Hypothesis: **Genomic data indicate that a potentially novel disease, possibly viral in origin, has been affecting a high proportion of juvenile and adult Fraser River sockeye salmon that may weaken fish and directly or indirectly enhance mortality of both smolts and adults**

Background:

DISCOVERY OF FATE-RELATED SIGNATURE IN ADULT SOCKEYE SALMON:

- In 2006, using a combined telemetry-genomics approach, adult sockeye salmon were biopsied and tagged in the marine approaches to the Fraser River and upon entry into the river and their migration was tracked to the spawning grounds through biotelemetry. We conducted a functional genomics study contrasting gene expression profiles between fish that made it to spawning grounds and those that went missing en route in the river, and identified in three separate experiments the same signature associated with enhanced mortality of adult salmon in the Fraser River. Approximately 50% of returning fish carried this signature in gill tissue in 2006, and were affected far as 300 km seaward of the Fraser River. Fish with this signature were termed "unhealthy".
- This signature was the most powerful physiological trajectory in the data (principle component 1)
- Salmon with the unhealthy signature in the ocean carried a 4x lower probability of reaching spawning grounds. The fate of 71% of the fish in river could be predicted based on this signature.
- A 30% reduction in survival was observed in Scotch Creek fish carrying the unhealthy signature in the lower river
- Fish carrying the unhealthy signature at the spawning grounds were twice as likely to die prematurely as those with a healthy signature.
- This study showed unequivocally that Fraser River sockeye salmon were entering the river in a compromised state, that survivorship was somewhat predictable based on gene expression >200 km before salmon reach the river, that stocks may be affected differently, and that the freshwater environment alone may not be sole source of the highly fluctuating mortalities of salmon in the river.

SAME SIGNATURE FOUND IN MULTIPLE TISSUES IN SMOLTS AND ADULTS:

- We have since observed this same powerful signature in brain, liver, and gill tissue (but not muscle) of sockeye adult salmon in all years over the past decade where samples were available (2003, 2005, 2006, 2007, 2008, 2009), with the proportion of affected fish varying in different years.
- We have also observed the signature in smolts in all years where samples were available (2007, 2008, 2009). A high proportion of smolts carry the signature before they leave natal rearing areas.
- In 2008, 60% of smolts left the Fraser River with the unhealthy signature in brain, 40% in liver, with 82% of fish affected in at least one tissue. There was a 30% reduction in brain prevalence of unhealthy signature fish from summer to fall in the ocean, and a 50% reduction in liver. Overall, there were 2.4 times as many healthy fish (both tissues) in the fall as in the summer (43% versus 18%).
- If these decreases in prevalence were due to mortality, and if we assume that 120 million smolts left the river in 2008 (there may have been more), we could account for the loss of >27 million salmon in 2008 associated with the unhealthy signature alone

- Prevalence of unhealthy livers in 2007 Fraser River sockeye smolts sampled in late June in the ocean was >90%. 2007 smolts also showed signs of hypoxia (lack of oxygen) and poor feeding relative to 2008 smolts.
- These data imply that Fraser River sockeye salmon are entering the ocean as smolts and the river as adults in an **already compromised condition**, suggesting that environmental conditions *alone* may not account for the high fluctuating mortalities observed.

POTENTIAL EFFECTS ON BEHAVIOUR:

- The unhealthy signature in brain is strongly associated with diversion of adults through Johnstone Strait (data from 2003 and 2005). It may also be associated with early entry of late-run sockeye salmon.
- The unhealthy signature in gill is associated with earlier river entry of Late-run sockeye salmon and faster travel speeds in the river to spawning grounds

FUNCTIONAL ANALYSIS POINTS TO INVOLVEMENT OF AN INTRACELLULAR PATHOGEN, LIKELY A VIRUS (this remains a *HYPOTHESIS* at this time, as we do not yet have a viral sequence or confirmed pathogen):

- 65% of the affected biological processes associated with unhealthy signature fish consistent with viral activity from other studies. Numerous key genes within these processes that are specifically targeted by viruses or in host response to viruses were differentially regulated.
- Stimulation of JAK-STAT, Interferons, and Th1 cellular immune response are all indications of a response to an intracellular pathogen, most notably viruses.
- Strong up-regulation of STAT1, MX, IFN, PRF1, TCRa, TAP2, MHC1 classically associated with viral activity
- Numerous genes involved in viral recognition, reception, entry, replication, integration, transcription, transport, encapsidation, and release were among those significant in the unhealthy signature fish.
- Some commonalities with IHN virus response in gill—Humoral immunity down, IFN induced JAK-STAT up
- Escalating immune response: Immunosuppressive/early recognition in SW to inflammatory/apoptotic in FW consistent with recent gill exposure to pathogen in SW escalating in pathogenicity in FW.
- The level of physiological response to this purported virus is very strong relative to the other stressors salmon are experiencing over migration, and relative to what we have observed with IHNV, something that would not be expected if it were innocuous. Challenge work with IHNV has shown that salmon that are more susceptible to succumbing to disease from this virus respond much more vigorously than those that are more resistant.

