Hypothesis:

Genomic studies suggest that some disease has infected sockeye and has become an important contributor to the Fraser River sockeye situation

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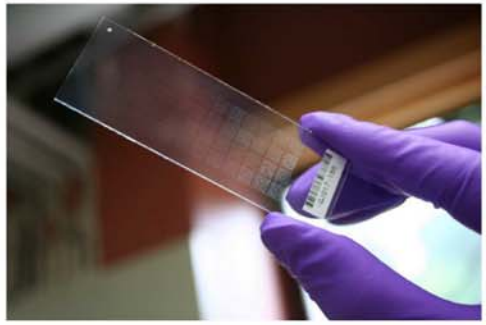
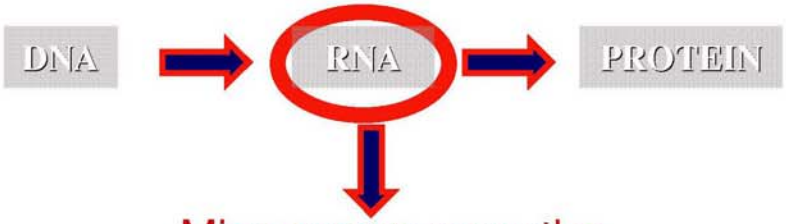
Functional Genomics: Assess Salmon Condition and Predict Fitness

Genome-wide scan of physiology





Microarrays scan the activity of 10's of 1000's of genes at once

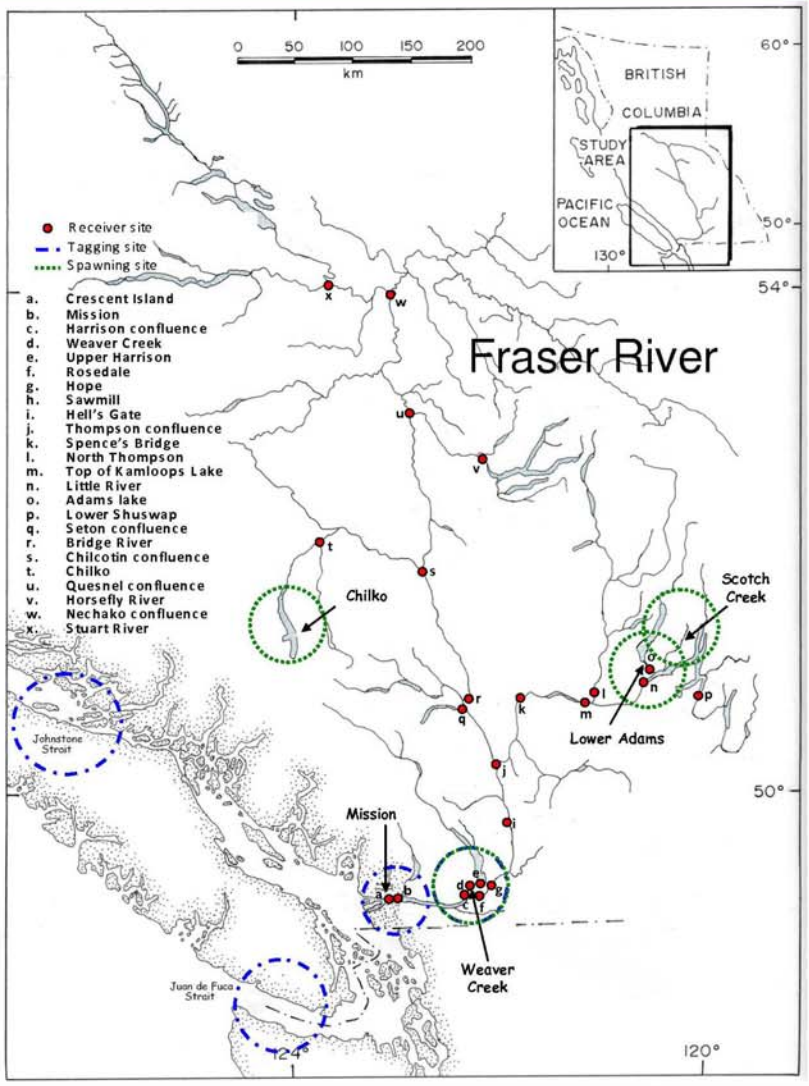
Identify the proportion of fish that are:

