salmon are changing physiologically as they migrate, they're senescing, they're maturing and they're encountering all kinds of different environments, et cetera. This signature is more powerful than even that. And this is what has caused us to really focus on this. And it is my view that if we do find that there is a viral pathogen that is causative of this signature, the strength of this signature alone suggests that it is potentially causing disease.

Q All right. And has your work and test results that you've obtained with respect to smolts changed your hypotheses in any way from what is set out in the 2011 paper?

DR. MILLER: I think it's really strengthened it. The fact that we're observing the same kind of tissue distribution in the presence of this signature, as we observed in adults. When we look at the

signature and we look at the genes that overlap between different tissues and between smolts and adults, if you only looked at those overlapping genes, and we have a paper in Paul Pavlidis' lab is sending out on this for adults. But we find that the linkages with viruses become even that much stronger, if you consider only the genes that are overlapping between these tissues and if you consider the same thing for smolts and adults. Basically, we're seeing very similar patterns in both of those life history stages but we're seeing that this is even much more prevalent in smolts than it is in adults and that the signature when smolts enter the ocean becomes even stronger in those fish.

And now we're working with this parvovirus. We are doing the work to determine whether or not that is associated or causative of this signature. We also observe the highest intensity, so the highest copy number of the parvovirus in smolts as they're entering the ocean, which is another piece of evidence to suggest that that is an important point in their life history where infectivity and an activity of this virus might be important.

Q Okay. Can you just clarify for the Commissioner, you said "ocean" a moment ago and "ocean" appears in various of your writings. What are you meaning by "ocean"? Where is the ocean starting in terms of your writings?

DR. MILLER: In the Strait of Georgia. We don't do a lot of work in the estuary but in the Strait of Georgia.

Q All right. So leaving the freshwater and going into the salt or vice-versa, Georgia Strait is captured by the word "ocean" in your writings, is it?

DR. MILLER: Correct.

Q Okay. Can you take a moment and just say, speaking as a scientist, how a hypothesis is developed?

DR. MILLER: I touched on that a little bit just a few comments back. Basically, the scientific approach is to take in information, whether that be from your own lab or from publications, and synthesize that information and develop hypotheses to explain that information and then to develop ways of testing those hypotheses. And you might have a

number of different hypotheses that one develops. And develop a way, a scientific approach, that would enable you to either validate that hypothesis as being correct or refute that hypothesis as most likely being incorrect.

And this is the process that we work with everyday. It can be little things that are new hypotheses or it could be very large things. And in the process, I think you can see I've provided a timeline in association with this inquiry, to provide information about how we were thinking about our various discoveries over time and why we generated specific hypotheses, on what basis of what data that we obtained made us take some different turns in the research that we were doing. I have to say that the hypothesis that this particular signature is associated with viral activity has never changed.

MR. TAYLOR: All right. Thank you. I'm going to cover off a couple of documents and mark them as exhibits before we proceed into questions. Could you please go to Tab 25 of Canada's documents, Mr. Lunn? Or Commission's documents, I'm sorry.

Q I think what you're going to see is a document you prepared come up, Dr. Miller. Do you recognize that?

DR. MILLER: Yes, I do.

MR. TAYLOR: Can you just scroll to the very end, Mr. Lunn, for a moment? I just want Dr. Miller to see what's at the end. Little before that end, I guess. Somewhere there's a date near the end. Just keep going up, I think. No, no.

DR. MILLER: Down.

MR. LUNN: DD.

MR. TAYLOR: DD is fine. It says in July 2011 and then if you go back to the beginning, Mr. Lunn.

Q You'll see that it says there "last revised May 19, 2011". What's the true date of this document?

DR. MILLER: I've been caught again on my dating issue. I take documents and I modify them and sometimes I forget to change the date at the top. So I prepared this, I believe, at the end of July/early August.

Q Of this year?

DR. MILLER: Of this year.

Q And it is what it says.

DR. MILLER: Or I revised it. I did prepare it

originally in May.
Q All right. I see. And then you updated it. And it is what the title says, a timeline of genomic research, is it?
DR. MILLER: Yes, this was suggested to me by Laura Richards, that it would be much easier to sort of understand our thinking and the changes in our thinking if I put together a timeline which showed when we discovered various things and how that resulted in some of the hypotheses that we put forward.
MR. TAYLOR: All right. May that be the next exhibit, please?
THE REGISTRAR: It's Exhibit 1517.

EXHIBIT 1517: Timeline of Genomic Research relating to the Mortality-related Genomic Signature Hypothesized to be associated with a potentially Novel Virus

MR. TAYLOR: And if you'd go to Tab 10 in Canada's list of documents, that is already an exhibit already, I think, although I don't know the number.
MR. LUNN: 1513.
MR. TAYLOR: Pardon me?
MR. LUNN: 1513.
MR. TAYLOR: Okay. This is Exhibit 1513.
Q If we go to page 13 of Exhibit 1513, you deal there with parvovirus but also retrovirus. Retrovirus is what?
DR. MILLER: Retrovirus is an RNA virus. Kyle might be better describing the actual viral families but they tend to be oncogenic viruses, which means that they tend to be associated with cancer. They have an ability to insert themselves in the host genome and become endogenous, which is a special feature of retroviruses but it's not the only viral family that does it but it certainly is the one that's most well-known for that.
Q Okay. And I probably should have asked, Dr. Garver, because you're the virologist, but do you want to add to that or describe retrovirus?
DR. GARVER: No, that's quite sufficient. It is an RNA virus and one of the key features and Dr. Kent alluded to this is so they transcribe their RNA into DNA and they use a special enzyme called "reverse transcriptase" so one of the common

things to look at for retrovirus is known as an "RT, reverse transcriptase activity". And so that's the only other addition.

Q Okay. There's two terms that we see in some of the writings and I'll ask this of whichever one of you is the right person to answer the question. The two terms are "molecular genomics" and "functional genomic studies". Is one of you able to give a sort of one or two-sentence answer or definition of what each of those is?

DR. MILLER: Functional genomics pertains to gene expression. And molecular genomics can pertain to a lot of different areas of genomics but I think the context, if I have use that terms, has been more sequencing level genomics.

Q All right. If we turn to Tab 22 of Commission's documents, which is also Exhibit 1512 now, you will see the document entitled "Hypothesis". This is something that you prepared, Dr. Miller. I'm not sure if you said when. You might have said this is for the June 2010 PSC symposium, is it?

DR. MILLER: Correct.

Q Okay. You begin this document by pointing out that in 2006 you first raised up what we're now talking about at a meeting in Oregon. You've spoken to some of this before but was there an instigating event or reason why you started into this line of work or this area?

MR. MARTLAND: I'm just going to clarify our understanding is it may be Nanaimo as opposed to Oregon, if that assists.

MR. TAYLOR: It's in the document, I think, but...

DR. MILLER: Oregon? Definitely Nanaimo.

MR. TAYLOR:

Q Okay, that's fine. In any event, in 2006, you first talked about the work that you've now been giving evidence about, as I understand it?

DR. MILLER: Correct. Our program really started in around 2005. We started purchasing equipment in about 2004 but our genomics program got up and going in 2005. And as I've said, the program was developed in response to the lack of predictability on salmon that in the return migration salmon that will successfully make it to the spawning grounds and salmon that would successfully spawn. It grew from that to working on smolts because there was a lot of interest in

that early marine mortality and what might be undermining performance of salmon in that early marine period. Now, that does not just extend to sockeye salmon. Early marine mortality has been increasing in chinook and coho salmon as well and I know that Dr. Beamish has already spoken to the Commission about that.

And so I developed a collaboration with Dr. Beamish and Dr. Trudel whereby we were able to obtain samples from their very extensive ocean cruises that are conducted every year in the Strait of Georgia and also into the high seas and with David Patterson, who is an absolute instrumental biologist here in DFO, who runs the Environmental Watch program and has from the very beginning of our program done all of the collections in the Fraser River and also put people in to do the collections on the smolts. So the program was developed in response to a lack of predictability.

The fact that the escapement models that are used in salmon management are not very accurate and so there's a lot of interest in (a) can we find ways of modifying those models with new information that might increase their accuracy? My program has been working in the area of salmon genetics for a lot of years and when I came into that program about 19 years ago, we were trying to develop a program to do genetic stock ID. And many of you might have heard of that. We have developed an incredible program that is used all the time now by managers for genetic stock ID. And the management of sockeye salmon is based on information that we provide on a real-time basis on what stocks are present in a given fishery.

The idea behind this program is managers can now know if they go out and catch fish in the marine environment what stocks of fish are present and they can make management decisions so that they can minimize impacts on stocks in need of conservation and maximize their targeted exploitation on stocks that can handle exploitation. The problem was that they still didn't know how many fish were going to make it back to spawning grounds.

And so the idea was if we could develop using genomics a program that piggybacks with that, that

looks at the health and condition of the fish, and adds that as another piece of information that managers can have, that might add greater predictability to their escapement models that they could not only know when they're out if they're looking at returning adults, they could not only know what stocks are present but what's the probability that those fish are actually going to survive to spawn?

That was what spurred the development of this program and it further developed into the smolt program in discussing this program with colleagues and the need for more information about health and condition of smolts in the early marine environment.

Q And that remains the driver for the work you're doing, I take it?

DR. MILLER: That is absolutely the driver. And this is a discovery program. We're using genomics to discover what kind of factors might be exacerbating their performance.

Q And as I understand it, it's fundamental to understand that this is a work-in-progress with much more to be done and learned?

DR. MILLER: Yes, I think it's a fairly unusual process to have this level of scrutiny on a program that is just in complete active research mode. And it's interesting but yes, this is absolutely research-in-progress. And we are taking many different angles to this research as we make new discoveries and as what we're doing with Kyle in terms of the disease challenge work.

Q All right. In the document that's up on the screen, Exhibit 1512 I think it is, if you turn to page 3, about halfway down there's a heading that starts with "Signature" and there's a bullet under that to do with affected tissue and under that it says "no muscle involvement". I understand that's significant and allowed you to rule out something. And I'm not sure if I should be asking you, Dr. Miller. This is your document. Or whether Dr. Garver is the one on this. But one or both of you, what's the significance about not seeing this in muscle?

DR. MILLER: Well, it isn't highly significant. It's significant that we don't see it in absolutely every tissue that we look at. And you know, where

that comes into play is that most viruses and most pathogens have specific tissues that they affect and Kyle should be the better one to speak to this but many viruses have a specific target tissue that they're generally seen in first and then they may move into other tissues at various stages of infection. The fact that we haven't picked up this signature in muscle tissue, I mean there are viruses that affect muscle tissue. That doesn't mean anything one way or the other about this being a pathogen or not being a pathogen but we also don't see it in hypothalamus tissue either. It just means it's not in absolutely every tissue in every individual.

Q And Dr. Garver, in terms of significance attached to it not being in muscle, do you have anything to say on that?

DR. GARVER: No, I think Kristi covered that pretty well. As far as viruses, they do have kind of a life cycle or an infectious cycle of how they progress through a host. And it ranges from the initial infection, which could be epithelial cells primarily with fish, but it could be different. And then it could go through a viremic state which then, as a viremic host, it's pretty much throughout the fish.

It's in the blood and then multiple tissues attach to the circulatory system and then either results in death of the host or may regress if the host is able to fight it off. And sometimes it's cleared and sometimes it actually even remains in tissues in a latent state or a carrier state. So yeah, from determining whether it's in the muscle or not, I don't think we can really conclusively say that this is linked to a pathogen or not.

