From:	Alex Morton
To:	Popham, Lana; Heyman.MLA, George LASS:EX; Meggs, Geoff PREM:EX
Subject:	ATIP documents for your review
Date:	Tuesday, February 13, 2018 10:51:09 PM
Attachments:	A-2016-01097-Marty corrects Proudfoot.pdf ATT00001.htm Jaundice results supressed A-2016-01097-DSP-FINAL (dragged).pdf ATT00002.htm Siah et al Formal Comment.pdf ATT00003.htm Siah et al Correction copy.pdf ATT00004.htm

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Dear Lana, George and Geoff

Attached are excerpts of ATIP documents I received today that concern your farm salmon health lab.

In one, we see reference to a BC Pathologist suppressing publication of the finding that piscine reovirus appears to be causing disease in BC Pacific salmon.

And second Dr. Gary Marty is trying to get a correction published in Maclean's Magazine's article on Dr. Kristi Miller. He reveals that "…B.C. veterinarians have long been aware of the disease [HSMI]."

Dr. Marty co-published 2 scientific papers with Marine Harvest stating he had not found HSMI in BC.

As a result 100's of millions of PRV-infected farm salmon have been transferred into sea pens on our wild salmon migration routes. This would have been prohibited by section 56 of the Fishery (General) Regulations - except that the BC lab in charge of BC farm salmon health was aware of the HSMI, but never diagnosed it. So it was never characterized as a "disease agent."

Now our coast is infected with a Norwegian virus that causes heart damage.

If you have been told PRV is not Norwegian, read the attached responses to Siah et al.

45% of wild salmon in Musgamagw territory are infected… (PLoS One Dec 2017), their rivers are nearly devoid of salmon, piscine reovirus has been detected in their Eulachons.

Please ensure these documents and this email are forwarded to your team investigating Dr. Gary Marty's lab.

The salmon farming industry can't survive *without* PRV because their fish are so infected, the growing evidence suggests wild salmon can't survive *with* PRV and the evidence of a government coverup is growing steadily.

Minister Heyman, in regards to the letter you sent me today - please don't send me a website informing me that the federal government has jurisdiction of salmon farms - I am the one who gave them the industry via my 2009 lawsuit.

You guys have a serious problem.

Choose reconciliation, announce you are not going to renew tenures where the industry has failed to secure agreements with their host nations, step away from the coverup you have inherited, throw wild salmon, our whales and entire coast a life line and stop this virus from spreading.

I don’t see any other way out of this for you.

Alexandra Morton
250-974-7086

McLeod, Patricia

From: Miller-Saunders, Kristi
Sent: January 16, 2017 8:54 AM
To: Taylor, Nathan
Subject: FW: Correction needed?

Kristi

From:
Sent: January-16-17 7:02 AM
To: Miller-Saunders, Kristi
Subject: Re: Correction needed?

Glad you liked the story and it's gotten good feedback! It got huge traction on Twitter and was the top story on our website for a few days.

thank you for explaining. I don't think a correction is necessary here so I'll just leave it.

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Maclean's magazine
Ottawa bureau
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From: "Miller-Saunders, Kristi" <Kristi.Saunders@dfo-mpo.gc.ca>
Date: Sunday, January 15, 2017 at 11:09 PM
To:
Subject: RE: Correction needed?

Sorry I only just saw this email from you. Nice article by the way. I have heard a lot of positive feedback on it.

I can assume that the email in question is from Gary Marty, a pathologist who works for the Provincial government who has published multiple papers (with scientists in DFO) stating that the disease HSMI is not present in British Columbia Our research is the first to *diagnose* the disease, HSMI (Heart and Skeletal Muscle Inflammation) in British Columbia. While Dr. Marty has occasionally noted the presence of heart lesions consistent with this disease, he has not diagnosed these lesions as being any specific disease and has discounted their linkage with the Norwegian disease HSMI, despite the virus known to associate with this disease being present in BC, and in fish carrying these lesions.

Hence, the statement that we are the first to diagnose the specific disease HSMI is not incorrect. The paper we are publishing does acknowledge that the heart lesions have been noted within the DFO audit program since 2008, but not diagnosed to a particular disease.

Kristi

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s.21(1)(a)
s.21(1)(b)

From:
Sent: January 9, 2017 12:24 PM
To: Miller-Saunders, Kristi
Subject: Correction needed?

Hi Kristi,

I just got a correction request through our letters editor, and I wanted to see what you thought, given that this is a sentence I ran past you for accuracy before publication. I'm wondering if this person is missing some nuance, or if there's something here that mitigates the apparent error they're pointing out. Let me know what you think, and I will amend the story as required.

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"In the 6 Jan 2017 article, "Unmuzzled government scientists are ready to talk", by Shannon Proudfoot, the article ends with, "Miller's program recently uncovered heart and skeletal muscle inflammation, a disease not previously known to be present in B.C. salmon."

This statement is not correct. A correct version would state something like, "Miller's program recently renamed a disease that has long been recognized among B.C. salmon by B.C. veterinarians."

This might seem like a minor point, but your article gives the impression that B.C. veterinarians were negligent in failing to diagnose a disease when, in fact, B.C. veterinarians have long been aware of the disease. I am not aware of any B.C. veterinarians that find the new name helpful, because the name used by Miller's group is the same name given to a Norwegian salmon disease that is sometimes much more severe than the B.C. disease.

Background: Inflammation in the Heart and skeletal inflammation is identified in the following 2015 DFO document based on information that I provided:
http://www.dfo-mpo.gc.ca/csas-sccs/publications/scr-rs/2015/2015_037-eng.pdf

Page 9 of this document includes, "For example, of the 1,013 Audit Program Atlantic Salmon sampled from 2014 and 2015, only two of the fish (0.2%) had both moderate skeletal muscle inflammation and significant cardiomyopathy."

I also reported fish with heart and skeletal muscle inflammation in 2013 in an expert report. That report is publicly available through the courts, but it is not available online. Since at least 2008 I have reported similar types of heart inflammation in B.C. salmon; I can provide a link on request.
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Maclean's magazine
Ottawa bureau
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Aquaculture and Disease Related Research (Pacific Region)

Draft July 29th, 2016

Outline/Table of Contents

Introduction

This draft document aims to provide a more detailed overview of Pacific Region programs that do Aquatic Animal Health research. While this research is related to some Cohen Commission recommendations, the department's responses to the Cohen Commission recommendations have been prepared in a separate package.

DFO is involved with numerous regulatory and non-regulatory research programs related to Aquatic Animal Health. The activities are organized at the level of programs that have broad research and/or monitoring objectives and within these, projects that address specific questions. Programs have specific deliverables that are dependent to some extent on funding obligations. Research in Aquatic Animal Health ranges from applied projects for monitoring and assessment activities, to collaborative research projects. Shellfish health research projects that are underway in the region are not discussed here. The following document provides an overview and summarizes current research activities in Aquatic Animal Health on fish in the Pacific Region at the program level, the questions being addressed by specific projects within those programs, and some key findings to date.

National Aquatic Animal Health Program (NAAHP)

The National Aquatic Animal Health Program (NAAHP) was implemented to be a co-delivered program between the Canadian Food Inspection Agency (CFIA), the lead regulatory and administrative authority for NAAHP under the authority of the Health of Animals Act and the Health of Animals Regulations, with DFO providing the diagnostic testing, research, and scientific advice to support the program. Specific infectious disease agents affecting fish, molluscs, and crustaceans are covered under NAAHP. Deliverables include diagnostic test results, test method protocols and research to support the CFIA/DFO priorities. The program has been rolled out in phases and is now fully implemented with an active surveillance program, import/export controls, a Domestic Disease Program and Response to Notification of Reportable Diseases in Canada. On the DFO side, our NAAHLS Laboratories are now fully accredited under the International standard ISO/IEC 17025:2005 and maintain a suite of fully validated tests to ensure diagnostic testing is fit for purpose.

Strategic Salmon Health Initiative, SSHI (Dr. K. Miller-Saunders)

DFO Science is collaborating with Genome British Columbia and the Pacific Salmon Foundation (PSF) to continue a research program to identify the spatial and temporal distributions of microbes associated with diseases in salmon worldwide present in wild and farmed salmonids in British Columbia. The multi-million dollar program is currently in the second of four phases and is co-led by Drs. Kristi Miller (DFO) and Brian Riddell (PSF). The program's long-term goal is to identify microbes in DFO's Pacific Region that may be undermining the performance and survival of wild salmon and may therefore warrant ongoing monitoring, and to better understand their possible origins and potential mechanisms of interaction. It consists of broad-scale microbe monitoring studies (quantify 46 microbes simultaneously across >28,000 fish) combined with multiple metrics to assess physiological and organismal impacts in order to identify microbes with the greatest pathogenic potential in BC salmon. An international team of investigators provide the diverse expertise (veterinary pathology, epidemiology, molecular biology, physiology, salmon ecology, salmon management) required to carry out the research program.

Development and validation of high throughput pathogen monitoring platform

Key Findings

The Fluidigm BioMark ™ platform was analytically validated for simultaneous monitoring of presence and abundance of 45 microbes known or suspected to cause disease in salmon worldwide. Cost reductions and efficiencies have been cited as some of the benefits when comparing this technology to single assay platforms.

The molecular assays do not perform any differently on this microfluidics platform than on traditional PCR platforms, but the platform was more sensitive to the detection of low copy number samples.

Piscine OrthoReovirus (PRV)- Heart and Skeletal Muscle Inflammatory Syndrome (HSMI)

provide access to regular sampling of live and moribund/dead salmon from four salmon farms over the entire ocean production cycle. This sampling occurred from 2013-2015, with ~2,500 Atlantic salmon collected for both molecular and histopathological analyses.

Key Findings

Pathological investigations revealed that moribund, dead and healthy fish from one of the farms carried a striking pattern of lesions diagnostic of (HSMI) that occurred over an 8 month period, peaking approximately 8-9 months after salmon had been transferred to the ocean farm site. Microbe monitoring revealed only three microbes present in >5% of heart tissues on the farm, and statistical analyses found that only PRV was statistically associated with the inflammatory lesions. Immunohistochemistry showed that PRV was localized within the inflammatory region of the heart and the affected cardiomyocytes in diseased fish, but was only present in blood cells in asymptomatic fish. The other two microbes (both parasites) were not localized within the inflammatory lesions.

. Discussions with the AMD and industry indicate that heart lesions consistent with HSMI have occurred sporadically on farms since 2002/2003 but that they did not follow up on these as they were not seen to be associated with significant mortality events. There have not, however, been any scientific analyses or follow-up on these data as research into novel diseases is not within the purview of the DFO audit program or the AMD. The SSHI epidemiology team is interested in following up with these analyses linking spatial and temporal variation in heart lesions with mortality data if the AMD will provide the data to them.

The study was not designed to determine a cause and effect relationship between PRV and HSMI, but did show the same correlations as have been noted in Norway and Chile.

Jaundice Study

In an ACRDP project funded in 2011, we undertook a study with Creative Salmon on a Jaundice syndrome that had been causing low losses overwinter on Chinook salmon farms on the West coast and for which the cause of the disease was poorly understood. The study combined host transcriptional profiling, histopathology, microbe monitoring, high throughput sequencing, and veterinary clinical evidence of disease to determine the disease etiology

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s.20(1)(b)
s.21(1)(a)
s.21(1)(b)

Key Findings

The study showed that the disease was likely infectious, most likely viral in origin. Microbe monitoring established an association of the disease with PRV, but was not designed to establish what the nature of the relationship with PRV may be (cause, opportunistic co-infection, or other). Unfortunately this was the first reported detection of PRV in BC, and the histopathologist from the province convinced the industry not to sign off on the report (after many iterations) if PRV was to be included in the analyses. Portions of this dataset, however, became public in Cohen

released the data to the CFIA. Since that time, two other farm studies performed in Norway on Rainbow Trout and in Chile on Coho salmon

have identified a jaundice-like syndrome (sometimes together with heart lesions resembling HSMI) in association with PRV. Jaundice was also identified in a number of Audit samples and preliminary assessments suggest that PRV loads are elevated in these samples. As such, the SSHI plans to bring this research, now in manuscript form, back to the table and pursue its publication.

The study was not designed to demonstrate cause and effect so the conclusions are all based on association. However no microbes other than PRV were associated with the Jaundice syndrome (although others were present in some samples), and deep sequencing also did not reveal any other viruses. OIE reportable viruses ISAV, IPNV, SAV, OMV and parasites *Gyrodactylus salaris* and *Myxobolus cerebralis* were not detected in any samples.

Audit Program Samples

We have conducted microbe monitoring and histopathology across all 930 samples provided by the Aquaculture Management Division (AMD) collected between 2011-2014. We are currently analysing the correlations between microbes and pathological lesions, and those data will be forthcoming in a few months. Many of the microbes detected are well known to the industry (*Piscirickettsia salmonis, Renibacterium salmoninarum, Vibrio anguillarum, Aeromonas salmonicida, Kudoa thyrsites, Loma salmonae*, PRV), but some are less so.

Key Findings

The most prevalent microbe was *Paranucleospora theridion*, a microsporidian parasite transmitted through sea lice and associated with proliferative gill disease in Norway, but only recently discovered in BC . Viral erythrocytic necrosis virus (VEN), a known endemic virus that has not been assessed previously on farms, was also moderately prevalent. *Parvicapsula pseudobranchicola*, a myxozoan parasite associated with gill disease in Norway, was also prevalent; this parasite was first detected by the SSHI in association with enhanced risk of predation of sockeye salmon smolts by Rhinoceros Auklets (Miller et al. 2014).

We did not find World Organization for Animal Health (OIE) reportable viruses ISAV, IPNV, SAV, OMV and parasites *Gyrodactylus salaris* and *Myxobolus cerebralis*, or the newly identified Pacific Salmon Parvovirus (reported at the Cohen Commission of Inquiry).

Adult Salmon Studies (UBC)

A range of holding and tracking studies have been carried on adult salmon returning to spawn and associations between microbes and survival, thermal response, and various physiological indicators of disease, at the molecular, protein, and cellular levels, have been assessed. This research is largely carried out with NSERC co-funding by academic collaborators (6 PhD students in Miller's lab).

Key Findings

Tracking and holding studies have identified a range of microbes (mostly microparasites and bacteria) associated with salmon survival as well as those that are more stimulated when additional stressors (thermal and handling stress) are present. Microparasites *Cryptobia salmositica, Ichthyophthirius multifiliis*, *Parvicapsula minibicornis*, and *Ceratomyxa shasta* and bacterium *Flavobacterium psychrophilum* have most commonly been associated with premature mortality and with physiological indicators of disease; all are known endemics to BC/Washington.

OIE reportable viruses ISAV, IPNV, SAV, OMV and parasites *Gyrodactylus salaris* and *Myxobolus cerebralis* were not found.

Hatchery-Wild smolt out-migration studies

The SSHI will be conducting microbe monitoring for over 20,000 outmigrating smolts, largely Chinook, Coho and Sockeye salmon. These data will be used in epidemiological analyses of the spatial and temporal variation in microbe prevalence and load, and to address specific hypotheses relating to microbe variation among species, stocks, seasons, hatchery enhanced vs wild fish, life-history variation, annual productivity, year-class strength, physiological indicators, and aquaculture-wild interactions. Microbe data for approximately 1600 Chinook salmon are currently being analyzed for the first manuscript, which will describe the spatial and seasonal variation in microbe distributions, highlighting variance by life-history types and documenting for the first time the full range of microbes originating in the freshwater environment and those first detected in the marine environment. Moreover, the study is identifying microbes for which abrupt shifts in prevalence/load occur in the marine environment which may be associated with host mortality.

Key Findings

We have found that half of the 32 microbes detected (of 45 surveyed) originated in freshwater, with the vast majority of microbes being microparasites (microsporidian, myxozoan, protozoan and flagellates). Strong shifts in microbe prevalence and abundance occurred seasonally, with a dozen or so with abrupt shifts that warrant further exploration (i.e. patterns could relate to mortality). We will follow up with physiological assessments associated with these microbes to determine if there is any evidence of a developing disease state. PRV was present in approximately 7% of smolts in the ocean, mostly off the west coast, and peaked in prevalence and load in the fall/winter samples . PRV is not highly prevalent in the Strait of Georgia.

Two studies linking infection status with risk of predation have been carried out using SSHI co-funding. The first, on Rhinoceros Auklets in the marine environment, showed that sockeye salmon smolts carrying any species of Parvicapsula parasite (there are three myxozoan species under this genus), smolts carrying a high load of any microbe, and smolts with high microbe diversity (two or more microbes detected) were at greater risk of predation by sea birds. The second study on Bull Trout predation in the Chilcotin tributary of the Fraser River found that sockeye smolts carrying the IHNV (infectious hematopoietic necrosis virus) were 34 times more likely to be eaten than those free of the

virus. This data explained the high losses associated with IHNV detected in a tracking study the previous year (Jeffries et al. 2014).

OIE reportable viruses ISAV, IPNV, SAV, OMV and parasites *Gyrodactylus salaris* and *Myxobolus cerebralis* were not found.

Wild/Farmed Interactions Migration Timing, Residency and Health of Juvenile Salmon in the Strait of Georgia and Discovery Islands(Dr. Stewart Johnson)

We are undertaking a series of linked projects that are designed to increase our understanding of the interactions between farmed Atlantic and wild juvenile salmon in the Discovery Islands. This information supports the Department's goal of assessing the cumulative impacts of multiple stressors on Fraser River Sockeye Salmon productivity and provides support DFO's risk assessment of disease transfer (IHN) from salmon farms. Although the focus is on Sockeye Salmon data on other species is also being collected.

To inform these processes we have been working collaboratively with Dr. Marc Trudel's group addressing the following questions:

1. When do Fraser River Sockeye Salmon and other juvenile salmon enter the Strait of Georgia (SOG) and for how long do they remain resident in this area?

2. How does the condition of juvenile salmon change during residency in the SOG?

3. Over what time period are juvenile wild salmon present in the Discovery Islands and for how long do they remain in the vicinity of fish farms?

4. What pathogens/parasites are carried by juvenile salmon prior to contacting salmon farms?

5. When and where do juvenile salmon become infected with sea lice?

6. With respect to all of the above questions how much variability is there between years?

Key Findings

This program has greatly improved our understanding how Fraser River Sockeye Salmon utilize the Strait of Georgia, as well as the timing of their migration past salmon farms in the Discovery Islands. With respect to the questions above the finding are:

1. Hydroacoustics and net based sampling are being used to examine migration timing and salmon behaviour in the Discover Islands.

2. Fraser River Sockeye salmon spend 6-8 weeks in the SOG.

3. The majority of Fraser River Sockeye Salmon pass through the Discovery Islands over a 3-4 week period in late June/early July. Note: there is year to year variability in the timing which is related to when Sockeye first enter the SOG.

4. Our data suggests that individual Sockeye spend only a short period of time in the vicinity of salmon farms.

5. Juvenile pink and chum salmon are present within the Discovery Islands over a longer period of time, which may be in part due to their origin from multiple watersheds around the SOG.

6. We cannot address the timing of Coho and Chinook Salmon migration through Discovery Islands, as these species remain in the Strait of Georgia until much later in the year.

We have examined Sockeye Salmon caught in the lower Fraser River and the SOG/Discovery Islands for the presence of pathogens using molecular diagnostics. Samples were collected from 2010-2016 and approximately 2500 fish have been examined to date. The pathogens we have screened for are infectious hematopoietic necrosis virus (IHNV), infectious salmon anemia virus (ISAV), viral hemorrhagic septicemia virus (VHSV), infectious pancreatic necrosis virus (IPN), piscine orthoreovirus (PRV), *Renibacterium salmoninarum, Myxobolus spp.* and *Parivcapsula* spp. For those pathogens listed as reportable we used the National Aquatic Animal Health Program (NAAHP) validated diagnostic tests. In 2010-2012, we also examine a subsample of Sockeye for evidence of disease by histology.

1. Viruses: Juvenile Fraser River Sockeye Salmon are carriers of IHNV (up to 24%; depending on year and stock of origin). Although infection with IHNV is common in Sockeye Salmon in freshwater, this is the first confirmed report of carriers in the marine environment. None of the fish we have examined have tested positive for ISAV, IPN, VHSV or PRV.

2. Bacteria: We have screened for only 1 bacterial pathogen, *Renibacterium salmoninarum* is the causative agent of bacterial kidney disease. We have not identified any Sockeye that are infected with this bacterium.

3. Parasites: Parasitic infections are common prevalence ranging from 29.5% to 60.8% (*Myxobolus arcticus*), from 5.7% to 22.1% (*Parvicapsula minibicornis*) and from 0 to 0.3% (*Ceratomyxa shasta*). Sea lice are commonly found in low abundance on juvenile salmon, threespined stickleback and herring in the SOG/Discovery Islands. *Caligus clemensi* is the dominant species which is present. With respect to Fraser River Sockeye there is a strong correlation between distance and prevalence and abundance of sea lice.

4. Histology: Histological examinations of Sockeye Salmon from fresh and marine waters (2010-2012) found no evidence of significant infectious disease problems or inflammation. When compared to molecular methods histology identified lower prevalence of infections with Myxobolus spp. and Parivcapsula spp. This is not surprising as molecular methods are much better suited for the identification of pathogens when present in low abundance.

PRV genotype collaborative work (Dr. S. Johnson)

The genetics of PRV on the West Coast of North America was examined in a project led by the BC Centre for Aquatic Health Sciences, Campbell River. This collaboration included fish health scientists from Canada, UK and USA. Marine Harvest Canada supported this work through contract with BC Centre for Aquatic Health Sciences. A-base funding was used to support DFO's participation in this project.

Two questions have been studied on the genotype of PRV found in BC waters:

1. How genetically similar is PRV from collected from different host species and different areas of the West Coast of North America?

2. How genetically similar is PRV from the West Coast of North America to PRV from Norway and Chile?

Key Findings

Questions 1 and 2

PRV shows little genetic diversity across a large geographic distance (Alaska to Washington State), and the sequence types were relatively stable over a 13 year period. There are genetic differences between PRV from the West Coast and PRV from Norway and Chile.

The results of this work have been published in a peer-reviewed journal, as well as presented at a number of scientific conferences.

Marine Parasitology Program (Dr. Simon Jones)

Research in the Marine Parasitology Program (MPP) addresses three aspects of parasite infections in fish: 1. The structural and genomic characterization of parasites assists in their identification, provides measures of variability within parasite populations and permits predictions concerning parasite interactions with the host; 2. Research explores defense responses of the host to better understand resistance to parasite infection; 3. Development and application of treatment and immunisation strategies that enhance resistance within a population through the use of novel therapeutic, vaccine or immuno-stimulant formulations is studied. Within DFO, the MPP collaborates with the National Aquatic Animal Health Program, the High Seas Salmon Program, the Molecular Genetics Laboratory and the Aquaculture Management Division. External collaboration are ongoing with members of the BC Salmon Farmers Association. Within DFO, the MPP collaborates with the National Aquatic Animal Health Program, the High Seas Salmon Program, the Molecular Genetics Laboratory and the Aquaculture Management Division. External collaborations are ongoing with members of the BC Salmon Farmers Association.

Marine reservoirs of infectious agents associated with proliferative gill disorders in farmed salmon

Proliferative gill diseases contribute to economically important production losses in Atlantic salmon aquaculture and the main goal of this research is to improve our understanding of reservoirs of infections with infectious agents associated with these disorders. The objectives of the proposed research are to: determine distribution of *P. perurans* and *D. lepeophtherii* in wild Pacific salmon and salmon lice at various locations relative to marine netpens; describe the occurrence of proliferative gill lesions in wild fish; characterize the genomic sequence of BC variants of *P. perurans* and *D. lepeophtherii*; conduct laboratory transmission studies to identify and quantify (host and environmental) parameters surrounding transmission of causative agent between candidate reservoir species and Atlantic salmon.

Key Findings

We have begun to analyse samples collected so far in this first season of the project. In this first season of the project we have collected several hundred gill samples from wild juvenile and sub-adult Pacific salmon and from farmed Atlantic salmon. The sample locations were centred on northwest Vancouver Island, the Discovery Islands area and near Klemtu on the central coast. Samples will be screened for evidence of infectious disease and for pathological changes. So far we have found evidence of pathology and 1 or more replicating agents in a small percentage of samples.

The effect of sea lice modulating salmonid susceptibility to virus

Previous gene expression research identified a significant loss of the anti-virus response capacity during sea lice infections in salmon. This is the first year of a multiyear project that studies the effects of controlled infections with salmon lice (*Lepeophtheirus salmonis*) on the capacity of Atlantic and Chinook salmon to respond to infection with Infectious Hematopoietic Necrosis Virus (IHNV). Working with Dr. K. Garver's group, a series of pilot studies have been conducted with lice and virus to establish the parameters for a reproducible co-infection model. Analysis of tissues from these trials is underway.

Key Findings

Evidence to date supports a significant shift in the salmon skin transcriptome suggesting a broad-spectrum reduction in the capacity of the sea lice-infested salmon to respond to virus infection. Preliminary evidence supports significant alterations in the physiology of sea lice–infested salmon consistent with increased susceptibility to infectious disease. More IHN virus-infected fish died among sea lice infested fish than non-infested controls.

We have not yet explored the specificity of the loss of anti-virus response: does it influence susceptibility to certain viruses and not to others? The research does not address the possibility that sea lice infestations affects susceptibility to infections with bacteria or other parasites, for which some evidence exists.

Probiotic bacteria and their bacteriocins to replace antibiotics in salmon aquaculture (NSERC-funded)

The overall goal of this project is to enable replacement of antibiotics and the anti-parasitic agent emamectin benzoate (SLICE) used in salmon aquaculture with benign and environmentally friendly probiotic bacteria and their bacteriocins (antimicrobial peptides). The project focuses on control of bacterial pathogens that cause disease in cultured Atlantic salmon (*Salmo salar*) and Pacific salmon (*Oncorhynchus* spp.), as well as killing of salmon lice (*Lepeophtheirus salmonis* and *Caligus* spp.). The research adopts a combination of in vitro and in vivo models.

Key Findings

This research has identified that a bacteriocin molecule produced by our model probiotic organism kills fish bacterial pathogens in vitro. However, the susceptibility of probiotic-fed salmon to bacterial infection was not different from controls. The probiotic organism does not survive well in the salmon gut and appears not to have a significant effect on normal gut microbial diversity.

We are not finding evidence that this bacterium is a particularly effective candidate as a probiotic in salmonids. Now that we have developed the appropriate analytical tools, an exploration of additional candidates is warranted.

The effects of smolt size on the intensity of *Kudoa thyrsites* infections in Atlantic Salmon

Our *Kudoa thyrsites* research program focuses on approaches which will assist in the management of this serious production disease in Atlantic salmon in British Columbia. The infection does not cause clinical signs of disease in Atlantic salmon. Parasite proteases rapidly deteriorate affected muscle during processing, resulting in reduced quality and economic loss. The results of this project will assist in the improved marketability of Atlantic salmon produced in British Columbia. Our collaborators are Marine Harvest Canada and Cermaq Canada.

Key Findings

Our laboratory studies utilise a reproducible method for exposure of salmon to the parasite that relies on the efficacy of ultraviolet irradiation of seawater. We have developed a method to quantify the level of infection in salmon and or the infective stage in seawater. Our research to date shows that prior exposure of salmon to the parasite or to treatment with certain anti-parasitic drugs reduces the risk of infection.

This addresses whether a particular set of risk factors influences infection with the parasite in a laboratory context. As with all research of this type, further collaborations with industry are required to ground-truth the laboratory findings in a production environment.

Viral diagnostics and characterization (Dr. K. Garver)

The overarching goal of the Virology research programs is to gain a better understanding of aquatic viral diseases of wild and cultured fish populations. Current aquaculture disease related research falls into two broad categories/programs: 1) Viral diagnostics and characterization and 2) Virus interaction of wild and farmed fish. Item 2 is discussed under the Virus interaction of wild and farmed fish heading below.

With respect to viral diagnostics and characterization, rapid and accurate diagnosis are paramount to avoiding disease spread in farmed populations. The virology laboratory has devoted significant efforts towards the development and validation of viral diagnostic protocols that are recognized at an international level.

National Animal Health Program (NAAHP) and Non-NAAHP diagnostic investigations.

Testing is conducted under a quality controlled environment meeting ISO 17025 requirements. In excess of 5000 tests are conducted annually and provide science to the Canadian Food Inspection Agency towards import/export, domestic movement, surveillance and disease response. Viral diagnostic services and advice are also provided to Fisheries and Oceans Salmon Enhancement Program, First Nations Organization, and Freshwater Fisheries Society of BC.

Key Findings

Through NAAHP surveillance and disease investigation, multiple regulated viral pathogens were discovered for the first time in Canada or in a new region of Canada. This work has led to two peer reviewed publications and numerous World Organization for Animal Health (OIE) Reports. Additionally through non-NAAHP research investigations three novel virus have been discovered in wild fish populations.

With the exception of viral hemorrhagic septicemia virus (VHSV) and Infectious Hematopoietic Necrosis virus (IHNV) , no reportable or novel viral pathogens have been discovered in farmed salmon populations.

Virus interaction of wild and farmed fish (Dr. Kyle Garver) -

Aquaculture within sea-cages leads to possible disease risks from the marine environment due to the generality that fish sharing water are likely to share diseases. Research is conducted to investigate viral transmission between wild and farmed fish, develop tools to evaluate potential for virus dispersal from farm sites, and investigate new and/or uncharacterized disease concerns of aquaculture.

Susceptibility of Sockeye salmon to viral hemorrhagic septicemia virus.

As noted above, surveillance of farmed Atlantic salmon has resulted in the occasional detection of VHSV in farmed Atlantic salmon. Although these infections of farmed Atlantic salmon do not cause serious disease there are concerns that VHSV in farmed Atlantic salmon may contribute to VHSV infections in wild salmonids. As a first step in determining risk of VHSV transfer farm to wild salmon, this project is determining the susceptibility of Sockeye salmon to infection and whether this species can develop disease.

Key Findings

Regardless of the how Sockeye salmon smolts were exposed to VHSV they did not appear susceptible to VHSV disease.

Laboratory trials failed to demonstrate VHSV infection through waterborne exposure suggesting transfer of VHSV from farmed Atlantic salmon to Sockeye salmon smolts is unlikely.

Development of an infectious hematopoietic necrosis virus (IHNV) dispersion model (collaboration with Institute of Ocean Sciences).

Understanding how pathogenic organisms spread in the environment is crucial for the management of disease, yet knowledge of propagule dispersal and transmission in aquatic environments is limited. Conducting laboratory studies on the aquatic virus, infectious hematopoietic necrosis virus (IHNV), we quantified infectious dose, shedding capacity, and virus destruction rates in order to better understand the transmission of IHN virus among Atlantic salmon marine net-pen aquaculture. Coupling the IHNV transmission parameter estimates with physical water circulation models, we've established a viral dispersion model which provides accurate geospatial predictions of risk for IHNV transmission from marine salmon sites.

