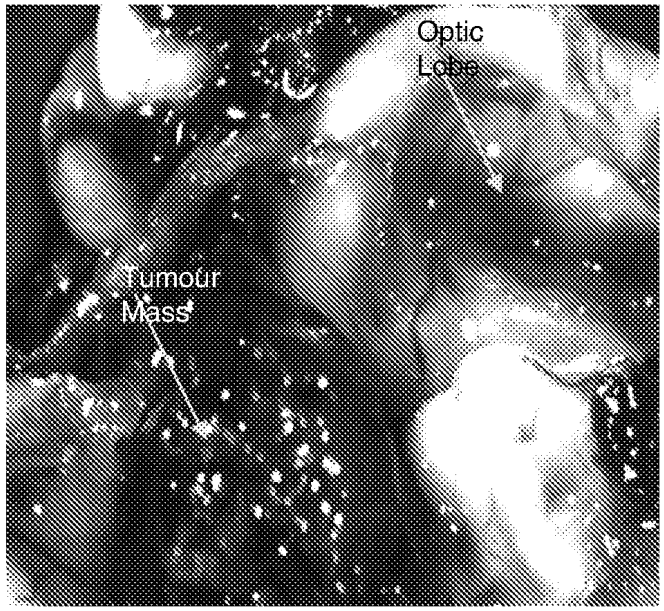
Epidemic of a novel, cancer-causing viral disease may be associated with wild salmon declines in BC



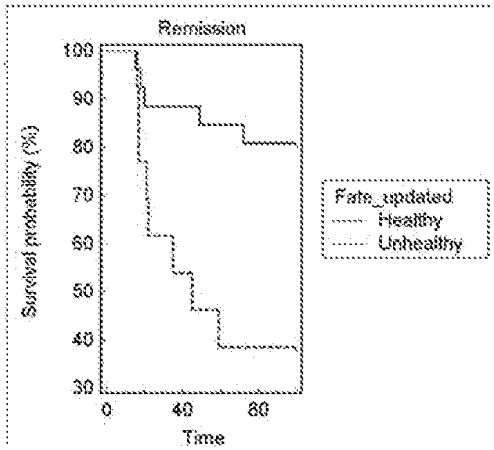
Kristi Miller
Molecular Genetics Lab
Karia Kaukinen, Shaorong Li, Tobi Ming, Norma Ginther, Angela Schulze

David Patterson, Jayme Hillis, and others….

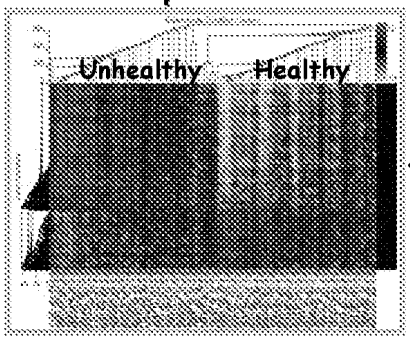
- Genomic Evidence
- Tumour Evidence
- Adults
- Smolts
- Sockeye, Coho and Chinook salmon

Sept 27, 2008

Genomics Research uncovers a viral-induced signature correlated with adult sockeye salmon mortalities in the river



Physiology in saltwater predictive of Fate In River



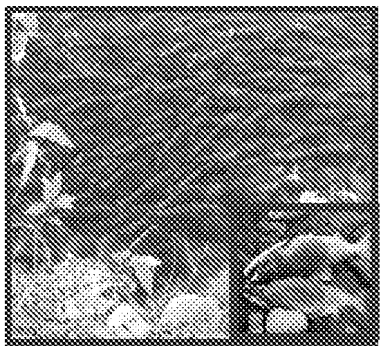
1881 genes

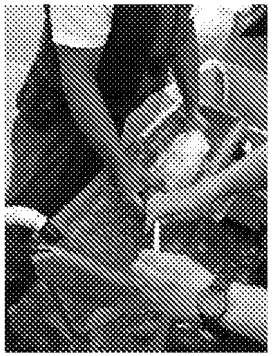
"Unhealthy Signature"
Induction of defence response
antigen presentation
membrane
Intracellular Pathogen Response
complement cascade
Stress Response
Post-translational modification
Ubiquitin-dep. Proteolysis
Cell to cell signalling
Neurological systems processes
TCA cycle
Inflammatory response/apoptosis (SW-FW)
*Osmoregulation—FW shift

Viruses evade host, aid replication

"Healthy Signature"
Protein Biosynthesis
Oxidative Phosphorylation

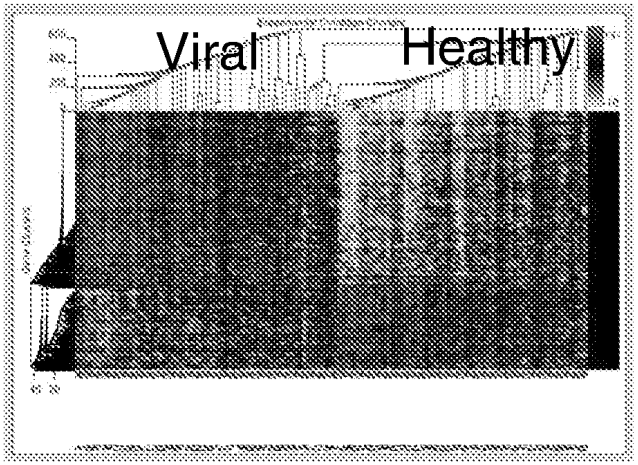
"Unhealthy" Signature
Is a Viral signature



