Epidemic of a Novel, Cancer-causing Viral Disease may be Associated with Wild Salmon Declines in BC

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I. High En-route losses of sockeye salmon stocks returning to spawn in the Fraser River may be linked to a retroviral disease

- Over the past 12 years, sockeye salmon stocks have experienced unprecedented levels of en-route and pre-spawning mortality during return migration in the Fraser River. Whereas historically losses in the river ranged from 15-25%, in recent years they have fluctuated between 30-95%. The lack of predictability in survivorship to spawning in the river has resulted in large variations in escapement, leading to outcries from stakeholders, ENGOs and the public. In some years (e.g. 2001, 2004), public inquiries have resulted.
- While elevated river temperatures are clearly associated with these losses (as deduced from radio-tracking data and modeling), there is growing evidence that a novel disease is also impacting survivorship in river. In 2006, sockeye salmon were biopsied and tagged on the marine approaches to the Fraser River and upon entry into the river, and their migration was tracked to the spawning grounds through biotelemetry. The Molecular Genetics lab at PBS conducted a functional genomics study comparing the gene expression profiles between fish that made it to spawning grounds and those that were lost in the high water temperature regions of the river (where losses were high and effects of fishing mortality were minimal). Genomic analysis revealed a viral-response profile that was associated with poor survivorship of adult migrating salmon in the river.
    - Salmon up to 300 km seaward of the Fraser River carrying this "viral" signature in gill tissue had a 16-times lower probability of arriving to spawning grounds than those carrying a "healthy" signature.
    - Combining expression profiles and additional physiological information, 85% of the losses in the river could be predicted in advance of river entry, increasing to 90% if river entry timing was also included. This suggests that the condition of fish upon entry into the river is a major factor in their subsequent survivorship to spawning.
    - There was also an association of the "viral" signature with pre-spawning mortality, with >80% of the pre-spawning mortalities (at spawning grounds) carrying the "viral" signature.
    - The "viral" signature has been observed in multiple tissues (gill, brain, liver) and has been observed in varying proportions of sockeye salmon in all years in which functional genomics studies have been carried out (2003, 2005, 2006). 60% of returning sockeye salmon in 2006 carried the "viral" signature in gill tissue.
    - Molecular screening of known viruses has not yielded a positive identification. However, elements of the viral signature are suggestive of a retroviral infection.

II. Brain tumours in migrating salmon are likely associated with the purported retroviral infection, and may impact behaviour and survivorship in multiple salmonid species

- Genomic profiling of brain tissue from adult migrating sockeye salmon in 2003 and 2005 revealed a profile highly correlated with the "viral" profile in gill tissue. The brain profile also showed a strong stimulation of the sensory region of the brain, potentially impacting visual and olfactory cueing systems and maturation processes, and stimulating of numerous pathways associated with tumour activity.
    - Brain dissections revealed that 30-40% of adult migrating sockeye salmon in 2006 contained tumour-like growths in their ocular lobe, a region that was highly stimulated in the brain profiles.
    - In 2008, a year of exceptionally high levels of en-route and pre-spawning mortality in the river, brain dissections revealed similar tumour-like growths in 50-60% of adult sockeye salmon in the marine approaches and lower Fraser River (over 250 dissected). The fact that less than 20% of fish at spawning grounds contained brain tumours implies a potential association of tumours with the extensive en-route losses.
    - In 2009, only 1.35 million of the expected 10 million returning Fraser River sockeye salmon arrived at the river. 2009 brain dissections have revealed the highest incidence of tumours thus far, with over 70% of the return-migrating salmon containing tumour-like growths in the marine approaches and 50% of fish in the lower river. These early data imply that the tumours may also be associated with losses in the Strait of Georgia. In 2005, the brood year for the 2009 returns, the "viral" signature was observed in 17% of gill, 40% of liver and 25% of brain tissues of returning adults, with only 26% of fish containing no viral signature in any tissue.
    - Given the high correlation between the gill and brain profiles, we hypothesize that the tumours may be caused by the same retroviral infection. If so, then the virus must have been present in these fish for some period of time during ocean migration (months at least). Retroviruses are often neoplastic (cancer-causing) and can be vertically transmitted (mother to egg), so we looked to the smolts to see if similar genomic signatures and tumour activity was present in this earlier life-history stage.
    - Genomic profiles of brains from sockeye salmon smolts leaving the river in 2008 revealed a dominate profile highly correlated with that observed in adult brains and virally affected gills. 50% of smolts in the lower river in May and the ocean in June contained the viral-tumour associated profile. Incidence in Cultus Lake hatchery, an assisted breeding facility for a stock in severe decline, was 30% in parr in November and 80% as smolts began their river decent. Brain dissections of sockeye smolts revealed a similar incidence of tumours, and the presence of tumours in stocks outside of the Fraser River.
    - Ocular Tumours were also observed in coho and Chinook salmon smolts sampled in the ocean in June and Sept/Oct of 2008. 37% of coho salmon smolts contained ocular tumours in stocks ranging from the Fraser, Skeena, Columbia, Central Coast, and Vancouver Island (N=20). 40% of Chinook smolts sampled in June contained ocular tumours, reduced to only 11% in Sept (N=30). These data

reflect a large range of stocks from the Northern Mainland, Nass, Stikine, Fraser, and Columbia systems. Similarly, 40% (N=58) of sockeye, coho and Chinook salmon smolts in 2009 had ocular tumours in June, with 0% with tumours (N=55) in September. This data indicate that the tumours are associated with early ocean mortality in all three species.