DR. MILLER: Okay. Can I just add to that? I'm actually looking at what's in front of me. I didn't actually look at it carefully. The point in this particular part of the document was, if you read the top, that this signature is not consistent with the general stress response, sea lice infection or parvicapsula infection because this is a question that I was often asked when I would talk about this work and about this signature, could this simply be a signature associated with parvicapsula? So now I see the significance of that in this particular document

is that sea lice affects muscle tissue.
Sea lice will bind to the skin and will cause a reaction in muscle tissue. And so what this was basically saying was there's no muscle involvement. We don't see the signature in muscle tissue. Really, the strongest part of what this argument shows is where the fish are affected. If this signature were something that was a response to sea lice then it shouldn't be emanating from the freshwater environment because salmon don't pick up sea lice until they enter the marine environment. So it is highly inconsistent with this being a response to sea lice. It's also not consistent with a parvicapsula infection because we see the signature as far as the Haida Gwaii in returning adult salmon. And returning adult salmon pick up parvicapsula when they enter the Fraser Estuary. And so where the tissues were affected, it has something to play. We don't expect there to be a strong involvement of the brain, for instance, for sea lice and we do see very strong involvement of the brain associated with this signature. But I think that the most important point here was where we see the fish affected.

Q All right. Did one or both of you put your mind to whether the signature had any relationship to well-known pathogens such as IHN or ISA and take steps to rule them in or out?

DR. MILLER: We conducted screening for all of the viruses that were in B.C. that had molecular markers for them so that we already had sequence information for. We applied the molecular markers that other labs had already developed to our samples and we found that none of those known characterized viruses were (a) present in any kind of prevalence like we have observed this signature, or (b) associated with fish that carried and didn't carry the signature. So in the early days, in fact, before we wrote the Science paper, had already discounted that. We couldn't find a virus or intercellular pathogen because we also tested a variety of intercellular pathogens that was correlated with the presence of this signature.

Q And is the viruses that were considered the ones that Dr. Kent reviews in his paper?

DR. MILLER: Yes.
Q All right.
DR. GARVER: I can add one thing to that as well. And I alluded to it earlier.
Q Add as many as you wish, Dr. Garver.
DR. GARVER: So again, this genomics profiling is not a definitive diagnostic and to be able to rule out other signatures of viruses, you need to know those signatures. And there has been some genomic work done. Kristi, in particular, has worked on IHN and has established a possible signature for IHN. However, there hasn't been much work outside of that to the other pathogens. So definitive signatures for ISA, VHS, all these other pathogens are not really well-known for fish. So to apply a signature in this case may not necessarily be appropriate without that information.
DR. MILLER: I don't believe that's actually what we were trying to do. What we identified from this signature was that it contained numerous elements that were consistent with known processes that were affected by viruses. The specifics about what virus it was really was when we took the molecular approach to look at the presence of known viruses and known viral sequences. I don't believe that I ever went and looked at this signature and asked, is this an IPN virus based on the signature? All we did with the signature was suggest that this was virally mediated and that there were components of the signature that were really highly similar to the types of things that could be affected by retroviruses but that was as far as that went.
Q Were MRS-positive tissues tested for ISA or other viruses?
DR. MILLER: We did test for ISA but we did not have a positive control for ISA. Those are tightly held because of the worry about infection. But we did test with ISA primers.
Q All right. Now, Dr. Garver, you have already testified to some of your work and involvement in the work of Dr. Miller. Is there anything more that you want to add to that in terms of your role and the role of your lab in Dr. Miller's work?
DR. GARVER: I think the main point is, in establishing now once we have a molecular diagnostic for the parvovirus and so now the real question is, is it

infectious and does it cause disease? And if so, what is the pathology associated with that disease? And then there's many other questions in relation to that. If we do prove it's infectious then what are the predisposing factors for disease? In other words, if you change the temperature of the water, does that predispose a fish to subsequent infections, if infection does occur to the parvovirus, or does smolting of the fish increase infection, or does multiple pathogens infecting that fish, does that make it more susceptible? So there's a whole line of questions to go down now but we really need to establish, is this sequence that we have right now, is it an actual agent and is it infectious to fish?

Q So with those questions, what is the current state and what are the next steps in timing for that work?

DR. GARVER: As I alluded to earlier, we just started a challenge yesterday. This is to determine the infectious nature of this sequence that we have right now.

Q And do you have a timeline for this work?

DR. GARVER: It typically takes up to several months. So we hope to have some answers maybe within two months or so.

Q And you don't know what the answers are, of course, but what sort of answer? What's the topic the answer would be on? What will you know then one way or the other?

DR. GARVER: Well, we'll have an idea under the challenge conditions that we're using whether it's infectious. If we don't see transmissibility through this challenge that we're doing then we might not just have what could be possibly happening in nature. So we need to then explore different challenge scenarios. But ultimately, we hope to have after two months a good idea of whether this is a transmissible agent or not.

Q So it sounds from what you're saying that as we move into the year 2012, you're going to have advanced some distance in the work you're doing as part of this genomic signature?

DR. GARVER: Yeah, once you have a challenge model to work with for this virus and take it down into the lab and actually start manipulating different

variables, then you really progress your science as far as disease progression and whether this is linked to disease.

Q Let me just -- sorry. Dr. Miller?

DR. MILLER: At some point, I'd just like to add a few comments about parvoviruses.

Q All right. Go ahead.

THE COMMISSIONER: Mr. Taylor, I wonder if we could take the lunch break and get into parvovirus after lunch.

MR. TAYLOR: Sure.

THE REGISTRAR: The hearing is now adjourned until 2:00 p.m.

(PROCEEDINGS ADJOURNED FOR NOON RECESS)
(PROCEEDINGS RECONVENED)

THE REGISTRAR: The hearing is now resumed.

MR. TAYLOR: Thank you, Mr. Commissioner.

CROSS-EXAMINATION BY MR. TAYLOR, continuing:

Q We're going to come to parvovirus in a second here. I just want to take a few minutes to put some documents in as exhibits. I'm mindful of the time. So I'm going to ask you, witnesses on the panel, if you can identify a document, whether you prepared it, what it is, and the approximate date, and then put it in as an exhibit. And I think in the interests of time, have to leave it there.

Canada's Tab 1, this is a question of Dr. Garver. Do you recognize that, Dr. Garver?

DR. GARVER: I do, yes.

Q Your mike's not on, I think.

DR. GARVER: I do recognize that document.

Q What is that?

DR. GARVER: This is a presentation that I gave at the April DFO workshop to give updates on hypotheses presented at the Pacific Salmon Commission workshop.

Q Okay. The April 2011, DFO meeting.

DR. GARVER: That's correct.

MR. TAYLOR: All right. May this be the next exhibit, please.

THE REGISTRAR: Exhibit number 1518.

EXHIBIT 1518: Garver, Hypothesis: Diseases in freshwater and marine systems are an important contributor to the Fraser sockeye situation, April 2011

MR. TAYLOR:
Q Then if we go to Canada's Tab 8, Mr. Lunn, please. Do you recognize that, Dr. Garver?
DR. GARVER: I do, yes. This is again a presentation that I gave at a Western Fisheries Research conference, Fish Health Disease conference. I believe that was in Utah.
Q All right.
DR. GARVER: I can't recall the date.
Q Is this just what it says there, some of the technician methodology that was used by you in some of the work you've been doing?
DR. GARVER: Yeah, it's some of the research that I've been doing with Dr. Tang and the technicians in my laboratory to validate the microarray detection method I spoke of for fish viruses.
MR. TAYLOR: Thank you. Then if we go to Canada's Tab 11, please.
THE REGISTRAR: Did you wish to mark Tab 8 first?
MR. TAYLOR: Oh, I'm sorry. I'm moving too fast, aren't I. Next exhibit, please.
THE REGISTRAR: Tab 8 will be marked as Exhibit 1519.

EXHIBIT 1519: Garver et al, Microarray-based Detection of Fish Viruses

MR. TAYLOR: Thank you. Tab 11, Mr. Lunn.
Q Dr. Miller, do you recognize that, and what is it?
DR. MILLER: This is a presentation, a private presentation I gave at the internal DFO workshop in April of 2011.
Q Thank you. And that's contrasting with the 2007 and 2008 results that you spoke about earlier, is it?
DR. MILLER: Correct.
MR. TAYLOR: Thank you. And then if we move to Tab 12, please, of Canada's documents. Oh, I'm sorry, I forgot to mark it again. May that be an exhibit, please.
THE REGISTRAR: That will be marked as Exhibit 1520.

EXHIBIT 1520: Miller, 2007 versus 2008 Genomics Contrast Study, April 2011

MR. TAYLOR:
Q And Tab 12, please. Do you recognize that, Dr. Miller? Do you need to see more of it?
DR. MILLER: Yes, I do. This is the talk I gave at the Pacific Salmon Commission in June of 2010.
MR. TAYLOR: All right. And may that be an exhibit, please.
THE REGISTRAR: Exhibit 1521.

EXHIBIT 1521: Miller, Hypothesis: Genomic studies suggest that some disease has infected sockeye and has become an important contributor to the Fraser River sockeye situation, June 2010

MR. TAYLOR:
Q Now, just before lunch, Dr. Miller, you were going to explain or elaborate on parvovirus. Could you take a couple of moments to do that right now, and in regard to that, may we have, please, Exhibit 1513, which is also Canada's Tab 10, page 12.
DR. MILLER: Can we move to page 11 first. Oh, okay, sorry, it was page 10. Okay, go back to 12, sorry. I'm sorry about that.
Q Do you want to have the -- do you want to have the full document in front of you?
DR. MILLER: The one on the contrast between -- between retroviruses and --
Q Oh, 13.
DR. MILLER: Oh, it was 13, I'm sorry.
Q Page 13.
DR. MILLER: Okay. I know I was asked about retroviruses and what retroviruses are, and in -- certainly one of the stages in my hypotheses about this MRS signature was that it could be elicited from a retroviral-like infection. We didn't end up identifying a retrovirus in association with that signature, but we did identify a parvovirus. And I just wanted to point out that there are some very interesting similarities between the two, despite the fact that one is a DNA virus and another is an RNA virus.
And one of which is parvoviruses can insert their genetic material into the host genome. This

is not something that is as well-known. We have done the work to establish that this is not the case for the sequence that we have. If they've inserted their genome into their host, every cell would have the same complement of DNA and we would see this virus in every cell within an individual, and we do not see that.

Interestingly, one of the most powerful aspects of the signature is in terms of a stress response, is a host DNA damage response. And both of these viral families require the elicitation of a DNA damage response in order to complete replication. And so that's something they have in common.

They both are associated with very strong immunosuppression of the host.

They both actually can cause leukemia-like disease.

Retroviruses obviously contain the leukemia viruses, which we know to be causative of leukemia, but there is a plethora of studies over the last 20 years about the role of parvovirus B19 in humans, in leukemia-like disease, and its role in exacerbating the outcome for people with leukemia. But there are some papers out that show that you can actually get a leukemia-like response from a parvovirus, which is something we also found in our data.

Both have been linked with cancer, but while retroviruses can cause cancer, parvoviruses are actually anti-cancer activities. So they target rapidly dividing cells to facilitate their reproduction, and so they have been used -- one kind of human one has been used to fight brain tumours, actually.

And they can both be involved in latent infections. And both viral families are known for remaining inactive for years to wait until the right conditions to begin to replicate and cause disease.