ADDITIONAL EVIDENCE OF POTENTIAL PATHOGEN INVOLVEMENT

- A VIRAL PATHOGEN?: In collaboration with BC Centre for Disease Control, we ran both healthy and unhealthy tissue RNA on a Viral Array (used to identify viral strains in humans and agricultural animals), and found the unhealthy tissue gave 6x higher intensity binding to the array than healthy tissue. There was a 3-fold over-representation of Retroviral family DNA.
- INFECTIVITY: We conducted a temperature holding study (14 and 19 degrees) of Chilko and Adams adult salmon returning to the Fraser River to spawn. Fish were sampled

before the experiment, as they became moribund, and at the end of 1 week holding and used these in a microarray study to assess response to temperature stress. Initial pre-holding sampling indicated 35% of fish were unhealthy. 69% were unhealthy after 1 week of holding. All mortalities were unhealthy. All unhealthy Adams fish died. Unhealthy Chilko survivors were mostly from the lower temperature holding group. This study indicates that healthy fish can become unhealthy when cohabitated in close quarters with unhealthy fish. Note, however, that as it was not designed to specifically assess infectivity, there was no negative control (i.e. unhealthy only fish held together).

ADDITIONAL POTENTIAL MECHANISMS LEADING TO POOR SURVIVAL

- Adult salmon carrying the unhealthy signature in brain and liver do not respond as strongly to changes in their environment as do healthy fish, which may impact their ability to adapt.
- Associated effects on osmoregulation in gill may affect tolerance to SW, which may be one mechanism to push adult salmon quickly into the river.
- Gas exchange may be negatively impacted when the unhealthy signature is present in gill
- The unhealthy profile in brain may affect the maturation process; late-stage maturation genes are turned on far too early (in QCI), but fish don't actually appear to be mature.

SIGNATURE NOT CONSISTENT WITH GENERAL STRESS RESPONSE, SEA LICE INFECTION, OR PARVICAPSULA INFECTION

- Affected Tissues:
    - No muscle involvement—as expected for sea lice
    - Strong involvement of the brain—not expected for sea lice
    - Variable nature of tissue involvement within fish and high conservation of signature between tissues not consistent with general stress
- Where fish are affected:
    - Smolts affected before leaving natal sites—sea lice marine, *Parvicapsula* picked up in lower river
    - Adults affected before entering FW, with escalation in FW gills—*Parvicapsula* picked up in lower river, sea lice fall off in FW
    - Would not expect such a consistent signature associated with "stress" over diverse environments, multiple tissues and multiple life-history stages
- Signature notably intracellular, not extracellular
    - Intracellular immune responses to intracellular pathogens—like viruses or intracellular parasites
    - Extracellular (humoral) immune response common for bacteria and extracellular parasites—e.g. *Parvicapsula* and sea lice
- We have already identified a *Parvicapsula* signature
    - Response to wounding, inflammatory response, stress response, cytokine production, blood coagulation, and homeostasis all up-regulated with *Parvicapsula*, not the unhealthy signature
- Most specific stress-related pathways (e.g. oxidative, temperature, chemical, toxin-responsive) are down-regulated in the unhealthy signature fish. The only specific stress-related response in the signature is a DNA damage response. This can be affected by a wide range of external and internal factors (including viral infections) and alone, this does not tell us much about the stressor.