- Food deprived/starving
- Consuming poor quality prey (low lipid content)
- Under stress—temperature, oxidative, hypoxia, disease, toxins, osmotic
- Growing fast or growing slow
- Active or inactive
- Ready or not ready for salinity shift
- Mature or immature

Identify genes/pathways or conditional states associated with:

- Migration timing and speed
- Poor survival

Independent tagging experiments in SW, Lower River, and Spawning grounds show linkage of same genomic signature with elevated mortality (en route and pre-spawning)



2006:
3 separate Telemetry studies

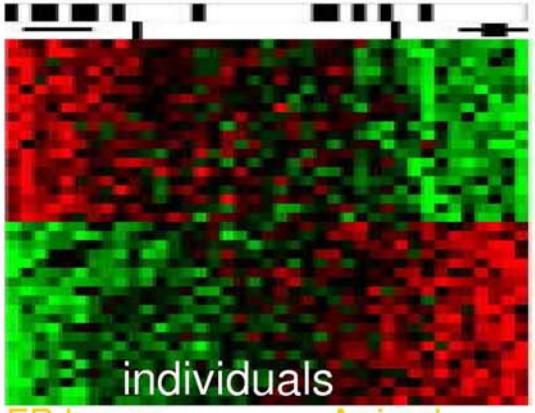
SW—JS/JDFS
FW—Lower River
FW—Spawning Grounds

Salmon Tagged

Non-destructive Gill Biopsy

Independent tagging experiments in SW, Lower River, and Spawning grounds show linkage of same genomic signature with elevated mortality (en route and pre-spawning)

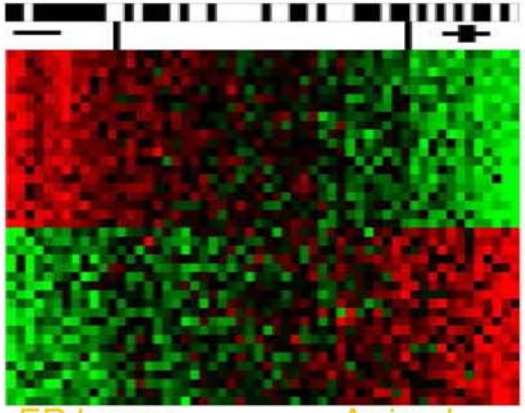
SW Tagging



13.5x lower probability

SVM=10 genes can predict outcome for 71% of fish

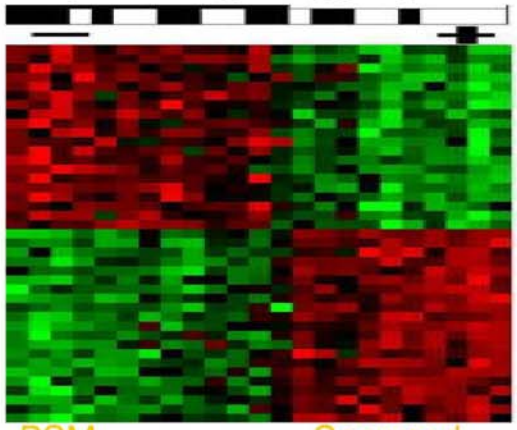
Lower River Tagging



10x lower probability

SVM=10 genes can predict outcome for 65% of fish

Spawning Tagging



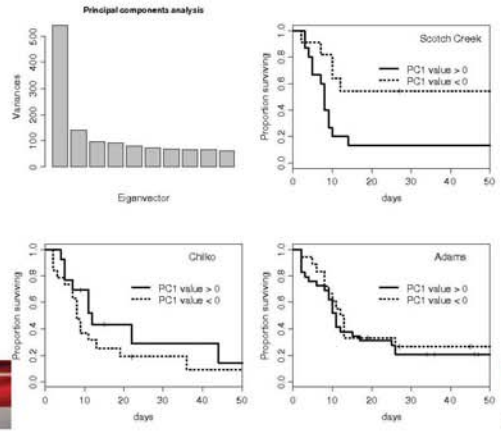
3.7x lower probability

Profile associated with High mortality termed "Unhealthy"