Key Findings

Viral dispersion models have illustrated that in the absence of disease mitigation measures; water-borne transport of IHNV from diseased sites to downstream sites can occur and can account in part for disease dispersal among neighboring farms.

Detectable levels of virus were not evident in non-diseased fish and if mitigation measures such as vaccination or depopulation were employed the infectious load and potential for IHNV transmission was greatly abated.

Aquaculture disease investigations.

In 2009, the expertise of the Virology laboratory was sought to investigate a recurrent Jaundice Syndrome that sporadically affects cultured Chinook salmon on the west coast of Vancouver Island. Throughout the world reports of jaundice has been described in numerous salmon and trout species with variable causes ranging from infectious agents, nutritional and toxic factors, as well as genetic abnormalities. In our studies, through two separate sampling events, no bacterial or viral agents were cultured from tissues of jaundiced Chinook salmon; however, piscine orthoreovirus (PRV) was identified via RT-qPCR in affected fish. Infectivity trials conducted to evaluate transmissibility of the Jaundice disease demonstrated that despite the intraperitoneal inoculation of Chinook, sockeye and Atlantic salmon with an organ homogenate from jaundiced Chinook salmon, the disease was not transmitted. Nevertheless inoculation did result in the transmission of PRV to these species, where the virus persisted for the 5 months duration of the experiment.

Key Findings

PRV was found in association with a recurrent Jaundice Syndrome that sporadically affects cultured Chinook salmon.

The farmed Chinook Jaundice Syndrome was not transmissible by injection of material from infected fish suggesting a low transmissibility of this condition from infected farm fish.

Investigations into the relationship of PRV with HSMI.

It is recognized that piscine orthoreovirus (PRV) is endemic and widespread throughout the Pacific Northwest and Alaska in a range of salmonid species and has repeatedly been detected from farmed Atlantic salmon in British Columbia as well as in wild Pacific salmon from both Canadian and US waters. Due to the association of PRV with the disease Heart and Skeletal Muscle Inflammation (HSMI) in farmed salmon, the detection of PRV in British Columbia raises concerns about the risk the virus may pose to wild and farmed salmon populations. To this end the Virology laboratory has pursued multiple lines of investigations to understand the disease causing potential of PRV and its impact on its host.

Key Findings

Under controlled laboratory challenge studies co-led by Drs. K. Garver and S.C. Johnson, PRV infection and replication were evident in Atlantic, Sockeye, Chinook, and Pink salmon. Further over a 41 week period, PRV persisted in laboratory challenged Atlantic and Sockeye Salmon without any histopathological signs of HSMI. Atlantic salmon appear to be slightly more susceptible to infection than Sockeye when challenged by co-habitation; however, both species are highly susceptible. Examination of transcriptional patterns of key genes involved in antiviral responses showed that both Atlantic and Sockeye Salmon have only a relatively limited immune signalling response to the presence of PRV. Further, transcriptome profiling by RNA-Seq during persistent PRV infections of sockeye subsequently challenged with Infectious Hematopoietic Necrosis Virus (IHNV) revealed little difference in the transcriptional response between PRV+ and PRV- Sockeye and demonstrated PRV infection does not affect how Sockeye salmon respond to subsequent infection.

Laboratory studies performed at PBS and in Washington (collaboration) using PRV from non HSMI diseased fish demonstrated a failure of fish to develop HSMI despite maintaining significant PRV infections. Although through collaboration with scientists in Norway, a pilot study exposing Norwegian Atlantic salmon to PRV material from BC showed evidence of heart inflammation suggesting the importance of host and/or environmental factors in disease development. This work requires repeating on a larger scale with appropriate controls but holds promise to uncover the true role of PRV in development of disease.

Related Projects

There are several projects in the region that do not specifically do research on animal health. However, they are important components of the overall effort to understand the effects of disease in wild fish populations, for example understanding disease transmission dynamics.

Risk Assessments - Pathogen transmission risk assessments (Fish Diagnostic Lab)

The Fish Diagnostic Lab uses traditional diagnostic methods (cytology, bacteriology, virology, serology) which, in combination with invertebrate disease, histology and molecular diagnostic expertise within the Aquatic Animal Health Unit, enables full diagnostic capabilities for aquatic animal diseases of regional concern.

The Fish Diagnostic Laboratory's main client is the DFO Salmonid Enhancement Program (SEP), supported through a broad range of services: disease investigation, diagnosis, prevention, management and treatment advice. This is not a surveillance based program. Lab submissions are triggered by a fish culturist's recognition of clinical signs of disease; broodstock screening for vertically transmitted pathogens; pre-release and pre-transfer pathogen screening to inform pathogen transmission risk assessments. These services are also of benefit to DFO Science; assisting Pacific Region Animal Care Committee animal welfare oversight, laboratory animal medical and DFO research site biosafety decisions.

Use of hydro-acoustic methods to assess the migration timing and distribution of juvenile salmon in Discovery Islands and Johnstone Strait (Dr. S. Gauthier)

This is the first year of a 3 year project. This study builds upon our existing project as described above. This project uses moored autonomous sounders at strategic locations within the Johnstone Strait / Discovery Islands area. The multiple acoustic frequencies available on these systems enable us to assess and discriminate a wide range of aquatic organisms at high temporal and spatial resolutions. These echo sounders enable us to monitor the movement of juvenile salmon and other fish, as well as changes in plankton productivity within the water column through the season. Research seining carried out at regular intervals in the area informs these acoustic observations by providing juvenile salmon species identification and stock composition.

Through these related projects we are refining our understanding of how juvenile salmon utilize the Strait of Georgia including the Discover Islands area. We are: 1. developing accurate estimates of residence time within the Strait of Georgia and Discovery Island areas on a stock-by-stock basis, 2. assessing the passage rates and residence time of wild salmon smolts in the vicinity of salmon aquaculture sites, and 3. assessing changes in water column usage and migration dynamics of juvenile salmon in response to local conditions (e.g. tidal cycles, temperature, plankton productivity).

Development of Finite Volume Coastal Ocean Models (FVCOM, Dr. M. Foreman and Dr. D. Stucchi)

Finite Volume Coastal Ocean Models have been developed and validated for two of the major salmon farming areas in BC. These are the Broughton Archipelago and Discovery Islands. These models have been combined with biological information to: provide geospatial predictions of risk for pathogen (IHNV, sea lice) transmission among salmon farms (farm conductivity) and distribution of pathogens within the environment

- aid in the development of siting criteria (salmonid biocapacity)
- evaluate effect of disease management strategies (i.e. vaccination)
- predict critical areas where impacts on wild salmon may occur

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CORRECTION

Correction: Piscine Reovirus: Genomic and Molecular Phylogenetic Analysis from Farmed and Wild Salmonids Collected on the Canada/US Pacific Coast

Ahmed Siah, Diane B. Morrison, Elena Fringuelli, Paul Savage, Zina Richmond, Robert Johns, Maureen K. Purcell, Stewart C. Johnson, Sonja M. Saksida

In "Piscine Reovirus: Genomic and Molecular Phylogenetic Analysis from Farmed and Wild Salmonids Collected on the Canada/US Pacific Coast," by Siah et al. [1], clarifications were needed in regards to the following: (a) the sampling population used in this paper versus that of Kibenge et al. [2] and; (b) the discussion of work by Kibenge et al. [2]. Additionally, there was an error in Fig 6 and Table 2. Here, the authors would like to provide some additional information about the methods used in the PLOS ONE article, clarify the discussion, and correct the aforementioned Figure and Table.

Comparison of the sampling population used in this article versus that of Kibenge et al. [2]

The authors would like to clarify the differences in the sampling population used in Siah et al. [1] versus Kibenge et al. [2], since such differences may contribute to the different conclusions reached in these articles:

In the present study [1], the samples were selected from extensive PRV surveys performed on the west coast of Canada and the US. Salmonids archived paraffin blocks from 1974 to 2008 (n = 363), fresh-frozen samples from 2013–2014 (n = 1,838) from wild and farmed fish collected in British Columbia, fresh-frozen samples from fish collected in Alaska (n = 295) and fresh or RNA-later preserved samples from fish collected in Washington State (n = 724) were analyzed with real-time RT-PCR [3]. Only samples (n = 71) with Ct values lower than 30 and from which the authors were able to amplify a PCR product were used for this study. Our work extended our knowledge of PRV sequence diversity across a larger geographical range. Additionally, the authors found that partial segment S1 sequence types derived from archived Atlantic and Chinook salmon samples collected in 2001 and 2005 were identical to some PRV sequence types obtained from samples collected in 2013–2014. The phylogenetic analysis of partial PRV S1 sequences from North American Pacific Coast indicated high genetic homogeneity, forming a subgroup within Group II. Little genetic differentiation was observed among sequence types since 2001.

In Kibenge et al. [2], the authors examined PRV segment S1 sequences variation within British Columbia salmon and trout samples (14 samples in total from western Canada) recently collected in 2012.

Citation: Siah A, Morrison DB, Fringuelli E, Savage P, Richmond Z, Johns R, et al. (2016) Correction: Piscine Reovirus: Genomic and Molecular Phylogenetic Analysis from Farmed and Wild Salmonids Collected on the Canada/US Pacific Coast. PLoS ONE 11(10): e0164926. doi:10.1371/journal.pone.0164926

Published: October 12, 2016

Correction to the Discussion, regarding work by Kibenge et al. [2]

In the last paragraph of the current study [1], Siah et al. conclude, "This suggests that the circulating virus sequence types are relatively stable in western North American Pacific waters and rules out a recent introduction of PRV into the western North Pacific as suggested by Kibenge et al [10]." The work by Kibenge et al. was instead done in the eastern north Pacific (off the western coast of Canada), not the western north Pacific. In addition, after careful reconsideration, the authors feel this conclusion is overstated. The authors would like to correct these two issues with the following revision to the final paragraph:

In previous study performed by Kibenge et al [10], the authors examined PRV segment S1 sequences variation within British Columbia salmon and trout samples recently collected in 2012. In the present study, we analyzed PRV sequences obtained from samples of wild and farmed salmonids collected across an expanded geographic range from Alaska to Washington State over 13 year period. The phylogenetic analysis of partial PRV S1 sequences from western North America Pacific Region indicated high genetic homogeneity and they form a subgroup within Group II. In addition, the results presented here suggest that salmonids from western North America Pacific waters carried PRV RNA sequences for at least 13 years with little genetic differentiation among sequence types in selected samples spanning 2001 to 2014. However, the mechanisms by which the virus is globally distributed, as well as transmission pathways remain to be elucidated.

Please see the correct Fig 6 here.

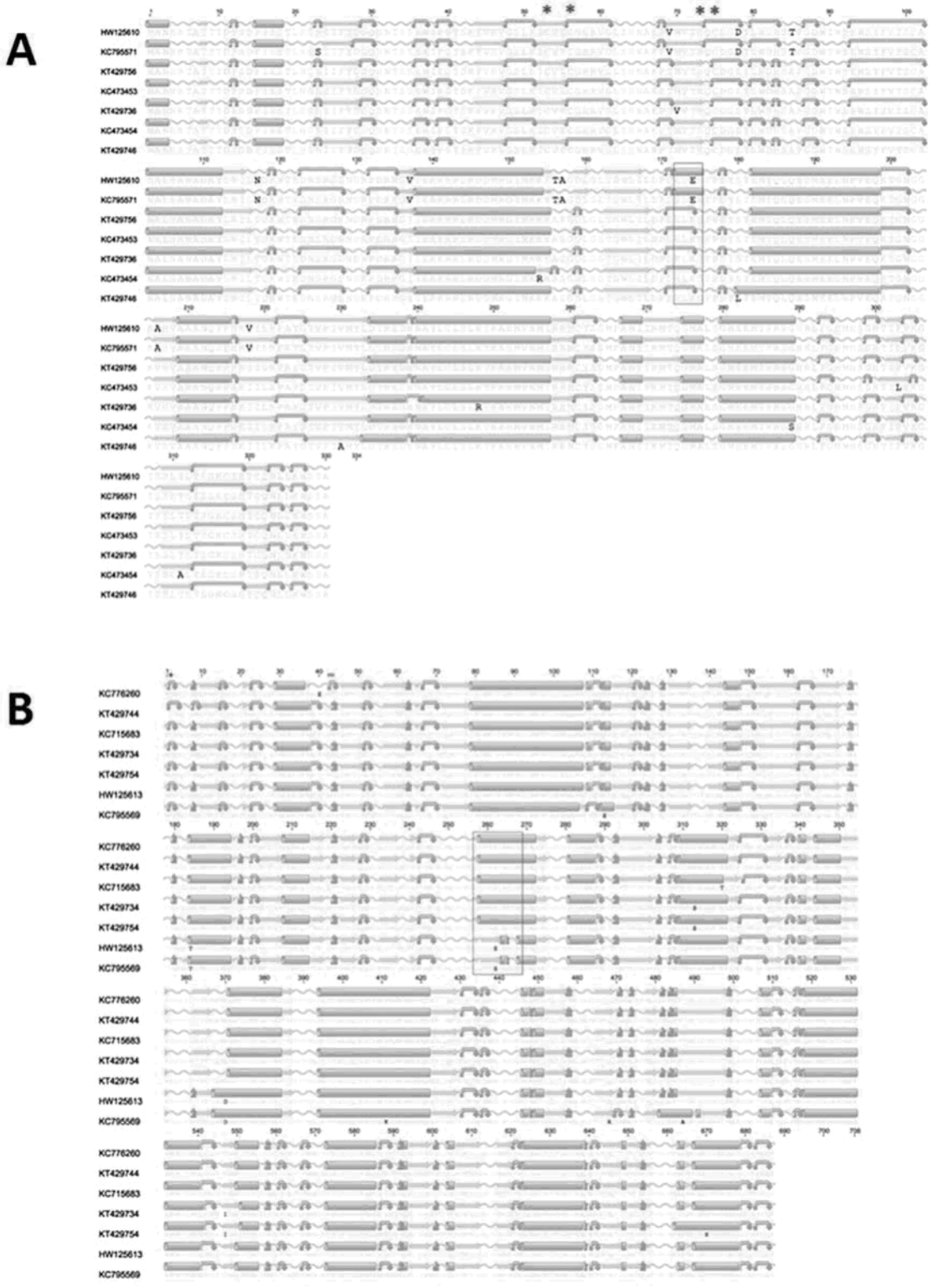

Fig 6. Amino acid alignment of open reading frame consensus sequences encoding the Piscine reovirus σ3 and μ1 protein. Secondary structure and transmembrane domains were predicted using EMBOSS 6.6.7 (Geneious software v6.1). Predicted secondary structure of alpha helix, beta strand, coil and turn are presented in purple cylinders, yellow arrows, grey sinusoids and blue curved arrow. Sequences are identified using the GenBank accession numbers. A/ represents ORF sequences encoding PRV σ3 amino acid alignment. Red stars are conserved Zn-finger motifs. B/ represents ORF sequences encoding PRV μ1 amino acid alignment. Red cross is myristoylation site in the MRV protein and green line is post-translational cleavage site in MRV and ARV [7].

doi:10.1371/journal.pone.0164926.g001

Please see the correct Table 2 here.

Table 2. Information on partial segment S1 sequenced from fish samples collected in Alaska, British Columbia and Washington State. Ten types of identical sequences have been identified and grouped in five clusters.

Clusters	Types	GenBank ID	Name	Host species (common name)	Collection Date	Tissue	Location (State, Country)
Cluster 1 (C1)	BCJ31915	KR558677	BC131_13	Farmed *Salmo salar* (Atlantic salmon)	May-13	Heart	DFO area 12 (British Columbia, Canada)
		KR781117	BC1310_13	Farmed *Salmo salar* (Atlantic salmon)	May-13	Heart	DFO area 12 (British Columbia, Canada)
		KR781118	BC1311_13	Farmed *Salmo salar* (Atlantic salmon)	May-13	Heart	DFO area 12 (British Columbia, Canada)
		KR558678	BC132_13	Farmed *Salmo salar* (Atlantic salmon)	May-13	Heart	DFO area 12 (British Columbia, Canada)
		KR558679	BC133_13	Farmed *Salmo salar* (Atlantic salmon)	May-13	Heart	DFO area 12 (British Columbia, Canada)
		KR558680	BC134_13	Farmed *Salmo salar* (Atlantic salmon)	May-13	Heart	DFO area 12 (British Columbia, Canada)
		KR558681	BC135_13	Farmed *Salmo salar* (Atlantic salmon)	May-13	Heart	DFO area 12 (British Columbia, Canada)
		KR558682	BC136_13	Farmed *Salmo salar* (Atlantic salmon)	May-13	Heart	DFO area 12 (British Columbia, Canada)
		KR558683	BC137_13	Farmed *Salmo salar* (Atlantic salmon)	May-13	Heart	DFO area 12 (British Columbia, Canada)
		KR558684	BC138_13	Farmed *Salmo salar* (Atlantic salmon)	May-13	Heart	DFO area 12 (British Columbia, Canada)
		KR558685	BC139_13	Farmed *Salmo salar* (Atlantic salmon)	May-13	Heart	DFO area 12 (British Columbia, Canada)
		KR872637	BC361_14	Farmed *Salmo salar* (Atlantic salmon)	May-14	Heart	Hatchery (British Columbia, Canada)
			BC362_14	Farmed *Salmo salar* (Atlantic salmon)	May-14	Heart	Hatchery (British Columbia, Canada)
			BC363_14	Farmed *Salmo salar* (Atlantic salmon)	May-14	Heart	Hatchery (British Columbia, Canada)
			BC364_14	Farmed *Salmo salar* (Atlantic salmon)	May-14	Heart	Hatchery (British Columbia, Canada)
			BC365_14	Farmed *Salmo salar* (Atlantic salmon)	May-14	Heart	Hatchery (British Columbia, Canada)
			BC366_14	Farmed *Salmo salar* (Atlantic salmon)	May-14	Heart	Hatchery (British Columbia, Canada)
			BC367_14	Farmed *Salmo salar* (Atlantic salmon)	May-14	Heart	Hatchery (British Columbia, Canada)
			BC368_14	Farmed *Salmo salar* (Atlantic salmon)	May-14	Heart	Hatchery (British Columbia, Canada)
		KR347084	BCJ24201_13	Farmed *Salmo salar* (Atlantic salmon)	Sep-13	Heart	DFO area 12 (British Columbia, Canada)
		KR347085	BCJ28529_13	Farmed *Salmo salar* (Atlantic salmon)	Apr-13	Heart	DFO area 7 (British Columbia, Canada)
		KR347086	BCJ28537_13	Farmed *Salmo salar* (Atlantic salmon)	Apr-13	Heart	DFO area 7 (British Columbia, Canada)
		KR347087	BCJ28545_13	Farmed *Salmo salar* (Atlantic salmon)	Apr-13	Heart	DFO area 7 (British Columbia, Canada)

(*Continued*)

Table 2. (*Continued*)

Clusters	Types	GenBank ID	Name	Host species (common name)	Collection Date	Tissue	Location (State, Country)
		KR347088	BCJ31910_13	Farmed *Salmo salar* (Atlantic salmon)	Oct-13	Heart	Hatchery (British Columbia, Canada)
		KR347089	BCJ31914_13	Farmed *Salmo salar* (Atlantic salmon)	Oct-13	Heart	Hatchery (British Columbia, Canada)
		KR347090	BCJ31915_13	Farmed *Salmo salar* (Atlantic salmon)	Oct-13	Heart	Hatchery (British Columbia, Canada)
		KR347091	BCJ31916_13	Farmed *Salmo salar* (Atlantic salmon)	Oct-13	Heart	Hatchery (British Columbia, Canada)
		KR347092	BCJ31920_13	Farmed *Salmo salar* (Atlantic salmon)	Oct-13	Heart	Hatchery (British Columbia, Canada)
		KR347094	BCJ35240_13	Farmed *Salmo salar* (Atlantic salmon)	Nov-13	Heart	Hatchery (British Columbia, Canada)
		KR347096	BCJ35249_13	Farmed *Salmo salar* (Atlantic salmon)	Nov-13	Heart	Hatchery (British Columbia, Canada)
		KR347098	BCJ35256_13	Farmed *Salmo salar* (Atlantic salmon)	Nov-13	Heart	Hatchery (British Columbia, Canada)
		KR347095	BCJ35246_13	Farmed *Salmo salar* (Atlantic salmon)	Nov-13	Heart	Hatchery (British Columbia, Canada)
		KR347097	BCJ35255_13	Farmed *Salmo salar* (Atlantic salmon)	Nov-13	Heart	Hatchery (British Columbia, Canada)
		KR347100	BCJ40723_13	Farmed *Salmo salar* (Atlantic salmon)	Nov-13	Heart	Hatchery (British Columbia, Canada)
		KR347102	BCJ40740_13	Farmed *Salmo salar* (Atlantic salmon)	Nov-13	Heart	Hatchery (British Columbia, Canada)
		KR347101	BCJ40731_13	Farmed *Salmo salar* (Atlantic salmon)	Nov-13	Heart	Hatchery (British Columbia, Canada)
		KR347105	BCJ402256_13	Wild *Oncorhynchus kisutch* (Coho salmon)	Nov-13	Heart	Quinsam Hatchery (British Columbia, Canada)
		KR347112	BCK14114_14	Farmed *Salmo salar* (Atlantic salmon)	Apr-14	Heart	DFO area 27 (British Columbia, Canada)
		KR347113	BCK14120_14	Farmed *Salmo salar* (Atlantic salmon)	Apr-14	Heart	DFO area 27 (British Columbia, Canada)
		KR347106	BCJ402276_13	Wild *Oncorhynchus kisutch* (Coho salmon)	Nov-13	Heart	Quinsam Hatchery (British Columbia, Canada)
Cluster 2 (C2)	BCJ18824	KR347081	BCJ18824_13	Wild *Oncorhynchus tshawytscha* (Chinook salmon)	Aug-13	Heart	DFO area 127 (British Columbia, Canada)
		KR347083	BCJ19943_13	Wild *Oncorhynchus kisutch* (Coho salmon)	Aug-13	Heart	DFO area 127 (British Columbia, Canada)
		KR347093	BCJ34056_13	Wild *Oncorhynchus kisutch* (Coho salmon)	Oct-13	Heart	Quinsam Hatchery (British Columbia, Canada)
		KR347103	BCJ378151_13	Wild *Oncorhynchus kisutch* (Coho salmon)	Nov-13	Heart	Quinsam Hatchery (British Columbia, Canada)
Cluster 3 (C3)	BCJ19323	KR347082	BCJ19323_13	Wild *Oncorhynchus kisutch* (Coho salmon)	Aug-13	Heart	DFO area 7 (British Columbia, Canada)
		KR347104	BCJ378241_13	Wild *Oncorhynchus kisutch* (Coho salmon)	Nov-13	Heart	Quinsam Hatchery (British Columbia, Canada)
		KR347099	BCJ37896_13	Wild *Oncorhynchus kisutch* (Coho salmon)	Nov-13	Heart	Quinsam Hatchery (British Columbia, Canada)
		KR347110	BCK1562_14	Wild *Oncorhynchus kisutch* (Coho salmon)	May-14	Heart	Quinsam Hatchery (British Columbia, Canada)
		KR347115	BCK15625_14	Wild *Oncorhynchus kisutch* (Coho salmon)	May-14	Heart	Quinsam Hatchery (British Columbia, Canada)

(*Continued*)

Table 2. (*Continued*)

Clusters	Types	GenBank ID	Name	Host species (common name)	Collection Date	Tissue	Location (State, Country)
		KR347111	BCK1566_14	Wild *Oncorhynchus kisutch* (Coho salmon)	May-14	Heart	Quinsam Hatchery (British Columbia, Canada)
		KR478634	WS1209_12	Wild *Oncorhynchus tshawytscha* (Chinook salmon)	Sep-12	Pool of gill, heart and kidney	Columbia River (Washington State, US)
		KR478637	WSKFH11_14	Wild *Oncorhynchus kisutch* (Coho salmon)	Mar-14	Blood	Columbia River (Washington State, US)
		KR478639	WSKFH13_14	Wild *Oncorhynchus kisutch* (Coho salmon)	Mar-14	Blood	Columbia River (Washington State, US)
		KR478636	WSKFH2_14	Wild *Oncorhynchus kisutch* (Coho salmon)	Mar-14	Blood	Columbia River (Washington State, US)
		KR478633	WS1207_12	Wild *Oncorhynchus tshawytscha* (Chinook salmon)	Sep-12	Pool of gill, heart and kidney	Columbia River (Washington State, US)
Cluster 4 (C4)	BCA1338	KR478642	BCA1338_01	Wild *Oncorhynchus tshawytscha* (Chinook salmon)	May-01	Multiple Organs	DFO Area 13 (British Columbia, Canada)
		KR478643	BCA1846_01	Farmed *Salmo salar* (Atlantic salmon)	Aug-01	Multiple Organs	DFO Area 18 (British Columbia, Canada)
		KR478644	BCA1848_01	Farmed *Salmo salar* (Atlantic salmon)	Aug-01	Multiple Organs	DFO Area 18 (British Columbia, Canada)
		KR347078	BCA1849_01	Farmed *Salmo salar* (Atlantic salmon)	Aug-01	Multiple Organs	DFO Area 18 (British Columbia, Canada)
		KR347079	BCA1850_01	Farmed *Salmo salar* (Atlantic salmon)	Aug-01	Multiple Organs	DFO Area 18 (British Columbia, Canada)
		KR347080	BCA1854_05	Farmed *Salmo salar* (Atlantic salmon)	Mar-05	Head kidney, trunk kidney, liver and spleen	DFO Area 18 (British Columbia, Canada)
		KR347107	BCJ402334_13	Wild *Oncorhynchus kisutch* (Coho salmon)	Nov-13	Heart	Quinsam Hatchery (British Columbia, Canada)
		KR347109	BCK1436_14	Salmo salar (Atlantic salmon)	Apr-14	Heart	DFO area 12 (British Columbia, Canada)
Cluster (C5)	AKJ20115	KR478640	AKJ20115_13	Wild *Oncorhynchus kisutch* (Coho salmon)	Aug-13	Heart	Copper River (Alaska, US)
		KR478641	AKJ20120_13	Wild *Oncorhynchus kisutch* (Coho salmon)	Aug-13	Heart	Copper River (Alaska, US)
		KR872635	BCINOC3_13	Wild *Oncorhynchus tshawytscha* (Chinook salmon)	May-13		DFO area 124 (British Columbia, Canada)
		KR478635	WSKFH1_14	Wild *Oncorhynchus kisutch* (Coho salmon)	Mar-14	Blood	Columbia River (Washington State, US)
		KR478638	WSKFH12_14	Wild *Oncorhynchus kisutch* (Coho salmon)	Mar-14	Blood	Columbia River (Washington State, US)
		KR347108	BCK1435_14	Farmed *Salmo salar* (Atlantic salmon)	Apr-14	Heart	DFO area 12 (British Columbia, Canada)
		KR347114	BCK14310_14	Farmed *Salmo salar* (Atlantic salmon)	Apr-14	Heart	DFO area 12 (British Columbia, Canada)
		KR872636	BCINOC12_13	Wild *Oncorhynchus tshawytscha* (Chinook salmon)	May-13		DFO area 124 (British Columbia, Canada)

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PLOS ONE

FORMAL COMMENT

Formal comment on: Piscine reovirus: Genomic and molecular phylogenetic analysis from farmed and wild salmonids collected on the Canada/US Pacific Coast

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Introduction

This formal comment is in response to Siah *et al.* [1], Piscine Reovirus: Genomic and Molecular Phylogenetic Analysis from Farmed and Wild Salmonids Collected on the Canada/US Pacific Coast, with a subsequent correction (Siah *et al.* 2016 [2]). Although a correction for this paper was published on Oct. 12, 2016, (Siah *et al.* 2016 [2]), there continues to be inadequate supporting evidence for the primary conclusion that PRV genetic sequences are temporally and spatially homogeneous in salmonid species across the northeastern Pacific region.

The evidence in this paper warrants thorough consideration. Piscine orthoreovirus (PRV) causes acute infection of the red blood cells in salmon (Finstad *et al.* 2014 [3]; Haatveit *et al.* 2017 [4]). It is the causative agent of the emerging farm salmon disease Heart and Skeletal Muscle Inflammation (HSMI) (Wessel *et al.* 2017 [5]) with clinical symptoms which can include lethargy, anemia, anorexia and mortality (Kongtorp *et al.* 2004 [6]). Palacios *et al.* [7] expressed concerns about the transfer of PRV from farmed to wild fish due to its contagious nature. PRV is now considered ubiquitous in farmed Atlantic salmon (Haatveit *et al.* 2017 [4]) and has an estimated 80% prevalence rate among BC farmed salmon (Kibenge *et al.* 2013 [8]). HSMI has recently been diagnosed in British Columbia (BC), Canada (Di Cicco *et al.* 2017 [9]). Hence, release of PRV from salmon farms into Pacific salmon habitat is a significant management concern in the eastern Pacific Ocean.

In the correction, Siah *et al.* [2] acknowledge that the conclusion that PRV has not been recently introduced to BC was overstated. However their supporting evidence that ". . . salmonids from western North America Pacific waters carried PRV RNA sequences for at least 13 years with little genetic differentiation among sequence types in selected samples spanning 2001 to 2014" remains insufficient.

Their conclusion appears to be highly dependent on six unique sequences of PRV segment S1, detected by Siah *et al.* [1]:

KR478642: collected in May 2001

KR478643: collected in Aug. 2001

KR478644: collected in Aug. 2001

towards high survivorship of wild salmon in BC's rivers and near-shore marine environment.

Competing interests: This work was supported by the Virology Research Laboratory at the Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PE, Canada. Van City supported the work by Alexandra Morton. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. This does not alter our adherence to PLOS ONE policies on sharing data and materials. Alexandra Morton is a director of the Pacific Coast Wild Salmon Society, whose goals include working towards high survivorship of wild salmon in BC's rivers and nearshore marine environment.

KR347078: collected in Aug. 2001

KR347079: collected in Aug. 2001

KR347080: collected in Mar. 2005

These six Siah *et al.* [1] sequences collected in 2001 and 2005 (submitted to GenBank April—May 2015) predate those collected by Kibenge *et al.* [10,11] by seven years and are nearly identical to the isolates Siah *et al.* [1] collected in 2013 and 2014. Thus, these six PRV isolates appear to be highly resistant to mutation over a 13-year interval 2001–2014, which is atypical for RNA viruses, generally known to exhibit a high mutation rate (Chao *et al.* [12]). Drake and Holland [13] estimate the genomic mutation rate (U) to be between 1 and 0.1 for most RNA viruses, where U is G x u, G is the genome size in nucleotides, and u is the per-nucleotide mutation rate.