III. The Salmon Leukemia Virus causing Plasmacytoid Leukemia—Best link to the genomic evidence

- There are only two known retroviruses in salmon, one that causes tumours in the swim bladder (not consistent with our observations), and another associated with tumours in the ocular lobe of Chinook salmon in BC and Washington (called the Salmon Leukemia virus [SLV] or Plasmacytoid Leukemia [PL]). This latter virus has been associated in the 1980's through 1990's with mortalities in cultured Chinook salmon in fresh and saltwater in BC and mortalities of Coho in a BC enhancement hatchery. Moreover, challenge studies show high susceptibility of Chinook, sockeye and coho salmon to the virus, low susceptibility of Atlantic's, and no measurable effect on Rainbow trout.
    - SLV causes severe anaemia (salmon farmers call it Marine anaemia), with primary infections involving the kidney and spleen. In advanced infections, proliferating plasmocyts move into secondary organs, including liver, pancreas, intestine, gill and brain. Involvement of secondary organs varies among individuals.
    - Linkages of SLV with our genomic and physiological data are thus far indirect but strong
        - Genomic signatures indicative of retroviral activity and tumourogenicity
        - Incidence of ocular tumours (predicted originally from brain profiles)
        - Observations of ocular tumours in Chinook, coho and sockeye salmon
        - Involvement of gill, brain and liver but not muscle tissue
        - Percentage involvement of secondary tissues similar to histological observations. In 2005, multiple tissue profiles of the same fish showed 40% of fish with viral profiles in liver, 30% in brain, and 20% in gill tissue. Importantly, only 25% of fish were negative in all three tissues. Given that these are all secondary organs, we predict that rates of infection are actually higher than 75%.
    - There are several elements of the history and timing of descriptions of PL/SLV that potentially implicate this virus in the large-scale declines of coho and Chinook salmon in BC, and may be suggestive of a role of hatcheries and aquaculture in this decline. Below is a timeline of viral observations and potential linkages (by date) with major events in coho, Chinook and sockeye. Note that the scientists involved in PL/SLV research left the province in the late 1990's, and no significant direct study of this disease been pursued since their departure.

Chinook Salmon

1974
PL discovered through histology at Washington State Hatchery
Fish released to ocean

1977
Chinook Declines Begin

1988
PL/SLV BC Net Pens in S. BC
Ocular tumours associated with SLV

1989-1992
SLV spreads to Net Pens in central BC
Large outbreak in 1991 from hatchery stock throughout VI, S/N BC, Yukon
Decreased growth and chronic losses throughout production cycle---most notable 1 year at sea (Aug-Sept)-Temperature stress?

1991-1992
PL/SLV in 6% of wild fish in the SOG

1993
Vertical transmission demonstrated
Some evidence for horizontal transmission

1994
Experimental SLV challenge
100% infected

2008
Ocular tumours in 50% of smolts in SOG

Coho Salmon

1983
Ramp up hatchery production in BC

1985
PL Big Qualicum Hatchery
12% mortality
Released to ocean

1986
PL Big Qualicum Hatchery
45% mortality
Released to ocean
Note behavioural shifts--reduced activity with energy bursts

Marine Challenges
poor survivorship in SW

1986
Coho Declines Begin

1993
Large crash in coho Begins

1994
Experimental SLV challenge
70% infected

1995-1996
PL/SVL in 5.9% of cultured Coho in Chile
Less common/virulent in Atlantics, but challenge from infected Atlantic caused disease in coho
(Atlantics as carriers?)

2008
Ocular tumours in 50% of smolts in SOG

2009
Ocular tumours in 40% of sockeye/coho/Chinook smolts in June, 0% in Sept. SLV-associated early ocean mortality of wild fish?

Sockeye Salmon

1991
PL/SLV in wild fish in the SOG

1994
Experimental SLV challenge
100% infected

1996-present
Sockeye Behavioural Alterations
Late-run Fraser R sockeye salmon
Entering the river 6 weeks early
High fluctuating losses in river
Role of Temperature

2003-2006
Genomic signatures suggestive of a retroviral infection associated with early river entry and mortality in the river
Brain signatures indicate presence of virus and tumour activity