If you can just go to the previous slide. I won't spend a lot of time on this.

But in terms of parvoviruses, some of the best known parvoviruses are parvoviruses in dogs, and they can have decimating effect on young -- on puppies and young dogs.

And there's a parvovirus that is quite

virulent in humans called B19. It's associated with fifth disease in children, but it requires hematopoietic cells to divide, and it's associated with severe anaemia. Again, this is -- anaemia is something that comes up over and over again in some of the observations that we have in salmon, not me personally, but people have been observing anaemia in salmon. And parvoviruses can actually cause anaemia in a variety of different organisms.

I think one of the most interesting things about this family of viruses is not only that they require rapid -- rapidly dividing cells to facilitate their own reproduction, but they can be oftentimes stimulated by stress. And there's a number of studies that have shown that one can induce, if you have cells that are not rapidly dividing, one can induce the proliferation of parvoviruses by simply stressing the cells.

And so I think this is interesting in the context of whether or not a virus like this could become more active and elicit more disease in a situation where salmon are known to be highly stressed, when they go between freshwater and saltwater and return back into the freshwater environment. That at this point I would say is speculation, but it is -- it is something that I am quite interested in terms of this particular viral family.

Q Okay, thank you. Dr. Garver, before lunch you gave some evidence about the lab studies that you have done, and then moving on, lab studies that you're currently embarking upon. Laboratory studies are in a controlled setting, of course. Can you clarify what next steps you see beyond those laboratory studies that you're about to take on right now?

DR. GARVER: Yeah, sure. I guess to back up just a little bit, I'll give you some thinking, rationale for our thinking on why we're progressing to laboratory studies. This is not a typical disease investigation. Typically when we approach a disease investigation, it's usually because we have some -- we actually have a disease or a pathology or even more specifically, mortality associated in a population.

So if there's mortality, if there's a die-off event in fish, we'll go out and then run a disease

diagnostic on those fish to identify the etiological agent that might be responsible. And to do that, that involves traditional methods of culturing, culturing the pathogens, and doing histology to identify the pathology associated. And that's important, because once you have the histological marker or signature of that disease, then you can actually see what damage is being done and get an idea of the impact of that pathogen and consequently the disease on the population. And so in this case, it's quite a bit of a reverse scenario.

And we first -- Dr. Miller first identified a genomic signature that might be linked to a negative impact on the fish. And so inferring upon that genomic signature, it was found that there could be a possible virus associated with it. I grant there was no mortality associated with that, or a disease associated with it, it's now a sequence of a virus. And so we have to identify is that an infectious agent and does it cause disease. And so to do that, you then take it back into the laboratory and do the transmission studies. So that's really the key on where we're going.

But it's nice to take into a laboratory study, because then you can obviously control the setting, control the parameters. And it has to be repeated, it has to be at least confirmed that you can do that over and over again. And so in that regard, once you establish the fact that it is infectious, it's all under the context that it's in the laboratory.

And then you, then once you have histological markers, then you can go out in the wild and start looking at it, using histological markers, using the molecular techniques that Dr. Miller is establishing, getting viral loads in these and really assessing is there an impact due to the infection of this pathogen on a population.

Q All right. And my final question, then, is of you, Dr. Miller, and Dr. Garver has just spoken to some of this, but -- a lot of this. Do you have anything to add to what's been said there about the approach you're embarking on and doing versus the traditional approach to studying a disease.

DR. MILLER: Yes. First of all, I would correct one

thing that Kyle said, that we -- that it's correct that we didn't have observable mass mortality events, and we don't have observable mass mortality events in the ocean. And we do have mortality in fish when they come back in the river, but we don't generally observe that mortality unless they're dying at the spawning grounds. When we originally identified this signature, it was associated with mortality in that initial study that's in the *Science* paper.

But, no, I think it's an important point. And you just have had heard two days of talks or of a panel of disease experts who have basically suggested that it is nearly impossible to study disease in wild salmon because we can't observe their mortality. And if we use the classical approaches of only -- only studying disease where we can observe massive mortality events, and assuming that we're going to pick up all diseases by only looking at massive mortality events in hatcheries, in one environment, in the freshwater environment where we can see these sorts of things, I think it's clear, given the lack of data that exists in terms of diseases in sockeye salmon, that that approach simply hasn't worked. And that the approach that we're taking is a different approach to -- and again we didn't set out to look for disease, but it is almost backwards of what -- of what a normal approach to studying disease would be.

In normal, microarrays are actually used in the human medical world all the time. They're used to study disease and host response to a wide variety of pathogens and as well as drug therapies. And that's the last thing one does in order to figure out what's the best, what's the best prophylactic treatment, what's the best vaccine treatment, how do we get an effective response so that animals are less prone to disease from specific pathogens.

You know, we flipped that on the head and said, let's just look at what the animals are telling us. Let's look at what the physiology of the animals can tell us about what's affecting them, and then we'll go back and hypothesize as to what could be causing that, and then we'll go back and do the laboratory studies to validate those

hypotheses, and to validate whether or not one can, at any stage in the development of salmon, elicit disease and mortality associated with what we now have as a candidate virus.

MR. TAYLOR: All right, thank you. Thank you, Dr. Garver, Dr. Miller. Those are my questions, Mr. Commissioner.

MR. MARTLAND: Thank you. Mr. Commissioner, next I have counsel for the Province at 55 minutes.

MS. CALLAN: Mr. Commissioner, Callan, C-a-l-l-a-n, initials T.E., appearing on behalf of Her Majesty the Queen in Right of the Province of British Columbia.

CROSS-EXAMINATION BY MS. CALLAN:

Q Dr. Miller, how was the common genomic profile defined, and specifically the MRS?

DR. MILLER: I'm not sure what your question is.

Q Specifically is it a specific cluster of genes?

DR. MILLER: Oh, okay, I'm sorry. Yes. The signature, actually, when we first uncovered the signature we had simply run a *t* test between 12 fish sampled in a marine environment but made it to the spawning grounds, and 12 fish that went missing. That was the very first time we uncovered that signature. However, we added more fish to our study. We did a study in the freshwater environment, as well, and when we added those additional fish, a simple *t* test didn't pull it out very well.

One of the reasons for that, and something that has to be recognized is that in return migrating salmon, there is not likely a single cause of all mortality that occurs in the river. And so we recognize at a very -- at the very outset that it may be difficult to simply assume that all fish that die in the river die of the same thing, and that really wasn't our assumption. We were looking for genomic signatures that were associated with poor performance, not necessarily causative of all mortality in the river.

We found that we were able to identify this signature with principal component analysis, and we've used principal component analysis as a method to identify the major physiological trajectories in the data. And then we looked at correlations between success and lack of success

in terms of migration along each of the principal components in principal component analysis.

I know that's very technical, but you asked.

Q And I get what I asked. I understand from your earlier evidence this morning that you stated that the power of the test strength of the signature alone might be evidence of disease. You would agree, however, that the signature alone will not cause disease, although it may be a marker?

DR. MILLER: The signature alone will not cause disease in and of itself. The signature indicates a lot of activities at a cellular level when salmon reach the freshwater environment. The signature does not cause the disease. Whatever's causing the signature could cause disease.

Q Now, I understand earlier you were talking about principled and unprincipled. Is that the same as supervised and unsupervised analysis?

DR. MILLER: No. Principal component analysis is an unsupervised analysis. So it's a way to simply let the data speak for itself and tell you what are the major trajectories in the data.

A supervised analysis is if we were to run a *t* test or an ANOVA, and say I want to contrast what genes are being turned on and off in this set of individuals, compared to this other set of individuals. So that is one of the analyses that we did try, which was to simply run a *t* test, comparing fish that made it to the spawning grounds and fish that didn't.

But in order for a *t* test to be -- to resolve anything very powerfully, you have to have -- it depends on your sample sizes, but you have to basically have, you know, a single signature that's associated with whatever you're looking for.

Q So to summarize it, in your ocean-tagging study, the supervised analysis was not statistically significant.

DR. MILLER: In our first -- in our first analysis of the ocean-tagging study, the first fish we ran we did actually get a significant *t* test. However, when we added more fish to that analysis, because we were just -- we were just looking -- we were looking, actually, at muscle tissue and gill tissue at the time. And we found that we didn't really get any signal associated with survivorship

in muscle tissue, and but when we did our first analysis of the gill tissue, we actually did. When we added more samples, it became more obscure. And but we did then pull it out with PCA analysis.

Q Now, in the principal component, or the unsupervised analysis, you did find gene expression patterns, and this was the basis for your statement in Exhibit 558, your paper that 60 percent of the fish contained a gene expression signature in seawater greater than 200 kilometres from the river that was predictive of an in-river fate.

DR. MILLER: That was -- it was associated with poor performance in the river.

Q Okay. So, Mr. Lunn, if we could turn to the top part of Figure 1A of Exhibit 555 -- 558. It's page 214, which would be the second page. How many fish are in the group with the mortality-related signature in Figure 5A?

DR. MILLER: You're not showing 5A.

Q Oh, sorry, 1A.

DR. MILLER: Well, I'd have to read through the paper again. Okay. So over all of those fish, there's somewhere around 40 fish in A -- is A what you're talking? I'd have to actually look at the paper to remember the exact numbers. Do you want me to look at the paper? Which tab is this?

Q This is --

DR. MILLER: It's outlined in the paper. Which tab is this?

MR. TAYLOR: It's 18 in the Commission's binder.

MS. CALLAN:

Q I can suggest to you that it was 10, but if you could --

DR. MILLER: Oh, I'm sorry. I misunderstood. I thought you meant in the whole study.

Q No, just speaking about the fish with the mortality signature in figure 1A.

DR. MILLER: Okay, where we've demarcated it. Yes, I believe there's ten.

Q Now, when I count the two groups, it looks like there's samples of five and five, and I understand from speaking with others that that's actually mistake, and it was six and four.

DR. MILLER: Yes. There was -- we've had a discussion about this with Gary Marty. The top bar that

demarcates survivors and upper river morts, there was a glitch in the way that that was put on, and there's one -- there's one fish that died that's missing on the -- on the left-hand side.

Q But certainly for the purposes of your analysis, you didn't use five and five, you used six and four, so it's just a glitch.

DR. MILLER: No, no. I know. Gary Marty has had all of the data and redone all of the analyses, and we noticed that we had a glitch on the top bar.

Q Okay. So for this purpose of my next set of questions, it's going to be about determining whether or not a diagnostic test can be derived from this, because I'm anticipating my friends might recommend that we could try to test for these fish using the genomic signature. So that's going to kind of form where I'm going.

So essentially 40 percent of the time you would be -- you would be incorrect if you tried to predict based on this test?

DR. MILLER: "Forty percent of the time you would be incorrect", I think there's something that is in the *Science* paper that I need to explain. And that is that what we found with this signature, and it makes obvious sense when you think about it, is that the individuals on the extremes of this signature, the individuals that are most highly affected, which are those on this -- on this figure that would be way to the left-hand side, as you go on this, on this almost continuous gradient, you have individuals that are highly affected, individuals that are something in the middle that don't really have any effect and, you know, have -- and then you have individuals on the other side.

If you envision this as individuals having exposure to a flu bug, and having the flu, and having pneumonia, right. So you have a very different probability of survival if you have pneumonia than if you simply were exposed to the flu bug. And this could be, it doesn't even have to be a disease scenario. If you had individuals that were starving and you were nutritionally deprived, and some that were well fed, you would only expect that there would be an effect on survivorship on the ones that are starving, not the ones that are just nutritionally deprived.