Weight of evidence for longer-term PRV presence in BC

Siah et al. [1] cite detection of PRV in a wild Steelhead trout (*O. mykiss*) collected in 1977 in support of longer-term PRV presence in BC. This result is cited from Marty *et al.* [14], who provided no S1 segment sequence information to verify the PRV strain identity as per the sequence groupings reported by both Kibenge *et al.* [8] and Garseth *et al.* [15]. The recent discovery of the widespread occurrence of PRV-2 across the North Pacific (Takano *et al.* 2016 [16]) raises the question: Was the 1977 steelhead infected with PRV-2 or PRV? In absence of S1 sequencing this uncertainty cannot be resolved.

Furthermore, this result could not be replicated by a second laboratory (Purcell and Thompson 2014 [17]) and therefore warrants qualification as a non-repeatable result and a suspect positive lacking sufficient robustness to provide evidence critical to the temporal presence of PRV in BC.

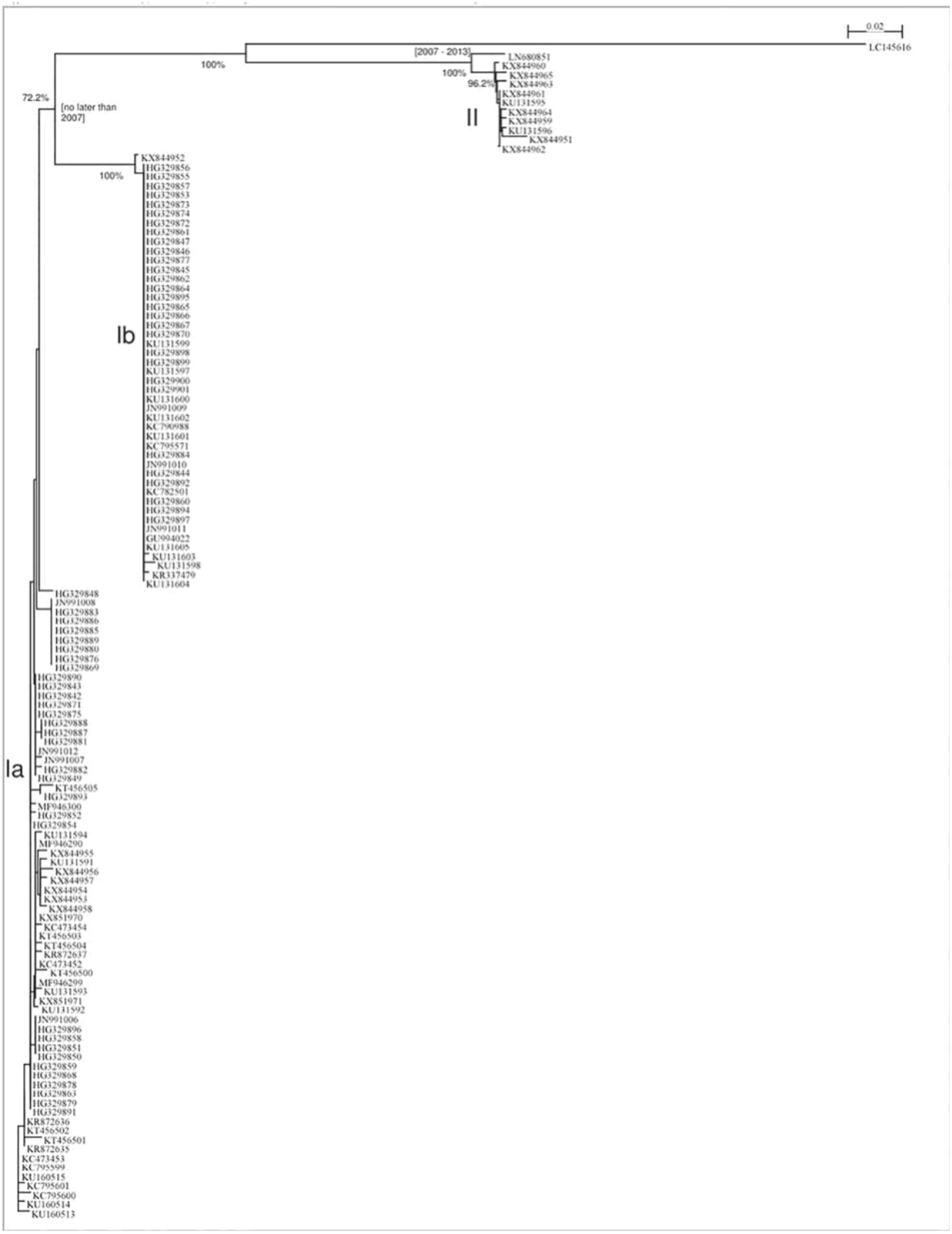
Phylogenetic comparative analysis

To illustrate our interpretation of the phylogenetic analysis of PRV isolates, we constructed a phylogenetic tree (Fig 1), of the 127 sequences described in S1 Table. The mutation direction was determined by an outgroup sequence (GenBank Accession No. AF004856). After the root of the tree was determined, the outgroup sequence was removed so that the details of the tree could be shown.

Fig 1 demonstrates that all PRV isolates can be classified into Genotypes I and II, with Genotype I further divided into sub-genotype Ia and sub-genotype Ib. Among these sub-genotypes, all Canadian isolates exist in sub-genotype Ia; this evidence provides information that PRV in BC-Canada is closely related to PRV found in Norway.

We estimated the divergence time between the genotypes and sub-genotypes (Ia, Ib and II) based on the collection time of each isolate described in S1 Table. Basic rules of logic were used in the estimation, such as the divergence time must not be later than the collection time of any isolate in all branches.

We estimated that the divergence time between sub-genotype Ia and sub-genotype Ib was 2007 or earlier. Because all sequences in S1 Table were collected in 2007 or later, we are not able to estimate a more accurate divergence time. We also estimated that the divergence time between Genotype II and the rest of the isolates was in the range of 2007 to 2013. The PRV-2 sequence GenBank Accession No. LC145616, from Japan (Takano *et al.* 2016 [16]), is quite different from all other isolates and it may constitute a second sub-genotype of Genotype II or a completely new genotype (Genotype III); but because we cannot find other evidence, we consider this sequence an outlier at the present time.


0.02
LC145616
[2007 - 2013]
100%
LN680851
KX844960
100%
KX844965
96.2%
KX844963
KX844961
KU131595
II
KX844964
KX844959
KU131596
KX844951
KX844962
72.2%
[no later than 2007]
100%
KX844952
HG329856
HG329855
HG329857
HG329853
HG329873
HG329874
HG329872
HG329861
HG329847
HG329846
HG329877
HG329845
HG329862
HG329864
HG329895
HG329865
HG329866
HG329867
Ib
HG329870
KU131599
HG329898
HG329899
KU131597
HG329900
HG329901
KU131600
JN991009
KU131602
KC790988
KU131601
KC795571
HG329884
JN991010
HG329844
HG329892
KC782501
HG329860
HG329894
HG329897
JN991011
GU994022
KU131605
KU131603
KU131598
KR337479
KU131604
HG329848
JN991008
HG329883
HG329886
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HG329876
HG329869
HG329890
HG329843
HG329842
HG329871
HG329875
HG329888
HG329887
HG329881
JN991012
JN991007
HG329882
Ia
HG329849
KT456505
HG329893
MF946300
HG329852
HG329854
KU131594
MF946290
KX844955
KU131591
KX844956
KX844957
KX844954
KX844953
KX844958
KX851970
KC473454
KT456503
KT456504
KR872637
KC473452
KT456500
MF946299
KU131593
KX851971
KU131592
JN991006
HG329896
HG329858
HG329851
HG329850
HG329859
HG329868
HG329878
HG329863
HG329879
HG329891
KR872636
KT456502
KT456501
KR872635
KC473453
KC795599
KU160515
KC795601
KC795600
KU160514
KU160513

Fig 1. Phylogenetic tree for sequences of piscine orthoreovirus (PRV) segment S1 listed in S1 Table. All 127 available robust isolates are included in this tree. The phylogenetic tree was constructed using the neighbor-joining method and Tamura-Nei genetic distances (Saitou and Nei 1987 [18]). Bootstrapping was performed 1,000 times. Bootstrap supports of topology of 70% or higher are shown at the nodes. The PRV grouping of Genotype I sub-genotypes Ia, Ib, and Genotype II are indicated. The mutation direction was determined by an outgroup sequence.

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Sampling inadequacies

The description of the temporal and spatial distribution of the samples reported on by Siah *et al.* [1] is inadequate to support the conclusion that PRV is endemic to BC. The reader cannot ascertain the degree of independence between sampled fish. For example, there are two groups of samples that generated PRV sequences from locations labeled "Hatchery (British Columbia, Canada)". One group was sampled in October 2013, and another eight samples were obtained in November 2013. Were these fish from the same, related (i.e. shared personnel and broodstock) or different hatcheries? Fish sampled one month apart from the same hatchery would presumably have an increased probability of being infected with the same strain of PRV and cannot be interpreted as independent samples.

Furthermore, all but eight of the partial sequences reported by Siah *et al.* [1] in their Table 2 were obtained in 2013 and 2014. Of the eight other sequences, two were from 2012, one from 2005, and the remaining five were from 2001 (four of which were reportedly sampled on the same day on one or more farms within DFO Statistical Area 18; an area with no reported marine salmon farms, suggesting these were from a hatchery). Thus, it appears that there were only three temporally separate sampling events for the time period 2001–2012. This sparse temporal coverage does not provide sufficiently extensive evidence to support a conclusion of long-term PRV genetic heterogeneity in BC.

There was also inadequate spatial and host-species coverage. Siah *et al.* [1], reported that over half (43/71) of the fish that produced partial PRV sequence information were farmed Atlantic salmon (Siah *et al.* 2015 [1] Table 2). Only two wild fish were sampled north of central BC (two Coho Salmon, *O. kisutch*, from the Copper River in Alaska), and only six Chinook Salmon, *O. tshawytscha*, were sampled (four from southern BC, and two from further south in the Columbia River, Washington State). No other Pacific salmon or trout species were included. Hence, the host species and spatial coverage of PRV sequencing presented by Siah *et al.* [1] are very sparse. Thus Siah *et al.* [1] do not provide sufficient evidence to draw reliable inferences either on the temporal stability or geographic homogeneity of PRV throughout the coastal eastern Pacific Ocean. Considerable variation over time or space could easily have been bypassed.

Furthermore, farm restocking methods could potentially account for at least some of the homogeneity in the Siah *et al.* [1] samples. Although BC farm salmon broodstock sourcing and the distribution of Atlantic salmon from specific hatcheries is not public information in BC, presumably farm salmon from the same hatchery could be transferred into farms hundreds of kilometers apart that are sited throughout wild eastern Pacific salmon migratory corridors. Repeat introduction of the same PRV variant across years and regions may be occurring from Atlantic salmon hatcheries that share broodstock and/or eggs. Thus, the appearance of genetic stability of PRV in migratory wild salmon could be the result of exposure to farm salmon from the same hatchery.

HSMI in BC

Siah *et al.* [1] state, citing Kibenge *et al.* [8] and Marty *et al.* [14], "PRV is known to occur in a wide variety of salmon species on the Pacific Coast of North America, *a region where HSMI*

has never been reported." (Emphasis added.) However, Kibenge *et al.* [8] do cite lesions identified as diagnostic of HSMI in BC farmed Atlantic salmon beginning in 2008. Furthermore, subsequent to both these publications, HSMI has been confirmed in BC (Di Cicco *et al.* 2017 [9]).

Conclusion

We conclude that the longer-term presence of PRV in BC prior to 2001 has not been adequately described and that the evidence that the virus was introduced from Norway is more robust than the hypothesis that PRV is endemic to the eastern Pacific Ocean.

Supporting information

S1 Table. Piscine orthoreovirus segment S1 nucleotide sequences analyzed in this study.
(DOC)

Author Contributions

Methodology: Yingwei Wang, Frederick S. B. Kibenge.

Writing – original draft: Molly J. T. Kibenge, Yingwei Wang, Alexandra Morton, Richard Routledge, Frederick S. B. Kibenge.

Writing – review & editing: Molly J. T. Kibenge, Yingwei Wang, Alexandra Morton, Richard Routledge, Frederick S. B. Kibenge.

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From:	Alex Morton
To:	Xia, Eveline ENV:EX; McGuire, Jennifer ENV:EX; Morel, David P ENV:EX; Popham, Lana; Heyman.MLA, George LASS:EX; Meggs, Geoff PREM:EX
Cc:	Tavish Campbell; s.22
Subject:	Blood Water Testing
Date:	Tuesday, February 6, 2018 8:00:59 AM
Attachments:	Siah et al Formal Comment.pdf ATT00001.htm The effect of exposure to farmed salmon.pdf ATT00002.htm

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Dear Eveline, Jennifer and Dave;

I received a link to an article reporting on your testing of the bloodwater samples from Tofino and Browns Bay. https://www.desmog.ca/2018/02/05/bloodwater-released-b-c-s-coastal-water-contains-deadly-fish-virus-government-tests-confirm

Can you forward a copy of your report to me?

Attached are two papers that I co-published in December, one on the spread of this virus through BC wild salmon and the other a Formal Comment published in PLoS One a paper stating they had ruled out that any strains of PRV found in BC came from Norway. The authors of Siah et al withdrew that statement in a correction, but we also present further information that at least one strain of PRV found spreading in wild salmon and causing HSMI in farm salmon is most likely from Norway.

The concern with PRV is not that it is outright lethal, but rather that it exists in fish in a low-grade chronic state which means that infected fish can travel with it. The evidence in my work below, and in Miller et al 2014 suggests this blood virus is impeding fish's success in reaching their spawning grounds. Research in Norway suggests that the high presence of the virus in the fish's red blood cells may reduce the cell's ability to transport oxygen to muscle tissue and thus reduce fitness required to catch prey, evade predators and ascend rivers. As well there are reports of jaundice associated with the virus in Pacific salmon and so HSMI may only be the final outcome of infection with this virus, which only occurs in farms where predators are unable to remove fish during the earlier stages of the disease.

This is certainly a pathogen of concern as it is durable and thus contagious and over 95% of farm salmon sold in markets are infected.

I am available to discuss this further, thank you for your investigation. I look forward to receiving your report.

Alexandra Morton

PLOS ONE

RESEARCH ARTICLE

The effect of exposure to farmed salmon on piscine orthoreovirus infection and fitness in wild Pacific salmon in British Columbia, Canada

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Abstract

The disease Heart and Skeletal Muscle Inflammation (HSMI) is causing substantial economic losses to the Norwegian salmon farming industry where the causative agent, piscine orthoreovirus (PRV), is reportedly spreading from farmed to wild Atlantic salmon (*Salmo salar*) with as yet undetermined impacts. To assess if PRV infection is epidemiologically linked between wild and farmed salmon in the eastern Pacific, wild Pacific salmon (*Oncorhynchus* sp.) from regions designated as high or low exposure to salmon farms and farmed Atlantic salmon reared in British Columbia (BC) were tested for PRV. The proportion of PRV infection in wild fish was related to exposure to salmon farms ($p = 0.0097$). PRV was detected in: 95% of farmed Atlantic salmon, 37–45% of wild salmon from regions highly exposed to salmon farms and 5% of wild salmon from the regions furthest from salmon farms. The proportion of PRV infection was also significantly lower ($p = 0.0008$) where wild salmon had been challenged by an arduous return migration into high-elevation spawning habitat. Inter-annual PRV infection declined in both wild and farmed salmon from 2012–2013 ($p \leq 0.002$). These results suggest that PRV transfer is occurring from farmed Atlantic salmon to wild Pacific salmon, that infection in farmed salmon may be influencing infection rates in wild salmon, and that this may pose a risk of reduced fitness in wild salmon impacting their survival and reproduction.

Introduction

Infectious viruses are imposing a significant impact on the global salmon farming industry [1], where high host density can elevate both pathogen production and virulence above levels generally found in wild salmon [2]. Reduction in wild salmon productivity has been related to the

Tides Foundation, The Vancouver Foundation, Vancity Credit Union and many smaller donors.

Competing interests: I have read the journal's policy, and the authors of this manuscript have the following competing interests: Alexandra Morton is on the board of the Pacific Coast Wild Salmon Society, Drs. Molly and Frederick Kibenge are married. This does not alter our adherence to PLOS ONE policies on sharing data and materials.

scale [3] and presence [4] of salmon farms, and pathogen surveillance provides useful insight into declining wild salmon populations [5]. Nonetheless, few studies have examined the relationship between exposure to salmon farms and the proportion of wild salmon infected with specific viruses [6].

The piscine orthoreovirus (PRV), discovered in 2010 [7], belongs to the family *Reoviridae*, subfamily *Spinareovirinae* [8], and is now considered ubiquitous in marine farmed Atlantic salmon (*Salmo salar*) in Norway and British Columbia (BC), Canada [9, 10]. PRV is the causative agent of the disease heart and skeletal muscle inflammation (HSMI) [11], which causes specific lesions in the heart and skeletal muscle and can result in anorexia and abnormal swimming behavior in affected fish [9, 10, 11]. An HSMI outbreak can cause 100% morbidity in a salmon farm [11, 12] with associated mortality between 0 and 20% [12]. Stressors, such as sea lice treatment, bacterial infection, and algae blooms, appear to trigger the development of HSMI in PRV-infected fish [13, 14].

PRV infection is also associated with melanized foci in white muscle in Atlantic salmon in Norway [15]. A PRV variant (genotype II) is associated with HSMI-like disease in farmed coho salmon (*Onchorhynchus kisutch*) in Chile [16] and rainbow trout (*O. mykiss*) in Norway [17, 18]. Recently, another related orthoreovirus (PRV-2) was demonstrated as the etiologic agent of erythrocytic inclusion body syndrome (EIBS), a condition associated with mass mortality in farmed juvenile coho salmon in Japan [19]. PRV sequences have also been detected in rainbow trout in Chile that were affected by idiopathic syndrome of rainbow trout (ISRT) [20], and another potential member of the PRV group was associated with epidemic mortality in wild largemouth bass (*Micropterus salmoides*) in the USA [21]. PRV and related orthoreoviruses of fish are therefore not only of major economic concern to the salmon aquaculture industry worldwide, but also with significant consequences for conservation and fisheries of wild salmon.

Recent virus challenge studies with Atlantic salmon show that initially PRV causes a transient acute infection of the erythrocytes (red blood cells), which are nucleated in fish, where it replicates rapidly infecting up to 50% of the red blood cell population [9, 22, 23, 24]. PRV becomes detectable in other organs subsequent to this initial blood-borne infection [11, 22, 23]. HSMI is not detectable within the first 8–10 weeks post challenge [25].

The science on the infection dynamics of PRV in wild fish populations is still emerging. Garseth et al. [5] provide molecular-based evidence that salmon farms play a significant role in the long-distance transport and transmission of PRV in Norway, speculating that pathogen exchange solely between wild salmon during the at-sea migration phase likely plays a minor role in PRV dispersal. While PRV infection in Norwegian sea trout (*Salmo trutta*) is low (1.9–3.0%), the species' persistence in the nearshore environment elevates exposure to salmon aquaculture. This heightens the possibility that sea trout could serve as an intermediary host for aquaculture-source PRV through habitat overlap with salmon during the freshwater spawning and juvenile rearing phases [26]. While no evidence of HSMI was detected in Norwegian wild salmonids [26], the researchers postulated that the impact of severe heart and skeletal muscle damage on a salmon's cardiovascular capacity could decrease the likelihood of an infected fish entering the riverine habitat where sampling was conducted. It is widely observed that diseased wild fish are typically difficult to sample because they are preferentially removed from the population by predators [27].

Most BC marine salmon farms, which are distributed in clusters along the southern half of the BC coast (Fig 1), raise Atlantic salmon, while steelhead (*O. mykiss*) are farmed in BC lakes. Although the Atlantic salmon eggs that entered BC may not have come directly from Norway [28], the dominant strain of Atlantic salmon farmed in BC is the Norwegian 'Mowi' strain [29].

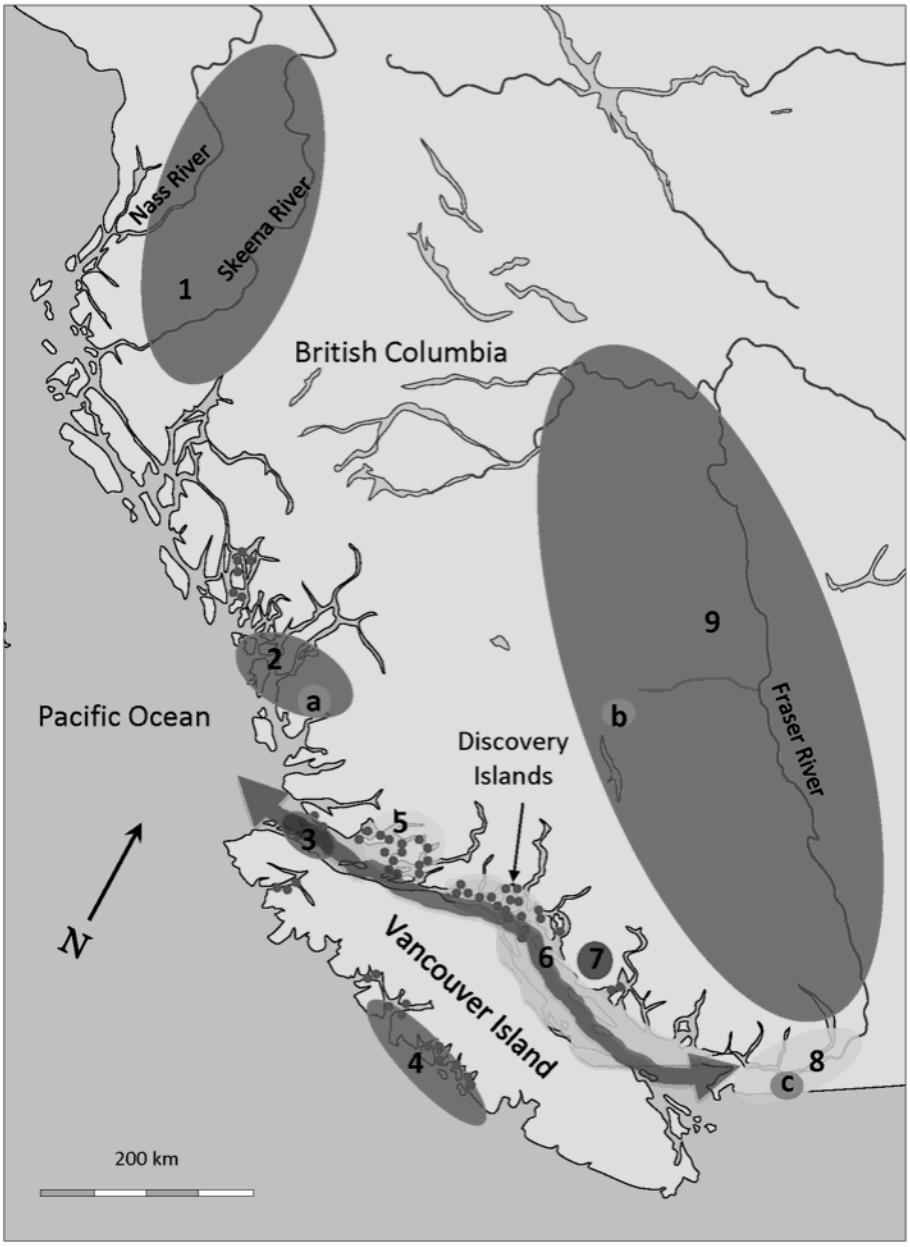

Fig 1. Map of BC with salmon farms and regions sampled. This map shows the following: (1) locations of salmon farms (red dots), (2) the 9 regions where wild salmon were sampled, (3) three lakes discussed in the text, (a) Oweekeno (elevation 15 m) (b) Chilko (elevation 1172 m) and (c) Cultus (elevation 47 m), and three river systems also discussed, the Fraser, Skeena, and Nass. Region color corresponds to the cluster analysis in Fig 4. The blue arrow represents the major Fraser River salmon migration route [38].

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The BC Ministry of Agriculture has reported that approximately 80% of BC farmed Atlantic salmon are infected with PRV [30]). Previous research on PRV infection in wild salmon in BC includes failure to detect the virus in 200 wild salmon collected in 2008 [31], but also a later report that the virus has been common in BC farmed and wild salmon at statistically similar rates of infection since 1987 [32]. Siah et al. [33] also reported a well-established PRV presence in wild and farmed salmon from Alaska through BC to Washington State.

Experimental challenge studies show that PRV will transmit readily from Atlantic salmon to conspecifics through cohabitation [9, 22, 23, 34], as well as sockeye (*O. nerka*) and chinook salmon (*O. tshawytscha*). These infections result in high viral loads in the erythrocytes and kidney [9, 22, 23, 24, 34, 35]. However, evidence of immune activation in response to PRV infection is mixed. Dahle et al. [34] and Haatveit et al. [9] find that PRV infection strongly induces a wide number of interferon-regulated antiviral and MHC class I genes in Atlantic salmon red blood cells. Garver et al. [23] report only a modest antiviral immune response in Atlantic salmon red blood cells, and they fail to find this response in head kidney tissues. Similarly, Polinski et al. [24], find no upregulated innate immune gene expression in sockeye salmon head kidney tissues. The cause for this discrepancy is unclear. However, Dahle et al. [34] and Haatveit et al. [9] challenged with PRV-infected tissues from a field outbreak of HSMI in Norway, while Garver et al. [23] and Polinski et al. [24] performed their studies with a strain of PRV from BC. The samples used in the present study have yielded 14 PRV isolates [16, 36]. The discrepancies between published findings as they relate to the induction of immune responses in host salmon have not been resolved.

While Garver et al. [23] reported that western North American PRV fails to cause HSMI, Di Cicco et al. [13] reported on two HSMI outbreaks in a salmon farm in BC. Hence, while earlier work reported that HSMI does not occur in BC [23, 32, 33], it is now understood that HSMI does occur in BC. However, HSMI has not been reported in wild or captive Pacific salmon.

Here, we report the results of PRV screening of a broad collection of wild salmonids sampled throughout much of BC in 2012 and 2013, and samples of farmed Atlantic salmon and steelhead reared in BC net pen facilities from the same time period. We assess these data for evidence of (i) a potential epidemiological link between farmed and wild salmon and (ii) potential impact of PRV infection on wild fish. In addition, we also present data, sampled from Oweekeno Lake between 2014 and 2016, on PRV infection status of wild salmonids including an endangered sockeye salmon population (S1 and S2 Tables).

Materials and methods

Sampling

As per restricted direct access to farm-specific Atlantic salmon, samples were obtained from markets selling fresh farmed salmon reared in BC marine net pen facilities. In 2012–2013, gill and head kidney samples were collected from 262 fresh farmed BC Atlantic salmon and 35 farmed Steelhead reared in freshwater net pens purchased from 10 BC market chains located in southwestern BC on 93 different dates. The fish suppliers confirmed that these farm salmon had been reared in the pens sited on the BC coast. There was no information as to the specific farm each sample was from. The "Best Before" date was used to select for the freshest samples.

In 2012–2013, gill, heart, head kidney, and spleen tissues were extracted from 601 wild Pacific salmonids (*Oncorhynchus* spp.) (Table 1) collected from marine and freshwater throughout southern British Columbia from the numbered Regions in Fig 1. Another 402 salmonids were sampled 2014–2016 from Oweekeno Lake, Region 2a (Fig 1, S1 Table). Because these were sampled during different years, they were analyzed separately.

We note that the sampling did not constitute an extensive, structured surveillance of wild salmonids in BC. Hence, we have not attempted to construct precise estimates of PRV prevalences in wild salmon with tight confidence limits. Our study, aimed at exploring potential geographic patterns and generating epidemiological evidence providing provisional support for key hypotheses, was more akin to those reported in [5] and [37].

Table 1. Numbers of wild salmon and trout collected in 2012 and 2013 by species and life stage. Numbers inside brackets are for the subset "exposed" to salmon farms, i.e. from Regions 5, 6, 7, 8 and 9.

Species	Juveniles	Adults	*Totals*
Chinook (*O. tshawytscha*)	22 (22)	77 (13)	*99 (35)*
Chum (*O. keta*)	23 (15)	44 (2)	*67 (17)*
Pink (*O. gorbuscha*)	32 (28)	76 (22)	*108 (50)*
Sockeye (*O. nerka*)	91 (3)	129 (74)	*220 (77)*
Coho (*O. kisutch*)	24 (23)	45 (8)	*69 (31)*
Steelhead (*O. mykiss*)	0 (0)	14 (9)	*14 (9)*
Kokanee (*O. kisutch*)	0 (0)	8 (1)	*8 (1)*
Trout (*O. mykiss/clarkii*)	0 (0)	16 (12)	*16 (12)*
Totals	**192 (91)**	**410 (141)**	***601 (232)***

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The 192 juvenile salmon sampled in 2012–2013 were obtained from weekly beach seines conducted to monitor the spring outmigration through the near shore marine environment in Regions 5 and 6. These were collected under Fisheries and Oceans Canada scientific collection permits. The juvenile salmon collected from Oweekeno Lake in 2014–2016 were obtained via fixed trap nets, purse seining, and surface trawling. Adult salmon collected in 2012–2013 were opportunistically collected from marine sport and commercial fisheries in Regions 3, 5 and 6, and were obtained as freshly dead specimens from rivers in Regions 1, 2, 3, 4, 5, 6, 8, and 9. The Kokanee (*O. nerka*) sampled 2012–2013 from Region 7 and the trout sampled in the same years from Regions 2a, 7 and 8c were obtained from sport fisheries. One to ten fish were taken from each sampling event. The adult fish sampled in Oweekeno in 2014–2016 were collected via angling and gillnetting under BC Provincial licenses and from aboriginal food fisheries.

Percussion to the head was used to euthanize live fish. Tissues were extracted within hours after specimens were obtained, whether live-caught, from fisheries or purchased from markets, using aseptic technique, including fresh, disinfected tools (a separate set for external vs. internal sample removal), and disposable work surfaces for each fish. Tissue samples were preserved in RNAlater® and shipped on ice to the Atlantic Veterinary College laboratory. No accompanying information on specific site identification or exposure classification was provided to laboratory analysts in order to minimize any bias. Cross contamination between samples from fisheries is expected and was minimized by sampling between different boats. In the case of sport-caught fish only 1–2 fish were sampled per boat.

Regions

While the regional source of the farmed salmon could only be identified as the southern half of BC where salmon farms are established, the wild salmonids were collected from nine distinct geographic regions across BC (Fig 1). These regions, shown in Fig 1, are grouped into two categories which differ with respect to exposure to Atlantic salmon farms.

Regions 1 and 2 are distant from salmon farms, while Regions 3 and 4, though closer to salmon farms, are directly flushed by open-ocean water. Collectively, Regions 1–4 were classified as experiencing low exposure to Atlantic salmon farms (369 fish).

Regions 5 and 6 are inshore archipelago environments with high fish farm density and retentive marine circulation [39]. Region 7 is a lake that is inaccessible to anadromous fish, where a steelhead farm is sited. Regions 8 and 9, divide the lower and upper Fraser River at the strong rapids in the Fraser Canyon. A large percentage of sockeye, the second most numerically abundant salmon species in the Fraser River system [40], migrate through Region 6 as they approach the river to spawn [41]. Salmon from Regions 5–9 were therefore classified as having a high exposure to farmed Atlantic salmon (233 fish).

