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

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Summary

A novel viral disease may be a key factor in high variability in performance and general declines in abundance of sockeye, Chinook, and coho salmon in Southern BC. Genomic data from gill, liver and brain tissue elucidated a powerful anti-viral signature in >75% of sockeye salmon returning to the Fraser River in 2005. This signature was associated with high levels of mortality in the Fraser River in 2006. Indications of tumour activity within the "anti-viral" brain profile led to the identification of ocular tumour-like growths in 30-40% of returning adults in 2006. Tumour incidence was increased to 50% of returns in 2008, but declined to less than 20% for fish at the spawning grounds, indicative of tumour-associated mortality in the river. In 2009, a year in which only 1.35 of the estimated 10 million sockeye returned to the river, tumour incidence in returning fish rose to 70% in the ocean, dropping to 50% upon river entry, indicative of tumour-associated mortality prior to river entry. A high incidence of ocular tumours have recently also been discovered in out-migrating smolts of sockeye, coho and Chinook salmon from a wide range of southern BC stocks, with levels declining significantly in the first few months in the ocean (from 40-50% to 10% June-Sept) in all three species. The salmon leukemia virus, the proposed causative agent of plasmacytoid leukemia in Chinook salmon in the late 1980's, is the best fit to the genomic and tumour evidence to date. Establishing a direct link to this virus, obtaining a viral sequence and a tool for molecular screening, and conducting disease challenge and epidemiological research is paramount if we are to establish the potential role of this virus in salmon declines and to develop mitigative measures for control.

I. High En-route losses of sockeye salmon stocks returning to spawn in the Fraser River 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. While elevated river temperatures are clearly associated with these losses, there is growing evidence that a novel disease is also impacting survivorship in river. By combining biotelemetry and genomics, the Molecular Genetics lab at PBS identified a viral-response profile 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. 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 in varying proportions of sockeye salmon in all years in which functional genomics studies have been carried out (2003, 2005, 2006). In 2005, only 26% of returning fish were negative for the "viral" signature in all tissues.

- The genomic profile from brain tissue was highly correlated with the "viral" profile in gill tissue, but also showed a strong stimulation of the sensory region of the brain (visual, olfactory), a more advanced maturation profile, and stimulation of pathways associated with tumour activity. Brain dissections (in 2009) 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 (in Sept 2009) revealed similar ocular tumours in 50-60% of adult sockeye salmon in the marine approaches and lower Fraser River, but only 20% of fish at spawning grounds (N=250), implicating the 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 revealed the highest incidence of ocular tumours thus far, with tumours in over 70% of salmon in the marine approaches and 50% in the lower river (N=63). These data imply that the tumours may also be associated with losses of adult salmon in the Strait of Georgia. Estimates put those losses as high as 0.9 million fish.
- The viral-tumour linkage in our data is suggestive of the presence of a virus that can induce cancer; viruses in the retroviral family are most commonly associated with tumours. There are additional elements in the expression signatures that are consistent with a retroviral infection, as a large number of genes associated with retroviral control of host immunity are stimulated. As retroviruses are often vertically transmitted (mother to egg), in September of 2009, we looked to the smolts to see if similar genomic signatures and tumour activity was present in this earlier life-history stage.
- In 2008, 50% of sockeye smolts in the lower river in May and the ocean in June contained the viral-tumour associated brain profile. Incidence in Cultus Lake hatchery, an assisted breeding facility for a stock in severe decline, was 30% in age-0 parr in November and 80% as smolts began their river decent (2-year olds). Incidence at Chilko, a large indicator stock for Fraser sockeye, was 50% in smolts leaving the lake. Brain dissections revealed a similar incidence level of tumours, and the presence of tumours in stocks outside of the Fraser River. These data imply that the viral infection associated with tumour activity is already active in natal rearing areas; hence, there is the potential for the virus to impact survivorship in all stages of salmon development.
- Ocular Tumours were also observed in coho, Chinook, and sockeye smolts sampled in the ocean in June and Sept/Oct of 2008 and 2009 (N=400). For each species in each year, there was a notable decline in tumour incidence from June to Sept/Oct (average 40% incidence in June, 10% in Sept/Oct). This data indicate that the tumours are associated with early ocean mortality in all three species.
- Importantly, in all three species, tumours were found in a wide range of stocks from the Fraser, Skeena, Nass, Stikine, Columbia, Central Coast, Vancouver Island, and Northern Mainland. These data imply epidemic levels of disease throughout southern BC, Washington, and parts of the North Coast.

III. The Salmon Leukemia Virus causing Plasmacytoid Leukemia—Best link to the genomic evidence, but not yet confirmed

- There is strong evidence to suggest that the genomic profiles and tumours observed in all three salmonid species are linked to Plasmacytoid Leukemia (PL) that is purported to be caused by a Salmon Leukemia Virus (SLV). This relatively unknown virus was associated in the 1980's through 1990's with mortalities in cultured Chinook salmon in fresh and saltwater in BC and potentially associated with mortalities of Coho in a BC enhancement hatchery. Moreover, challenge studies showed a high susceptibility of Chinook, sockeye and coho salmon to the virus, low susceptibility of Atlantic's, and no measurable effect on Rainbow trout. Most importantly, the virus is associated with ocular tumours in a proportion of affected fish.
- SLV causes severe anaemia (salmon farmers call it Marine anaemia), with primary infections involving the kidney and spleen. In advanced infections, proliferating plasmablasts 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 sockeye salmon with viral profiles in liver, 30% in brain, and 20% in gill tissue. Importantly, only 26% of fish were negative in all three tissues. Given that these are all secondary organs, we predict that rates of infection could exceed 90%.

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.
- 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. Decisions to screen could be based on prevalence levels in returning fish each year. Viral screening would require a DNA sequence of the virus, something we do not as yet have. Husbandry practices could also be improved to minimize horizontal transfer of the virus. There are anti-viral treatments that can be done on the eggs as well. 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 sea lice, 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. A research plan has been developed, and a team of DFO scientists from Molecular Genetics and Fish Health is ready to carry out the research pending funding.