And so this is the way that we have looked at this data, and others have, as well, in other kinds of studies. What we're looking for are the physiological extremes. And so you're absolutely right, there is no predictive power for fish that are intermediate in this signature.

Q Now, Dr. Garver, you have experience in developing diagnostic tests for developing viruses.

DR. GARVER: That's correct.

Q Would you use or recommend a diagnostic test from this data.

DR. GARVER: To determine a virus?

Q Correct.

DR. GARVER: A specific virus from a genomic signature?

Q That's right.

DR. GARVER: I think if you had a biomarker for that virus and you had validated it in a lab, then, yes, you could use genomics to identify that virus.

Q Okay. Now, in this case where the ocean-tagging studies only predicted 60 percent of the time, is this data that you would find suitable to create a diagnostic test from?

DR. GARVER: Well, like I said, you have to identify that signature to ensure that it is definitively just to that virus. For a diagnostic answer, you have ensure specificity and sensitivity. So in other words you want to ensure that you have no false positives, and to do that, you need a specific biomarker for that virus.

So if you're able to identify a specific signature for virus A, then, yes, you could use a genomic profile to diagnose that. But typically, if you know what the agent is, you're going to seek the agent, you're going to look for the agent. You're not going to use genomics as a diagnostic. You potentially could, but why would you if you know what virus you're looking for, you're going to look for the virus.

Q Okay.

DR. MILLER: And I'd like to add to that, and I think I've iterated this a couple of times. The genomics approach that we use is for discovery, and that's its sole purpose. It wasn't that we were going to run microarrays on every fish and predict their survivability. The point was we don't understand, or we didn't understand what was

undermining performance in the river. We used genomics to try to understand more about potential mechanisms that may be involved.

And in this paper it's very clear that we're not expecting to find a single physiological component that can predict all mortality. It would be completely unrealistic. Fish are hit with all kinds of other things in the river.

The important point of this work was that for a segment of the population that was coming back into the river they were so ill-affected that there was an effect, even before they hit the river, on their subsequent survival.

Q Now, the genomic signature affects different sockeye stocks differently in freshwater, I understand?

DR. MILLER: What we found was that we, in this freshwater one, we actually had more -- we had a larger sample size, we had more fish that were tagged. So we were able to derive a study that contrasted three different stocks, and the reason we were interested in that was that there's a lot of work that shows, you know, that stocks are differentially affected by different kinds of physiological components; disease being one of them, but we didn't actually set out to do disease here. We were interested in if we found something that was predictive of survivorship or premature mortality, how well did -- how predictive was that across different stocks.

What you need to understand about the three stocks that we looked at, was that they have difference in their susceptibility to high water temperature stress. And so I think the backdrop to everything that we have here is that the signature alone or the -- even a virus alone, in certain environments, may not have a negative impact. But when you put something that might compromise fish on top of stressful conditions in a river, like high water temperature stress - and my colleagues have done a lot of work on high water temperature stress - you have a greater potential of having a negative impact.

And so the stocks that would be most susceptible to high water temperature stress were the Lower Adams, which is a late run stock that's been entering the river early, and the Scotch

Creek. Chilko, and there's a publication out of Tony Farrell's lab, is a superfish. It can take high water temperature stress and it can have no ill effects that are measureable.

And so the stock that really showed the least proclivity to responding in a negative way to this signature was the Chilko fish, which -- which didn't have the double whammy of carrying the signature and being stressed by high water temperature. And that was one of our hypotheses for why that stock was much less affected. I have others, but we don't need to go into them.

Q So to summarize, for the freshwater study the survival of Scotch Creek fish correlated with the genomic signature, and Chilko, and I'm getting from your paper, Late Shuswap, as well, didn't correlate?

DR. MILLER: In the freshwater environment there was not a correlation between survivorship of those two stocks.

Q So you'd agree, then, that the freshwater tagging study is not consistent?

DR. MILLER: You know what's interesting about this signature is that -- is that the signature in the marine environment is suggestive of a very early stage recognition of a pathogen. It's an immunosuppressive signature.

The signature that we have in freshwater, although there are enough elements to find a good correlation between those signatures, is something of a change, in that -- in that you move from a very early stage recognition signature to a full-blown apoptotic, which means cell death, and stimulation of inflammatory response. And so it's more likely that it's at that point that if there is disease, that that's when disease is starting to come about.

And so one of the things that we hypothesize, and I think we might say something about that in this paper, is that at the point when fish are entering freshwater and swimming through freshwater, we hypothesize that it's possible that if there is a virus, that that virus might be being transmitted at that point. So when we're picking up fish at that stage, you know, there's fish that are already affected, but there may be more fish affected as they migrate.

And so if -- the point is that if we sample them as early as in the marine environment, if they already have that signature in the marine environment, they're more doomed. They would have had it for a longer period of time.

Q Have you done any follow-up studies on freshwater environments and similar to your *Science* paper in subsequent years?

DR. MILLER: we're doing that right now in 2010, and we have a 300-fish study, the same three stocks, so we'll be able to look at this relationship again in those same three stocks. The difference is we tagged all of the fish in the marine environment.

So, you know, for -- again bringing this back to practicalities, one of the points in doing this was to provide something that might be useful to managers. Managers want to know if there is predictability on -- on what the effects on salmon returns might be, they would like to know that before they hit the river. They would like to know that in the marine environment. This study was encouraging, that we could identify a signature associated with poor performance in the river before they made it to the river. So that's before they opened the major fisheries in Johnstone Strait and Juan de Fuca Strait.

And so we decided this year to focus, or in 2010 to focus our efforts on the marine environment, but still looking at mortality in the freshwater environment. Because if we were to develop tools from this, that's where the -- that's the point where managers want that ability to predict.

Q And do you know when those studies will be published or be available?

DR. MILLER: We just ran the microarray study. We actually got some -- and it's a -- the unfortunate thing is it's a new array, it's not the same array that we used. The array that we used here is no longer available, so we're using a different array which has a different complement of genes. But the results I've seen so far are very encouraging that we do have some important signatures associated with survivorship.

Q Now, I also understand you did a spawning ground study, as well?

DR. MILLER: The spawning ground study was -- it was a

bit of an add-on. There was a graduate student in Scott Hinch's group who was looking at factors associated with pre-spawning mortality at the spawning grounds. And so, yes, there was a tagging study and we were able to get her fish and use them in our study, as well. This came quite a bit later than the other studies, and this is what held up publishing this, because we wanted to wait for it to look at that third environment.

Q And I understand that 3.7 was the odds ratio for that study, and that as a result they were not statistically significant?

DR. MILLER: It was a very -- it really was we were looking to see whether or not it was consistent, but the sample sizes for that study were significantly smaller, and because that's all the fish we could get. So recognizing that we were really on the limits of being able to find something that was truly significant, the trend was still the same.

Q So then for the three studies, again, the genomic signature isn't predictive for a diagnostic test for predicting mortality. You'd agree with that?

DR. MILLER: I would say that the point, the first and foremost point of this study was to uncover potential mechanisms associated with poor performance in the river, and also to have a better understanding of whether or not salmon were already physically compromised, physiologically compromised before they reached the river. I would say we have absolutely done that.

I would say in terms of the predictive nature of this, this is a one-year study, and clearly one needs to do this in more years and with more fish in order to really develop something that's truly predictive. So I wouldn't want to go out tomorrow and say I have a test, you know, to predict mortality in any year in any stock.

Q Have you done any analysis on whether or not the differences in gene expression are a result of chance, or not a result of chance?

DR. MILLER: I don't know what kind of studies you're imagining. But randomizing samples, are you thinking technical, or I don't -- I don't understand the question.

Q Oh, I'm trying to find out the confidence level.

DR. MILLER: That there is a signature?

Q Right.
DR. MILLER: Well, I mean, we've demonstrated this same signature in gill tissue in multiple years. We've demonstrated a highly correlated signature in other -- in other tissues that do not correlate with any kind of technical effects of running microarrays. Those can, I mean, we can get correlations of over .95 between different studies for this signature. So I do think that we have validated that this is something biological as opposed to technical.
Q If we could turn to Figure 3 of Exhibit 558. As I see it there is -- maybe if you could explain this figure for the Commissioner.
DR. MILLER: Okay. This figure shows biological processes and the biological processes are shown on the left. Pro-virus integration is a biological process. These kinds of biological processes are called gene ontologies, and for every -- for every gene it is involved -- each gene is involved in a number of different biological processes. And so when we do an analysis that is called a functional analysis, we're looking at all of the genes that are present on the array, and what are all of the biological processes or GO terms, as what -- as people call them, that they are involved in. And then we're looking if we have a list of genes that defines a signature, what among those biological processes are statistically over-represented in that list of genes. And so that's what this figure shows are the biological processes that are found statistically associated with this particular signature.

So on the bars on the graph, the ones that go to 0 to -3, those are processes that are down-regulated, so that are -- that think of as pathways that are being turned off in fish with the MRS signature, and the ones that go from 0 to 3 are pathways or GO terms that turned on in the fish with the signature relating to higher mortality.
Q Now, I understand you had some complex results and in seven of the 40 biological processes some were at the same down-regulated and up-regulated?
DR. MILLER: That means you really can't describe, you really can't prescribe which direction the pathway

goes. That is quite common in microarray studies that, you know, sometimes it's very clear what your directional. But what you have to understand about -- about the technique is there are genes that can be negative effectors and genes that can be positive effectors of a pathway. So you can have a gene that actually when it's turned on, it's turning the pathway off. Right? And then you can have other genes that when it's turned on, it's turning the pathway on. So it is quite complex.

So when you get this -- when you get this mixed pattern where it's not really clear that most of the genes are being stimulated, then you have to go and say, okay, what's the effector of each of these genes? What do they do, and is it that you have ones that are being, you know, turned off are actually the repressor. So it can be quite complicated.

We didn't go into that detail here and most people don't. Most people simply show which ones are obviously being activated and deactivated, and that's what we've done.

Q I understand for the purposes of your *Science* paper the samples were taken from the gills, and that's not the ideal sample, and specifically the ideal sample are heart, kidney or brain. And I understand you've done that subsequently?

DR. MILLER: Yes. The reason we have to take samples from a gill when we're doing radio tracking is because it is a non-destructive tissue. You can't take the brain of a fish and have it swim to the spawning ground. So it really is, it really is our only choice. We can use gill, we can use skin, we can use muscle, but you really -- it's absolutely true that you are limited into what, if you're going to combine with radio tracking, you're limited in what kinds of physiological processes you can look for. And we have followed this up using destructive samples from other tissues.

Q Okay. So my question is I understand that you were only getting inconsistent results on an individual level where some would have brain tissue that showed the MRS and others would show heart tissue only that had the MRS?

DR. MILLER: I don't understand the term "only". But,

yes, we do find, and this really was highlighted in our 2005 studies, where we looked at the same fish over three different tissues and showed that the signature, the highly correlated signature was present in each of those, but the prevalence in each of those was really quite different. And the prevalence in that study, I believe was highest in the brain and lowest in the liver.

Q And I'm assuming there will be more research on that in the future, that you'll narrow that down and...

DR. MILLER: Absolutely. I mean, right now we're doing a lot of work on adults, other than the work that combines the radio tracking with the genomics. But we are doing a lot of work on smolts. So we will have a lot of information about -- most of -- we're running smolt studies using multiple tissues where we'll be able to say at an individual level what proportion of individuals carry this signature in one, two and three tissues.