Impact on survival varies among stocks

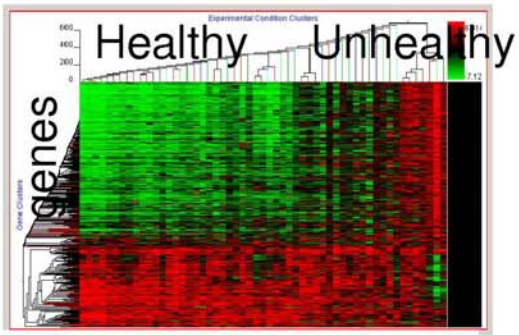
Red up-regulated

Green down-regulated

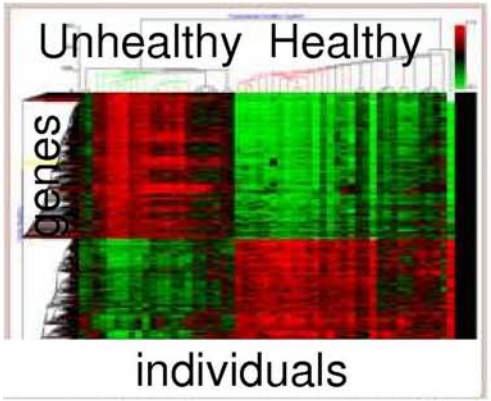


Healthy/Unhealthy profile present in at least three tissues (liver, brain, gill), with high overall prevalence rates

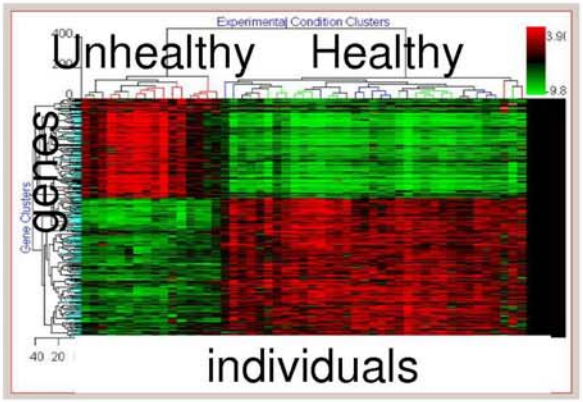
Profiles in Adult salmon returning to the Fraser River in 2005



Gill: 13% prevalence
Early Stage



Liver: 40% prevalence
Advanced Stage



Brain: 30% prevalence
Advanced Stage

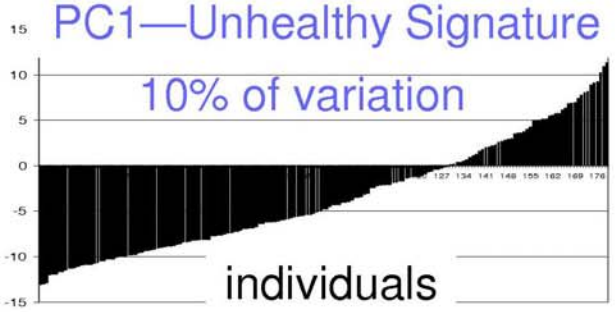
NOTE: A fish can be "Unhealthy" in one tissue and not in another

Overall (three tissues) prevalence in 2005 was 75% unhealthy fish

Red up-regulated Green down-regulated

The same powerful Unhealthy genomic signature present in smolts before they leave FW, with prevalence decreasing during ocean residence