NOTE: **Molecular screening for known viruses and intracellular parasites has not yielded a positive identity. Hence, the unhealthy signature does not appear to be the result of a known or well characterized pathogen. We are working to obtain a sequence of the *purported* pathogen. Until then, the involvement of a pathogen, and specifically a virus, is *speculative*, and awaits confirmation. Even so, the genomic data do show that the condition of a large proportion of Fraser River sockeye salmon may be compromised *before* they enter the ocean as smolts and the river as adults, and that perhaps we should not be looking solely at the environment for solutions to the declines. We hypothesize that the interaction between the compromised condition of salmon entering an increasingly poor and stressful environment is likely to be key to the declines.**

Four Questions

1. **Explanatory power**
   a) Our data provide a plausible mechanism to account for significant levels of mortality of smolts and adults in the ocean
   b) Potential impact on behaviour and survival throughout salmon lifecycle
   c) High but variable prevalence in adults since 2003
   d) Very high prevalence in 2005 returning adults (75% of returning adults affected in at least one tissue) and 2007 smolts (>90% livers affected—note small sample size available)
   e) Present also in Chinook and coho stocks that are in decline (smolts)
   f) In smolts in 2008, while 40% of livers and 60% of brains of fish sampled in the SOG in June carried the unhealthy signature, 82% of smolts carried the signature in at least one of these tissues, with 24% affected in both tissues. We observed a 1.45-fold reduction in the overall prevalence of the unhealthy signature in the ocean from June to Sept/Oct in 2008, and a 2-fold reduction in fish with signatures in both tissues. These data are consistent with disease-related mortality in the ocean (as the most strongly affected fish "disappeared" at a greater rate). This was a year whereby ocean conditions were perceived to be optimal; we expect losses could be higher in suboptimal years. If no more disease-related mortality or expansion of infection occurred during ocean residence, we would expect that returning adults would carry an infection rate of 50-60% upon their return. In 2005, if we limit our analysis to only these two tissues, prevalence rates of returning fish was 53%.
   g) A viral disease has a strong potential to impact survival of the magnitude observed, but we need long-term dataset to assess associations with long term trends and more research on the purported pathogen

2. **Direct or Indirect evidence?**
   a) We do not have a long-term dataset for this hypothesis, and our experiments have not encompassed the same tissues in all years, meaning that a full assessment of prevalence among years is not yet available.
   b) Viruses do not always directly cause mortality, but rather weaken fish and make them more vulnerable to other stressors or pathogenic insults. We do not know at this point whether the mortality we associate with the unhealthy signature is direct or indirect, and have not confirmed that a virus is responsible, but it is clear, at least in the gill, that the virulence of this signature increases over migration of adults in the river. If viral involvement is confirmed, it is highly likely that the interaction between prevalence and intensity of viral-infection and levels of environmentally induced stress could provide the

most explanatory power in terms of survivorship. There are other disease studies that have been carried out in Oregon and Washington that point to a disease-environmental interaction in the ocean. Both prevalence and intensity of infection were noted to be important indicators in these studies (that focussed on parasites and BKD infections in Chinook salmon).

3. **What specific research needs to be done to change the degree of belief in the hypothesis that you have been asked to address?**
   a) Identify an infectious agent associated with the unhealthy signature
   b) Develop a molecular marker and expand screening
   c) Establish whether prevalence has shifted over time during the decline, whether there is a correlation with recruitment/escapement, and impact of environmental conditions
   d) Challenge studies to determine whether the pathogen (if obtained) causes mortality directly or simply weakens fish and how it is transmitted
   e) Confirm behavioural impact of pathogen-infected fish in more years/samples
   f) Establish whether stocks that are doing well (e.g. Harrison) are also affected and prevalence in sockeye outside the Fraser River
   g) Establish effects on other salmonid species, in hatcheries versus wild fish, and in Atlantic salmon
   h) Identify potential mitigation measures to control infection-levels or viral-mediated mortality in the wild

4. **Can any management actions reduce the effect on Fraser sockeye salmon of the hypothesized mechanisms that you have been asked to address?**
   If viral pathogen, or a pathogen of any kind is confirmed,
   a) the main potential for mitigation would be in the hatcheries
      - Difficult to mitigate directly on sockeye salmon, but if hatcheries are an important reservoir in Chinook/coho, rapid molecular-based broodstock screening could be used to minimize vertical transmission
      - Improved husbandry to minimize horizontal transmission
      - requires information on viral transmission
   b) Potential for Enhanced Forecasting
      - Molecular screening to establish levels of infection smolts/adults
      - Modelling to establish relationship with year-class strength/escapement and interaction with other variables
   c) Not likely an effect of salmon aquaculture due to presence before sockeye smolts leave the river, but there could be linkages with the expansion of hatchery production. This is only *speculation* and requires *research* to assess.

   If the signature is the result of something other than a pathogen
   a) Still the potential for enhanced forecasting and modelling
   b) Mitigation would depend upon the mechanism