I should add that with the virus, the parvovirus that we've identified in tissue that contains this signature, kidney is actually probably the best tissue we could have been using.

Q Now, just moving on to the histology samples that Dr. Marty analyzed for yourself.

DR. MILLER: Yes.

Q I understand that his conclusion was that they -- the lesions were related to blunt force trauma.

DR. MILLER: You're talking about the brains.

Q That's right.

DR. MILLER: So we sent in 2009 when we first observed what we thought were abnormalities in the brain, we had histological slides made of those. Those were from -- the slides were made in our Fish Health group. They took one -- they took two slices from the middle of the brain, and made slides into them -- or made them into slides. They took 12 brains total. All of those brains came from the spawning grounds.

You know, one of the issues is that when we're doing our genomics, as I said before, we're doing the genomics and we use the whole brain. So and the other thing is that when we're doing -- when we're doing the dissections or even the collections, we don't collect histology-grade brains. We collect this -- these samples for

doing RNA work, and the kinds of the chemicals one uses for that are different from the kinds of chemicals you would use for histology.

So the only reason I'm bringing that up is that those were the only brains that we had that were collected in -- that were either collected in a chemical and never frozen, which you can't freeze if you're going to do histology, or they were collected in histology chemicals. So although we'd seen these what we thought was anomalous in smolts and in other -- in other points along the migration, the only samples we had available to run histology on were spawning ground samples.

And so Dr. Marty got 12 brains that we had classified according to whether or not they contained these what we thought again were lesions. And when he read those slides, he concluded that they were likely arising from haemorrhages and they were likely arising from haemorrhages from as a sampling artefact, as an artefact of the handling of the fish.

Q I just want to make clear, I mean no criticism by that question at all.

DR. MILLER: No, I'm just being clear.

Q Yes.

DR. MILLER: I'm just trying to be clear.

Q Exactly. And I just wanted to clarify that point so we could nail down the brain tumour issue.

DR. MILLER: That's fine. Yeah.

Q Now, at this point you're not in a position to definitively identify the cause of the MRS as a novel virus.

DR. MILLER: The cause of the MRS as it -- are we -- okay, yes. We have not definitively established that the parvovirus causes the MRS. That is something that is the topic for our disease challenge work.

Q And at this point parvovirus in fish, this will be the first time it's ever been identified, if it is ultimately identified by yourself?

DR. MILLER: That is correct. This is the first time a parvovirus has been identified in a fish. They have been identified increasingly in lower vertebrates in the last decade. generally associated with mortality events.

Q And other parvoviruses have been identified in

humans, dogs and sea lions?
DR. MILLER: Oh, and shrimp and insects and ducks and geese, and a variety of other lower vertebrates, as well, and snakes.
Q And Dr. Garver, what are your thoughts on whether parvovirus is linked with the MRS?
DR. GARVER: I agree with what Kristi says. At this time we don't have the actual link to the fact that it is the cause of the MRS and that's what we're working towards.
Q Now, I understand that you have identified a 2,214-base pairing sequence?
DR. MILLER: That's correct.
Q And that's about 50 percent of the parvovirus genome?
DR. MILLER: Yes.
Q Does a partial signature necessarily mean the full sequence is there, or is it a possibility that it could be chance?
DR. MILLER: I don't -- I guess I don't see how it can be chance, given the kinds of data that we're seeing. Chances of exactly what? It's certainly not endogenous in the salmon genome. It's not something that is -- that is there in the DNA of the salmon. So I don't know where you'd pick up a partial viral sequence by chance.
Q And at this point have you done any histopathology to determine if the genomic signature or parvovirus is associated with disease?
DR. MILLER: That again is something that we're going to concentrate on with the disease challenge work. We have done a little bit of histology, taking some fish that were parvovirus positive from -- that were sampled from smolts sampled in the marine environment. The thing to recognize is that when we sample fish in the marine environment, at the time that we're sampling them, we're sampling live fish. We're not sampling at the time of death.

And I am not a histologist, but from what I understand of histology, the histology will become a lot stronger and more powerful and easier to detect if you're sampling fish at a later state of -- at the latest state of disease. And most -- a lot of histology that's done in concert with disease, but not all - Gary Marty has a study on herring that looked at wild herring - has

concentrated on fish that -- moribund fish, or fish that are sampled close to death.

So when he -- he processed these, we only looked at about ten or 12 samples and he didn't see anything that -- that through histopathology was really conclusively suggested that there was a histological feature that would be associated with mortality.

Q Now, while I understand you're getting closer to identifying parvovirus as the cause and studies are ongoing, there still are alternative hypotheses or differentials that it could be related to.

MR. TAYLOR: I just rise because the way the question's framed doesn't seem to accord with the evidence, and says "getting closer to finding that 'X' is the cause", as I heard you.

MS. CALLAN:

Q Well, what I meant by the question was studies are starting to -- there's different possibilities still. They're looking at one hypothesis, but at the other time considering alternative hypothesis.

DR. MILLER: I would say that's correct. I mean, at the moment we have enough to keep going with the parvovirus and keep going with the research to establish whether or not the parvovirus is (a) associated with the signature, (b) causative of the signature. If we find that it is not, then obviously we have a couple of things to do, one of which is to go back and say, well, okay, what else might be there that we're missing, that might be associated with this signature. And maybe there's, you know, another -- another infective agent that we have missed.

But I think we will still continue on with our work on this parvovirus, because we've identified a virus in sockeye salmon that is at a very high prevalence, and at a very high prevalence at the time point when sockeye salmon are entering the marine environment and undergoing some of the highest mortality that they do in their life history. And so I think that in and of itself makes -- warrants further study into this virus, even if in the end it doesn't happen to correlate with the MRS.

Q And, Dr. Garver, do you have anything to add?

DR. GARVER: No, just to the fact that we're following

the scientific methodology and approach. You take one step at a time. You need to confirm route "A" before you can go to "B" a lot of times. So that's the project that we're -- that's the track that we're on right now.

Q Now, I understand in your timeline document, and I'm specifically referring to provincial Tab 20, which is the earlier one from May, as opposed to the later one, that you identified a declining prevalence of the signature in the ocean, but could point to a potential impact of the signature or -- and then goes on a little bit to -- or early marine mortality, and then goes on, but cannot discount the possibility that some individuals recovered from the signature.

DR. MILLER: That's absolutely correct. What we're doing at this point in addition to doing the challenge work is to start -- starting to accumulate the information about how prevalent is this, and do we see shifts in prevalence and over space and time, or in years where we have strong year class strength and weak year class strength. When you're working with wild fish, looking at shifts in prevalence is one of the indicators that people use to try to pinpoint what factors might be involved in declines.

I should point out that there's studies on disease that have taken place in Oregon on wild -- on wild chinook and coho salmon from the Columbia system. And one of the things that they found, they look at BKD, and they look at -- they look at various parasites. And I believe Mike Kent was even involved in some of these studies. It's out of Kym Jacobson's lab. And what they have found over ten years, so they've been at this longer than we have, is that -- and they only sample -- and they only, I should caveat, they only sample fish in June in the ocean. What they have found over ten years is that they can't -- in years where ocean conditions are poor, they can't find a fish with BKD in the ocean when they go out and sample in June. Now, recognizing that when they go out and sample in June, the fish have been in the ocean for a month and a half or two months, if they go out into the ocean when the conditions are good, and good for growth of smolts, they can find up to 70 percent of the smolts that contain very

mild infections with BKD.

And they found a very similar result when it came to -- when it came to parasites. They can't find fish that have a heavy parasite load when the ocean conditions are poor. They can find fish with three or more parasites when the ocean conditions are good.

And what they have concluded is that fish disease is not tolerated when the ocean conditions are poor. Those fish that carry disease, simply don't survive. What they've missed in their studies, however, is that they don't link it to the freshwater, so they don't really know on an annual basis how many diseased fish might have gone out into that environment. And I would say, you know, that's something that we're interested in, as well.

So right now, with the declining prevalence, we're looking for patterns, and the signature in 2008 and in 2007 - mind you, small sample size - showed a declining pattern in the prevalence of the signature.

We have since now also focused on this parvovirus and we see the same thing. We see when we see the parvovirus that the highest prevalence is in the river. We see a declining prevalence in the early ocean environment. But hints to that decline may vary in different years, so the degree of decline that we observe may not be the same in every year. And that is what I'm focused on, is how much does it decline.

But you're absolutely right in that we do need to establish whether or not fish could recover, and/or or whether a decline is always going to be associated with mortality, and we have not established that.

The other thing that I would just like to say is that we, with the parvovirus, we have been sampling a broad range of stocks and we've sampled a broad range of stocks in the Fraser River. In last year, in 2008, there were over 400 fish collected in the marine environment in May and June in the ocean, and we looked at the presence of this parvovirus sequence in those fish, and the only stock that we could not find the parvovirus in that we had a sample size of over 15 fish for was Harrison. And we had 51 Harrison fish.

Harrison is the one stock in the Fraser River that is increasing in productivity. Why that's important is that we're looking for patterns. Okay? We don't have all of the data to show disease and to show mortality yet. We're working towards this. We are working with wild organisms. But everywhere we turn we see indications that indicate that what we're looking at could have a negative impact at this life history stage.

MS. CALLAN: Thank you, those are all my questions.

MR. MARTLAND: Mr. Commissioner, next on the list of counsel, I have counsel for the B.C. Salmon Farmers Association at 30 minutes.

Indeed, maybe just to clarify the record, then. I think there was a document on screen that may not have been marked. And perhaps I can just, by way of a interjecting question without taking anyone's time, I hope, confirm, Dr. Miller, is that an earlier draft of the timeline document that had been provided and put into evidence as 1517, Exhibit 1517?

DR. MILLER: I can't tell if it's early till you see the end.

MR. MARTLAND: All right. Maybe we can. Is the best way to do that to go to the very end, and perhaps the equivalent of "DD".

DR. MILLER: Yes, if it says July, that's the most recent one.

MR. MARTLAND: Okay.

DR. MILLER: That's the old one, because I see it ends in May.

MR. MARTLAND: Thank you. If this might be marked, then, as the next exhibit.

THE REGISTRAR: It will be marked as 1522.

EXHIBIT 1522: Miller, Timeline of Genomic Research relating to the Mortality-related Genomic Signature Hypothesized to be associated with a potentially Novel Virus, May 2011

MR. BLAIR: Mr. Commissioner, for the record, Alan Blair appearing for the B.C. Salmon Farmers Association. I note the hour. It's three o'clock, and I'm in the Commissioner's hands whether we take a break now or later.

THE COMMISSIONER: You can carry on, Mr. Blair.

MR. BLAIR: Very well, thank you, then.

CROSS-EXAMINATION BY MR. BLAIR:

Q Drs. Garver and Miller, I'll start firstly with you, if I may, Dr. Miller. We've been speaking of course in this Commission about the effect on Fraser River sockeye. But I believe that the studies you've done and the reports that you've been discussing today indicate that you found this signature in a variety of salmon stocks, not only in sockeye; is that correct?

DR. MILLER: That is correct. We have observed it in chinook and much less powerfully so in coho.

Q And in any of the other species, or have you looked?

DR. MILLER: We haven't looked, but we have -- we now have some sample collections of pink and chum, so we will be looking at them.

Q And what about the distribution, where were these fish from that you found the signature?