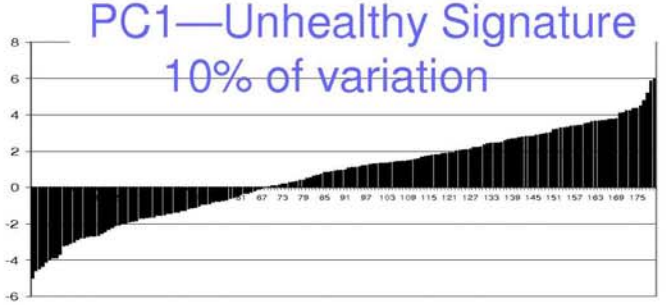
Smolt Brain 2008

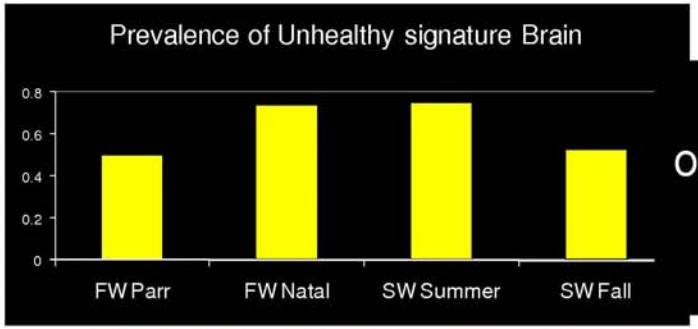


Healthy

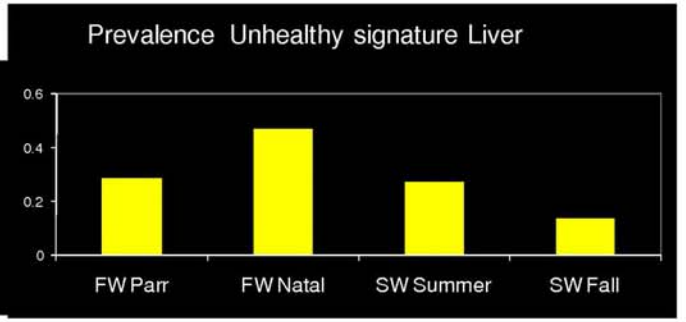
Unhealthy

Smolt Liver 2008





Smolts show signs of unhealthy signature well before they leave the river



30% reduction in prevalence Summer-Fall

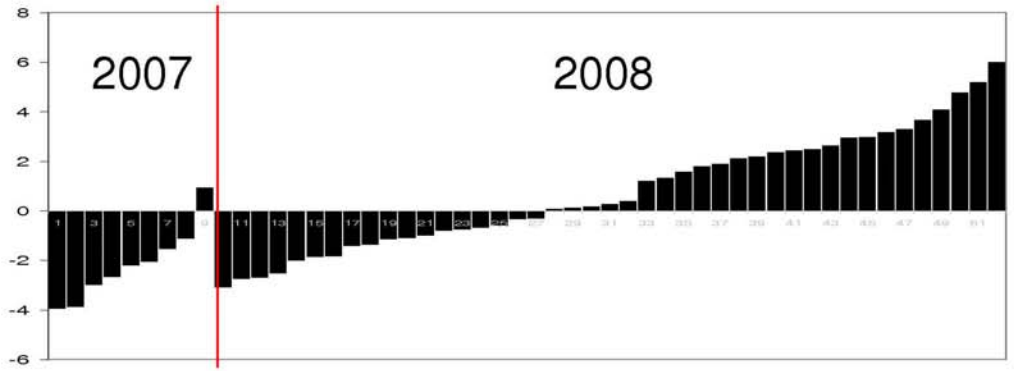
50% reduction in prevalence Summer-Fall

Assuming 120 million smolts left the river

If mortality occurred only on the Unhealthy fish

Disappearance of 27 million fish in first 3 months in the ocean associated with this signature alone

Contrast 2007 and 2008 sockeye smolts sampled in June
Liver tissue



Healthy

Unhealthy

90% prevalence of Unhealthy fish late June in the ocean in 2007

40% prevalence of Unhealthy fish late June in the ocean in 2008

Other indications that 2007 fish were in more compromised physiological condition than 2008 fish