Migration challenge

Fish sampled from the upper reaches of substantial watersheds (the Fraser, Skeena, and Nass, Fig 1) were deemed to have overcome significant migration challenges. The two largest of these watersheds are the Fraser and Skeena. For the Fraser, the most significant restriction is at Hells Gate in the Fraser Canyon (elevation about 100 m, but with the majority of the samples above this restriction taken from elevations of over 300 m). The primary salmon rivers in the Skeena watershed are the Babine, with major restriction in the vicinity of the 1951 Babine Slide (elevation around 400 m) [42], and the Bulkley, with major restriction at Moricetown Canyon (elevation around 380 m). Fish sampled from above these restrictions, and from above 300 m in another tributary, were placed in the high-challenge category. All fish sampled from the Nass were obtained from the Meziadin Lake watershed above the rapids in the Nass River, and were therefore also placed in this category.

Viral screening

The laboratory was provided with a unique identification code for each sample which did not include information on the site or exposure classification. When the laboratory returned the results for the statistical analysis, the identification codes were used to link viral status to sampling location, species, and life stage.

RNA was manually extracted from fish tissues and quality was based on the OD A260/A280 ratio and quantitative reverse transcription polymerase chain reaction (RT-qPCR) amplification of either Atlantic salmon ELF-1α (GenBank accession number BT072490) or chinook salmon ELF-1α (GenBank accession number FJ890356) as an internal control. RNA was considered suitable for viral testing if amplification of ELF-1α yielded cycle threshold (Ct) values <30. Primers, probes, and RT-qPCR thermal cycling parameters were as described in Kibenge et al. [36]. All samples were screened for PRV targeting the L1 gene segment as described in Kibenge et al. [36]. In brief, Ct values ≤ 40 were considered positive.

Statistical analyses

The data files used in the following analyses are available in S3 and S4 Tables.

The relationship between the viral screening results and exposure to salmon farms was first examined using a cluster analysis on the proportions of PRV-positive test results within farmed fish (Atlantic salmon and steelhead) and the nine wild fish regions (all species combined). Additionally, logistic regression analyses were used to: (a) probe for potential underlying causes for the geographic patterns in these proportions, (b) generate leads for further investigation, and (c) check for the potential that any apparent patterns could be attributable to other causes. The focus in the logistic regression analysis was on levels of exposure to salmon farms, return migration challenge, and host species. Lastly, the proportions of Atlantic salmon testing positive for PRV were assessed for inter-annual variation using likelihood-based inference.

Because so little is known about the potential epidemiological interactions between farmed and wild salmon in the North Pacific, an exploratory approach to our analyses was used. Thus, in keeping with the spirit of exploratory data analysis [43], we adopted a flexible approach to the selection of statistical methods and models, and put forward our conclusions as hypotheses worthy of further attention.

Cluster analysis. To perform the cluster analysis on the regional proportions of PRV results we applied the agglomerative, hierarchical clustering method based on cluster centroids as implemented in the SAS® CLUSTER procedure, SAS software, Version 9.4. In keeping with commentary in SAS 2013, the centroid method was selected to avoid giving too much influence to the much larger proportion of PRV positive fish in the farmed Atlantic salmon category.

Logistic regression analysis. The logistic regression analysis was conducted solely on the wild fish. The factors of primary interest were: salmon farm exposure, migration challenge, and host species.

The number of categories was restricted to avoid the potential for over-parameterization. Farm exposure and migration challenge were categorized as low or high as described above. Host species were reduced to four taxonomic units among the wild fish by combining lineages that had not diverged prior to approximately 7.5 million years ago [44, 45]–chinook-coho salmon with 168 samples, chum-pink salmon with 175 samples, sockeye salmon with 220 samples, and rainbow-cutthroat trout, with 38 samples.

Two other factors, life stage and year, were included in the logistic regression analysis to probe for potential confounding effects. The wild salmon life stages were divided into 2 categories: juveniles (192 fish) and adults (409 fish).

Observations used in the logistic regression analysis were limited to 2012 and 2013, the years for which farmed and wild salmon were concurrently sampled in sufficient numbers. There was insufficient data to extend formal inferences to other years. There were too few degrees of freedom, and the standard assumption of independence between years that underlies the usual models for random effects would have been compromised if, for example fish returning at ages 4 and 5 from the same cohort were both exposed to the same PRV source at an earlier life stage. Furthermore, Taksdal [46] highlights the potential both for differences in virulence between virus subtypes, and for relatively abrupt changes in viral-subtype presences that could produce sudden jumps in the proportions of positive tests. Both of these events would reduce the comparability of years in which only farmed Atlantic or wild salmon were collected. Such complex behavior calls for more elaborate modelling. Hence, inferences have been limited to 2012–2013, year effects were treated as fixed, and Oweekeno Lake data was not included in the analysis.

Furthermore, there were sufficient numbers of observations to assess the main effects of each of the factors, but not necessarily for interactions between them (see S1 File for further explanation).

Finally, a random effect associated with the within-cluster correlation of fish obtained from the same location and year was included to account for potential dependency in PRV presence among fish sampled from the same effective host population. This term additionally compensates for cross-contamination within a sampling event, as this would have had a comparable impact to the contagious spread of virus within a school of fish before they were caught.

A more formal description of the statistical model is provided in S1 Text.

Model selection. We used a stepwise approach to our logistic regression (starting with a full model) to screen for potentially influential factors. To reduce the likelihood of deleting potentially important variables in this exploratory analysis, we planned to remove, at each deletion step, the variable with the highest p-value from the model only if its p-value exceeded

0.10. Competing methods based on AIC and other similar measures of goodness of fit were complicated by occasional cases of missing information on some variables. Hence, the stepwise approach was more appropriate, and generated a preferred model after only two deletion steps. All mixed-effects logistic regression inferences were performed using the SAS GLIMMIX procedure as implemented in SAS software, Version 9.4.

Comparative analysis of test results on farmed Atlantic salmon. We also formally compared the proportions of PRV-positive tests for the farmed Atlantic salmon between 2012 and 2013. Because multiple fish were purchased from the same outlet on the same day, we needed to account for potential dependence within such clusters of sampled fish generated by factors such as a common farm of origin and cross-contamination in processing and handling during harvest. We did so by incorporating a random effect term similar to that used in the logistic regression model. Details are provided in S2 Text.

Results

The farmed fish generated slightly higher ELF-1α Ct values, in keeping with the unavoidable delay in tissue preservation of market-sourced fish; however, tissue quality was suitable for RT-qPCR testing across all samples (Fig 2).

Raw proportions of PRV-positive tests are shown in Fig 3. PRV infection was highest among the farmed salmon categories; Atlantic salmon (95%) and steelhead (69%). The highest proportions of PRV-infected wild salmonids were from the high exposure regions, *i.e.*, Regions 5–8, including the lake with a steelhead farm and the highly exposed inshore archipelago environments (37–50%). The proportion of PRV infection declined between the highly exposed lower (41%) and upper (22%) Fraser River. The lowest proportions were in Regions 1 and 2, furthest from salmon farms (5%). In addition, Cultus Lake trout were highly infected with PRV (76%) (Lake c, Fig 1), while only 3% of the salmonids in Oweekeno Lake were infected with PRV (Lake a, Fig 1, S1 Table).

A complementary perspective emerged from the cluster analysis on these proportions (Fig 4). The two farmed fish species each formed distinct, single-element clusters. All high exposure regions, except Region 9 (post high migration challenge) appear in the yellow cluster. The green, less homogeneous cluster includes the high migration challenge Region 9 with all the low exposure regions.

Details of the stepwise logistic regression procedure are summarized in S5 Table Summary of stepwise regression process. Two factors were dropped in the stepwise regression: "species group" and "life stage", and then the algorithm terminated.

All of the fixed factors in the preferred model (year, farm exposure, and migration challenge) were significant ($p < 0.01$, Table 2).

The proportion of PRV-infection in both wild and farmed salmon declined substantially between 2012 and 2013. For highly exposed wild salmon that had not faced a major migration challenge, the least-squares mean estimate of this proportion declined from 0.564 in 2012 to 0.129 in 2013 (Fig 5). The corresponding decline for farmed Atlantic salmon, from 0.974 to 0.790, was also strongly significant ($p = 0.002$ from the modelling procedure outlined in S2 Text).

In addition to year, the effects of the other two factors, exposure and migration challenge, were also estimated to be large, though with substantial standard errors (Fig 5). The estimated effect of the most significant of all three factors, migration challenge, was also the largest. Fig 5 shows that, for high-exposure wild salmon in 2012, there was over a six-fold decline in the estimated proportion of PRV-positive test results from (a) fish in the low-challenge category to (b) those in the high-challenge category. This estimated decline is commensurate with the

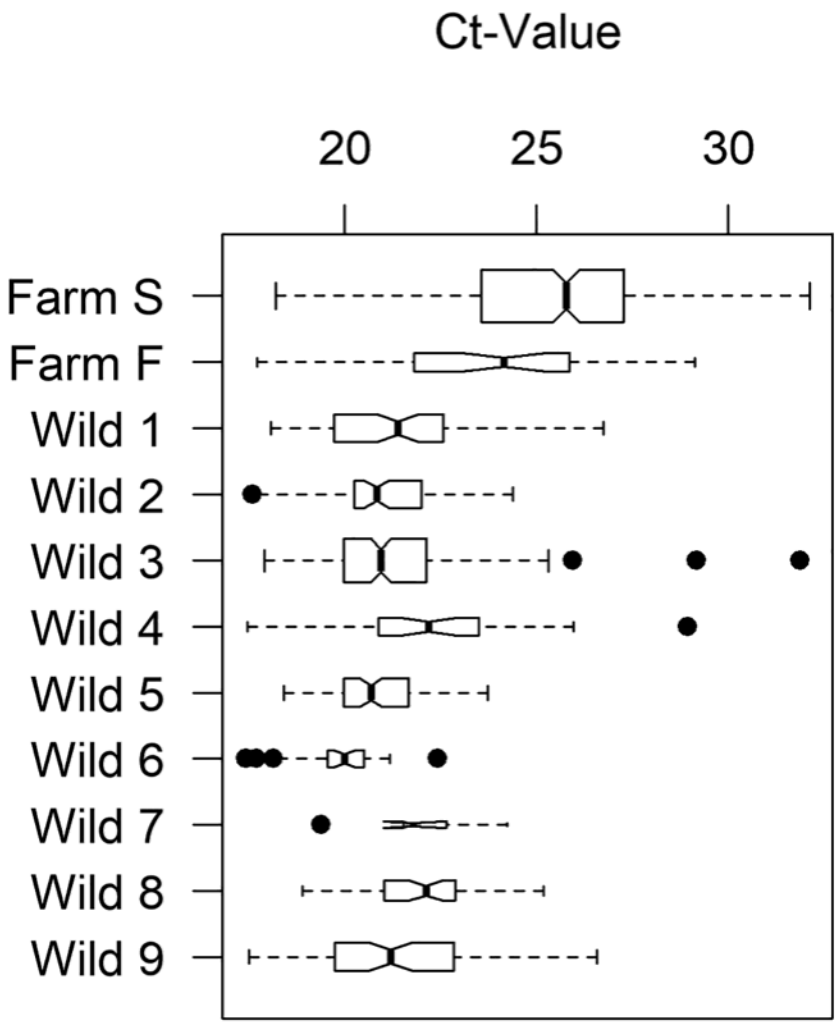

Fig 2. Internal control ELF-1α Ct values indicating sample quality. Ct values <30 are considered of sufficient quality for RTqPCR viral screening.

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observed declines (i) between Regions 8 and 9 (the lower and upper Fraser River areas) and (ii) between the lower and higher elevations in Region 1 in northern BC (Table 3). Fig 5 also shows that, for low-challenge wild salmon in 2012, there was over a two-fold decline in the estimated proportion of PRV-positive test results from (a) fish in the high-exposure category to (b) those in the low-exposure category.

Discussion

The results of this work suggest that exposure to salmon farms has a strong association with increased risk of PRV infection in wild salmonids, and that the proportion of PRV-infected wild vs. farmed salmon can vary synchronously between years. In addition, the decline in PRV infection between the low and high migration challenge groups suggests that PRV infection may reduce a host's capacity to complete a challenging upriver migration, thereby reducing reproductive fitness. We stress the correlational nature of the present findings, but believe, in keeping with the Precautionary Principle, that they warrant further research attention due to the high ecological, economic, and cultural value of wild Pacific salmon.

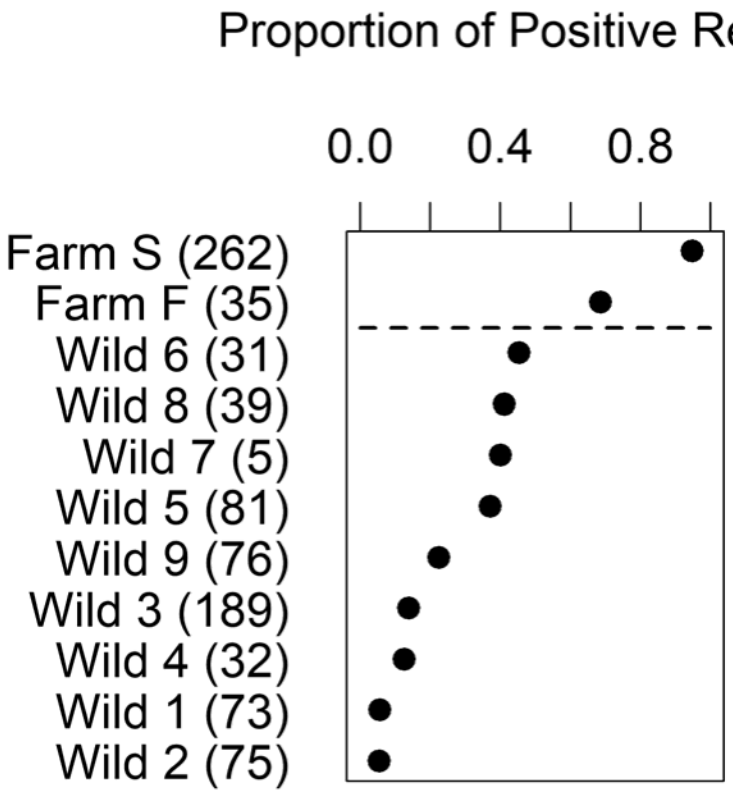

Fig 3. Proportions of PRV RT-qPCR-positive results. Results are arranged in decreasing order. The "Wild" designations reflect the Region numbers in Fig 1; i.e., Wild 1 is from Region 1. Numbers of fish sampled are provided in parentheses on the horizontal axis labels. Relevant estimates and confidence limits for key differences in this figure were generated by the logistic regression modelling where the effects of potential confounding variables could be filtered out (Fig 5).

https://doi.org/10.1371/journal.pone.0188793.g003

The hierarchical cluster analysis of PRV-infection proportions showed a clear separation between the more highly infected farmed Atlantic salmon and all categories of wild Pacific salmon (Fig 4). This demonstrates the significantly greater potential infection pressure imposed by farmed salmon in comparison to wild salmon. Further, the logistic regression analysis has demonstrated that higher exposure to farmed salmon is associated with a significant increase in the proportion of PRV infected wild salmon. This is a plausible result as 79–95% of farmed salmon tested positive for PRV and wild fish in the regions where salmon farms operate among retentive currents would likely experience a higher contact rate with infectious PRV particles than wild salmon elsewhere. This result is in keeping with other research findings [6,47]. Deterministic modeling of water-borne infectious particles demonstrates that a high number of shedding hosts elevates the localized concentration of infectious particles thereby increasing the rate of infection in susceptible hosts [47]. This model is well supported by the empirical evidence that farmed salmon epizootics tend to cluster in both space and time (as reviewed in [6]). PRV has also been shown to be highly infectious both among and between species with transmission occurring from Atlantic salmon to both Atlantic and Pacific salmon through experimental cohabitation challenges [9, 22, 23, 24, 34]. While the exact mechanism of PRV transmission remains unknown, Hauge et al. [48] show that faecal virus shedding may release a significant amount of infectious particles into the water. While the heightened proportions of PRV-infection in wild salmon from high exposure regions provides some epidemiological evidence of PRV transmission between farmed and wild fish, the presence of PRV in

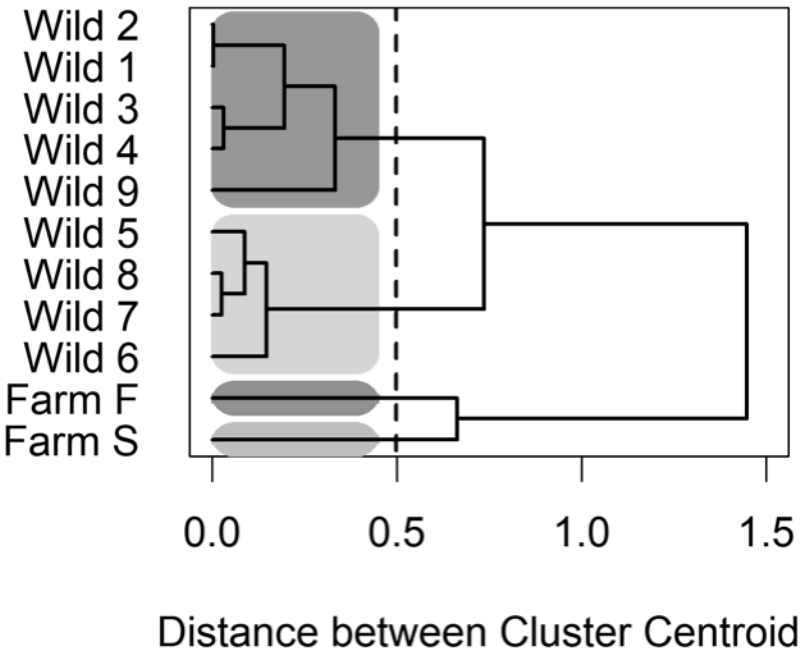

Fig 4. Hierarchical cluster analysis of test results by region and farmed categories.

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low exposure populations suggests that transmission may also occur between individuals of wild populations in the open Pacific, though perhaps at a lower frequency. Together, these findings raise the concern that point-source pathogen release from aquaculture facilities may affect both populations directly exposed, and those that are not directly exposed to salmon farms. Siah et al. [33] also suggested that wild-to-wild transmission best explains the homogenous distribution of PRV S1 sequence types in the eastern Pacific.

By contrast in Norway, Garseth et al. [5] proposed that PRV transmission between low density wild Atlantic salmon during their at sea phase likely plays a minor role in infection rates. However, it is possible that the more abundant wild salmon populations in the northeastern Pacific may provide better opportunities for PRV transmission. Our data provides some evidence for PRV transmission between wild fish, as low exposure populations also carry PRV. However, the higher PRV infection rates among those wild salmon in closer contact with Atlantic salmon also provides provisional evidence of PRV transmission in at least one direction between wild and farmed salmon. Additionally, the significant effect of year on the PRV infected proportion, which acts in the same direction for both wild and farmed salmon, also appears to corroborate the hypothesis that PRV prevalence in wild salmon is epidemiologically linked to prevalence in farmed Atlantic salmon. Garver et al.'s [23] findings that PRV can be

Table 2. Summary of results for the preferred model. The SAS-generated table shows results of tests generated by dropping each factor from the model containing all three factors, with each factor replaced in the model before the next deletion. Degrees of freedom were calculated by SAS with a Satterthwaite correction. Estimates of the odds ratios were obtained by exponentiating the estimated coefficients for the log-odds ratios.

Type III Tests of Fixed Effects							
Effect	**Coeff.**	**SE**	**Num. DF**	**Den. DF**	***F***	***P***	**Odds Ratio**
Year	2.17	0.615	1	98.3	12.40	0.0007	8.72
Exposed	1.55	0.587	1	91.5	6.97	0.0097	6.97
Challenged	2.60	0.764	1	189.8	11.57	0.0008	13.44

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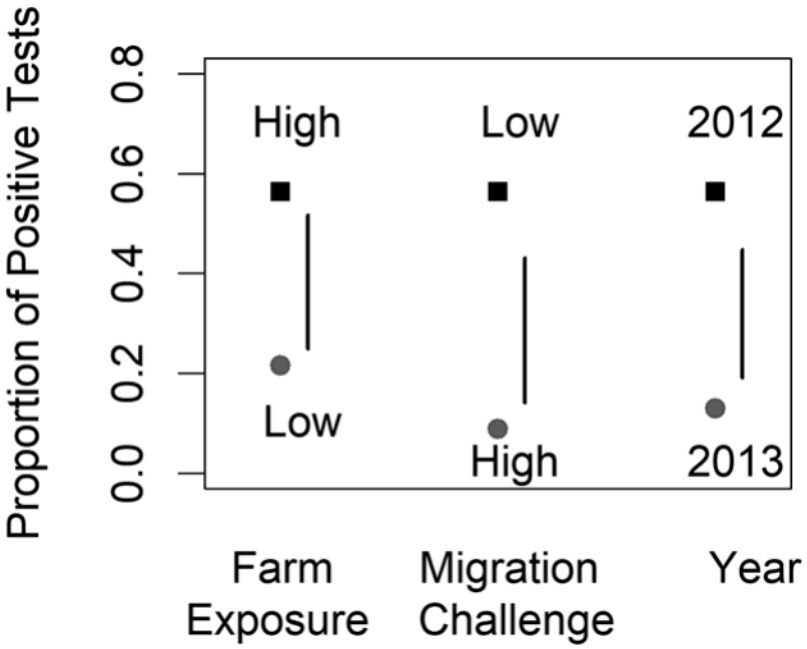

Fig 5. Least-squares mean proportions for RT-qPCR positive test results. The black squares provide a reference estimate for fish at high farm exposure level, and low migration challenge level in 2012 (the 'common reference'). The blue circles are least-squares mean proportions with each of these factors switched in turn to the opposite level, with the other factors left at the common reference level. The vertical bars cover approximately 2 standard errors. Where a vertical bar does not span the gap between the two estimates, the difference is significant at approximately the 5% level in a test against a two-sided alternative.

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transmitted from Atlantic salmon to Pacific salmon but not vice versa provides support for a dominant farmed-to-wild transmission route.

Additionally, this study demonstrates strong evidence generated collectively from two regions in BC of a negative association between increased migratory challenge and PRV-positive proportions in return-migrating wild adult salmon. Fewer infected adults of any species were detected at higher vs. lower elevations in the Fraser River, as well as tributaries of the Skeena and Nass rivers in northern BC. This association points to a cost of infection from PRV to the fitness of wild Pacific salmon. While the pathogenicity of PRV in wild Pacific salmon has been questioned (e.g., [26, 32]), PRV-associated disease states (i.e., HSMI [13] and Jaundice Syndrome [16, 17]) are characterized by lethargy and erratic swimming behaviour [12], which would have more serious consequences for wild Pacific salmon than for farmed Atlantic salmon in net pens. The statistical modelling performed accounted for potential confounding effects from year, exposure level, salmonid host species, and life stage, and still found strong

Table 3. Observed proportions of positive PRV tests by migration challenge level for regions with substantial numbers of migration-challenged fish (low exposure region 1 and high exposure regions 8 & 9). Numbers in brackets reflect numbers of positive tests per fish sampled.

	Migration Challenge	
Region(s)	**Low**	**High**
1	0.429 (3/7)	0.018 (1/56)
8 & 9	0.410 (16/39)	0.224 (17/76)

https://doi.org/10.1371/journal.pone.0188793.t003

evidence of a decline in the infected proportion of salmon at higher elevations. However, it is possible that some other factor not included in the model could account for this change in proportions. Further investigations employing the tracking of the in-river fates of individual salmon by biotelemetry, as has been demonstrated by Jeffries et al. [49], and Miller et al. [37, 50], can better resolve confounding variation possibly associated with the migration timing of specific stocks and the timing of sampling events. However, with the geographic scale and numbers of fish used in the present study, it was infeasible to employ such technologies. Nonetheless, the evidence of lower PRV presence in salmon at higher elevations has important potential implications regarding fitness costs and population impacts of PRV on wild salmon. Similar findings were also reported by Miller et al. [37], who found PRV infection to be significantly associated with en-route migration losses for Chilko Lake sockeye salmon, which are challenged by an arduous 1,172m elevation gain in their return migration (Region 9, Lake b, Fig 1). In contrast, these authors reported that PRV infection was not significantly associated with migration losses into the lower elevation Shuswap Lake watershed (elevation 350m).

The PRV infections detected in salmonids in low-lying lakes, Cultus (elevation 47 m) and Oweekeno (elevation 15 m), and in particular the higher proportion of positives in Cultus Lake trout where anadromous salmon entering the lake have been highly exposed to farmed salmon potentially on both seaward and return migrations, provide a contrast to the observed reduction in PRV in fish sampled at higher elevations. This contrast suggests the following hypotheses for future research: (i) PRV-infected wild fish are less able to meet the challenge of migrating into higher elevations above sea level, (ii) easily accessed, low-lying lakes lack the infection filtering effects of return migrations with greater challenges and may be more vulnerable to the introduction of aquaculture-source viruses via infected anadromous salmonids than high elevation habitat, and (iii) resident trout or other fish species in these lakes may act as viral reservoirs increasing the complexity of PRV transmission dynamics and potentially exposing successive generations of salmonids to infection.

PRV has previously been shown to have a broad host range among salmonids in the Pacific and Atlantic [this study, 5, 23, 26, 32, 33, 36], including a first report in this study of a positive test for Dolly Varden char (from Oweekeno Lake, Fig 1, Lake a, S1 Table). Positive PRV results have been reported for some non-salmonid marine fish in coastal Norway as well [51]. The consequences of these potentially complex host-pathogen dynamics for sustaining infections in wild salmon populations are unknown, but their prospect raises important questions regarding the vulnerability of low-elevation salmonid populations to viral disease. Future work should attempt to identify competent host species, and to characterize viral reservoirs in addition to Atlantic salmon farms, particularly in light of the collapse of both the low-lying Cultus and Oweekeno Lake sockeye salmon populations to less than 1% of their historic spawner returns with no clear cause, despite significant restoration efforts [52, 53].

Recent research on PRV points to mechanisms through which the virus might impact the capacity of a salmon to complete a challenging migration to reach its spawning grounds [13, 22]. PRV has been found to proliferate in the erythrocytes, with possible implications for oxygen transport and swimming performance [22]. Research on PRV infection in Atlantic salmon hosts has also shown that PRV has a transient acute infection stage during which innate antiviral pathways are strongly upregulated [9]. Activation of these immune system pathways has been shown to have both direct and indirect energetic costs to a host [54]. While a similar level of immune activation in response to PRV infection has not been shown for Pacific salmon species [24], this could have other explanations beyond a total lack of pathogenicity, specifically: differences in pathogenicity among described and uncharacterized PRV strains, host species/virus strain interactions, and inferential complications arising from the current inability to culture PRV in fish cell lines.

Histopathological examination of samples has value in confirming disease state and reinforcing the association between a condition and any impact to fitness; however, this approach was not employed in the present study as it is considered unlikely that wild salmon will progress to clinical disease before being targeted by predation [55]. A potentially more profitable approach employed by Miller et al. [55] uses modern molecular methods to predict the pathogenic outcomes of infection for salmon at early stages of infection. These authors have found that gene expression biomarkers for active virus infection can differentiate between both Atlantic salmon with HSMI and Pacific salmon species with Jaundice Syndrome (also strongly associated with PRV [16, 17]) from virus-negative fish and from fish with clinical diseases caused by other pathogen types [55]. It is hoped that greater numbers of future studies will take this approach in order to strengthen or refute the associations found herein, and more fully understand the consequences of viral pathogens like PRV for the fate of infected wild salmonids.

Conclusions

This study provides the first evidence that (i) exposure to farmed Atlantic salmon is associated with infection of wild Pacific salmon with PRV, a virus of significant concern to both the aquaculture industry and wild fisheries management, and (ii) that PRV infection may impair the capacity of wild salmon to complete a challenging spawning migration, with the potential for population-level impacts. The evidence, based solely on molecular screening tests from this observational study, and constrained by limited access to farmed Atlantic salmon samples of known provenance, cannot be definitive. Nonetheless, we view it as providing an early warning sign of a potentially serious problem that warrants immediate and ongoing research. Research into the fitness impacts to wild Pacific salmonids of farmed salmon pathogens is needed in wild fish populations in addition to controlled laboratory environments, and could provide valuable insights useful for the management of critically declining wild salmon populations.

Supporting information

S1 Table. Oweekeno Lake salmonid samples.
(DOCX)

S2 Table. Oweekeno Lake data file.
(XLSX)

S3 Table. Wild salmonid data file.
(XLSX)

S4 Table. Farmed fish data file.
(XLSX)

S5 Table. Summary of stepwise regression process.
(DOCX)

S1 File. Numbers of 2012–3 sampled salmonids by species group and migration challenge categories.
(DOCX)

S1 Text. Logistic regression model summary.
(DOCX)

S2 Text. Technical details of comparative analysis on farmed Atlantic salmon.
(DOCX)

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Project administration: Alexandra Morton.

Resources: Alexandra Morton.

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Writing – review & editing: Alexandra Morton, Richard Routledge, Stacey Hrushowy, Molly Kibenge, Frederick Kibenge.

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FORMAL COMMENT

Formal comment on: Piscine reovirus: Genomic and molecular phylogenetic analysis from farmed and wild salmonids collected on the Canada/US Pacific Coast

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Introduction

This formal comment is in response to Siah *et al.* [1], Piscine Reovirus: Genomic and Molecular Phylogenetic Analysis from Farmed and Wild Salmonids Collected on the Canada/US Pacific Coast, with a subsequent correction (Siah *et al.* 2016 [2]). Although a correction for this paper was published on Oct. 12, 2016, (Siah *et al.* 2016 [2]), there continues to be inadequate supporting evidence for the primary conclusion that PRV genetic sequences are temporally and spatially homogeneous in salmonid species across the northeastern Pacific region.

The evidence in this paper warrants thorough consideration. Piscine orthoreovirus (PRV) causes acute infection of the red blood cells in salmon (Finstad *et al.* 2014 [3]; Haatveit *et al.* 2017 [4]). It is the causative agent of the emerging farm salmon disease Heart and Skeletal Muscle Inflammation (HSMI) (Wessel *et al.* 2017 [5]) with clinical symptoms which can include lethargy, anemia, anorexia and mortality (Kongtorp *et al.* 2004 [6]). Palacios *et al.* [7] expressed concerns about the transfer of PRV from farmed to wild fish due to its contagious nature. PRV is now considered ubiquitous in farmed Atlantic salmon (Haatveit *et al.* 2017 [4]) and has an estimated 80% prevalence rate among BC farmed salmon (Kibenge *et al.* 2013 [8]). HSMI has recently been diagnosed in British Columbia (BC), Canada (Di Cicco *et al.* 2017 [9]). Hence, release of PRV from salmon farms into Pacific salmon habitat is a significant management concern in the eastern Pacific Ocean.