DR. MILLER: Most of our work has been about the Fraser River, but some of our coho and chinook have extended to East Coast of Vancouver Island and Burrard Inlet stocks. We have in our chinook salmon work extended as far as the Columbia River system, and we do see this signature in the Columbia in chinook.

Q I have a note here, and perhaps you can just confirm whether this is correct or not. But have you also found the signature in some of the rivers to the north? I'm thinking the Skeena, the Nass, Stikine.

DR. MILLER: We have not. I believe we might have looked at a fish or two, but we really haven't got -- we haven't looked at a lot of samples to the north. We have some. Dr. Trudel conducts high seas surveys every year, multiple times a year, that go up to southeast Alaska. So we do collect and we run stock ID, so we know where the fish from those collections come from. And so we do have some fish that are from more northerly stocks and we will be running them, but we haven't really done a lot of work on them yet.

Q So are you able to say whether you found the signature in any of these northern stocks, or that work is yet to be done?

DR. MILLER: I am not able to say that right now.
Q And I think earlier in the day we heard reference to Haida Gwaii, that's of course a jurisdiction, and it's also an area that people often refer to in terms of where the stocks migrate past. Have you received information in terms of a signature in the Haida Gwaii area?
DR. MILLER: In returning adult salmon, we do see the signature in fish in the Haida Gwaii, yes.
Q And what about the Strait of Juan de Fuca?
DR. MILLER: Yes, we see the signature there, as well.
Q Now, of course everyone's been very curious about your work, and that includes my client, the B.C. Salmon Farmers Association. And is it true to characterize the discussions you've had with the B.C. Salmon Farmers generally, and maybe more specifically with Mary Ellen Walling, the Executive Director of the Salmon Farmers Association, that you've indicated to the association that the data you have to date doesn't point to a strong involvement of salmon net pens in the transmission of the virus to migrating salmon?
DR. MILLER: We have no direct data on aquaculture fish. However, the finding that fish are leaving the river with the highest prevalences of this would stand to suggest that a lot of the transmission of this virus - and I'm talking the virus right now, but one could say the signature, as well - because the highest prevalence of the signature is also in freshwater, seems to emanate out of the freshwater environment. That doesn't mean that there couldn't be transfer in a marine environment, but it does mean that we don't have data pointing to that.
Q And also in your discussions with the people at the BC Centre for Aquatic Health Sciences, sometimes referred to by its acronym, CAHS, you've also had discussions noting that the signature present in the returning adult salmon migrating through Haida Gwaii, the signature has shown up before they would have encountered the salmon farms closer down, further south?
DR. MILLER: That is correct.
Q Now, there was a reference just a few moments ago about the Harrison stock, and I think I understand that in the samples you've done of the Harrison

stock, you've not found the signature in that stock?
DR. MILLER: We've looked at 156 samples now. I only talked about one, what we looked at last year at kidney tissue, but we've also looked at liver tissue and brain tissue, and we've looked at 156 different fish, and we haven't found a single positive smolt from the Harrison.
Q Now, the Harrison sockeye have some of the shortest residence time in freshwater in the Fraser system?
DR. MILLER: Yes, they do.
Q And is it true to say that in terms of the relative prevalence rates, your studies have shown the highest -- amongst the highest prevalence rates in those sockeye salmon from the upper reaches of the Fraser, in other words, those with the longest residence time in the freshwater environment?
DR. MILLER: In 2010, certainly that did appear to be the trend, that the higher prevalence was in -- was in stocks that were further up the river.
Q And so are you able to draw any conclusions, or have you drawn any conclusions in terms of the relationship to the relative prevalence and the residence time in the freshwater systems?
DR. MILLER: Well, the unfortunate thing is, and maybe this will be easier in chinook where we have more stocks within the Fraser River that have those alternative life histories. I mean, Harrison fish are the only Fraser River stock with a life history that puts them in the river for less than a year. And so that chinook salmon, they, you know, we have ocean type and stream type chinook salmon stocks and we are interested in that question, whether or not that relates to the difference in the life history strategy, or something unique about Harrison.
I should say that we did find positives in the Birkenhead system, which Birkenhead fish actually swim by Harrison, by Harrison Lake in order to get to Birkenhead, and we do see positives in Birkenhead.
Q And is that an anomaly at present you're not able to explain, or just not sufficient evidence?
DR. MILLER: We need -- we need more data to try and understand it. But I think, you know, we're doing

a study right now, which is -- which is contrasting Harrison and Chilko in sockeye, and a variety of chinook salmon, stream type and ocean type stocks. And we're not only looking for this signature, we're looking for other physiological factors that may differentiate them. Because just like in Harrison and the other stocks in the Fraser River, in the chinook salmon, the stocks that are in the worst decline tend to be those that have a life history more like the bulk of the sockeye salmon. So the fish that spend less time in freshwater tend to be doing better than those that spend more.

Q Now, you made reference to the phrase "lifecycle", and you also referred earlier today to the recent communication, and I believe also communication with the salmon farmers that was not so recent, you've been endeavouring to coordinate the sampling with the assistance of the salmon farmers, and you now understand that fish will be coming from the various companies that make up the B.C. Salmon Farmers Association?

DR. MILLER: Yes. That's absolutely correct.

Q And I think it was Dr. Garver who spoke about the protocols necessary for doing work, it's your intention and your understanding that the B.C. Salmon Farmers will cooperate and provide a whole series of lifecycle stages of fish from a variety of different farms across the spectrum of the industrial salmon farms. Is that your understanding? I see you looking to Dr. Garver.

DR. MILLER: Oh, I thought you were asking him.

Q No, no, I was asking you.

DR. MILLER: I don't think he knows, because he wasn't involved in those initial discussions.

Q All right.

DR. MILLER: So I was looking at him, wondering if he was going to answer that.

Q Well, he might be, he could try.

DR. MILLER: No, that is my understanding. Again, you know, I've really only emailed back and forth with Mary Ellen Walling. I haven't spoken with the different vets. But I am told that that they are on board with providing those samples, yes.

Q So within the lifecycle and also from multiple farms.

DR. MILLER: And I did make a mistake, I called them

the samples from the river, and I meant from the hatcheries, from freshwater and in the marine environment --

Q Yes.

DR. MILLER: -- previously.

Q I wonder if we could just, Mr. Lunn, pull up Exhibit 1521, and go to page 13, please. This document, before he flashes past the front page, is you've told us when this was written, Dr. Miller. I don't have a note of it. Do you recall?

DR. MILLER: This was provided to the Pacific Salmon Commission in June of 2010.

Q All right. And at the bottom of page 13, please, Mr. Lunn.

DR. MILLER: Hopefully I don't have a date problem here again, but...

Q Yes, the last sentence, you've written:

> Given the high prevalence before fish leave the river, salmon aquaculture is not likely a main route of transmission to wild salmon.

We've covered that point already. I just wanted to -- firstly, these are your words, this is your report, correct?

DR. MILLER: Yes.

Q Nods don't always transcribe quite as well.

DR. MILLER: Sorry. Yes, it is.

Q Quite all right. Lawyers are usually guilty of that. So this is -- this was your opinion back in June of 2010.

DR. MILLER: Yes.

Q And it really accords with your current view, as well, as a result of the recent discussions you've had with the salmon farmers, you've repeated this, you've not changed your point of view in this regard, have you?

DR. MILLER: Not particularly. It doesn't dismiss the potential of transfer back and forth between wild and aquaculture fish when they're passing salmon farms, but again, I would say that the main time point of transmission appears to be occurring in freshwater.

Q And the last comment about not removing that possibility, you say that, but it's purely speculative because to date you --

DR. MILLER: Absolutely. We have no information about Atlantic salmon aquaculture fish.
Q Even whether they possess the signature.
DR. MILLER: Even whether they possess the signature, or the virus. That is what we're hoping to gain by working with the industry.
MR. BLAIR: Thank you. Mr. Commissioner, did you want to take a short break now?
THE COMMISSIONER: Yes, thank you.
MR. BLAIR: Thank you.
THE REGISTRAR: The hearing will now recess for 15 minutes.

(PROCEEDINGS ADJOURNED FOR AFTERNOON RECESS)
(PROCEEDINGS RECONVENED)

THE REGISTRAR: Order. The hearing is now resumed.
MR. BLAIR: Thank you, Mr. Commissioner.

CROSS-EXAMINATION BY MR. BLAIR, continuing:

Q Dr. Garver, these questions are for you, and they relate to IHN. My question, in a general sense, is there any evidence that the prevalence of IHN stocks in B.C. sockeye salmon have changed since the 1990s?
DR. GARVER: So a predecessor of mine, Garth Traxler, began a surveillance program for IHNV in various sockeye salmon stocks, and so we have -- it's actually one of the few diseases or pathogens that we have a very long-term monitoring program for, and he started this back in 1986. And what we found is that the prevalence values vary considerably from year to year and between stocks, and since that monitoring period there were a few outbreaks in salmon farms. And when we compare those times during the outbreaks to the stocks that we are looking at for IHN prevalence, it didn't appear to change the prevalence in the wild stocks. In other words, it wasn't a driving factor for the occurrence IHNV in the wild stocks.
Q And in that work, sir, did you find whether there was any correlation in the IHNV prevalence as between adults and its occurrence in fry?
DR. GARVER: No. And that was the big motivation behind beginning the monitoring program, is to establish something where we could predict the

occurrence of IHN disease in our wild stocks, and so Garth Traxler had looked at the adult -- the prevalence in adults, and in the subsequent year the fry from those adults, looked at the prevalence in there. And when we run the correlations, there is no correlations between the prevalence in adults and those that occur in its offspring the following year.

Q And I think another part of your work in your summary could be summarized as this; that is, that your work suggests -- has suggested that IHNV is not a major contributor to the long-term decline of these two stocks, and by the two I'm referring to the Weaver Creek and Nadina River?

DR. GARVER: That's correct. There has been episodic events which have caused catastrophic mortality, particularly in the Weaver Creek. Garth Traxler documented this in, I believe it was, a 1987 publication that the outbreak occurred in 1986, and it killed that Weaver Creek, it killed about 50 percent of the fry, so there was a dramatic impact at that, but it was epizootic in that it wasn't reoccurring every year. And so from what we have to date is dated to suggest that, yeah, if we're looking at a long-term trend where IHN or reduced productivity in the Fraser stocks, the sole factor wouldn't be IHN.

Q So noting the outbreaks as you've just done, it is correct to characterize that IHNV was not a major contributor to the long-term decline of the stocks, but you had spikes when it went through those two systems?

DR. GARVER: There are spikes, that's correct. And the problem is, it's very difficult with diseases. There could be compounding factors. So if you have other diseases or other environmental factors that increases the susceptibility to that disease, a lot of those we don't have determined and IHN would fall into one of those categories we don't know all the predisposing factors to disease.

MR. BLAIR: Thank you, Dr. Garver. Thank you, Dr. Miller. Thank you, Mr. Commissioner.

MR. MARTLAND: Mr. Commissioner, counsel for the Aquaculture Coalition is next, with 65 minutes. That'll run us till 4:00 and then continue tomorrow, I expect.

MR. McDADE: Dr. Miller, Dr. Garver, my name is Gregory

McDade, and I am counsel for Dr. Alex Morton and for the Aquaculture Coalition.

CROSS-EXAMINATION BY MR. McDADE:

Q Just in starting, Dr. Miller, my client has instructed me to say that we want to thank you for your courage and for the fascinating work that you've done on these studies. It's obviously very important.