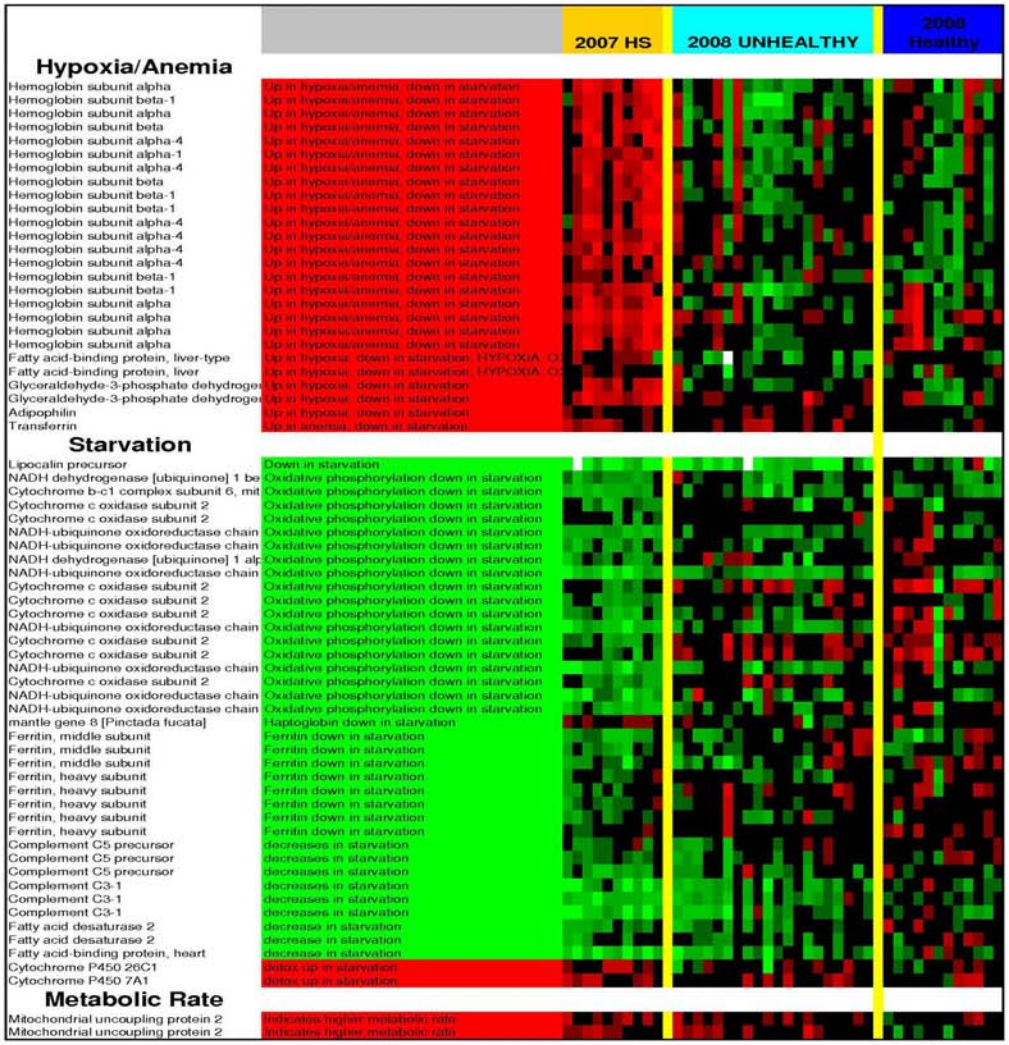
2007 smolts in Hecate Strait all carry indications of:

Hypoxic
Induced by harmful algal blooms?

Starvation/reduced feeding
Poor ocean feeding conditions?

Higher metabolic rate
Less efficient energy conversion

This is not a good formula for survival!