In the correction, Siah *et al.* [2] acknowledge that the conclusion that PRV has not been recently introduced to BC was overstated. However their supporting evidence that ". . . salmonids from western North America Pacific waters carried PRV RNA sequences for at least 13 years with little genetic differentiation among sequence types in selected samples spanning 2001 to 2014" remains insufficient.

Their conclusion appears to be highly dependent on six unique sequences of PRV segment S1, detected by Siah *et al.* [1]:

KR478642: collected in May 2001

KR478643: collected in Aug. 2001

KR478644: collected in Aug. 2001

towards high survivorship of wild salmon in BC's rivers and near-shore marine environment.

Competing interests: This work was supported by the Virology Research Laboratory at the Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PE, Canada. Van City supported the work by Alexandra Morton. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. This does not alter our adherence to PLOS ONE policies on sharing data and materials. Alexandra Morton is a director of the Pacific Coast Wild Salmon Society, whose goals include working towards high survivorship of wild salmon in BC's rivers and nearshore marine environment.

KR347078: collected in Aug. 2001

KR347079: collected in Aug. 2001

KR347080: collected in Mar. 2005

These six Siah *et al.* [1] sequences collected in 2001 and 2005 (submitted to GenBank April—May 2015) predate those collected by Kibenge *et al.* [10,11] by seven years and are nearly identical to the isolates Siah *et al.* [1] collected in 2013 and 2014. Thus, these six PRV isolates appear to be highly resistant to mutation over a 13-year interval 2001–2014, which is atypical for RNA viruses, generally known to exhibit a high mutation rate (Chao *et al.* [12]). Drake and Holland [13] estimate the genomic mutation rate (*U*) to be between 1 and 0.1 for most RNA viruses, where *U* is *G* x *u*, *G* is the genome size in nucleotides, and *u* is the per-nucleotide mutation rate.

Weight of evidence for longer-term PRV presence in BC

Siah et al. [1] cite detection of PRV in a wild Steelhead trout (*O. mykiss*) collected in 1977 in support of longer-term PRV presence in BC. This result is cited from Marty *et al.* [14], who provided no S1 segment sequence information to verify the PRV strain identity as per the sequence groupings reported by both Kibenge *et al.* [8] and Garseth *et al.* [15]. The recent discovery of the widespread occurrence of PRV-2 across the North Pacific (Takano *et al.* 2016 [16]) raises the question: Was the 1977 steelhead infected with PRV-2 or PRV? In absence of S1 sequencing this uncertainty cannot be resolved.

Furthermore, this result could not be replicated by a second laboratory (Purcell and Thompson 2014 [17]) and therefore warrants qualification as a non-repeatable result and a suspect positive lacking sufficient robustness to provide evidence critical to the temporal presence of PRV in BC.

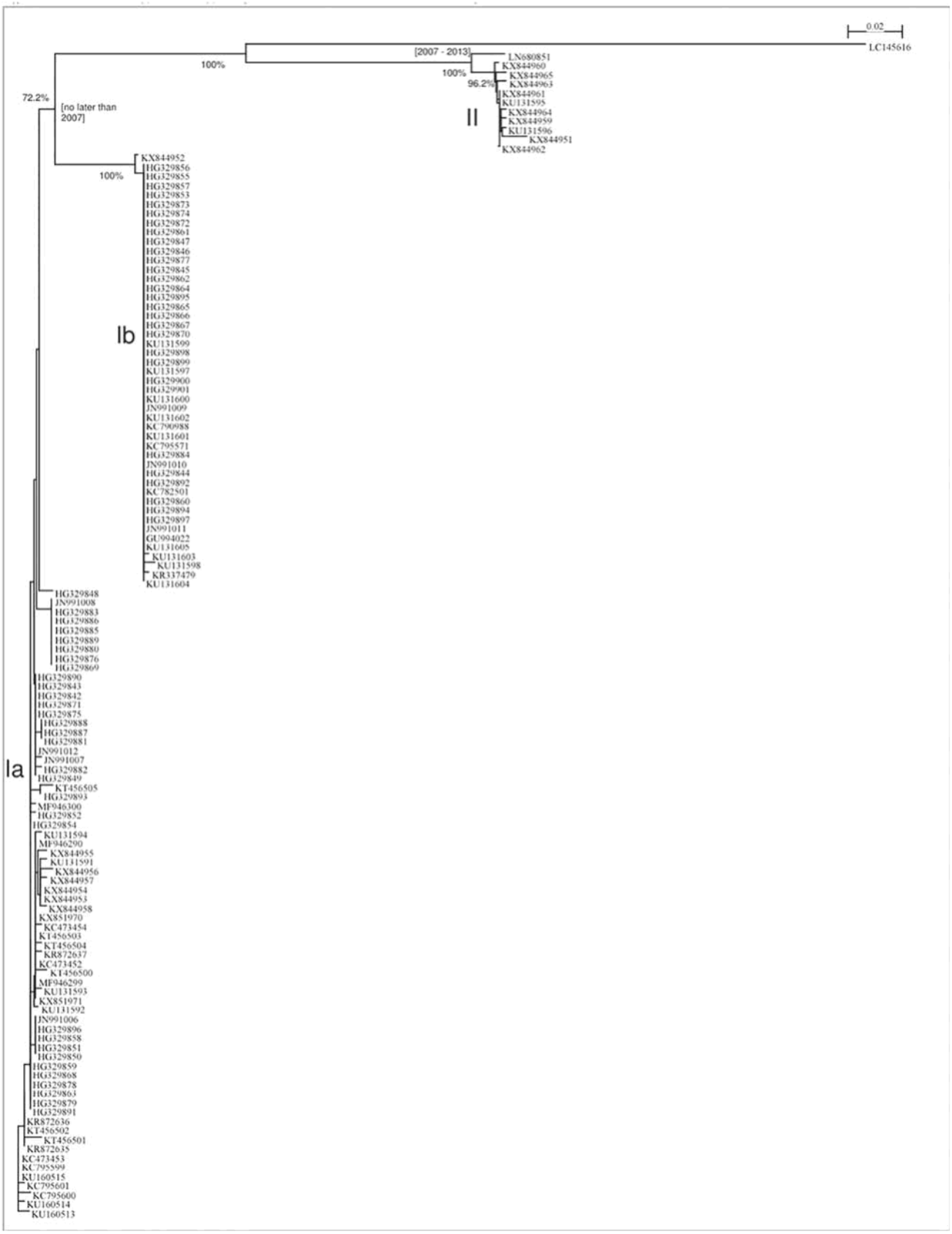
Phylogenetic comparative analysis

To illustrate our interpretation of the phylogenetic analysis of PRV isolates, we constructed a phylogenetic tree (Fig 1), of the 127 sequences described in S1 Table. The mutation direction was determined by an outgroup sequence (GenBank Accession No. AF004856). After the root of the tree was determined, the outgroup sequence was removed so that the details of the tree could be shown.

Fig 1 demonstrates that all PRV isolates can be classified into Genotypes I and II, with Genotype I further divided into sub-genotype Ia and sub-genotype Ib. Among these sub-genotypes, all Canadian isolates exist in sub-genotype Ia; this evidence provides information that PRV in BC-Canada is closely related to PRV found in Norway.

We estimated the divergence time between the genotypes and sub-genotypes (Ia, Ib and II) based on the collection time of each isolate described in S1 Table. Basic rules of logic were used in the estimation, such as the divergence time must not be later than the collection time of any isolate in all branches.

We estimated that the divergence time between sub-genotype Ia and sub-genotype Ib was 2007 or earlier. Because all sequences in S1 Table were collected in 2007 or later, we are not able to estimate a more accurate divergence time. We also estimated that the divergence time between Genotype II and the rest of the isolates was in the range of 2007 to 2013. The PRV-2 sequence GenBank Accession No. LC145616, from Japan (Takano *et al.* 2016 [16]), is quite different from all other isolates and it may constitute a second sub-genotype of Genotype II or a completely new genotype (Genotype III); but because we cannot find other evidence, we consider this sequence an outlier at the present time.


0.02
LC145616
[2007 - 2013]
LN680851
KX844960
KX844965
KX844963
KX844961
KU131595
KX844964
KX844959
KU131596
KX844951
KX844962
II
100%
100%
96.2%
72.2%
[no later than 2007]
100%
KX844952
HG329856
HG329855
HG329857
HG329853
HG329873
HG329874
HG329872
HG329861
HG329847
HG329846
HG329877
HG329845
HG329862
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HG329897
JN991011
GU994022
KU131605
KU131603
KU131598
KR337479
KU131604
Ib
HG329848
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HG329883
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HG329871
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HG329893
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HG329852
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KC473454
KT456503
KT456504
KR872637
KC473452
KT456500
MF946299
KU131593
KX851971
KU131592
JN991006
HG329896
HG329858
HG329851
HG329850
HG329859
HG329868
HG329878
HG329863
HG329879
HG329891
KR872636
KT456502
KT456501
KR872635
KC473453
KC795599
KU160515
KC795601
KC795600
KU160514
KU160513
Ia

Fig 1. Phylogenetic tree for sequences of piscine orthoreovirus (PRV) segment S1 listed in S1 Table. All 127 available robust isolates are included in this tree. The phylogenetic tree was constructed using the neighbor-joining method and Tamura-Nei genetic distances (Saitou and Nei 1987 [18]). Bootstrapping was performed 1,000 times. Bootstrap supports of topology of 70% or higher are shown at the nodes. The PRV grouping of Genotype I sub-genotypes Ia, Ib, and Genotype II are indicated. The mutation direction was determined by an outgroup sequence.

https://doi.org/10.1371/journal.pone.0188690.g001

Sampling inadequacies

The description of the temporal and spatial distribution of the samples reported on by Siah *et al.* [1] is inadequate to support the conclusion that PRV is endemic to BC. The reader cannot ascertain the degree of independence between sampled fish. For example, there are two groups of samples that generated PRV sequences from locations labeled "Hatchery (British Columbia, Canada)". One group was sampled in October 2013, and another eight samples were obtained in November 2013. Were these fish from the same, related (i.e. shared personnel and broodstock) or different hatcheries? Fish sampled one month apart from the same hatchery would presumably have an increased probability of being infected with the same strain of PRV and cannot be interpreted as independent samples.

Furthermore, all but eight of the partial sequences reported by Siah *et al.* [1] in their Table 2 were obtained in 2013 and 2014. Of the eight other sequences, two were from 2012, one from 2005, and the remaining five were from 2001 (four of which were reportedly sampled on the same day on one or more farms within DFO Statistical Area 18; an area with no reported marine salmon farms, suggesting these were from a hatchery). Thus, it appears that there were only three temporally separate sampling events for the time period 2001–2012. This sparse temporal coverage does not provide sufficiently extensive evidence to support a conclusion of long-term PRV genetic heterogeneity in BC.

There was also inadequate spatial and host-species coverage. Siah *et al.* [1], reported that over half (43/71) of the fish that produced partial PRV sequence information were farmed Atlantic salmon (Siah *et al.* 2015 [1] Table 2). Only two wild fish were sampled north of central BC (two Coho Salmon, *O. kisutch*, from the Copper River in Alaska), and only six Chinook Salmon, *O. tshawytscha*, were sampled (four from southern BC, and two from further south in the Columbia River, Washington State). No other Pacific salmon or trout species were included. Hence, the host species and spatial coverage of PRV sequencing presented by Siah *et al.* [1] are very sparse. Thus Siah *et al.* [1] do not provide sufficient evidence to draw reliable inferences either on the temporal stability or geographic homogeneity of PRV throughout the coastal eastern Pacific Ocean. Considerable variation over time or space could easily have been bypassed.

Furthermore, farm restocking methods could potentially account for at least some of the homogeneity in the Siah *et al.* [1] samples. Although BC farm salmon broodstock sourcing and the distribution of Atlantic salmon from specific hatcheries is not public information in BC, presumably farm salmon from the same hatchery could be transferred into farms hundreds of kilometers apart that are sited throughout wild eastern Pacific salmon migratory corridors. Repeat introduction of the same PRV variant across years and regions may be occurring from Atlantic salmon hatcheries that share broodstock and/or eggs. Thus, the appearance of genetic stability of PRV in migratory wild salmon could be the result of exposure to farm salmon from the same hatchery.

HSMI in BC

Siah *et al.* [1] state, citing Kibenge *et al.* [8] and Marty *et al.* [14], "PRV is known to occur in a wide variety of salmon species on the Pacific Coast of North America, *a region where HSMI*

has never been reported." (Emphasis added.) However, Kibenge *et al.* [8] do cite lesions identified as diagnostic of HSMI in BC farmed Atlantic salmon beginning in 2008. Furthermore, subsequent to both these publications, HSMI has been confirmed in BC (Di Cicco *et al.* 2017 [9]).

Conclusion

We conclude that the longer-term presence of PRV in BC prior to 2001 has not been adequately described and that the evidence that the virus was introduced from Norway is more robust than the hypothesis that PRV is endemic to the eastern Pacific Ocean.

Supporting information

S1 Table. Piscine orthoreovirus segment S1 nucleotide sequences analyzed in this study.
(DOC)

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Writing – review & editing: Molly J. T. Kibenge, Yingwei Wang, Alexandra Morton, Richard Routledge, Frederick S. B. Kibenge.

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From:	Alex Morton
To:	Heyman,MLA, George LASS:EX
Cc:	Popham, Lana; s.22 s.22 s.22 Tavish Campbell; s.22 Meggs, Geoff PREM:EX; s.22 s.22
Subject:	Enterococcus Urgent Questions
Date:	Friday, February 9, 2018 11:34:02 AM
Attachments:	Enterococcus.pdf ATT00001.htm

Dear George Heyman;

In follow-up to my last email on this issue, attached please find a short briefing on the extremely high enterococcus counts that were detected by the province of BC at the fish farm processing plants in Tofino and Browns Bay. This is in addition to the confirmation of PRV in these samples as originally reported by the Kibenge lab at the Atlantic Veterinary College.

As you will see in my report, 30 - 501cfu/100ml is considered the safe level of enterococcus in marine waters, however the plants are discharging in excess 60,000cfu/100ml. They report there were too many bacteria to count. This discharge is going into water frequented by people and raises the concern that this bacteria of human health concern is also being discharged from individual salmon farms coast-wide where people are exposed in a variety of ways.

From a brief review of the literature I find that t**he salmon farming industry has been experimenting with feeding this bacteria to their fish** to produce a probiotic effect, increase growth, and increase resistance to sea lice. Is the high enterococcus count in the blood water a result of this practice?

There are urgent questions that arise from these test results:

1 - have you tested the beach areas adjacent to the Browns Bay and Tofino packing plants?
2 - what species of enterococcus was detected?
3 - what is the antibiotic resistance profile of the bacteria collected?
4 - are BC salmon farmers feeding this bacteria to the fish in their pens?
5 - what is the explanation for these high counts?
6 - what are the enterococcus levels found around salmon farms where people are fishing?

Can you let me know who will be taking the lead in answering these questions? In the meantime I will initiate testing for enterococcus around salmon farms.

Thank you so much for performing these tests,and alerting us to this concern.

Alexandra Morton
250-974-7086

Enterococcus

The presence of the enterococcus bacteria is used as an indicator of fecal pollution in marine waters. The level considered safe by the US Environmental Protection Agency in marine waters is 104 – 501 cfu/100mL for a single sample[1] and ≤70 enterococci/100 ml as per the Canadian marine recreational water quality guidelines[2]. Further action should be initiated if this guideline value is exceeded. Minimum action should consist of immediate resampling of the site (or sites). In addition, a swimming advisory may be issued should the responsible authority identify that the area is not suitable for recreational water use.

Both water samples taken by the BC provincial government from the Tofino and Browns Bay processing plants reported >60,000 enterococci/100ml, noting there were more bacteria than they had the ability to count. The samples reported by the BC Ministry of Environment were not taken in the ocean, so we don't know what these very high output levels have diluted down to in the marine waters where people are at risk of coming into contact, however both processing plants are in areas frequented by people.

Enterococci are not always considered harmful to humans, but their presence in the environment may indicate that other disease-causing agents such as viruses, bacteria, and protozoa may also be present. Significant amounts of enterococci in a water body can negatively affect the recreational and economic value of the aquatic resource. Overabundance of fecal bacteria in the water can cause beach closures, swimming and boating bans and closures of fishing and shell fishing areas[3].

While enterococci are used simply as an indicator species to alert regulators to fecal contamination of marine waters, i.e. to provide warning that other pathogens are likely present, the bacteria itself can also cause serious and often life-threatening disease.[4] Enterococci are the second leading cause of bacterial blood infections.[5]

Many studies correlate increasing concentrations of environmental enterococci with gastrointestinal and dermatological illnesses. As a result, the Environmental Protection Agency suggests it is urgent to more thoroughly define ecological reservoirs, understand host and bacterial traits that promote colonization, and clarify mechanisms for transmission that enhance the spread of multi-drug resistant enterococci[6]. The Province of BC testing raises the question – are the salmon farms throughout BC "ecological reservoirs."

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[1] https://www.epa.gov/sites/production/files/2015-09/documents/ecoli.pdf
[2] https://www.canada.ca/en/health-canada/services/publications/healthy-living/guidelines-canadian-recreational-water-quality-third-edition.html
[3] https://www.epa.gov/national-aquatic-resource-surveys/indicators-enterococci
[4] https://www.ncbi.nlm.nih.gov/books/NBK190429/
[5] https://www.ncbi.nlm.nih.gov/pubmed/18947320
[6] https://www.ncbi.nlm.nih.gov/books/NBK190429/

Treatment of enterococcal infections can be difficult because *Enterococcus* species are intrinsically resistant to many antimicrobial agents and have the capacity to acquire resistance genes and mutations. Use of antibiotics in the BC salmon farming industry is second only to Chile[7] with BC using an average of 1.75 antibiotic treatments per grow out cycle in 2014. Sixteen percent of enterococcus isolates from farmed salmon were found to be resistant to the antibiotic tetracycline[8]. Release of antibiotic resistant strains of enterococcus would pose additional threat.

Surprisingly research is underway to feed enterococcus to farm fish to achieve a probiotic effect. When Enterococcus was introduced to rainbow trout feed, fish growth accelerated.[9] The FDA has approved no enterococcal feed additive. However, on a DFO webpage entitled "**Thinking Out of the Box: Exploring Strategies to Reduce Sea Lice Infestations in Salmon Farms[10]**" dated 2017-01-19 we see that DFO is exploring the use of beneficial bacteria to feed to increase resistance to sea lice

Questions for BC regulators:

1. Given the very high levels of this bacteria in the blood waste pouring from farm salmon processors into the marine environment what testing has been done around the plants to ensure the water is safe for recreational activities?
2. What species of Enterococcus was detected?
3. What is the antibiotic resistance profile of the bacteria?
4. Are BC farm salmon being fed enterococcus bacteria in an effort to reduce the BC salmon farming industry's large consumption of antibiotics, or to reduce sea lice populations which have been escalating again in Musgamagw territory since 2015[11]?
5. What is the explanation for the astronomical levels of the bacteria, i.e. more than could be counted?

Alexandra Morton
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250-974-7086

[7] https://www.undercurrentnews.com/2015/10/27/bc-salmon-antibiotic-use-second-highest-in-world-further-reductions-planned/
[8] http://jfoodprotection.org/doi/pdf/10.4315/0362-028X.JFP-15-463?code=fopr-site
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From:	Alex Morton
To:	OfficeofthePremier, Office PREM:EX; Donaldson.MLA, Doug LASS:EX; Heyman.MLA, George LASS:EX; Minister, FLNR FLNR:EX; Popham, Lana; Meggs, Geoff PREM:EX; Trevena.MLA, Claire F LASS:EX; Weaver.MLA, Andrew LASS:EX; Furstenau.MLA, Sonia LASS:EX; Olsen.MLA, Adam LASS:EX; fin.donnelly@parl.gc.ca; Eby.MLA, David LASS:EX; Chandra Herbert.MLA, Spencer LASS:EX; Farnworth.MLA, Mike LASS:EX; Fleming.MLA, Rob LASS:EX; Leonard.MLA, Ronna-Rae LASS:EX; Fraser.MLA, Scott LASS:EX; Rice.MLA, Jennifer LASS:EX; Routley.MLA, Douglas G LASS:EX; Mark.MLA, Melanie LASS:EX; Dix.MLA, Adrian LASS:EX; Simons.MLA, Nicholas LASS:EX; James.MLA, Carole A LASS:EX; Simpson.MLA, Shane L LASS:EX; Mungall.MLA, Michelle LASS:EX; Routledge.MLA, Janet LASS:EX
Subject:	Fish farm frontlines update - Mar 9
Date:	Saturday, March 10, 2018 6:23:20 AM

Hello

In follow up to my last email video, showing Marine Harvest wrestling with a young woman, Marine Harvest was given the opportunity to serve Ernest Alfred legal papers.

Nine nations showed up to voice their opinions. Young Karissa Glendale received a knee injury in her struggle with Marine Harvest employees. Obviously this has raised the ire of the powerful Glendale family.

Marine Harvest was told to never step foot in Alert Bay again.

https://vimeo.com/259440895

I feel it is important that you be kept on notice of what is happening as a result of no decision on the expiring tenures for 22 salmon farms. 1/4 of the BC industry, which is sitting in territories where they never received permission from First Nations. This video was taken **day 198 of the fish farm occupations.** Perhaps this is what you have been waiting for before you feel you can stand up to these aggressive companies, or you are waiting for this to die down. The evidence suggests this is a rapidly escalating situation in which the First Nations are showing remarkable bravery, honour and endurance.

Alexandra Morton

250-974-7086

From:	Alex Morton
To:	OfficeofthePremier, Office PREM:EX; Donaldson.MLA, Doug LASS:EX; Heyman.MLA, George LASS:EX; Minister, FLNR FLNR:EX; Popham, Lana; Meggs, Geoff PREM:EX; Weaver.MLA, Andrew LASS:EX; Furstenau.MLA, Sonia LASS:EX; Olsen.MLA, Adam LASS:EX
Subject:	Fish farm frontlines update
Date:	Thursday, March 8, 2018 7:50:04 AM
Attachments:	Bateman et al. 2016 (sea louse outbreak).pdf Dept Wild Salmon Oct 8 copy.pdf.pdf ATT00001.txt

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Hello

I saw the article on the CBC website, that you are very interested in moving open-net fish farms onto land.

Thank you for that, an important step.

However, I think you should see the iphone video shot yesterday at Swanson Island, as Marine Harvest hunts for hereditary leader Ernest Alfred, who they are in phone contact with, who told them he was not on site. Over 30,000 people have viewed this live-feed on facebook and the number is rising rapidly. This young woman is the kind of leader we need more of and she deserves contact with you.

https://vimeo.com/259102708

In a few weeks I will be documenting the damage the farms in this territory is doing to wild salmon. This will be my 18th year of this work. Attached is my most recent co-publication on the, a failure to protect wild salmon from sea lice.

Next week Marine Harvest cross-examines me in my case to make the Minister of Fisheries obey a Federal Court ruling and the laws of Canada to test farm salmon for piscine reovirus. Then Cermaq is going to do the same, while DFO just sits there and lets them fight to keep putting diseased fish into the ocean. You have got to see the perversity of this.

The longer you wait to tell Marine Harvest and Cermaq whether or not their tenures will be renewed, the more bad, ugly, disturbing news will come of this. Soon it will be tourism season in the Broughton - an industry twice as big as salmon farming, an ambassador to this country and one that you never seem to value enough. People from Germany will be watching desperate bears starving for lack of salmon and it would be better for them and all if the operators could say - yes it is terrible but my government has done the right thing and taken the salmon farms out. Wild salmon will no longer be eaten to death by sea lice, and infected with piscine reovirus.

I can see most of you are all too afraid to speak to me, you have bought into the industry slander, but I still have the right to speak to you. There are brilliant solutions - see attached Dept Wild Salmon document, we have incredible opportunity to grow the economy, mature the aquaculture industry and restore wild salmon, and yet all of this is being suppressed, squandered and ignored while you dare to believe what the salmon farmers are doing is the best use of our coast.

Look closely at how Marine Harvest is treating a young indigenous woman, now we see who they really are.

Alexandra Morton
250-974-7086

Page 066 to/à Page 074

Withheld pursuant to/removed as

s.3

PROBLEM:

Salmon farms are exposing our wild salmon stocks to unnecessary risks. Farms must be removed from migration routes or, even better, removed from the ocean all together.

An ad from the current Salmon Farmers' Campaign

OUR GOAL: INCREASE THE PUBLIC'S INTEREST IN INCLUDING THE SALMON FARM ISSUE ON THE POLITICAL AGENDA.

CRITERIA FOR CREATIVE APPROACH:

- *Visual imagery should connect "salmon" and "food."*
- *Without impersonating a govt agency, messaging should look like it came from an official, reputable source.*
- *Messaging should be fact-based and supportable. Should be easily corroborated through an Internet search.*
- *To elevate engagement with moms, creative concept should enable a "discovery" about a potential danger in their family's food sources.*
- *Simple, easily understood images that can be used across different mediums, especially for social media sharing.*

"Drugs"

MacLAREN McCANN

Image creates "discovery" of danger in family's food.

Simple image for all mediums, especially social media sharing.

Kitchen cues for "food."

Fact-based and supportable. Emulates an official look.

"Drugs"

MacLAREN McCANN

"Pork"

MacLAREN McCANN

"Gas Mask"

MacLAREN McCANN

ASSUMING A LOWER MAINLAND FOCUS,WE WOULD ESTIMATE THE MEDIA COST FOR 6-8 WEEK CAMPAIGN TO BE IN THE $150-200K RANGE.

THANK YOU

CONTACT:

Hagan Ainsworth, Senior Vice President, General Manager 604 601 8561
hagan.ainsworth@maclaren.com


TRUTH
WELL TOLD

From:	Alex Morton
To:	OfficeofthePremier, Office PREM:EX
Cc:	Meggs, Geoff PREM:EX; Popham, Lana; Heyman.MLA, George LASS:EX; Minister, FLNR FLNR:EX; president@ubcic.bc.ca; Furstenau.MLA, Sonia LASS:EX; Weaver.MLA, Andrew LASS:EX; Trevena.MLA, Claire F LASS:EX; Pamela Goldsmith-Jones
Subject:	Investigation in BC lab - update?
Date:	Tuesday, March 6, 2018 12:43:51 PM
Attachments:	Dr. Marty May 23, 2017.pdf ATT00001.htm Marty 2008 ATIP A2016-203final (dragged) 1 copy 3.pdf ATT00002.htm

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Dear Premier John Horgan:

I am writing to ask for an update on your investigation into the "integrity" of Dr. Gary Marty's lab. When can I expect an announcement?

There are serious concerns that failure by this lab to diagnose HSMI, the disease caused by piscine reovirus, has led to the Federal government permitting the transfer of tens of millions of farm salmon infected with the disease agent piscine reovirus, into wild salmon habitat.

In 2016, Dr. Marty co-published with Marine Harvest in a top-flight international scientific journal (PLoS One) that piscine reovirus does not cause the salmon heart disease HSMI in BC, "*Western North American PRV Fails to Cause HSMI*"

Then just a few months later he wrote two private emails suggesting that he knew the HSMI was occurring in BC salmon farms since 2008 and in fact was asking for credit in its discovery (attached).

His lab is the firewall between BC farm salmon health and what the public is allowed to know about BC farmed salmon health and unless there is significant explanation regarding the difference between the public and private conclusions of this lab I don't see how we can trust anything coming from the BC government on farm salmon health.

I can see this is a difficult process for you, but a lot of people and over 100 species of animals are potentially being impacted by the affect of this virus on wild salmon.

I have tried to make it easy to understand with this 5 minute video, which over 60,000 people have viewed between facebook and Vimeo https://vimeo.com/258135790

Such great solutions exist to this problem to the benefit of our economy, ecology, and the evolving relationship between the indigenous and non-indigenous people of BC, but like most problems, first your government has to realize there is a problem here. This has reached a state of extreme non-confidence.

All the best

Alexandra Morton
250-974-7086

Miller-Saunders, Kristi

From: Brian Riddell <briddell@PSF.CA>
Sent: May-24-16 11:02 AM
To: Miller-Saunders, Kristi
Subject: RE: Comments for Public Interest Panel meeting of the Strategic Salmon Health Initiative (SSHI)

I can reply to this

From: Miller-Saunders, Kristi [mailto:Kristi.Saunders@dfo-mpo.gc.ca]
Sent: May 23, 2016 9:39 PM
To: Brian Riddell <briddell@PSF.CA>
Subject: FW: Comments for Public Interest Panel meeting of the Strategic Salmon Health Initiative (SSHI)

Kristi Miller
Head, Molecular Genetics
Pacific Biological Station
Nanaimo BC, Canada
kristi.miller@dfo-mpo.gc.ca

-----Original Message-----
From: Marty, Gary D AGRI:EX [mailto:Gary.Marty@gov.bc.ca]
Sent: Mon 5 23/2016 12:45 PM
To: Miller-Saunders, Kristi; 'Brian Riddell'
Subject: Comments for Public Interest Panel meeting of the Strategic Salmon Health Initiative (SSHI)

Hi Kristi and Brian,

I received an invitation to attend a meeting of the Public Interest Panel on June 7. I responded, "On June 7, I will be traveling to the annual meeting of the Fish Health Section of the American Fisheries Society, so I will not be able to attend."

My input would be to ask for clarification about what the SSHI project has discovered.

In an e-mail that I sent to Kristi last Thursday, I provided public information about inflammation in the heart and skeletal muscle of BC fish that was reported up to three years before Friday's press release. This includes information that was reported in 2013 based on examination of fish from the same farm and the same outbreak as that reported in the press release. The information reported publicly in 2013 included, "these fish had inflammation of the heart and skeletal muscle, which are two features of HSMI".

Disease (definition) = any deviation from normal structure or function of a part, organ, or system of the body: the cause might be unknown; and, the mere presence of an infectious agent does not mean that the infectious agent is causing disease.

s.19(1)
s.21(1)(a)
s.21(1)(b)

My understanding from our meeting on Wednesday is that the pathologists (Ferguson, DiCicco, and Marty) agree that the fish from the affected farm had inflammation of the heart and skeletal muscle, and we agree that these are the two morphologic features of HSMI. The only difference is that the SSHI team seems to be using a case definition for HSMI that is different from the HSMI case definition used over the past decade by the BC veterinarians, which could be stated in this way:

BC veterinarians - HSMI is diagnosed based on characteristic abnormalities in the heart and muscle, AND characteristic clinical signs (e.g., fish are lethargic, eat poorly, and growth is slower than normal).

SSHI researchers - HSMI is diagnosed based on characteristic abnormalities in the heart and muscle.

[redacted] when I read things like the following in a CBC news report (emphasis mine):

"A feared viral disease proven deadly in Norwegian fish farms has been confirmed for the first time by federal scientists studying farmed salmon in B.C.