It's a bit of a detective story, as I hear it, unwinding some of this, and clearly we're in the middle of a scientific process. So as I understand it, you weren't looking for a disease or a virus when you started this work, you were looking for the explanation for early entry?

DR. MILLER: Early entry and for survivorship in the river, yes.

Q And what you found is what is likely but not proven to scientific certainty yet, some sort of new virus?

DR. MILLER: That is correct. We have identified a novel virus, meaning it hasn't been described before. The sequence of a novel virus in salmon that contained the signature that we identified in the *Science* paper.

Q And your current leading, if I can put it, suspect in this matter is the parvovirus?

DR. MILLER: At the moment, that is our candidate virus.

Q And you haven't confirmed it's parvovirus, that's what you're working on?

DR. MILLER: If the question is, we haven't confirmed it's parvovirus that causes the MRS --

Q Yes.

DR. MILLER: -- that is correct. That is what we hope the disease challenge work will do.

Q And for a couple of years, or certainly in a lot of your early material, your leading suspect was salmon leukemia virus?

DR. MILLER: Yes, it was.

Q And as I understand it, you haven't ruled out salmon leukemia virus, at this point?

DR. MILLER: No, I have not. It has to be clear that the salmon leukemia virus, itself, has never been isolated. There's no sequence information for it. So there is a postulated virus associated with

plasmacytoid leukemia, and the work, unfortunately, of the investigators of plasmacytoid leukemia never identified a specific viral agent associated with that disease. It is still possible that this parvovirus could somehow relate to that.

Q Right. Because SLV was never actually -- what was your term?

DR. MILLER: It's never been isolated in sequence, so there is no cell culture of it, there is no sequence of a virus, there's no confirmation that a virus actually existed, direct confirmation.

Q And similarly, you haven't successfully cultured parvovirus?

DR. MILLER: We have had equally difficult and lack of success in terms of culturing the parvovirus yes.

Q So we're really in the same place with those two viruses, at this point?

DR. MILLER: At this point, we certainly don't have evidence that it's not that, but we don't have any evidence that it is, directly.

Q And the symptoms, what led you to first suspect salmon leukemia virus is that the symptoms you were finding were quite a bit similar?

DR. MILLER: Yes. You know, some of the symptoms that I talk about are things that I hear from the field. People who are on the ground sampling sockeye salmon, David Patterson is my collaborator that's on the ground, and his team, and oftentimes they have noted, you know, associated with these mortalities in the river, you know, the fish look really healthy, they look really good externally, sometimes they have pale gills, sometimes they see to have bleeding disorders, but not looking through histology but just simply looking at the condition of the fish from an external standpoint, they look really good and healthy, and those are sometimes the kinds of things that people would say when fish were, at least that I had heard, when fish were dying of marine anemia, that they were fish that actually looked good, externally, not necessarily through histology, that had pale gills and they were simply dying.

And so I thought that that was an interesting parallel. And the other interesting parallel was that, you know, the pale gills is an indicator of anemia and the marine anemia, or plasmacytoid

leukemia, you know, is an anemia-related disease. We've seen anemia-like symptoms in sockeye salmon as well.

Q And really, the primary similarity is immunosuppression, if I've pronounced that correctly. They're both diseases of immunosuppression.

DR. MILLER: A large number of viruses, and Kyle can speak to this probably better than I can, but, I mean, many viruses can induce immunosuppression but, you know, yes, that is potentially another comment feature.

Q And I understand that the suspect salmon leukemia virus was a retro virus, which -- and the parvovirus is a DNA-based virus?

DR. MILLER: As far as I understand it, and you had the two experts sitting here the last two days, and you will have another expert, Sonja Saksida from CAHS, here in another week, week and a half, who will be testifying. She did a masters degree on plasmacytoid leukemia as well. And as I understand it, the evidence that it was a retro virus and not some other kind of virus was two-fold. One, that they had positive RT assays; and, two, that they thought that they observed tumours behind the eyes of the fish that carry plasmacytoid leukemia. Now, I'm sure you were listening when Mike Kent was testifying in the last couple of days, and he seems to have backtracked on whether or not those lesions behind the eyes were, in fact, tumorous, or whether they could have been inflammatory cells, and I was quite -- that was the first time I'd ever heard that mentioned.

So I guess I'm not -- he's not, now -- he doesn't appear to be strongly convinced that it is a retro virus anymore, and so I'm a little bit less convinced that it has to be a retro virus associated with that and not something -- some other kind of virus.

Q So is it fair to say that at this point you haven't ruled out a retro virus or a DNA virus, it could be either?

DR. MILLER: We, in sequencing about 250,000 different reads of RNA, we did not uncover any retro viral sequences that were not already endogenous in the salmon genome. But that's not, you know, that's

the most intensive sequencing one can do. When heart and skeletal muscle inflammatory disease, when they identified a real virus in association with that, out of a couple hundred thousand reads, they only got one 240-base sequence one time out of that, that turned out to be important and they went back and they did another 500,000 reads to actually get more of the virus. So it's not impossible that there could be other viruses, you know, contained in fish that carry that signature, but right now my feeling is we need to follow through the parvovirus, see whether that could be causative. If it's not, we'll go back and see what else there might be.

Q And HSMI, or heart and skeletal muscular, is currently a disease causing significant problems in Norway's fish farms?

DR. MILLER: Yes, it is, and it's a disease that has been under study for over a decade and caused a lot of problems for over a decade, and it is only -- and they have been trying to isolate and trying to identify a pathogen associated with it, and they finally came up with a sequence. There's some, still, question as to whether this particular virus is absolutely causative as well. This stuff takes time. But it's only because they used a, really, a genomics approach that they were able to obtain a sequence, finally, after 10 years of studying this.

Q So is it possible it could take us a number of years to actually nail this virus down?

DR. MILLER: I'm sure hoping not. And, you know, we've cut a lot of corners and I think we've come really far and really fast, but there are some experimental studies that have to be done before we can move too far forward.

Q Dr. Garver, is it possible it could take a year or longer to identify this virus, if ever?

DR. GARVER: Have you had a science class, because that is science. That is pretty much the definition of science. It will take a considerable amount of time, yes.

Q Well, it's been a considerable amount of time since I've had a science class.

DR. MILLER: Well, I should just mention, we do have a candidate virus, so if you're saying, "Identifying a virus," we have identified a candidate virus at

this time.
Q So it's possible that, as I understood your earlier answers, it's also possible that the disease that was being identified, or the virus that was being researched by Dr. Kent back in the nineties might, in fact, have been parvovirus?
DR. MILLER: That is definitely possible. The difficulty with trying to relate that disease or that syndrome with the parvovirus is that there don't appear to be tissue samples of fish that carry marine anemia available to compare to the samples that we have. And because there is no one studying that particular syndrome or disease - usually they're called a syndrome unless you have an etiological agent, and then they can be called a disease; I think we learned that in the last couple days - but, you know, it makes it difficult. And I guess if I -- if we can't find someone who's actually studying that and diagnosing marine anemia, it will be very difficult to determine whether or not they are the same thing. Perhaps with histology, if we can do the challenge work and find disease and mortality, perhaps one can look at the histological signatures from the parvovirus and determine if they're anything like what's been observed in marine anemia. That, at the moment, is the only sort of indirect way we've got.
Q Okay. So whether this is parvovirus or SLV -- well, let me ask it this way: If this is parvovirus, it's never been seen in fish in B.C. prior to this time?
DR. MILLER: We did not know if its existence prior to this.
Q In fact, I think you're probably on the cutting edge here. It's really the first time it's been identified in fish?
DR. MILLER: Parvovirus, yes, it has never been identified in fish.
Q So when you get to the point, if you do, of sequencing this, it will be a new virus?
DR. MILLER: When we have the full sequence and -- yes.
Q So you'll get to give it a name, I suppose? If it's like astronomy, it gets to be called Miller Virus?
DR. MILLER: It will get a name when we have a full sequence.

Q All right. Because parvovirus is just a generic type of virus, right? It'll be called something --
DR. MILLER: It'll have something to do with salmon, probably.
Q Right. All right. So whatever its name, whether we call it Miller Virus or something else, it is quite -- what we do know, from your work in science and the last four years of research, is what we do know is that it is associated with a whole early entry phenomenon and the en route mortality?
DR. MILLER: There is an association in the 2006 study of the MRS signature with more rapid entry into the river and actually faster migration to the spawning grounds. That study needs to be repeated in other years to ensure that that signature is related to rapid entry into the river in other years, and that's something that we will have from our 2010 data. We have not shown that the parvovirus, itself, is associated with that but we certainly have the samples to do that.
MR. McDADE: Mr. Lunn, if I might just put two or three documents up on the screen. They're all related, I think. Let's start with Exhibit 1516, which we looked at earlier today. Now, this document has been identified, and I understand the comments in it were Dr. Garver's, so this is a draft of a document that was later finalized. Can we have Commission document 21 up on the screen.
MR. TAYLOR: Just on 1516, I'm not sure if it's ever been finalized or if there's evidence of that.
MR. McDADE: Well, that's what I'm about to ask about, I think.
MR. TAYLOR: Well, you just started by saying it was later finalized.
MR. McDADE:
Q Well, I think this is the final version, is it not, Dr. Miller? This is a version dated October 7th, 2009. It seems to be a very close correlation with the document we just looked at. I think this is the latest version that I've seen, but I stand to be corrected.
DR. MILLER: This was not the final version of a briefing note, if that's what you're asking.
Q All right. In any event, this document was prepared by you on October 7th, 2009?

DR. MILLER: It was. It was prepared in conjunction with the talk that I gave, the intradepartmental talk that I gave associated with the same title.
MR. McDADE: Mr. Lunn, could we put up DFO 59898. That's one of the later documents that have -- were produced this week by the Conservation Coalition.
MR. LUNN: 598, I think it's a six-digit code. There's a digit missing.
MR. McDADE: Sorry, I'll get it for you. 598981.
MR. LUNN: There are three files associated with that. I'll bring up the first one to start.
MR. McDADE: Yes, that's the one I'm looking for.
Q That's also prepared by you, Dr. Miller?
DR. MILLER: Yes, that looks to be a slightly earlier version.
MR. McDADE: So could we have those two versions marked as exhibits.
THE REGISTRAR: Tab 21 will be marked as 1523. DFO 598981 will be marked as 1524.