Cumulative Evidence for a Pathogen, and specifically A Viral Pathogen, responsible for eliciting the Unhealthy signature

1. Escalating immune response from SW to FW in gill

Immunosuppressive/early recognition in SW to inflammatory/apoptotic in FW consistent with recent gill exposure to pathogen in SW

2. Functional signature

65% of affected biological processes consistent with viral activity

>260 differentially regulated genes linked to viruses, some anti-viral, others pro-viral

STAT1, MX, IFN, PRF1, TCRα, TAP2, MHCI classically assoc with viral infections

Some commonalities with IHN response in gill—humoral down, IFN induced JAK-STAT up

The anti-viral nature of the signature is strengthened in genes overlapping among tissues

3. Viral Array

"Unhealthy" tissue gave 6x higher intensity binding than "healthy" tissue

3-fold over-representation of Retroviral family oligos—consistent with leukemia links

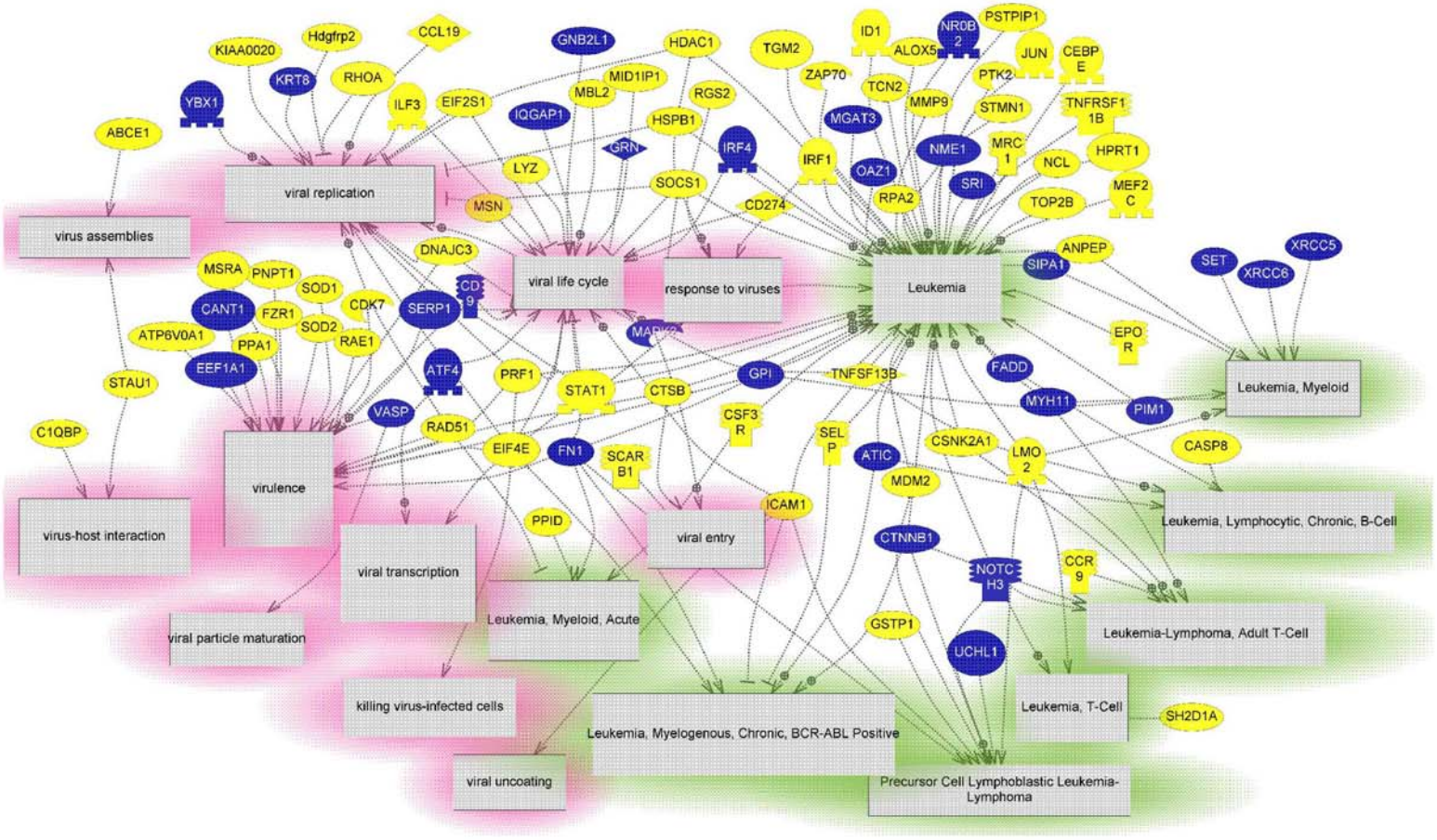
4. Temperature Holding Study--infectivity