Heart and Skeletal Muscle Inflammation (HSMI) has been linked to the deaths of up to 20 per cent at some Norwegian farms.

'The concern is that it is a disease that hasn't previously been detected in B.C. and at the present time we really don't have sufficient evidence to know if it causes mortality or is a production issue here,' said Kristi Miller, part of a team of federal scientists studying farmed fish samples from sites along the B.C. coast."

http://www.cbc.ca/news/canada/british-columbia/farmed-salmon-bc-disease-hsmi-aquaculture-1.3593958

I suspect that the first sentence ("confirmed") [redacted] And, I understand that reporters do not always get quotes correct, and they sometimes take words out of context. However, readers are likely to assume that the quote correctly captures what was said, and they are likely to assume that the quote is representative of the scientific integrity of the SSHI team. Also, it is unlikely that reporters from two independent organizations would make the same mistake. Today's article in the Globe and Mail has similar statements (emphasis mine):

"But it was a surprising diagnosis nonetheless, because the disease had not been detected before in B.C., although it is found in aquaculture operations globally.

It might be argued that HSMI should have been discovered much earlier, given all the testing routinely done on farmed fish on the West Coast.

But Dr. Miller, head of molecular genetics for Fisheries and Oceans Canada, said the disease may have escaped detection because it was not fatal to the infected fish, and did not affect the productivity of the farm - that is, the sick fish looked healthy.

She has found it now, however, as part of a long-term study of fish health that is using revolutionary DNA sequencing technology developed in her lab."

I do not want the SSHI to be seen as a project that takes credit for discoveries that were previously reported by other scientists. My understanding is that the SSHI team confirmed the presence of a disease that has long been detected in BC. And, microscopic features of the 2013 outbreak reported by DFO last Friday were first reported publicly by another researcher (me) in 2013. Reports from 2013 and 2015 have described disease in the heart and muscle of BC farmed Atlantic salmon, and pathologists at Wednesday's meeting seemed to agree that it is the same disease as reported in the DFO press release. However, the BC disease was not previously

called HSMI. What seems to be new is that the SSHI team now calls the BC disease HSMI.

Best regards,

Gary

Gary D. Marty, Senior Fish Pathologist
Animal Health Centre
Ministry of Agriculture
1767 Angus Campbell Rd.
Abbotsford, BC, V3G 2M3
604-556-3123

-----Original Message-----
From: DiCicco, Emiliano
Sent: Sat 5/21/2016 9:00 PM
To: Miller-Saunders, Kristi
Subject: I: HSMI diagnoses in BC

Hi... look at the following...

I look forward to reading Hugh's reply, though...

Talk you soon,

Emiliano

****

Emiliano Di Cicco DVM PhD
Fish Health Researcher
Molecular Genetics Lab - Pacific Biological Station Department of Fisheries and Oceans, Canada
3190 Hammond Bay Rd, Nanaimo, BC V9T 1K6 - Canada
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s.19(1)
s.21(1)(a)
s.21(1)(b)

-----Messaggio originale-----
Da: Marty, Gary D AGRI:EX [mailto:Gary.Marty@gov.bc.ca]
Inviato: sab 21/05/2016 12.36
A: DiCicco, Emiliano; 'ferguson@fishpathology.com'
Oggetto: HSMI diagnoses in BC

Hi Hugh and Emiliano,

It was nice to see you at the meeting on Wednesday. I appreciate that conflict is a part of science. In this case, some additional information might help clarify some things.

In particular, I want to clarify how HSMI has been defined in BC since I began working in my current position in 2004. It was not long after I started that I began seeing occasional fish with epicarditis, endocarditis, and variable amounts of myocardial necrosis. When I first diagnosed those cases, I provided a general comment that these lesions were

consistent with systemic disease. In February 2008, provided BC vets a continuing education session that summarized the pathology of emerging European diseases in farmed Atlantic salmon. When she showed images of HSMI, I immediately recognized the lesions as similar to what I had been seeing microscopically in some BC fish. However, the aquaculture veterinarians said that they were not seeing a clinical pattern that was consistent with Norwegian HSMI (all the Atlantic salmon companies have Norwegian connections, so I assume that they are well aware of the clinical signs of HSMI). Therefore, we decided that what I was seeing was probably not the same as Norwegian HSMI. We understood HSMI to be the name of a disease syndrome, and that characteristic clinical signs were needed for a diagnosis of HSMI (i.e., similar morphologic lesions without clinical signs did not warrant a diagnosis of HSMI). After that session, when I saw inflammatory heart lesions that were similar to HSMI, I started adding to my comments a note that the lesions were similar to lesions in Norwegian fish with HSMI, but that HSMI had never been seen in BC.

The expert report that I produced in an ongoing Canadian legal case provides a good example:

Public reporting: Affidavit of Dr. Gary D. Marty sworn October 30, 2013, in Morton v. Minister of Fisheries and Oceans et al, Federal Court No. T-789-13

21. Have you tested fish for PRV and/or HSMI with results that contradict the results of your testing for MHC?

I have not tested fish for PRV and/or HSMI with results that contradict the results of my testing for MHC, but I have tested fish in which the suite of lesions was different than the groups of fish I examined from MHC or DFO.

As described in my answer to question #4, among all the testing I have done for HSMI (e.g., the BC Fish Health Auditing and Surveillance Program), I occasionally diagnose "unexplained heart lesions" as the cause of death. However, the prevalence of PRV in tested cases (80%) is the same as the prevalence of PRV among (i) groups of fish that die of other causes and (ii) healthy fish that are sampled for pretransfer screening.

In two cases submitted directly by a BC fish farm company other than Marine Harvest (one case in 2011 and one case from a different farm in 2013), I diagnosed unexplained heart lesions as the cause of death in all of the fish in the sample group. These cases were not tested for PRV, but based on other data there is an 80% chance that they would be PRV positive. In this year's case, I requested a second submission that included skeletal muscle for histopathology (skeletal muscle is not included in routine submissions for diagnostic purposes). One of the 10 fish included in the second submission had severe heart lesions but no skeletal muscle inflammation; therefore, this fish did not have HSMI. Three other fish had moderate to severe heart lesions along with mild inflammation of skeletal muscle; therefore, these fish had inflammation of the heart and skeletal muscle, which are two features of HSMI. However, the farm's veterinarian told me that the fish did not have clinical signs consistent with the description of the European syndrome HSMI (see Dr. Nylund's expert report, answer to his question 24). Because these BC fish did not have all features of the European syndrome HSMI (i.e., clinical features are different), it is not appropriate to diagnose HSMI in these fish. Without consistent clinical signs, a diagnosis of HSMI in these fish is likely to result another example of the diagnostic "confusion" described by Dr. Nylund in his expert report (i.e., the response to his question 22). The submission form submitted with the second BC sample included a history that stated, "As environmental conditions improved, mortality

dropped significantly. Mortality is now low normal with no clinical signs of disease." The cause of the heart lesions in these fish remains unknown, but all the information I have better fits "transient adverse environmental conditions" (e.g., exposure to algal toxins) as the cause of disease rather than PRV. Also, if BC strains of PRV were causing HSMI, it is not plausible to have 80% of BC Atlantic salmon infected with PRV every year since 2006, but have only two cases of HSMI during that same period.

This expert report was entered into evidence and is available to the public. In the 2.5 years since I produced this document, I have not seen any information that compels me to change my response to this question (# 21). After our meeting on Wednesday, informed me that the 2011 and 2013 cases in my expert report

I think that the information above supports the conclusion that I diagnose inflammation in the heart and skeletal muscle when it occurs; however, I do not diagnose HSMI in these fish because the submitting veterinarians tell me that their fish do not have clinical signs consistent with HSMI. As a referral veterinarian, I would need some very strong justification to diagnose a syndrome contrary to the information provided by my referring veterinarians.

To summarize, I provided information in a public document 2.5 years ago that stated, "these fish had inflammation of the heart and skeletal muscle, which are two features of HSMI". My understanding from our meeting on Wednesday is that we do not disagree on these two features of HSMI. My understanding is that the fish I examined and the fish you examined were from the same farm and from the same outbreak. I reported "inflammation of the heart and skeletal muscle" publicly in 2013. Your findings of the same lesions from the same outbreak were reported yesterday (2.5 years later). when I read things like the following in a CBC news report (emphasis mine):

"A feared viral disease proven deadly in Norwegian fish farms has been confirmed for the first time by federal scientists studying farmed salmon in B.C.

Heart and Skeletal Muscle Inflammation (HSMI) has been linked to the deaths of up to 20 per cent at some Norwegian farms.

'The concern is that it is a disease that hasn't previously been detected in B.C. and at the present time we really don't have sufficient evidence to know if it causes mortality or is a production issue here,' said Kristi Miller, part of a team of federal scientists studying farmed fish samples from sites along the B.C. coast."

http://www.cbc.ca/news/canada/british-columbia/farmed-salmon-bc-disease-hsmi-aquaculture-1.3593958

s.19(1)
s.20(1)(b)

Best regards,

Gary

P.S. In my experience, reporters will make changes to stories if errors are pointed out quickly. I recognize that the information I highlight from the CBC story was not included in the press release, and that it might have been influenced by discussions with other people (e.g.,).

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

Senior Fish Pathologist
Animal Health Centre
Ministry of Agriculture
1767 Angus Campbell Rd.
Abbotsford, BC, V3G 2M3
604-556-3123

s.19(1)

From:	Alex Morton
To:	OfficeofthePremier, Office PREM:EX
Cc:	s.22 s.22 fin.donnelly@parl.gc.ca; Pam.Goldsmith-Jones@parl.gc.ca; Popham, Lana; Meggs, Geoff PREM:EX; Heyman.MLA, George LASS:EX s.22 s.22 s.22 Ken.Hardie.P9@parl.gc.ca; s.22 s.22 andrew.thompson@dfo-mpo.gc.ca; andrew.thomson@dfo-mpo.gc.ca; Rebecca Reid; s.22 Nathan.Taylor@dfo-mpo.gc.ca; s.22
Subject:	ISA virus - deleted, lost and misplaced
Date:	Wednesday, January 17, 2018 8:36:31 AM

Dear Premier John Horgan:

As you ponder whether to renew 1/4 of BC salmon farm tenures that are expiring in Musgamagw and Namgis territories, there is something you should know. As the landlord to these three Norwegian companies, you will decide which runs of wild salmon are exposed to the pathogen effluent from this industry.

Piscine reovirus and your lab's failure to diagnosis its disease have been in the media lately, but today I am writing about ISA virus, a farm salmon influenza virus, that is internationally reportable because countries are keen to protect themselves from it.

In 2011, I began sending samples of BC farm salmon for ISA virus testing and the virus was detected.

In 2016, I co-published a paper on ISA virus in BC in a top-tier scientific journal.

DFO and Provincial fish farm pathologist, Dr Gary Marty, tried and failed to have the paper "retracted". https://virologyj.biomedcentral.com/articles/10.1186/s12985-015-0459-1

But here is why I am writing to you.

In 2012, shortly after we announced the first detection of ISAV in BC, the Canadian Food Inspection Agency (CFIA) put *"a stream of commerce*"in place for one year.

In May 2012, Alfred Bungay, DFO National Manager for Aquatic Animal Health explains in an internal email:

"*Until then* [December 10, 2012] *the permit requirement at the border will not be strictly enforced. That is to say if a shipment* [of Atlantic salmon eggs] *arrives at the border without a CFIA permit or it does not meet all of the requirements the CFIA may still allow the shipment to enter Canada.*"

The most lethal salmon virus known is detected in BC and the CFIA lowers farm salmon importation standards, elevating risk to Pacific salmon!

I filed a request for information on who made this high-risk decision and why. I recently received this reply:

"*.. the records were deleted as transitory, some were lost as a result of a computer malfunction and some hard copy files were misplaced.*" (Manager for the CFIA Access to Information and Privacy).

The CFIA didn't say there were *no* records of their inexplicable decision, they said the records were - **deleted, lost *and* misplaced.**

I would welcome the opportunity to argue the evidence that ISA virus is present in BC, but this goes beyond the presence of this virus, this smacks of regulatory capture and significant government failure to protect Canadian interests. Lowering Canada's first line of defence against a virus spreading worldwide in farm salmon is not in the public interest.

The demands of the salmon farming have become dangerous to political careers.

Minister LeBlanc, who hopes for a legacy of science and protection of wild salmon, is so tangled up serving the Norwegians he is actually fighting me in court (round 2) to continue breaking Canadian law by permitting transfer of infected farm fish into BC waters because Marine Harvest would be "severely impacted" if they were denied this right.

You are a politician who wants to be seen as committed to reconciliation with First Nations, but your image is at risk as you decide whether to honour Musgamagw and Namgis demands not to renew fish farm tenures in their territories?

One quarter of BC salmon farms are operating in territories without agreements with First Nations, against their wishes. and members of these nations continue their 148-day vigil on Marine Harvest facilities

I understand that biological safeguards against fish farm pathogens are a federal government responsibility, but when the federal government dramatically shirks this responsibility, this responsibility falls to you. You will decide what Norwegian viruses critically depressed wild salmon populations are exposed to.

The ongoing fish farm scandal continues to unfold.

Respectfully,

Alexandra Morton
250-974-7086

From:	Alex Morton
To:	Popham, Lana; Meggs, Geoff PREM:EX; OfficeofthePremier, Office PREM:EX
Subject:	Marine Harvest Delivers Ultimatum
Date:	Thursday, November 9, 2017 11:12:11 AM
Attachments:	Marine Harvest Ultimatum to First Nations.pdf ATT00001.htm

Hello

Marine Harvest appears to insist on taking the low road and are moving to evict First Nations from their own territory by 5pm today. Perhaps they are making it easier for you to pursue the course that you are on, to stand behind Canada's status as a signatory to UNDRIP and refuse to renew these licences.

I know the industry has slandered me to the point people actually believe them, but my science is being published in top journals and the return of the sea lice infestations and the pattern of PRV infection in wild salmon points to salmon farms as a significant cause of loss of wild salmon. This is no small matter and is the greatest reversible impact on our fish stocks.

My MLA Claire Trevena is MIA on this issue, even though she ran on a promise to support these nations in their effort to protect their fish stocks and remove salmon farms and so I am turning to the three of you. The wild salmon numbers are so low in the Broughton that extinction is certain on this trajectory. I know the industry likes to say I have falsely predicted this before, but what they fail to mention is that the province of BC enacted the Pink Salmon Action Plan which called for farm salmon delousing prior to the juvenile salmon outmigration. This worked for a number of years, which I published on this, however it is no longer working and I have published on that: http://www.nrcresearchpress.com/doi/abs/10.1139/cjfas-2016-0122#.WgSlloZrxE4

I am headed back out to witness the heavy hand of Marine Harvest on two young Musgamagw women who embody the bravery, integrity and beauty we wish for all young women.

You have taken some remarkable steps and I am sure you are under threat from Marine Harvest as they do that so well. They run around threatening everyone when they don't get their way. Their industry is known for this back in Norway. They threaten reporters all the time who call me in a panic. They have threatened us with injunctions repeatedly and withdrawn them.

So power to all of us. When this industry is removed from the Broughton my field station will record the response in wild salmon. This will be an incredible legacy for your government, but now is the moment that decides your relationship with First Nations.

Please watch this if you have 5 minutes look at the effort of the past 3 months - https://vimeo.com/241939771

Thank you,

Alexandra Morton

SHOWDOWN IN THE BROUGHTON ARCHIPELAGO

Marine Harvest issues ultimatum to First Nations protestors
Get off by Thursday 5pm

November 8, 2017 (Port McNeill, BC)
It's showdown time in the Broughton Archipelago where two young indigenous women, among others, are occupying a Marine Harvest industrial fish farm that First Nations maintain is trespassing on their traditional territory.

On November 7, 2017 Marine Harvest delivered an unsigned letter to Karissa Glendale and Molina Dawson currently on the Midsummer fish farm in the Broughton Archipelago - ***"We demand that you leave the Midsummer facility by 5pm on Thursday, November 9, 2017 and not return to this or any other Marine Harvest property."***

This ultimatum comes amid investigation into the government lab responsible for farm salmon health, a lawsuit against the Minister of Fisheries for allowing diseased farm salmon into BC waters, and a warning to Marine Harvest that the BC government is committed to the UN Declaration on the Rights of Indigenous People.

First Nations and other observers have been living on salmon farms in the Broughton Archipelago for the past 77 days, documenting the poor health of the fish. Marine Harvest states that First Nations are trespassing, but the Musgamagw, Namgis and Mamalilikulla nations stand united in declaring that it is the industry which is in trespass. They demand the companies remove the fish and leave the territory. Wild salmon are in collapse in the region, leaving these nations without food fisheries.

Marine Harvest halted restocking this site two months ago, amid clashes with First Nations. Karissa and Molina, the young Musgamagw women occupying the farm, believe the ultimatum letter served to them by Marine Harvest, signals the company's intention to resume operations - despite warnings from the provincial government to halt restocking until indigenous issues are resolved.

"We are not going anywhere until we have concrete evidence that these farms are going to shut down," says Karissa Glendale.

"Our ancestors not only survived on this coast, they thrived and we will again when we get these fish farms out, I am prepared to spend the entire winter on this farm," says Molina Dawson.

"The world is watching," says Musgamagw organizer Carla Voyageur.

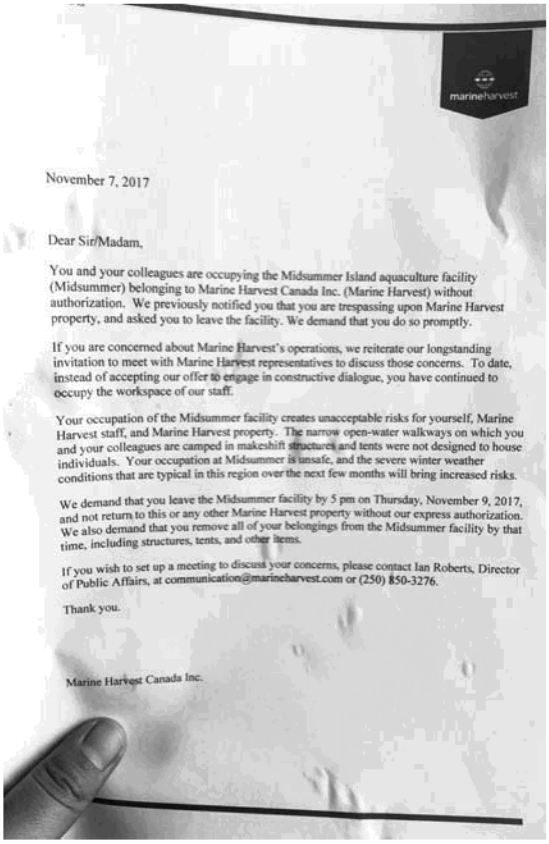
Contacts:

Carla Dawson Voyageur – 204-292-1098

Molina Dawson – 778-348-1976

Alexandra Morton 250-974-7086

IMAGES OF THE UPRSING: https://vimeo.com/241939771



marineharvest

November 7, 2017

Dear Sir/Madam,

You and your colleagues are occupying the Midsummer Island aquaculture facility (Midsummer) belonging to Marine Harvest Canada Inc. (Marine Harvest) without authorization. We previously notified you that you are trespassing upon Marine Harvest property, and asked you to leave the facility. We demand that you do so promptly.

If you are concerned about Marine Harvest's operations, we reiterate our longstanding invitation to meet with Marine Harvest representatives to discuss those concerns. To date, instead of accepting our offer to engage in constructive dialogue, you have continued to occupy the workspace of our staff.

Your occupation of the Midsummer facility creates unacceptable risks for yourself, Marine Harvest staff, and Marine Harvest property. The narrow open-water walkways on which you and your colleagues are camped in makeshift structures and tents were not designed to house individuals. Your occupation at Midsummer is unsafe, and the severe winter weather conditions that are typical in this region over the next few months will bring increased risks.

We demand that you leave the Midsummer facility by 5 pm on Thursday, November 9, 2017, and not return to this or any other Marine Harvest property without our express authorization. We also demand that you remove all of your belongings from the Midsummer facility by that time, including structures, tents, and other items.

If you wish to set up a meeting to discuss your concerns, please contact Ian Roberts, Director of Public Affairs, at communication@marineharvest.com or (250) 850-3276.

Thank you.

Marine Harvest Canada Inc.

From:	Alex Morton
To:	Morel, David P ENV:EX
Cc:	Xia, Eveline ENV:EX; McGuire, Jennifer ENV:EX; Popham, Lana; Heyman.MLA, George LASS:EX; Meggs, Geoff PREM:EX; Tavish Campbell; s.22 Zacharias, Mark ENV:EX
Subject:	Re: Blood Water Testing
Date:	Tuesday, February 6, 2018 9:53:31 AM

David

Thank you,

Alex

On Feb 6, 2018, at 8:34 AM, Morel, David P ENV:EX <David.Morel@gov.bc.ca> wrote:

Hi Alex
Thanks for the email and sharing your research. Will pass your email on to our compliance team and they can forward relevant materials to you.
David Morel
Assistant Deputy Minister
Environmental Protection Division
Ministry of Environment and Climate Change Strategy

From: Alex Morton [mailto:alexandramorton5@gmail.com]
Sent: Tuesday, February 6, 2018 7:59 AM
To: Xia, Eveline ENV:EX; McGuire, Jennifer ENV:EX; Morel, David P ENV:EX; Popham, Lana; Heyman.MLA, George LASS:EX; Meggs, Geoff PREM:EX
Cc: Tavish Campbell; Tony Allard
Subject: Blood Water Testing

Dear Eveline, Jennifer and Dave;
I received a link to an article reporting on your testing of the bloodwater samples from Tofino and Browns Bay.
https://www.desmog.ca/2018/02/05/bloodwater-released-b-c-s-coastal-water-contains-deadly-fish-virus-government-tests-confirm
Can you forward a copy of your report to me?
Attached are two papers that I co-published in December, one on the spread of this virus through BC wild salmon and the other a Formal Comment published in PLoS One a paper stating they had ruled out that any strains of PRV found in BC came from Norway. The authors of Siah et al withdrew that statement in a correction, but we also present further information that at least one strain of PRV found spreading in wild salmon and causing HSMI in farm salmon is most likely from Norway.
The concern with PRV is not that it is outright lethal, but rather that it exists in fish in a low-grade chronic state which means that infected fish can travel with it. The evidence in my work below, and in Miller et al 2014 suggests this blood virus is impeding fish's success in reaching their spawning grounds. Research in Norway suggests that the high presence

of the virus in the fish's red blood cells may reduce the cell's ability to transport oxygen to muscle tissue and thus reduce fitness required to catch prey, evade predators and ascend rivers. As well there are reports of jaundice associated with the virus in Pacific salmon and so HSMI may only be the final outcome of infection with this virus, which only occurs in farms where predators are unable to remove fish during the earlier stages of the disease.

This is certainly a pathogen of concern as it is durable and thus contagious and over 95% of farm salmon sold in markets are infected.

I am available to discuss this further, thank you for your investigation. I look forward to receiving your report.

Alexandra Morton

From:	Alex Morton
To:	Marty, Gary D AGRI:EX
Subject:	Re: Data Availability request - reminder
Date:	Wednesday, January 17, 2018 8:52:52 AM
Attachments:	Marty 2008 ATIP A2016-203final (dragged) 1.pdf ATT00001.htm Dr. Marty May 23, 2017.pdf ATT00002.htm

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Dear Gary Marty:

It is my personal opinion that the scope of your request goes beyond scientific pursuit and I do not wish to collaborate with you.

Specifically I am concerned that you co-authored, Garver et al 2016, that repeatedly states "*Western North American PRV Fails to Cause HSMI*" when on May 21, 2016 you sent emails stating that you recognized HSMI-type muscle and heart lesions in BC farm salmon since 2008 (attached). You go on to explain that you did not make an HSMI diagnosis on instruction from industry vets who gave you direction that it was not HSMI.

In my view, your novel HSMI diagnostic method should have been described in Garver et al 2016 published by PLOS ONE. Otherwise, readers may understand your work as reporting that HSMI heart and skeletal muscle lesions do not occur in PRV-infected BC farm salmon, when in fact they do, according to you.

We are free to develop new methods of course, but I believe it is our responsibility to describe all novel methods fully in our papers, particularily in a case like this where the diagnosis of a new disease in BC was likely delayed until a different team had the opportunity to examine BC farm salmon and made the HSMI diagnosis immediately.

Of significance, if *Western North American PRV Fails to Cause HSMI,* then PRV is not a "disease agent" in BC and therefore not subject to section 56 of the Fishery (General) Regulations which prohibits transfer of fish carrying a disease agent into BC marine waters. If PRV *is* causing disease in BC, which we now know it is, (DiCicco et al 2017) then according to the laws of Canada, PRV-infected salmon are prohibited from transfer into marine pens.

Your work has been used by the Minister of Fisheries as a defence against testing BC Atlantic farm salmon for PRV, and to justify the ongoing transfer of millions of Atlantic salmon into the Pacific infected with a highly contagious blood virus with strong genetic linkages to Norway.

Alexandra Morton

-----Original Message-----
From: DiCicco, Emiliano
Sent: Sat 5/21/2016 9:00 PM
To: Miller-Saunders, Kristi
Subject: I: HSMI diagnoses in BC

Hi... look at the following...

I look forward to reading Hugh's reply, though...

Talk you soon,

Emiliano

****

Emiliano Di Cicco DVM PhD
Fish Health Researcher
Molecular Genetics Lab - Pacific Biological Station Department of Fisheries and Oceans, Canada
3190 Hammond Bay Rd, Nanaimo, BC V9T 1K6 - Canada
Phone: office (250) 756 7045
Cell
e-mail: Emiliano.DiCicco@dfo-mpo.gc.ca

s.19(1)
s.21(1)(a)
s.21(1)(b)

-----Messaggio originale-----
Da: Marty, Gary D AGRI:EX [mailto:Gary.Marty@gov.bc.ca]
Inviato: sab 21/05/2016 12.36
A: DiCicco, Emiliano; 'ferguson@fishpathology.com'
Oggetto: HSMI diagnoses in BC

Hi Hugh and Emiliano,

It was nice to see you at the meeting on Wednesday. I appreciate that conflict is a part of science. In this case, some additional information might help clarify some things.

In particular, I want to clarify how HSMI has been defined in BC since I began working in my current position in 2004. It was not long after I started that I began seeing occasional fish with epicarditis, endocarditis, and variable amounts of myocardial necrosis. When I first diagnosed those cases, I provided a general comment that these lesions were

consistent with systemic disease. In February 2008, provided BC vets a continuing education session that summarized the pathology of emerging European diseases in farmed Atlantic salmon. When she showed images of HSMI, I immediately recognized the lesions as similar to what I had been seeing microscopically in some BC fish. However, the aquaculture veterinarians said that they were not seeing a clinical pattern that was consistent with Norwegian HSMI (all the Atlantic salmon companies have Norwegian connections, so I assume that they are well aware of the clinical signs of HSMI). Therefore, we decided that what I was seeing was probably not the same as Norwegian HSMI. We understood HSMI to be the name of a disease syndrome, and that characteristic clinical signs were needed for a diagnosis of HSMI (i.e., similar morphologic lesions without clinical signs did not warrant a diagnosis of HSMI). After that session, when I saw inflammatory heart lesions that were similar to HSMI, I started adding to my comments a note that the lesions were similar to lesions in Norwegian fish with HSMI, but that HSMI had never been seen in BC.

The expert report that I produced in an ongoing Canadian legal case provides a good example:

Public reporting: Affidavit of Dr. Gary D. Marty sworn October 30, 2013, in Morton v. Minister of Fisheries and Oceans et al, Federal Court No. T-789-13

21. Have you tested fish for PRV and/or HSMI with results that contradict the results of your testing for MHC?

I have not tested fish for PRV and/or HSMI with results that contradict the results of my testing for MHC, but I have tested fish in which the suite of lesions was different than the groups of fish I examined from MHC or DFO.

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

Senior Fish Pathologist
Animal Health Centre
Ministry of Agriculture
1767 Angus Campbell Rd.
Abbotsford, BC, V3G 2M3
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As described in my answer to question #4, among all the testing I have done for HSMI (e.g., the BC Fish Health Auditing and Surveillance Program), I occasionally diagnose "unexplained heart lesions" as the cause of death. However, the prevalence of PRV in tested cases (80%) is the same as the prevalence of PRV among (i) groups of fish that die of other causes and (ii) healthy fish that are sampled for pretransfer screening.

In two cases submitted directly by a BC fish farm company other than Marine Harvest (one case in 2011 and one case from a different farm in 2013), I diagnosed unexplained heart lesions as the cause of death in all of the fish in the sample group. These cases were not tested for PRV, but based on other data there is an 80% chance that they would be PRV positive. In this year's case, I requested a second submission that included skeletal muscle for histopathology (skeletal muscle is not included in routine submissions for diagnostic purposes). One of the 10 fish included in the second submission had severe heart lesions but no skeletal muscle inflammation; therefore, this fish did not have HSMI. Three other fish had moderate to severe heart lesions along with mild inflammation of skeletal muscle; therefore, these fish had inflammation of the heart and skeletal muscle, which are two features of HSMI. However, the farm's veterinarian told me that the fish did not have clinical signs consistent with the description of the European syndrome HSMI (see Dr. Nylund's expert report, answer to his question 24). Because these BC fish did not have all features of the European syndrome HSMI (i.e., clinical features are different), it is not appropriate to diagnose HSMI in these fish. Without consistent clinical signs, a diagnosis of HSMI in these fish is likely to result another example of the diagnostic "confusion" described by Dr. Nylund in his expert report (i.e., the response to his question 22). The submission form submitted with the second BC sample included a history that stated, "As environmental conditions improved, mortality

dropped significantly. Mortality is now low normal with no clinical signs of disease." The cause of the heart lesions in these fish remains unknown, but all the information I have better fits "transient adverse environmental conditions" (e.g., exposure to algal toxins) as the cause of disease rather than PRV. Also, if BC strains of PRV were causing HSMI, it is not plausible to have 80% of BC Atlantic salmon infected with PRV every year since 2006, but have only two cases of HSMI during that same period.

This expert report was entered into evidence and is available to the public. In the 2.5 years since I produced this document, I have not seen any information that compels me to change my response to this question (# 21). After our meeting on Wednesday, informed me that the 2011 and 2013 cases in my expert report

I think that the information above supports the conclusion that I diagnose inflammation in the heart and skeletal muscle when it occurs; however, I do not diagnose HSMI in these fish because the submitting veterinarians tell me that their fish do not have clinical signs consistent with HSMI. As a referral veterinarian, I would need some very strong justification to diagnose a syndrome contrary to the information provided by my referring veterinarians.