2006
Discovered ocular tumours in 40% of fish

2008
Huge losses in the Fraser River
of adult salmon returning to spawn
Many stocks affected

2008
Ocular tumours observed in 60% of returning salmon, only 20% at spawning

2009
Sockeye salmon don't show up
Only 1.3 of the expected >10 million
Sockeye return to the Fraser River to spawn

2009
Ocular tumours in 70% of returning fish, but only 50% upon river entry---marine mortality?

IV. Potential Good News and the Way Forward

- The evidence provided in this document indirectly links disease associated with the Salmon Leukemia Virus to shifts in behaviour and mortality events in sockeye, coho, and Chinook salmon both in freshwater and saltwater environments. We hypothesize that this disease may be a major factor both in the highly fluctuating annual returns and in the general decline of many stocks of these Pacific Salmon species in BC and Washington State.
- We cannot discount the fact that much of the documentation of mortality associated with this disease is from cultured fish (hatcheries and aquaculture facilities); hence the public and media could be quick to conclude that this is a "hatchery" or "aquaculture" impact on wild fish. It is certainly possible that these high density rearing environments can increase the incidence of disease. Moreover, in at least two hatchery cases, fish that were dying of SLV/PL were released into the ocean. However, it is important to realize that there is no regular disease/fish health screening on wild salmon. Hence, linkages of PL/SLV with cultured fish may be an artefact of sampling, as fish health experts are only brought in when we observe fish dying. In wild fish, we don't observe mortality events (especially in the ocean), the fish simply disappear.
- Recent media attention has implicated salmon aquaculture in the declines of wild salmon. While much of this attention focuses on sea louse, the biological evidence for a sea louse involvement is relatively weak compared to other potential factors (e.g. SLV, ocean carrying capacity, seal predation). Importantly, it is clear that Atlantic salmon, the key farmed salmonid species in BC, is not susceptible to SLV. Direct challenge studies did not result in significant disease in Atlantics, and there have been no reports of mortality associated with SLV in Atlantic salmon world-wide. The size of the Chinook aquaculture industry in BC is infinitely smaller than that for Atlantic salmon; given the fact that broodstock for this industry is generally obtained from BC enhancement hatcheries, it is unlikely that these small scale farming operations are directly linked to expansion of this disease.
- The good news is that if disease were the issue in the declines of Chinook and coho salmon, there may be ways to mitigate the impact in future. The most plausible mitigative action would be to screen hatchery broodstocks for the virus, and to select only fish that are virus-free for breeding. If we had a DNA sequence of the virus, we could design rapid molecular screening tests that could be performed in a matter of hours at the hatcheries. Even if all broodstock were not screened, the incidence of the virus could be tracked each year by screening a fraction of the return, and decisions to screen could be based on prevalence levels. Husbandry practices could also be improved to minimize horizontal transfer of the virus. While we cannot directly control disease incidence in the wild, reducing the incidence in hatchery fish would, over time, likely also reduce levels in the wild. Moreover, regular screening of smolts and adults would allow managers to adjust escapement estimates according to infection levels. Given the indirect evidence that this disease is most virulent when salmon are stressed, for this approach to work optimally, we need to develop a better understanding of the link between the viral-associated disease and the environment (e.g. temperature, salinity, food availability).

- DFO has been accused of mismanagement by various stakeholders and the media for the rapid decline and high variance in annual returns of Pacific Salmon stocks. Despite numerous inquiries, the situation has not been resolved. Blame has also been levied on fisheries stakeholders and First Nations groups, citing over-fishing and illegal fisheries as route causes of the declines. Disease, other than salmon louse, has rarely been considered as a key factor in salmon declines. Even with concerted field study, SLV's impact on wild fish would have been difficult to predict with the tools available to us even 5-10 years ago. Viruses are among the most elusive of all pathogenic diseases, because their virulence can depend upon the level of stress of the fish, and because they tend to weaken immunity and increase the susceptibility of fish to other pathogens and to predation. It is because of power of new genomics tools that the potential involvement of SLV was discovered. Novel molecular approaches will also be required to fully characterize the virus, its relationship with the declines, and strategies for mitigation.
- If an SLV epidemic is shown to be a major factor in salmon declines, it would mean that we are dealing with a new environmental playing field. To some extent, this knowledge could lesson tensions between stakeholders, while demonstrating the power of DFO science to resolve major issues associated with the aquatic resources under our management. You cannot mismanage something that is completely new and unforeseen, but once it is resolved, the key to success will be how quickly and effectively in new scientific findings are integrated into policy and management.
- Given the intensity of public debate about salmon issues in BC, we believe that these findings are of strategic importance and immediate interest to DFO. However, as this potential linkage is only recently discovered, there is no funding in place to gain direct evidence linking SLV to high incidences of mortality in wild salmon. It is imperative that a DNA sequence for the Salmon Leukemia Virus be obtained to establish this link and move forward with mitigative actions.
- Below is a schematic of the research required to characterize this relatively new, poorly understood viral disease, to establish its potential role in the widespread declines in salmon stocks in the Pacific Northwest, and to development mitigative measures to control the disease and reduce impacts in future generations. While this research could change the way we view enhancement and manage fisheries, ultimately it could lead to greater returns from both hatcheries and wild stocks.
- There exists a team of scientists within DFO that can carry out this research, as well as external collaborators willing to fill in the gaps. The four-year plan could be funded in a stepwise manner, with annual support dependent upon the data and evidence each year. If we are to move forward with this plan, we would need to move quickly to obtain fresh isolates from field samples for histology, culture, viral isolation, and disease challenges. All evidence suggests a high prevalence of SLV this year, but the smolts will begin migrating northward and adults will have spawned out by the end of October, leaving little time to lose.