Initial sampling indicated 35% of fish "unhealthy", after 1 week of holding, 69% unhealthy

All mortalities were unhealthy, no surviving Adams fish unhealthy, unhealthy Chilko survivors mostly from lower temperature holding

Striking linkages of genes with Leukemia

Linkages of Gill FW Genes (p<0.001) with Leukemia, Viruses and Pathogen Virulence



Four Questions

1. Explanatory power of Novel Disease Hypothesis

Appears to impact behaviour and survival throughout salmon lifecycle

- High but variable prevalence since 2003
- Very high prevalence in 2005 adult brood and 2007 smolts
- Present also in Chinook and coho stocks that are in decline
- Have observed in stocks outside the Fraser, but low sample sizes

A viral disease has a strong potential to impact survival of the magnitude observed, but need long-term dataset to assess associations with long term trends and more research on the purported pathogen

2. Direct of Indirect evidence

Insufficient data to assess relationship with indicators of mortality over multiple years

- Viral pathogens can kill fish directly, but more often weaken fish and enhance their susceptibility to other sources of mortality
- Expect an interaction with environmental conditions

Four Questions

3. Specific Research required to establish a viral role

Identify an infectious agent associated with the Viral signature

Develop a molecular marker and expand screening

Establish whether prevalence has shifted over time during the decline, whether there is a correlation with recruitment/escapement, and impact of environmental conditions

Challenge studies to determine whether the virus causes mortality directly or simply weakens fish and how it is transmitted

Confirm behavioural impact of virally-infected fish in more years/samples

Establish whether stocks that are doing well (e.g. Harrison) are also affected and prevalence in sockeye outside the Fraser River

Establish effects on other salmonid species, in hatcheries versus wild fish, and in Atlantic salmon

Identify potential mitigation measures to control infection-levels or viral-mediated mortality in the wild

Four Questions

4. Management Actions

Potential for Enhanced Forecasting

- Molecular screening to establish levels of infection smolts/adults
- Develop models that integrate disease prevalence data with environmental variables

Potential for Mitigation

- Additional pathogen for molecular-based brood stock screening of hatchery fish (coho, Chinook) to minimize vertical and horizontal transmission

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

Additional Information on the Unhealthy "Viral" signature

Chinook salmon from the Columbia River are positive for the viral signature

Viral signature affects more genes and biological processes than any other Measured variable -- stronger than shifts associated with salinity transfer, maturation, smoltification, thermal stress, IHN response, etc…

Adult salmon that carry the viral signature in liver or brain tissue do not respond as strongly to changes to their external environment

As the Viral signature is present before smolts leave FW natal sites, there is little likelihood aquaculture facilities are important in transfer to wild stocks—we have no information on this signature in Atlantic salmon

Prevalence rates are high enough to exert a very strong effect on populations

It appears that some stocks may be more resistant than others to adverse effects

Molecular screening for known viruses and intracellular parasites has not yielded a positive identity, but employment of a viral array pointed to a retroviral family virus, consistent with our leukemia-like signature

Evidence that this is not simply *Parvicapsula* or sea lice or a simple stress response

1. Affected Tissues

No muscle tissue involvement (expected for sea lice)

Strong involvement of brain (not expected for sea lice)

Variable nature of tissue involvement and high conservation of signature between tissues not consistent with a general stress response

2. 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 (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

3. Signature is 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

The only consistent stress indicator in signature is DNA damage response

3. 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 viral-signature