To summarize, I provided information in a public document 2.5 years ago that stated, "these fish had inflammation of the heart and skeletal muscle, which are two features of HSMI". My understanding from our meeting on Wednesday is that we do not disagree on these two features of HSMI. My understanding is that the fish I examined and the fish you examined were from the same farm and from the same outbreak. I reported "inflammation of the heart and skeletal muscle" publicly in 2013. Your findings of the same lesions from the same outbreak were reported yesterday (2.5 years later). when I read things like the following in a CBC news report (emphasis mine):

"A feared viral disease proven deadly in Norwegian fish farms has been confirmed for the first time by federal scientists studying farmed salmon in B.C.

Heart and Skeletal Muscle Inflammation (HSMI) has been linked to the deaths of up to 20 per cent at some Norwegian farms.

'The concern is that it is a disease that hasn't previously been detected in B.C. and at the present time we really don't have sufficient evidence to know if it causes mortality or is a production issue here,' said Kristi Miller, part of a team of federal scientists studying farmed fish samples from sites along the B.C. coast."

http://www.cbc.ca/news/canada/british-columbia/farmed-salmon-bc-disease-hsmi-aquaculture-1.3593958

s.19(1)
s.20(1)(b)

Best regards,

Gary

P.S. In my experience, reporters will make changes to stories if errors are pointed out quickly. I recognize that the information I highlight from the CBC story was not included in the press release, and that it might have been influenced by discussions with other people (e.g.,).

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

Senior Fish Pathologist
Animal Health Centre
Ministry of Agriculture
1767 Angus Campbell Rd.
Abbotsford, BC, V3G 2M3
604-556-3123

s.19(1)

Miller-Saunders, Kristi

From: Brian Riddell <briddell@PSF.CA>
Sent: May-24-16 11:02 AM
To: Miller-Saunders, Kristi
Subject: RE: Comments for Public Interest Panel meeting of the Strategic Salmon Health Initiative (SSHI)

I can reply to this

From: Miller-Saunders, Kristi [mailto:Kristi.Saunders@dfo-mpo.gc.ca]
Sent: May 23, 2016 9:39 PM
To: Brian Riddell <briddell@PSF.CA>
Subject: FW: Comments for Public Interest Panel meeting of the Strategic Salmon Health Initiative (SSHI)

Kristi Miller
Head, Molecular Genetics
Pacific Biological Station
Nanaimo BC, Canada
kristi.miller@dfo-mpo.gc.ca

-----Original Message-----
From: Marty, Gary D AGRI:EX [mailto:Gary.Marty@gov.bc.ca]
Sent: Mon 5 23/2016 12:45 PM
To: Miller-Saunders, Kristi; 'Brian Riddell'
Subject: Comments for Public Interest Panel meeting of the Strategic Salmon Health Initiative (SSHI)

Hi Kristi and Brian,

I received an invitation to attend a meeting of the Public Interest Panel on June 7. I responded, "On June 7, I will be traveling to the annual meeting of the Fish Health Section of the American Fisheries Society, so I will not be able to attend."

My input would be to ask for clarification about what the SSHI project has discovered.

In an e-mail that I sent to Kristi last Thursday, I provided public information about inflammation in the heart and skeletal muscle of BC fish that was reported up to three years before Friday's press release. This includes information that was reported in 2013 based on examination of fish from the same farm and the same outbreak as that reported in the press release. The information reported publicly in 2013 included, "these fish had inflammation of the heart and skeletal muscle, which are two features of HSMI".

Disease (definition) = any deviation from normal structure or function of a part, organ, or system of the body: the cause might be unknown; and, the mere presence of an infectious agent does not mean that the infectious agent is causing disease.

s.19(1)
s.21(1)(a)
s.21(1)(b)

My understanding from our meeting on Wednesday is that the pathologists (Ferguson, DiCicco, and Marty) agree that the fish from the affected farm had inflammation of the heart and skeletal muscle, and we agree that these are the two morphologic features of HSMI. The only difference is that the SSHI team seems to be using a case definition for HSMI that is different from the HSMI case definition used over the past decade by the BC veterinarians, which could be stated in this way:

BC veterinarians - HSMI is diagnosed based on characteristic abnormalities in the heart and muscle, AND characteristic clinical signs (e.g., fish are lethargic, eat poorly, and growth is slower than normal).

SSHI researchers - HSMI is diagnosed based on characteristic abnormalities in the heart and muscle.

[redacted] when I read things like the following in a CBC news report (emphasis mine):

"A feared viral disease proven deadly in Norwegian fish farms has been confirmed for the first time by federal scientists studying farmed salmon in B.C.

Heart and Skeletal Muscle Inflammation (HSMI) has been linked to the deaths of up to 20 per cent at some Norwegian farms.

'The concern is that it is a disease that hasn't previously been detected in B.C. and at the present time we really don't have sufficient evidence to know if it causes mortality or is a production issue here,' said Kristi Miller, part of a team of federal scientists studying farmed fish samples from sites along the B.C. coast."

http://www.cbc.ca/news/canada/british-columbia/farmed-salmon-bc-disease-hsmi-aquaculture-1.3593958

I suspect that the first sentence ("confirmed") [redacted] And, I understand that reporters do not always get quotes correct, and they sometimes take words out of context. However, readers are likely to assume that the quote correctly captures what was said, and they are likely to assume that the quote is representative of the scientific integrity of the SSHI team. Also, it is unlikely that reporters from two independent organizations would make the same mistake. Today's article in the Globe and Mail has similar statements (emphasis mine):

"But it was a surprising diagnosis nonetheless, because the disease had not been detected before in B.C., although it is found in aquaculture operations globally.

It might be argued that HSMI should have been discovered much earlier, given all the testing routinely done on farmed fish on the West Coast.

But Dr. Miller, head of molecular genetics for Fisheries and Oceans Canada, said the disease may have escaped detection because it was not fatal to the infected fish, and did not affect the productivity of the farm - that is, the sick fish looked healthy.

She has found it now, however, as part of a long-term study of fish health that is using revolutionary DNA sequencing technology developed in her lab."

I do not want the SSHI to be seen as a project that takes credit for discoveries that were previously reported by other scientists. My understanding is that the SSHI team confirmed the presence of a disease that has long been detected in BC. And, microscopic features of the 2013 outbreak reported by DFO last Friday were first reported publicly by another researcher (me) in 2013. Reports from 2013 and 2015 have described disease in the heart and muscle of BC farmed Atlantic salmon, and pathologists at Wednesday's meeting seemed to agree that it is the same disease as reported in the DFO press release. However, the BC disease was not previously

called HSMI. What seems to be new is that the SSHI team now calls the BC disease HSMI.

Best regards,

Gary

Gary D. Marty, Senior Fish Pathologist
Animal Health Centre
Ministry of Agriculture
1767 Angus Campbell Rd.
Abbotsford, BC, V3G 2M3
604-556-3123

From:	Alex Morton
To:	Popham, Lana
Cc:	Shoemaker, Wes AGRI:EX; Meggs, Geoff PREM:EX
Subject:	Re: The Legal Memo
Date:	Monday, September 25, 2017 7:05:58 AM

Lana

Thank you so much. I am hoping there can be a positive outcome for your government and for the women standing on the farms on this windy day. In my view they are making it easier for your government to make a move that will benefit the entire coast present and future.

So hoping.

Alex

> On Sep 24, 2017, at 10:36 PM, Popham, Lana s.17 wrote:
>
> Good Evening Alex,
>
> Thanks for your email.
>
> I am copying this to my Deputy Wes Shoemaker for follow up.
>
> I noticed that the email you are using for Geoff Meggs is not his correct email. The email you have used has never been his email so he may not have recieved the info you have sent him.
>
> I'm also copying Geoff so he has your information as well.
>
> Cheers, Lana
>
> _____
> From: Alex Morton <alexandramorton5@gmail.com>
> Sent: Saturday, September 23, 2017 9:13 AM
> To: Popham, Lana; geoff.Meggs@leg.bc.ca
> Subject: The Legal Memo
>
> Der Lana Popham and Geoff Meggs:
>
> By now you have received the legal memo on the actions of the Ministry of Agriculture's Animal Health Centre in Abbotsford. This document is based on emails that I received via a Freedom of Information request last December. I realized this information was more serious than I could handle personally and thus the memo you received was produced by a law firm.
>
> In particular there were two jealous emails from Dr. Marty when Dr. Miller went public with her findings.
>
> These emails reveal that when Dr Marty realized he was seeing Heart and Skeletal Muscle Inflammation (HSMI) in 2008, he consulted with industry and together they decided it was not HSMI, because, the industry people said, farm salmon in BC do not exhibit the behaviour associated with this disease. Dr. Marty admits in an email to me never attended these farms, which suggests he was relying in industry information. I now have hours of underwater footage from every farm this summer from Campbell River to Alert Bay and the lethargic, emaciated behaviour described for this disease is widespread in these farms. Furthermore, the ATIP documents I have contain information from other government scientists suggesting the same observations as I have made.

>
> Dr. Marty and Marine Harvest and some DFO scientists co-published three scientific papers wherein they state that the virus that causes HSMI, piscine reovirus - PRV, is endemic to BC and benign… even though Dr. Marty was already aware (as per his own emails) that according to the accepted diagnosis used for HSMI in Norway, HSMI was occurring in BC. He never stated in his papers that he had altered this definition with industry, to reach the opposite conclusion. This is a serious omission in scientific publications.
>
> In 2013, I published that PRV found in farm salmon from Superstore is Norwegian and I sent the late filmmaker Twyla Roscovich to Norway to ask the scientists there, who had received samples of BC farmed salmon from me, if the virus was Norwegian. Dr. Are Nylund confirms that it is https://www.youtube.com/watch?v=3scxcIDuEOo
>
> Using Dr. Marty as an expert witness the Federal Minister of Fisheries is fighting me in court to make it legal for the salmon farming industry to put highly contagious PRV-infected farm salmon into net pens coast wide throughout BC without even testing them. Marine Harvest and Cermaq joined as defendants and provided information to the court that 5/6 of Marine Harvest's farm salmon hatcheries are infected and thus the industry would be "severely" impacted if they couldn't put PRV-infected farm salmon into the farms. If I win this lawsuit, again, the industry will be in severe trouble, if I lose PRV-infection will continue to go viral in BC.
>
> There are less than 200 pink salmon in the Ahta River today, a river that should have thousands and I have studied the sea louse and PRV infection that happens to them as they pass the Marine Harvest farms in Musgamagw Dzawada'enuxw territory. There is a paper coming out on this. There is no guesswork here. Viruses have fingerprints they are traceable to source and the virus in BC matches the virus sequenced in farm salmon with HSMI in Lofoten Norway.
>
> What you have is a BC Ministry of Agriculture cover up that has resulted in millions of PRV-infected fish pouring into the very territory of the Nations who are now standing on these farms demanding respect for their rights and removal of the industry after 30 years of being ignored. This has grown into a human rights issue.
>
> I have tried since last November to meet with the BC NDP to warn you of this situation, but you have all refused.
>
> Piscine reovirus is a highly contagious salmon blood virus that does not break down at temperatures that exceed human body temperature. Meanwhile the salmon farming industry in BC has exempted itself from any requirement to freeze farm salmon before it is served as sushi, so people are eating it raw straight from the farm. Wild salmon exposed to salmon farms are highly infected, while those distant from farms are not, the virus is Norwegian, and wild salmon exposed to salmon farms are in such a state of collapse, most First Nation food fisheries are closed.
>
> Both of you have long been aware of the problems with salmon farms and you have both met with me on this prior to the NDP forming government.
>
> The BC NDP is the landlord of the salmon farms in Musgamagw Dzawda'enuxw, Namgis, Mamalililkulla territory, these nations have never given you permission to put farm here. Glen Clark, Moe and Corky approved a lot of these farms even as I was talking to them then, explaining the risks known in Norway. Norwegian members of government came to Canada and are recorded in the Hansard warning Canada about the farmers intent to do as they like in Canada, and the Licences you hold are expiring soon. The industry has millions of eggs in their hatcheries slated to go into these very farms, so you need to give them a heads up on your intentions ASAP. Over a $million was spent trying to ease the industry into closed containment with government subsidies and they refused. Now time has run out for wild salmon and you are holding this mess.
>
> Marine Harvest has ignored the First Nation demand not to put further fish into their territory and sent the Norwegian registered Viktoria Viking to Midsummer loaded not only with salmon, but also herring, which they are not allowed to have in their boats. It is now a stand-off. Young women are living in very uncomfortable conditions on this farm to protect the future of their children. Marine Harvest knows winter and high winds are coming soon and are cruelly just waiting them out, but a wood stove arrived on site yesterday. No one is giving up now.
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> There is an entire Cermaq farm (Sir Edmund) that is holding only herring. The salmon have been harvested, but the company has been holding the herring at least since the 9th of August. This farm is not licenced as a herring farm. Furthermore every farm has thousands of herring in and around the farm and they are infected with PRV.

>
> There is a strong sense of lawlessness around what is going on here. DFO shows up to protect the industry as they violate FN requests, but no longer even bother to count the collapsing herring and salmon populations here.
>
> Geoff, you expressed your deep concern about this industry in 1984 when you wrote “The days of common property fishing are over.” And here we are. The only fish boats moving are the farm packers working for Norwegian companies. Thousands of jobs have been lost even as Alaska and Russia enjoy huge runs of salmon in absence of salmon farms. This is exactly the effect salmon farms have had world wide, wild salmon go into exceptional collapse (Ford & Myers 2008) and local communities suffer. If wild salmon exposed to salmon farms in BC were not collapsing, it would be nothing short of a miracle, everyone would be excited to know why. But here we are suffering the second lowest Fraser sockeye run in a row and evidence that these fish are vanishing as they pass the farms on their way to sea. Cohen has given the industry until 2020 to prove they are having less than minimal impact in the Discovery Islands before those farms should cease to operate. Attached is my paper on this
>
> You have a short window of opportunity to get out ahead of this scandal. Claire Trevena campaigned in this territory on the promise to get salmon farms out of this territory and if you don’t do that by next spring's wild salmon out-migration you risk being the government that allowed extinction to occur in BC’s most southern virgin watershed and many other rivers.
>
> This problem is only going to get bigger and there is only one course of action. Make a public announcement that out of respect for the First Nations of BC, you are rescinding the Licences of Occupation for salmon farming in Musgamagw Dzawada’enuxw, Namgis and Mamalililkulla territories and the migration route within these territories i.e. Tribune, Burdwood, Sir Edmund and Fife Sound must be empty before March 2018. Wild salmon can only live or die, they can’t negotiate. I will ensure that the numbers on what happens when salmon farms are removed from this route again are published. I already did this for the fallow route enacted by the Province in 2003, which resulted in the best survival of pink salmon ever recorded.
>
> Geoff - you know how dangerous and aggressive this industry is and you understand everything I am saying here. I have not advised the chiefs of this situation yet as there was false hope of response by either the Minster of Fisheries or yourselves, but all of you have failed us and I will be forwarding this email shortly to the people whose territory I live in because I can’t silently watch extinction. I am giving you a heads up so you can collect your thoughts.
>
> All the best,
>
> Alexandra Morton
>
> 250-974-7086
>

From:	Alex Morton
To:	Popham, Lana
Cc:	Shoemaker, Wes AGRI:EX; Meggs, Geoff PREM:EX
Subject:	Re: The Legal Memo
Date:	Tuesday, September 26, 2017 7:45:09 AM
Attachments:	Screen Shot 2017-09-24 at 5.05.51 PM.png ATT00001.htm Salmon runs 2017.docx ATT00002.htm

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Hello

Just to further update you. The Broughton pink and chum salmon are collapsing. While DFO decided in 2014 not to count these runs anymore, we have maintained the records. There are less than 200 pink salmon in the once bountiful Ahta River, and less than 100 chum salmon in the Viner River. Both of these rivers shoud have runs in the 1,000s and both are directly impacted by salmon farms where 17 years of research have shown the impact of sea lice on these fish when they first leave the rivers weighing less than one gram and before they develop scales.

In 2007 we predicted sea lice from salmon farms would cause extinction of the pink salmon of the Ahta and nearby rivers. The Province of BC stepped in with the Pink Salmon Action Plan, which fallowed their major migration route and the fish rebounded immediately (Morton et al and Beamish et al). Then the province enacted a plan wherein the farmers had to remove their lice before the wild salmon went to sea and that worked for a while. However, since 2015 we have recorded high levels of lice again on the juvenile wild salmon. This is exactly what happens everywhere. While DFO claims there is no lice problem I have now seen hours of footage in the farms that the Ahta and Viner River salmon are exposed to and they do have lice, furthermore DFO permitted the farms to triple in size and so it takes fewer lice per farm salmon to cause lethal infections on wild fish. Today, the extinction trend is happening on your watch. Ignore it if you like, but I won't be ignoring it, nor will the nations and their allies standing on the farms.

Furthermore, you should be aware of the research by Dr. Miller and her recent public statements which fully support my published works that the piscine reovirus in BC is from Norway, arrived recently and is causing disease - all of this is contrary to the work copublished by your Ministry and Marine Harvest - a company that has told the court they cannot survive without piscine reovirus-infected fish. I am not sure if you can see it but when government and industry produce statements opposite to everyone else studying the same thing, statements that benefit the companies - it just doesn't look good.

In 2013, I posted this blog on your articles, Geoff Meggs, your insightful observations on the arrival of salmon farming in BC and you wrote "*The days of common property fishing are over.*"

That is exactly where we are today. The only fish boats moving on this coast are the handful that are being paid by three Norwegian-run companies

http://alexandramorton.typepad.com/alexandra_morton/2013/01/salmon-feedlots-this-was-not-a-mistake.html

Government and the salmon farming have been playing musical chairs, but the music has

stopped, the social and biological impact of this industry is exploding and the BC NDP are holding the hot potato, which is kind of fitting as you put the industry in these waters. However, I still don't think you need to go down with it.

Yesterday the bishop of the Anglican Church came out here and stood on the salmon farms with the young indigenous women who are on the frontlines

- Tell Minister LeBlanc to stop fighting to make it legal to put piscine reovirus into the farm tenures you are leasing to industry
- Tell the Musgamagw Dzawada'enuxw, Namgis and Mamalililkulla you have informed industry their leases are expiring without renewal
- Figure out how you are not going to wear the indiscretions of the staff you inherited

Hopefully you noticed the 50,000 people walking for reconcilliation with First Nations. Standing up to restore the food of the First Nations of BC is the perhaps the most visible act of reconiliation you can take. Go to the landbased fish farmers and find out what they need and make a show of supporting them. But DO NOT WAIT until the industry feels like getting into a tank - they will stall forever as they subsidize this research in Norway by farming as cheaply in Canada as possible.

Hope you are reading this, as the uprising here is strengthening and I am trying to help you avoid a collision. The attached photo breaks my heart - we are an example of what the industry. Please see attached list of reports on salmon runs in BC, Alaska and Russia - clearly we are doing something very very wrong in BC.

alex

NOW
LOOK WHAT
THEY'VE DONE
TO B.C. !!

On Sep 24, 2017, at 10:36 PM, Popham, Lana <s.17 > wrote:

Good Evening Alex,

Thanks for your email.

I am copying this to my Deputy Wes Shoemaker for follow up.

I noticed that the email you are using for Geoff Meggs is not his correct email. The email you have used has never been his email so he may not have recieved the info you have sent him.

I'm also copying Geoff so he has your information as well.â€‹

Cheers, Lana

_____
From: Alex Morton <alexandramorton5@gmail.com>
Sent: Saturday, September 23, 2017 9:13 AM
To: Popham, Lana; geoff.Meggs@leg.bc.ca
Subject: The Legal Memo

Der Lana Popham and Geoff Meggs:

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Using Dr. Marty as an expert witness the Federal Minister of Fisheries is fighting me in court to make it legal for the salmon farming industry to put highly contagious PRV-infected farm salmon into net pens coast wide throughout BC without even testing them. Marine Harvest and Cermaq joined as defendants and provided information to the court that 5/6 of Marine Harvestâ€™s farm salmon hatcheries are infected and thus the industry would be â€œseverelyâ€ impacted if they couldnâ€™t put PRV-infected farm salmon into the farms. If I win this lawsuit, again, the industry will be in severe trouble, if I lose PRV-infection will continue to go viral in BC.

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The BC NDP is the landlord of the salmon farms in Musgamagw Dzawdaâ€™enuxw, Namgis, Mamalililkulla territory, these nations have never given you permission to put farm here. Glen Clark, Moe and Corky approved a lot of these farms even as I was talking to them then, explaining the risks known in Norway. Norwegian members of government came to Canada and are recorded in the Hansard warning Canada about the farmers intent to do as they like in Canada, and the Licences you hold are expiring soon. The industry has millions of eggs in their hatcheries slated to go into these very farms, so you need to give them a heads up on your intentions ASAP. Over a $million was spent trying to ease the industry into closed containment with government subsidies and they refused. Now time has run out for wild salmon and you are holding this mess.

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Geoff, you expressed your deep concern about this industry in 1984 when you wrote â€œThe days of common property fishing are over.â€ And here we are. The only fish boats moving are the farm packers working for Norwegian companies. Thousands of jobs have been lost even as Alaska and Russia enjoy huge runs of salmon in absence of salmon farms. This is exactly the effect salmon farms have had world wide, wild salmon go into exceptional collapse (Ford & Myers 2008) and local communities suffer. If wild salmon exposed to salmon farms in BC were not collapsing, it would be nothing short of a miracle, everyone would be excited to know why. But here we are suffering the second lowest Fraser sockeye run in a row and evidence that these fish are vanishing as they pass the farms on their way to sea. Cohen has given the industry until 2020 to prove they are having less than minimal impact in the Discovery Islands before those farms should cease to operate. Attached is my paper on this

You have a short window of opportunity to get out ahead of this scandal. Claire Trevena campaigned in this territory on the promise to get salmon farms out of this territory and if you donâ€™t do that by next spring's wild salmon out-migration you risk being the government that allowed extinction to occur in BCâ €™s most southern virgin watershed and many other rivers.

This problem is only going to get bigger and there is only one course of action. Make a public announcement that out of respect for the First Nations of BC, you are rescinding the Licences of Occupation for salmon farming in Musgamagw Dzawadaâ€™enuxw, Namgis and Mamalililkulla territories and the migration route within these territories i.e. Tribune, Burdwood, Sir Edmund and Fife Sound must be empty before March 2018. Wild salmon can only live or die, they canâ€™t negotiate. I will ensure that the numbers on what happens when salmon farms are removed from this route again are published. I already did this for the fallow route enacted by the Province in 2003, which resulted in the best survival of pink salmon ever recorded.

Geoff - you know how dangerous and aggressive this industry is and you understand everything I am saying here. I have not advised the chiefs of this situation yet as there was false hope of response by either the Minster of Fisheries or yourselves, but all of you have failed us and I will be forwarding this email shortly to the people whose territory I live in because I canâ€™t silently watch extinction. I am giving you a heads up so you can collect your thoughts.

All the best,

Alexandra Morton

250-974-7086

From:	Alex Morton
To:	Joe.Knight@dfo-mpo.gc.ca; Rebecca Reid; Nathan.Taylor@dfo-mpo.gc.ca; Minister / Ministre; karen.calla@dfo-mpo.gc.ca
Cc:	s.22 Popham, Lana; OfficeofthePremier, Office PREM:EX; s.22 Pam.Goldsmith-Jones@parl.gc.ca; Ken.Hardie.P9@parl.gc.ca; s.22 rachel.blaney.a1@parl.gc.ca; Heyman.MLA, George LASS:EX; s.22 : Trevena.MLA, Claire F LASS:EX; s.22 s.22
Subject:	Your Comments re ISAV
Date:	Wednesday, December 20, 2017 9:55:56 AM
Attachments:	CFIA Email Thread highlighted.pdf ATT00001.htm

Dear Joe Knight,

When a DFO employee tells people that the ISAV results from Kibenge's lab have been discredited I realize that DFO staff don't know the full story. I understand this story is hard to follow, because most of it has played out behind closed doors, but I have pursued it for many reasons.

There are two things that would be helpful for you to know: 1.) no lab ever reported a different result than Kibenge's, 2.) his results are peer-reviewed and published. These are significant points that I suspect DFO has not circulated to staff.

The first round 2011 ISAV positive results were retested and all the labs got the same result - Dr. Miller, Ms. Nellie Gagne, Dr. Kyle Garver, Dr. Are Nylund. These results are all exhibits from the Cohen Commission ISAV hearings in Dec 2011. After that, while the DFO and CFIA told the public ISAV samples were being retested, CFIA staff informed me this was not the case. See attached email thread with the CFIA. His statements were a surprise to me.

It is important that you consider that despite the DFO and CFIA evaluation of the results, we went on to publish them results in a solid virology journal.
https://virologyj.biomedcentral.com/articles/10.1186/s12985-015-0459-1

Dr Gary Marty and Ms Nellie Gagne (DFO Moncton) contacted the journal and tried to have this paper retracted, thinking they had found flaws in the work. However, the journal did not accept their analysis or their request and the paper remains published. I doubt this information was circulated within DFO.

Dr. Fred Kibenge was the OIE reference lab for ISAV in the Americas, he wrote the OIE standard on ISAV testing and Marine Harvest went to him at the onset of their 2007 ISAV outbreak in Chile and he diagnosed it for them. Norway used him to audit their labs. It wasn't until he detected ISAV in two "ISAV-free" regions; BC and New Zealand in 2011, that he was discredited. However this was a political response, not a scientific response as no one ever demonstrated that any of his results were wrong.

Kibenge developed an ISAV test specific to the BC strain and detections became more common, but he was not permitted to forward those results to me, or in any way make them public. I only found them through a Freedom of Information request in which the data was embedded.

Some form of ISAV is in BC, Dr. Marty notes the classic lesions, five labs have detected it and genomic profiling of a farm salmon testing PCR-positive for the virus revealed the fish's

immune system exhibited classic *influenza response.* ISAV is in the influenza family (Cohen Commission exhibit 2052). These are all scientific facts.

I don't fault you for your misinterpretation of the situation as DFO under the Harper government did manufacture an alternate reality, however I continue to hope that we are in a new era, because our Minister of Fisheries is mandated to harness science to protect Canada's fisheries.

I mean no offence to you personally, but I view discrediting the Kibenge lab as groundless slander and an impediment to rebuilding wild salmon stocks. I have read tens of thousands of pages of ATIP emails between many of you in DFO and the CFIA, ISAV discussions with universities in New Zealand, PRV researchers in Norway who are skeptical of DFO experiments, the CFIA's "stream of Commerce" implemented after the ISAV findings that dropped our standards on egg imports, jealous emails between BC and DFO scientists over PRV and so much more. Did you know that after we published on ISAV in BC that the CFIA removed all ISAV surveillance samples from their labs and sent to Dr. Marty despite significant internal angst over this unprecedented move? Did you know the CFIA preserved all their ISAV samples in RNALater, which prevented confirmation of the virus as per Canadian standards? Why they do that? For any interested I can provide the government documentation on all of this.

I really do understand the pressure you are under to make any aquaculture-related concerns disappear. I have seen the endless drafts of Media Lines to that effect. However, remarkably none of the scientists involved have backed down, viruses have fingerprints and BC wild salmon in Area 12, a heavily salmon farmed region, are in full collapse. DFO doesn't count salmon in many rivers today so the scope of this collapse may not be easily apparent, but when there are less than 200 pink salmon in the Ahta River, an unlogged watershed, with no commercial fishing pressure, you know there is a serious problem beyond habitat or fishing. As you are aware, I have counted sea lice on thousands of Ahta River salmon at the Marine Harvest and Cermaq salmon farms over the past 17 years and have published extensively on the damage of this impact and, as well as, on the PRV infection in these fish, so I can't help but view salmon farms as significantly implicated in the collapse of Ahta River and other salmon.

The international experience with salmon farms is the same as we see in Musgamagw Dzawada'enuxw territory. Clearly DFO is not producing the results that Canadians expect from your department, something is very wrong with DFO management and unless we all pull together with as much honesty as possible the collapse of wild salmon will occur on our watch, similar to what DFO did to the North Atlantic cod.

Thank you for reading through this, and for your work in DFO. I hope that we can move beyond the myths that were developed during the previous government and use the available science to guide us.

All the best for the New Year,

Alexandra Morton

Email communications between CFIA investigator, Gary Kruger and Alexandra Morton March 20 – April 3, 2013

Alexandra Morton <gorbuscha@gmail.com> 3/19/2013 3:50 pm >>>

Dear Dr. Klotins:

I am writing to inquire about the CFIA's test results on my sample that tested positive for HPR0?

Thank you,

Alexandra Morton

From: **Kim Klotins <Kim.Klotins@inspection.gc.ca>**
Subject: Re: ISA confirmation?
Date: 20 March, 2013 4:29:37 AM PDT
To: Alexandra Morton <gorbuscha@gmail.com>
Cc: Gary Kruger <Gary.Kruger@inspection.gc.ca>

Good Morning,

I am forwarding your question for answer by the Western Area of CFIA Operations as they are conducting the investigation.

Regards, Kim

Alexandra Morton <gorbuscha@gmail.com> 3/19/2013 3:50 pm >>>
Dear Dr. Klotins:

I am writing to inquire about the CFIA's test results on my sample that tested positive for HPR0?

Thank you,

Alexandra Morton

From: **Gary Kruger <Gary.Kruger@inspection.gc.ca>**
Subject: Re: ISA confirmation?
Date: 20 March, 2013 11:07:53 AM PDT
To: Alexandra Morton <gorbuscha@gmail.com>
Cc: Kim Klotins Kim.Klotins@inspection.gc.ca

Hi Alexandra

Thank you for your enquiry. Since we will never be able to determine the exact origin of the samples from a regulatory perspective, due to chain of custody issues, we will not be conducting any further testing to confirm this finding. The best we can do is to try and narrow down the origin through DNA testing, but I have not received those results yet.

Thanks
Gary

Dr. Gary Kruger
P: (403) 292-5825 F: (403) 292-6629
Email: Gary.Kruger@inspection.gc.ca
Area Program Specialist (Aquatics) - Western Operations
Canadian Food Inspection Agency
1115 57 Ave NE, Calgary, AB T2E 9B2
www.inspection.gc.ca

Alexandra Morton <gorbuscha@gmail.com> 3/20/2013 1:17 pm >>>
Dr. Kruger:

I am astonished. When a person becomes ill from eating tainted food, the CFIA is known to step in and trace the food to source and stop the spread of the infectious agent, even though the victim does not have chain of custody.

In this case, we have an incident with a "reportable" disease, that is being sold to the United States and elsewhere, and this sample of Skuna Bay fish has tested positive for ISAv. Skuna Bay fish is being shipped over the border with the understanding that British Columbia is ISA - free.

Attached is the label on the box that this fish came from, it names the farm and the date the fish was harvested.

The CFIA is responsible for preventing pandemics, ISA virus is known to spread and kill large amounts of salmon.

I do not understand your position and request that you immediately return my samples to Dr. Kibenge's lab because they were requested by the CFIA for testing.

On the Skuna Bay website it says: Tamper proof and traceable seals on the box indicates that after our approved salmon selectors have sealed up the carton, the

next person to touch the fish is the chef. No person intervenes in this chain.