EXHIBIT 1523: Epidemic of a Novel, Cancer-causing Viral Disease may be Associated with Wild Salmon Declines in BC, by Kristi Miller, dated October 7, 2009

EXHIBIT 1524: Epidemic of a Novel, Cancer-causing Viral Disease may be Associated with Wild Salmon Declines in BC, by Kristi Miller, dated September 27, 2009

MR. McDADE: Let's just stick with that particular document for a few minutes.
Q So in the first -- in the bullet in the middle of the page, the first open bullet, you note that the salmon starting from 300 kilometres seaward had a 16 times lower probability of arriving to spawning grounds in terms of the healthy signature. And in the second bullet, that there is -- it may be associated with losses of up to 90 percent, if you count river entry timing losses.
Have you made a calculation of how many fish that might actually involve in the -- we're talking about many, many millions of fish, aren't we?
DR. MILLER: Yes. This was based on the prevalence of fish containing the signature and I'm trying to --

it's the second bullet you're talking about, right?
Q Yes, thank you.
DR. MILLER: Oh, yeah, well, and it's also to the additional physiological information, so Scott Hinch and his group have also found physiological indicators associated with advance entry timing and losses in the river, so it was including sort of his estimates as well.
Q And can we go to document 15 -- Exhibit 1512. And if we could scroll down to the final bullet there. We looked at this document this morning as well. I see there that you've done a calculation saying if the decreases were really from the causes of mortality, in 2008 there may have been as many as 27 million salmon --
DR. MILLER: In order to see the decrease in prevalence that we observed, if that decrease in prevalence were to be due to mortality, and that was something that still needs to be demonstrated, that that were how many fish basically that were missing that we didn't see in our second -- in the second sample period.
Q And can we go to Exhibit 1513 and go to page 6. We also looked at this, this morning. I just want to try to understand this. And this is a comparison between 2007 and 2008. So in 2007, you found a much heavier prevalence of the MRS in the smolts than you did in the 2008 smolts.
DR. MILLER: That's correct. It was a small sample size, because that's all that was available to us, but most of the fish that we sampled in the ocean at the end of June contained this signature in 2007, whereas it was less than 40 percent in 2008. We have actually, since, amplified parvovirus out of these same fish and we see the same phenomena.
Q The same phenomena was --
DR. MILLER: We see a much higher prevalence in 2007 than we do in 2008.
Q And if, in fact, the mortality is related as we just discussed, that would seem to indicate to me that the impacts in the 2007 smolts or the 2009 fish, would be much heavier than that of the 2008 smolts, 2010 fish?
DR. MILLER: Yes, potentially.
Q So we could be talking about many, many millions of fish here?

DR. MILLER: I did a calculation somewhere in one of these talks, but yes, we're talking in the order of, I can't remember what it was, three or four times more fish, in the least, between those different years. We're talking millions of fish, yes.

Q And so is it fair to suggest that this particular MRS, if it turned out to be the virus and if it turns out to have the mortality that you've speculated about, really could be a very, very significant explanation for the 2009 decline?

DR. MILLER: If we can demonstrate that this virus causes disease and has -- and mortality of fish in the early marine environment under certain circumstances, it doesn't necessarily have to be every year, I certainly expect that the role of the environment will be a strong one, but if we demonstrate that when fish are entering the ocean and they become stressed in the ocean and they carry a high load of this virus, that we see significantly enhanced mortality, they're certainly given the prevalence rates of fish that we see in certain years with this parvovirus there is certainly the potential that this virus could have a major impact on salmon declines.

Q And if, in fact, that's the case, using the terminology that we heard yesterday, this, in fact, may be the smoking gun for the 2009 declines?

DR. MILLER: It could be the smoking gun.

Q And we have heard you, I think, say, although this matter is not proven, yet, to be a virus that causes disease, you're prepared to say that's your strong speculation that, in fact, that will be proven?

DR. MILLER: I have some level of confidence that we will find disease with this virus, but we do have to do the work.

Q Now, if I could go back to 1524, and if I could go to page 3 of that document, and if I just look at the last bullet on the page, which is, I think, the end of the document -- oh no, sorry, the end of that section:

> There are several elements of the history and timing of descriptions of PL/SLV that potentially implicate this virus in the

large-scale declines of coho and Chinook salmon in BC, and may be suggestive of a role in hatcheries and aquaculture in this decline.

You wrote that at the time, didn't you?

DR. MILLER: I think I should be clear. I was a reviewing a literature that mostly came from Mike Kent and Bill Eaton and others who had done this -- who studied this disease. I wouldn't -- I'm not an expert on plasmacytoid leukemia, but in my purviewing, and I think you've seen the document that I made when I was originally interested in this disease, looking at the timing of various events and looking at the timing of when this was discovered, et cetera, it was my view, at the time, that it was a very interesting disease and it was largely overlooked, and I was interested in whether or not, (a) it could be related to what we were observing in sockeye, and if it was related to what we were observing in sockeye, whether or not it could be a factor in declines of multiple species.

Q So could I turn over the page. And you prepared a chart on the next page.

DR. MILLER: That's the one I'm talking about.

Q Yes. And when you went -- when you were speaking at this time and to the PSC, I've seen on a number of documents that you refer to it as the timing issues. This is one of the arguments at the time you considered in favour of the SLV hypothesis is the correlations in timing between these various matters happening at the same time, isn't it?

DR. MILLER: Yeah, the one thing that, given what we know, now, that would need to be removed from this, however, is that we didn't have ocular tumours, and so all references to that, since we saw haemorrhaging in the ocular lobe as opposed to tumours, that that data would not relate, or would not be validated at this point, or would not be accurate.

Q All right. That seems quite reasonable. But the key issue about the timing here, as I understand it, is that the connection that was present in your mind then, and is still in your mind today, with early entry, that's a behaviour that goes back to the early nineties?

DR. MILLER: Yes. 1996, really. The early entry behaviour in sockeye salmon started in 1996.
Q Right. And so that would have been the generation of the brood stock from 1992?
DR. MILLER: That's correct.
Q And the declines in productivity that we've seen in the sockeye salmon that is behind this Commission's mandate really dates back to about 1992 as well, doesn't it?
DR. MILLER: In the focus on sockeye salmon and early entry and for --
Q The decline --
DR. MILLER: The decline --
Q The decline of productivity.
DR. MILLER: I think it goes about that far. Now, one thing I would also like to correct here, is that - and Mike Kent is the one that corrected this - that they actually did not observe positive sockeye salmon in 1991 in their surveys. That was unclear to me; I thought that they had.
Q Because they never looked for it; is that right?
DR. MILLER: They did a very cursory look.
Q But there's no question that marine anemia or plasmacytoid leukemia, or whatever that disease was, if it was parvovirus at the time, it was killing huge amounts of Chinook fish in fish farms from 1988 to 1991; that was an important fact to you at the time, wasn't it?
DR. MILLER: That was of some import to me, but I'm not the one who observed that, so I'm probably not the one who should report on it. But yes, that it had been killing fish, Chinook salmon, during those periods of time, yes, it was something that I thought was important.
Q Now, today I heard you say that you'd tend to suggest that aquaculture might not be directly implicated because of the fact that the smolts coming out of the river have this MRS. And I can see the logic behind that. But that doesn't answer the question of where this disease came from in the first place, does it?
DR. MILLER: It absolutely doesn't, no.
Q And it's quite possible that the -- because you find the adults who have come past the fish farms, or sorry, let's just say the adults coming back to the river show this MRS in a group to a great deal and they're the parents of the smolts, right?

DR. MILLER: That's correct. They show the signature regardless of which route they take around Vancouver Island, but yes, they show the signature coming back.
Q So that suggests two possibilities to me. One, is the possibility you refer to in this document, which is the disease is vertically transmitted; that is, it's transmitted from the adult fish, through the eggs to the young fish. That's a possibility, isn't it?
DR. MILLER: It certainly is not unusual for parvoviruses to be transmitted vertically. However, there was a -- it was an interesting review, I think, that the B.C. Salmon Farmers Association put in by Dr. Lewis, who's a virologist, who suggested that he felt that the probability for vertical transmission was low, because in other species where vertical transmission with parvoviruses was a common route of transmission, you saw loss of the fetus, and he concluded that you would have losses of eggs. Kyle could really respond to this better than I could. We have discussed this. I would say we really don't have any data on this, and it would be pure speculation.
Q It is pure speculation. It could be vertically transmitted; it may not be. But that would be one mechanism which would explain why the adults had it and the babies had it?
DR. MILLER: Yes, and that is something that we are looking at, earlier life history stages, to find out how early we can identify this parvovirus out of fry.
Q And right now the earliest you've identified is in smolts; isn't that right?
DR. MILLER: In terms of the signature, the earliest we've identified it is in November before a fish is going to smolt, in their natal rearing areas. So before they leave their natal lakes.
Q So that would tend to suggest it's vertically transmitted, wouldn't it?
DR. MILLER: It doesn't, necessarily. It can still be horizontally transmitted in the natal lakes.
Q From adults?
DR. MILLER: Want to jump in, Kyle?
Q Yes, go ahead, Dr. Garver.
DR. GARVER: I'll just step back here a little bit. As

a scientist, I'm really concerned with all the speculation that's going on here. We have a parvovirus sequence. We don't have it linked to a disease. We don't have it linked to mortality. We don't know how it's transmitted. We don't know if it causes disease. We don't have any pathology associated with it. So if we're sitting around discussing scientifically hypothesis, this is fine, but if we're actually trying to get to some answers, it's pure speculation.

DR. MILLER: Yes.

DR. GARVER: Now, in addition to the other questions that are being asked, yes, there could be multiple reservoirs. Just because we're finding it in salmonids doesn't mean it's not in other fin fish that reside in a lake. So yes, it could potentially be in other species in a lake and could, therefore, be transmitted horizontally. But again, this is pure speculation since we don't even know if it's transmitted, nor do we know if it's infectious.

Q All right. Well, I apologize, Dr. Garver, if we're not yet meeting the scientific standards that you have for proof, but it's equally pure speculation that it's not coming from aquaculture, then, isn't it?

DR. GARVER: We don't know.

Q That's right.

DR. GARVER: We don't know where it is --

Q No.

DR. GARVER: -- and what species it's in. Right now, we don't even know if it's a true virus, other than the fact that we have a sequence.

Q Dr. Miller, it must have caused great consternation in the DFO when you put that paragraph in connecting it to aquaculture, in 2009, didn't it? You got some blowback on that, didn't you?

DR. MILLER: What paragraph are you talking -- I'm not sure --

Q Can we go back to Exhibit 1524, then. Just back to that page, just the previous page. That last paragraph there. The first sentence.

DR. MILLER: I would say there was concern, but I don't think there was a large pushback.

Q Well, if we cam go to 1523, which is -- could we go to the same place in that document, just above

number 4, which would be page 3. Now, what I see here is that particular -- when I compare these two documents -- Mr. Lunn, can we put this up, both documents up on the screen at the same place? So that should be enough. What we have there is document one, on September 27th, we have the same five bullets and then a paragraph, and in document two we have the same five bullets and no paragraph. It seems to have miraculously disappeared. Was that because of pressure you received inside the Department?

DR. MILLER: I think there was some concern over the speculative nature of that comment in the first one. I honestly don't remember the dialogue that occurred associated with that but I think that many felt that to be highly speculative and not really well supported.

MR. McDADE: This would be an appropriate time to break, Mr. Commissioner.

MR. MARTLAND: Mr. Commissioner, with respect to our timing, I've been canvassing and continually looking at our schedule. I'd suggest that we convene at the regular time of 10:00 a.m. tomorrow, please. Thank you.

THE REGISTRAR: The hearing is now adjourned for the day and will resume at ten o'clock tomorrow morning.

(PROCEEDINGS ADJOURNED TO AUGUST 25, 2011, AT 10:00 A.M.)

I HEREBY CERTIFY the foregoing to be a true and accurate transcript of the evidence recorded on a sound recording apparatus, transcribed to the best of my skill and ability, and in accordance with applicable standards.

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Irene Lim

I HEREBY CERTIFY the foregoing to be a true and accurate transcript of the evidence recorded on a sound recording apparatus, transcribed to the best of my skill and ability, and in accordance with applicable standards.

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Karen Acaster

I HEREBY CERTIFY the foregoing to be a true and accurate transcript of the evidence recorded on a sound recording apparatus, transcribed to the best of my skill and ability, and in accordance with applicable standards.

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Pat Neumann

I HEREBY CERTIFY the foregoing to be a true and accurate transcript of the evidence recorded on a sound recording apparatus, transcribed to the best of my skill and ability, and in accordance with applicable standards.

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Karen Hefferland