Please let me know when you will be returning my samples to Dr. Kibenge's lab

Alexandra Morton

From: **Gary Kruger <Gary.Kruger@inspection.gc.ca>**
Subject: Re: ISA confirmation?
Date: 21 March, 2013 8:57:45 AM PDT
To: Alexandra Morton <gorbuscha@gmail.com>
Cc: Kim Klotins <Kim.Klotins@inspection.gc.ca>, Ray Fletcher Ray.Fletcher@inspection.gc.ca

Hi Alexandra

Thank you once again for your message. As you know, the CFIA takes reportable animal diseases very seriously and follow up with an investigation when we are notified of these diseases. Unfortunately, we are unable to confirm ISA from the samples that you have submitted to Dr. Kibenge for reasons previously provided. Please feel free to review section 7.2 of the OIE Manual of Diagnostic Tests for Aquatic Animals which describes how ISA can be confirmed. You will notice that it refers to the farm of origin among many other factors. Since there is no fish left at the farm of origin, we cannot obtain further samples.
http://www.oie.int/fileadmin/Home/eng/Health_standards/aahm/2010/2.3.05_ISA_2009%20.pdf

Dr. Kiberge's laboratory is a private laboratory and is not approved by the CFIA for the purposes of conducting work for the National Aquatic Animal Health Program. While we do follow up on these lab reports, it is critical for the CFIA to be able to confirm these findings prior to taking on eradication efforts, etc. In addition, I'd like to remind you that after thousands of tests, the CFIA has not been able to confirm the presence of this disease in BC. The CFIA has undertaken a surveillance initiative in wild salmon last year and the plan is to include farmed salmon this year. Testing for ISA by DFO (research projects) and the industry via other private laboratories continues as well, with no notifications of suspicious positive results to the CFIA.

I do believe that we share a common goal as part of the CFIA's mandate to ensure the protection of the animal resource base, including wild and farmed salmon in BC. I would therefore encourage you to continue to work with us in collaboration, so as to ensure that we meet this goal.

If I may, I'd like to make a few more comments:

- There is absolutely no evidence that ISA is a zoonotic disease within the scientific community

- You are completely correct: The CFIA has a responsibility for preventing pandemics and the ISA virus is known to kill cultured Atlantic salmon. Since ISA is not zoonotic and since BC is free of this disease, no country (including the US) has placed any restrictions on BC salmon.

- The exact same principal applies here as what the CFIA would do with tainted food. We have the responsibility to trace it to source whether it is a food safety related issue or, in this case, an animal health related issue. We, were provided with the pictures that you had attached and followed up with the Skuna Bay Company. No fish were left from that harvested population to sample. The CFIA will continue it's surveillance program for finfish on the west coast.

The CFIA has the authority to obtain samples for testing and the samples from Dr. Kibenge's laboratory no longer belong to him. It is now the CFIA's property and the CFIA has the authority to determine how this sample will be disposed of after we have conducted our testing. I do not see any reason why we would not allow the return of the samples to Dr. Kibenge from a regulatory perspective, as the material is non infectious having been preserved in ethanol. However, there will most likely be nothing left after our testing is completed. Dr. Kibenge may request in writing to have the samples returned after completion of our testing.

I hope this further helps to address your concerns and if you have any other questions please feel free to contact me again.

Thanks
Gary

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Alexandra Morton <gorbuscha@gmail.com> 3/21/2013 1:16 pm >>>
Dear Gary

I really appreciate your willingness to explain, and I have further questions.

I remain unclear, are you running PCR testing for ISA virus in my SK samples or not? From your first message it sounded like you are not, but now you are saying you are going to use up all the tissue in testing.
Can you tell me what tests you are going to do to use up such a large amount of tissue? You have samples that were frozen and samples in RNA Later, that is a lot of tissue for PCR testing.
It seems to me that since Grieg rears its fish right there in a local hatchery and likely produced the fish that went into the Williamson site, that you should be testing that hatchery. Rumours reached me that they just killed a large number of fry. I can't verify that, but you could. You could also test adjacent sites as this virus is known to spread horizontally.
I would like to see the tests the CFIA has done on all of my samples. There has to be a reason so many labs have reported positives, but the CFIA cannot. I would really like to know what protocol the CFIA is using.
Have you seen the attached email from Dr. Miller? Apparently Dr. Saksida reported ISA positives to the CFIA in BC farmed chinook salmon. Are you aware of this? Also see the large number of positives reported by DFO's Kyle Garver and Miller. In addition, have you seen the email where Gagne reported a weak positive in the highly degraded Rivers Inlet samples (attached)? A weak positive in degraded samples is not a "negative."

Gary, there is something not right here. The CFIA cannot keep brushing off the positive results from so many labs, including those who have tracked this virus around the world and DFO's own labs, without telling us exactly what tests are being used. Dr. Gary Marty, for example, uses an ISAv testing protocol validated only within BCMAL. He is the only lab I know of that has tested BC salmon for ISAv and cannot find any positives, weak or otherwise. BCMAL, of course, is the agency that developed salmon farming in British Columbia. They are a well known advocate for the industry, sometimes at the expense of the facts. For example, while Dr. Mark Sheppard worked for BCMAL, as the vet in charge of farm salmon health, he advised the Minister in charge of salmon farms that no live Atlantic salmon eggs had been imported to BC so there was no concern about ISA virus reaching BC as it had Chile. This is simply wrong, according to the DFO website ~27 million presumably live Atlantic salmon eggs had entered BC by that time and Sheppard was the man in charge of their health! I have attached Sheppards "confidential" briefing as it has become an exhibit so you can read his words. So an in-house BCMAL validated ISAv test that is producing results contrary to the rest of the scientific community bears scrutiny.

I asked Con Kiley for the negative results on my ISAv positives in the Fraser River and he sent results for Salmo salar…. none of the salmon I tested in the Fraser were Salmo salar. I wrote back to him and never heard back.

I think it is very important that the CFIA test my SK samples. Please confirm that you are going to do so and forward the
protocol, which lab,
who signed off on the results and the results.

Thank you so much,

Alexandra Morton

From: **Gary Kruger <Gary.Kruger@inspection.gc.ca>**
Subject: Re: ISA confirmation?
Date: 21 March, 2013 4:02:43 PM PDT
To: Alexandra Morton <gorbuscha@gmail.com>
Cc: Kim Klotins <Kim.Klotins@inspection.gc.ca>, Ray Fletcher Ray.Fletcher@inspection.gc.ca

Hi Alexandra

Thank you for the additional information. I want to assure you that we are not brushing off the "positive" results from so many labs. You may not even be fully aware of the massive investigation that we launched in 2011 based on suspicious results from Dr. Kibenge's lab. I think part of the problem is that laboratories that deals with reportable aquatic diseases have not been properly educated in the use of appropriate terminology and seems to have a tendency to incorrectly report their suspicious findings as positive. This inaccurate and inappropriate use of the word "positive" unfortunately seems to alarm the media and the public, because they do not understand what it means to confirm a disease. They also do not seem to understand the limitations of the PCR test. All of the suspicious initial findings for ISA in BC could never be confirmed by the CFIA. We have enough data and scientific evidence that we do not currently have ISA in BC, and that all the suspicious findings to date were just that - suspicious, unconfirmed false positives.

We do not, however, take this disease lightly. Between April 2012 and December 2012, over 4,200 wild salmon samples were collected from B.C.

waters, as well as from processing plants and hatcheries (wild salmon). To date, more than 3,500 of those samples have been tested so far, all with negative results. This is part of our official CFIA surveillance program - not done in private labs. And as I mention, the plan is to expand on this by including farmed salmon.

I can literally spend hours and hours explaining how diseases are confirmed, and for ISA it certainly is not done through screening tests such as PCR. The previous link (from the OIE website that I provided below) plus this link, explains how ISA can be confirmed:
http://www.dfo-mpo.gc.ca/media/back-fiche/2011/20111108-eng.htm
I also thought to add the old well known methodology developed in 1884 to demonstrate the complexity of disease confirmation:
http://en.wikipedia.org/wiki/Koch's_postulates
We are a fact-seeking regulatory science based agency. It would be unacceptable and immature to implement certain measures based on assumption and suspicion (just like with any other regulatory agency).

I have looked at so many lab reports regarding ISA in the last two years, that I cannot say with certainty whether I have seen that report from Dr. Miller (PCR results) or remember Dr. Saksida's suspicious report, but all these laboratories are well aware of their legal obligation to notify us, so if this was withing the last two years, we would have received it and followed up on it. As for Dr. Gagne's email, yes I have seen that, and once again these are PCR tests. All it means is that it would prompt the lab to try and confirm whether that is a false positive or whether they really have a virus. In that case they tried to confirm it and couldn't - which means that it wasn't ISA.

My apologies for the confusion about what we are doing with your recent samples. We collected the homogenates from Dr. Kibenge. I think he still has the rest of the samples. We rendered these homogenates non infectious by preserving it in ethanol. We are not interested to try and run a PCR test on these samples and as described before we will not be able to confirm the disease any ways. So as such, all we are able to do is try and narrow down the origin of the samples by running DNA analysis. I can let you know of the results once we obtain them.

I appreciate that it might appear as if some facts are not adding up, but it is mostly due to inappropriate use of language that leads to confusion. As I mentioned before, there is no hidden agenda. We will continue to look for ISA and if it is confirmed we will notify the OIE (under international law), immediately notify our trading partners (so as to prevent the possible spread of disease to their countries) and

take whatever other measures ar
e appropriate and necessary at the time.
I personally are much more concerned with IHN than ISA.

Canada is the 5th largest exporter of seafood in the world. One would think that if any of these countries had a concern with ISA in BC, they would have banned our salmon by now. But they haven't, so they must be just as confident as we are that we do not have the disease. I really appreciate working with you, and as before please do not hesitate to contact me or share any information you may have.

Thanks
Gary

Dr. Gary Kruger
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Email: Gary.Kruger@inspection.gc.ca
Area Program Specialist (Aquatics) - Western Operations
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From: **Alexandra Morton <gorbuscha@gmail.com>**
Subject: Re: ISA confirmation?
Date: 21 March, 2013 11:07:11 PM PDT
To: Gary Kruger <Gary.Kruger@inspection.gc.ca>
Cc: Kim Klotins <Kim.Klotins@inspection.gc.ca>, Ray Fletcher <Ray.Fletcher@inspection.gc.ca>

Dear Gary

Sorry, now I am very confused about what testing the CFIA has done on my Skuna Bay samples.

In the first email you said:

"there will most likely be nothing left after our testing is completed"

This made it sound like the CFIA was going to run confirmatory tests for ISA virus

But in the next email, you say the CFIA took the homogenate of my samples from Kibenge's lab and put it in ethanol to make it non-infectious and then decided

" *We are not interested to try and run a PCR test on these samples and as described before we will not*
be able to confirm the disease any ways."

Why did you take the samples if you are not interested in running tests? Why did you say the tests would use up the whole sample?

In Canada, confirmation of ISAv requires culturing the virus, and this can only be done with live virus.

When you put my sample in ethanol, didn't that kill the virus? Making it impossible to culture?

When you said in the previous email that *" we are unable to confirm ISA from the samples that you have submitted to Dr. Kibenge "* that would be because the samples were put in ethanol?

But what really is confusing me, Gary, is that the CFIA did not take the homogenate from the lab, they took the fresher samples.

Apparently the fresher samples were the ones put in ethanol? Has this been the procedure with all my samples?

When I wrote Dr. Klotins to ask about the results of the CFIA confirmation tests for ISA virus in my Skuna Bay samples, she forwarded my request to you, indicating you are leading the investigation. But the information you got from the east coast about what was taken from the lab is incorrect.

Furthermore, the positive PCR results was for the HPR0 variant of ISAv, which cannot be cultured. Thus a positive HPR0 variant of ISA virus is never going to be confirmed in Canada.

So just to confirm, the CFIA did no testing on my Skuna Bay samples?

alex

From: **Gary Kruger <Gary.Kruger@inspection.gc.ca>**
Subject: Re: ISA confirmation?
Date: 22 March, 2013 9:19:51 AM PDT
To: Alexandra Morton <gorbuscha@gmail.com>
Cc: Kim Klotins <Kim.Klotins@inspection.gc.ca>, Ray Fletcher Ray.Fletcher@inspection.gc.ca

Hi Alexandra

My apologies for the confusion. Initially I was told that they are going to be taking the homogenates, but then after that I was no longer in the loop because our inspector communicated directly with the lab and they must have decided that it is better to send the fresh samples, which is actually better. So to confirm, we are not doing any diagnostic testing and will not be doing any diagnostic testing for ISA whatsoever. No PCR testing, no virus isolation, no further diagnostic testing because such testing will be of no value to the CFIA at this time.

What we are doing is that we are doing DNA testing to narrow down the origin of the fish. This is part of any thorough investigation where the chain of custody was lost. If we are able to find a DNA match it will put us in a better position to focus on this site for possible future ISA testing.

Thanks
Gary

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Alexandra Morton <gorbuscha@gmail.com> 3/22/2013 11:36 am >>>
Gary

Why did the CFIA go the lab to take my Skuna Bay samples, if they did not intend to use them?

You can understand I have doubts about this investigation.

First you said there would be no testing due to chain of custody issues
Then you said the entire sample would be used up in testing
When I asked how you could use so much sample tissue you came back that only the homogenate was taken
When I pointed out this was wrong, the homogenate was left and the other tissue taken
you said the lab chose to give my samples away to the CFIA
You also talked about what it takes to confirm ISAv, which is culture

of the live virus, but the CFIA apparently had my samples put in ethanol, which will kill the virus, making culture impossible.
Now we are back to square one, CFIA is not going to do any testing of the Skuna Bay samples, due to lack of chain of custody. Why did you tell me earlier that the sample would be all used up in CFAI testing and there would be nothing to send back to the lab?

I would like to daw your attention to the Skuna Bay website:
http://www.skunasalmon.com/

"Tamper proof and traceable seals on the box indicates that after our approved salmon selectors have sealed up the carton, the next person to touch the fish is the chef. No person intervenes in this chain."

Indeed the name of the farm is on the box and in the pictures forwarded to you.

If the CFIA is not going to test a farm salmon sample that has tested positive for the ISA virus from a traceable, sealed box, from a known site and company, and the CFIA is not testing farm salmon themselves how will we ever know if farm salmon have ISA virus or not? I would like to know if this is what happened to all my samples taken from the lab by the CFIA. Were they all put in ethanol and never tested due to lack of chain of custody?

Alexandra Morton

From: **Gary Kruger <Gary.Kruger@inspection.gc.ca>**
Subject: Re: ISA confirmation?
Date: 25 March, 2013 11:03:47 AM PDT
To: Alexandra Morton <gorbuscha@gmail.com>
Cc: Kim Klotins <Kim.Klotins@inspection.gc.ca>, Ray Fletcher Ray.Fletcher@inspection.gc.ca

Hi Alexandra

Yes, there is a complete lack of chain of custody. For more information, please see:
http://en.wikipedia.org/wiki/Chain_of_custody
http://en.wikipedia.org/wiki/DNA_profiling

Thanks
Gary

Dr. Gary Kruger
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Alexandra Morton <gorbuscha@gmail.com> 3/25/2013 12:25 pm >>>

Gary

Is this true of all my samples that have produced positive PCR results
for the ISA virus? The CFIA has not tested any of them because I don't
have chain of custody?

alexandra morton

On 2013-03-26, at 9:28 AM, Gary Kruger wrote:

Hi Alexandra

Yes, that is true. Members of the public cannot submit samples for reportable disease diagnostics. If you suspect a reportable disease,
please contact the CFIA so that we can collect and submit samples to the
appropriate laboratories if necessary.

Thanks
Gary

Dr. Gary Kruger
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Alexandra Morton <gorbuscha@gmail.com> 3/26/2013 10:31 am >>>

Gary

What I am asking is when the CFIA takes samples that belong to me from the AVC lab in PEI that have produced positive PCR test results, does the CFIA run its own tests on these samples?

Alexandra Morton

From: **Gary Kruger <Gary.Kruger@inspection.gc.ca>**
Subject: Re: ISA confirmation?
Date: 27 March, 2013 10:08:23 AM PDT
To: Alexandra Morton <gorbuscha@gmail.com>
Cc: Kim Klotins <Kim.Klotins@inspection.gc.ca>, Ray Fletcher <Ray.Fletcher@inspection.gc.ca>

Hi Alexandra

Thank you for your message. Yes, as explained a couple times below, the CFIA is running tests on these samples for DNA profiling.

Thanks
Gary

Dr. Gary Kruger
P: (403) 292-5825 F: (403) 292-6629
Email: Gary.Kruger@inspection.gc.ca
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Alexandra Morton <gorbuscha@gmail.com> 3/29/2013 12:00 am >>>

Dear Gary

Once you have run the DNA, what are the next steps the CFIA is going to take with these samples?

Will you be running any tests for ISAv?

Alexandra Morton

From: **Gary Kruger <Gary.Kruger@inspection.gc.ca>**
Subject: Re: ISA confirmation?
Date: 3 April, 2013 9:04:11 AM PDT
To: Alexandra Morton <gorbuscha@gmail.com>

Hi Alexandra

It is too early to tell whether we would do any further tests. If there are any tissue left, we can return it to Dr. Kibenge, but as you know, they are in ethanol.

Thanks
Gary

Dr. Gary Kruger
P: (403) 292-5825 F: (403) 292-6629
Email: Gary.Kruger@inspection.gc.ca
Area Program Specialist (Aquatics) - Western Operations
Canadian Food Inspection Agency
1115 57 Ave NE, Calgary, AB T2E 9B2
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From: **Alexandra Morton <gorbuscha@gmail.com>**
Subject: Re: ISA confirmation?
Date: 3 April, 2013 9:25:48 AM PDT
To: Gary Kruger <Gary.Kruger@inspection.gc.ca>

Gary

The CFIA's role is to confirm, or not whether ISA virus is in this sample of Skuna Bay salmon raised near Gold River, in the Williamson farm site. The only way to confirm ISA virus in Canada is to culture it and this is only possible if the virus is active, "alive." When the sample was put into ethanol, as per the CFIA's instructions, the CFIA made it impossible to confirm ISA virus in this sample.

Why is the CFIA putting reportedly ISA virus - positive tissue into a solution that inactivates the virus, destroying any possibility of confirming the virus?

And you do realize that you have contradicted yourself again, earlier you said:

"to confirm, we are not doing any diagnostic testing and will not be doing any diagnostic testing for ISA whatsoever. No PCR testing, no virus isolation, no further diagnostic testing because such testing will be of no value to the CFIA at this time."

Why was the ISAv - positive sample put into ethanol?

Alexandra

From: **Gary Kruger <Gary.Kruger@inspection.gc.ca>**
Subject: Re: ISA confirmation?
Date: 4 April, 2013 12:03:38 PM PDT
To: Alexandra Morton <gorbuscha@gmail.com>

Hi Alexandra

Thank you for your message. I actually did not contradict myself, but realize that the wording was not clear. As I mentioned before "we are not doing any diagnostic testing and will not be doing any diagnostic testing for ISA whatsoever. No PCR testing, no virus isolation, no further diagnostic testing because such testing will be of no value to the CFIA at this time." We may, however, do more DNA analysis if needed, which is what I meant.

I should point out that it is indeed not the CFIA's role to confirm, or not whether ISA is in the sample of Skuna Bay that you had submitted to Kibenge. We do not test samples for reportable diseases that was submitted by members of the public. We will, however, always follow up on these suspicious findings and determine the best course of action. Once again, if you suspect a reportable disease at the origin, please contact the CFIA so that we can determine whether to collect samples ourselves for further testing. Members of the public cannot participate in sample collection for reportable diseases in Canada or any other country.

Thanks
Gary

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From: **Alexandra Morton <gorbuscha@gmail.com>**
Subject: Re: ISA confirmation?
Date: 4 April, 2013 1:09:27 PM PDT
To: Gary Kruger Gary.Kruger@inspection.gc.ca

Gary

Thank you for this.

The lab which got the positive ISAv result in Skuna Bay salmon is the OIE lab for the Americas. It is the lab that diagnosed Chile with ISAv when no one else knew ISA was in Chile. That outbreak led to a $2 billion loss. Here in BC, the stakes are extremely high because the wild salmon economy stands to be affected.

If the CFIA won't go to the farms, won't retest ISA virus positives from one of only two OIE reference labs for ISA virus, but went to the lab took the samples and has destroyed the evidence for ISAv confirmation, how exactly do you know the Skuna Bay salmon are not carrying ISA virus into the US, a country that has gone on the record specifically stating they do not want ISAv-infected salmon? "Morgan Lascinsky of the U.S. Food & Drug Administration said salmon with the virus would not be allowed across the border because American law prohibits the importation of any diseased animal." http://www.thestar.com/news/canada/2013/02/01/infected_salmon_declared_fit_for_human_consumption_by_canadian_food_inspection_agency.html

How exactly does the CFIA know the Skuna Bay salmon from the Williamson farm, harvested in December 2012 did not enter the United States infected with ISA virus?

Alexandra Morton

From:	Alex Morton
To:	Popham, Lana
Cc:	Meggs, Geoff PREM:EX; OfficeofthePremier, Office PREM:EX; s.22
Subject:	Your stand on UNDRIP
Date:	Tuesday, October 24, 2017 10:40:12 AM
Attachments:	Minister Popham Oct 23.pdf

Dear Lana Popham,

Please find the attached open letter. I see you have a serious problem, but when a scientist refuses to reveal his methods in a high ranking scientific journal and that benefits an industry whose pratices are in the spotlight - you have a really serious problem.

Attached is my open letter to you.

Thank you for your fortitude so far.

Alex

October 24, 2017

Dear Minister of Agriculture Lana Popham:

Thank you for your brave response to the First Nation's effort to get salmon farms out of the Broughton Archipelago, an effort that has been ongoing for them for 30 years. You are the first politician to stand up for their rights against the Norwegian-run salmon farms.

With the collapse of food fisheries, the problem with salmon farms has grown beyond biological chaos to also become a human rights issue. Reconciliation can't happen between cultures when one is destroying the food resources of the other.

You directed very powerful words at Marine Harvest when they breached the First Nation directive to remove all farm fish and began restocking a farm in Knight Inlet where the tenure will expire one year before the newly-stocked fish are ready of harvest.

"Whatever operational decisions you [Marine Harvest] *should choose to make, the Province retains all of its rights under the current tenure agreements, including potentially the requirement that you return possession of tenured sites at the end of the current terms."* The Honourable Lana Popham to Vincent Erenst, Marine Harvest

In 2016, I won a lawsuit that revealed that the Marine Harvest Dalrymple Hatchery is infected with piscine reovirus, a highly contagious Norwegian salmon blood virus. DFO was directed to test farm salmon for this virus issuing transfer permits into ocean pens, but DFO still refuses to test and so we are back in court. Marine Harvest admitted to the court that most of their hatcheries are infected and complained that *they* would be "severely" impacted if they can't grow infected fish in BC waters. What about people who don't want to eat salmon infected with a virus that damages the fish's heart, who rely on salmon, who have no food fish. What about the much larger wild salmon economy? What about future generations?

Scientists in your ministry decided *with industry* that piscine reovirus is harmless and this flawed decision allowed millions of infected farm salmon to pour into farms on BC 's wild salmon migration routes over the past ten years. Now the virus is spreading. Your ministry knew the virus was causing heart disease and did not release this evidence. Why?

As I photographed the transfer of Atlantic salmon smolts from Marine Harvest's Dalrymple Hatchery last week, thousands were dying. Many rolled over to reveal haemorrhages on their bellies and at the base of their fins. Marine Harvest poured these dead and dying farm salmon into Musgamagw Territory. See pictures below.

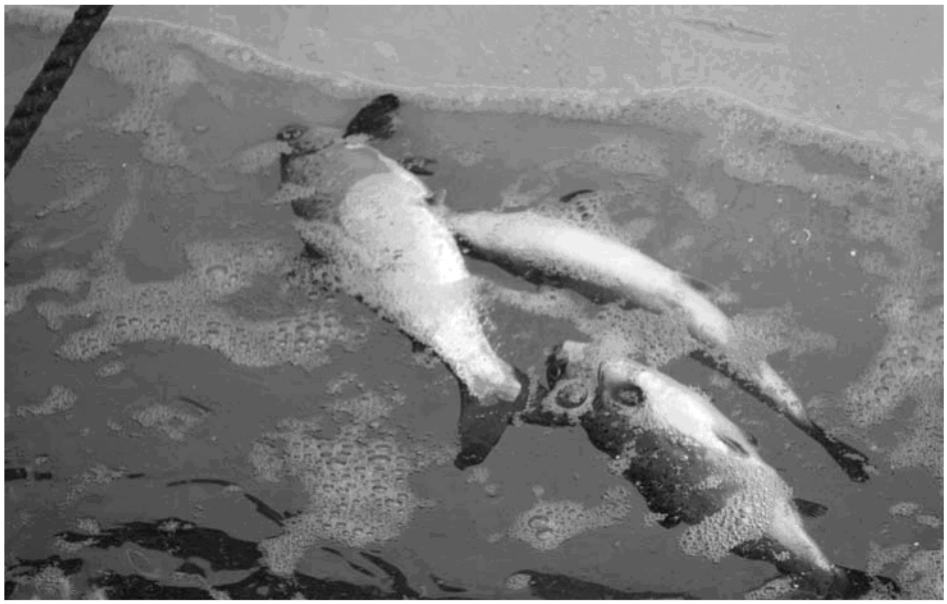
No government worldwide has stood ground against the salmon farming industry, even in the face of unmistakable devastating impact on wild salmon.

In their typically aggressive manner, Marine Harvest gambled and transferred 1.7 million potentially infected farm salmon into a farm where the tenure is going to expire one year before the fish will be harvested and you responded as a government should.

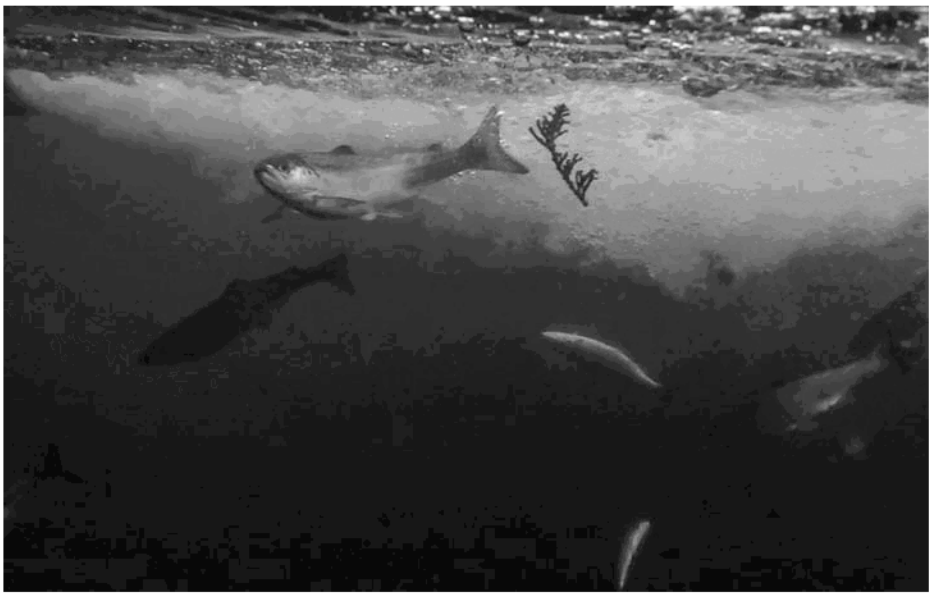
You are clearly under enormous pressure and yet your words have given many of us hope that wild salmon will be allowed to exist in BC and that the relationship between BC and First Nations can heal and become an example to the world of how humans should behave. The wild salmon economy remains more than twice the size of the salmon farming industry, there is no reason the salmon farming industry should be allowed to fill BC waters with diseased fish.

I stand with you Minister Popham,

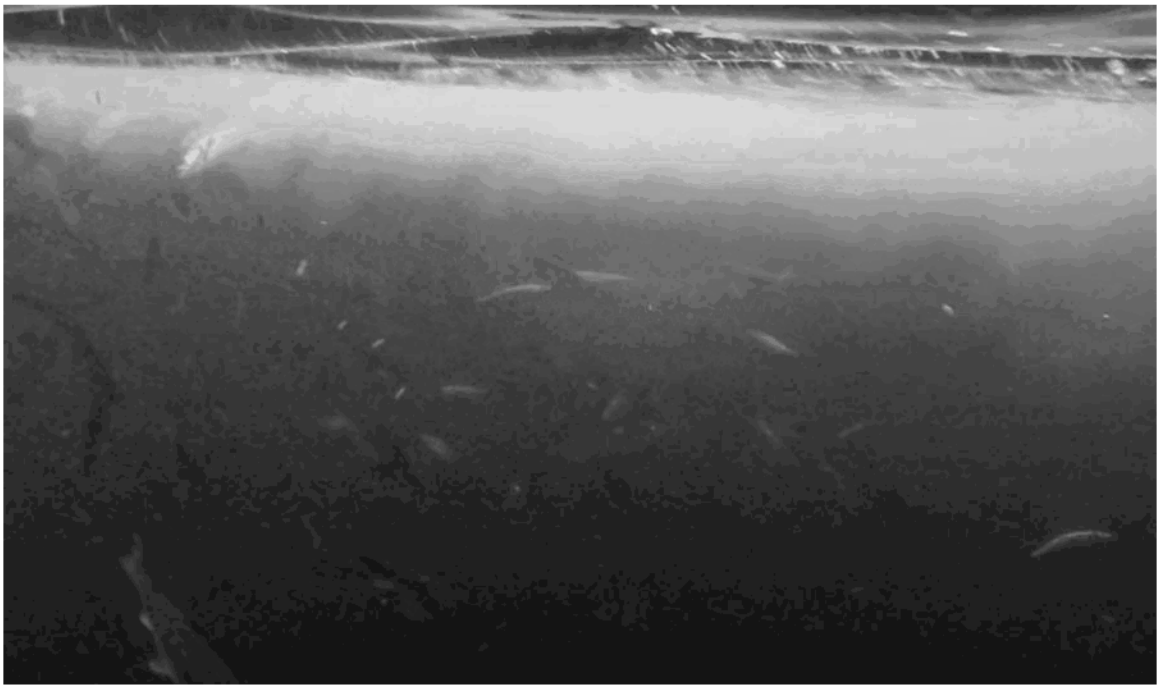
Alexandra Morton
Gwayum'dzi
Independent Biologist



Dead Atlantic salmon smolts from the Sayward Dalrymple hatchery in packer *Roy Kristian*, destined for Port Elizabeth, Knight Inlet. October 17, 2017



Dying Atlantic salmon smolts pouring into Musgamagw territory from the *Orca Chief* October 15, 2017.



Dead Atlantic salmon tumble from the Marine Harvest vessel *Orca Chief.*