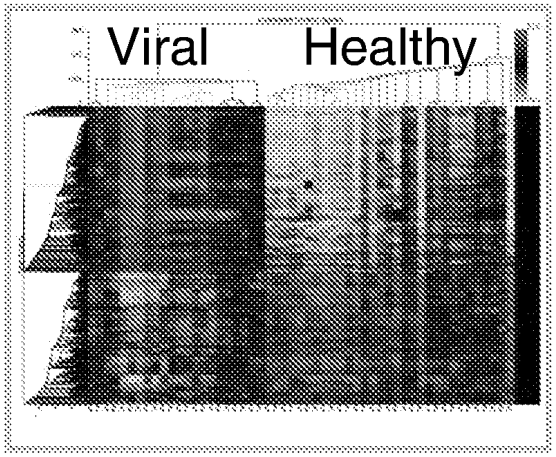


"Unhealthy" sockeye entered the Fraser River faster and suffered 9-16x higher mortality en route to spawning grounds than healthy fish
At spawning grounds, only 18% of "unhealthy" fish spawned (Weaver)

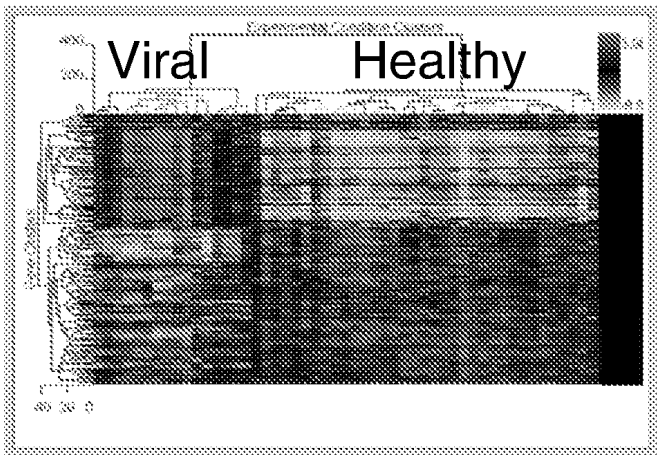
Viral Signature Observed in Multiple Tissues



Gill 2006: 60%



Liver 2005: 40%

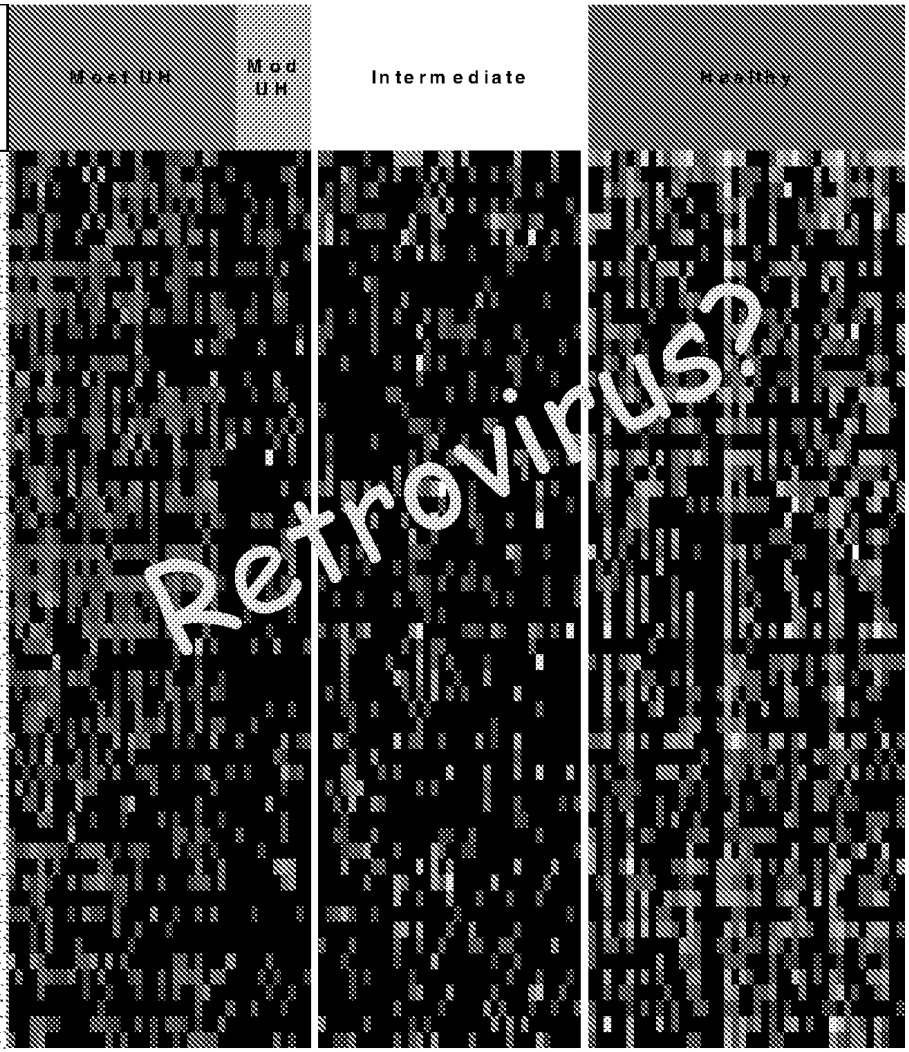


Brain 2005: 30%

- Viral profiles highly correlated among tissues, but there are some notable differences
  - Gill profiles congruent with early stage infection enhanced from SW-FW
  - Liver profiles are already at an advanced infection stage in SW – no sig Change SW-FW
  - Brain profiles at an advanced infection stage in SW, but also contain stimulation of the sensory region of the brain (visual, olfaction, taste), enhanced maturation signals, and tumour-associated signalling

“Viral” signature: stimulation of genes associated with retroviral infections in mammals

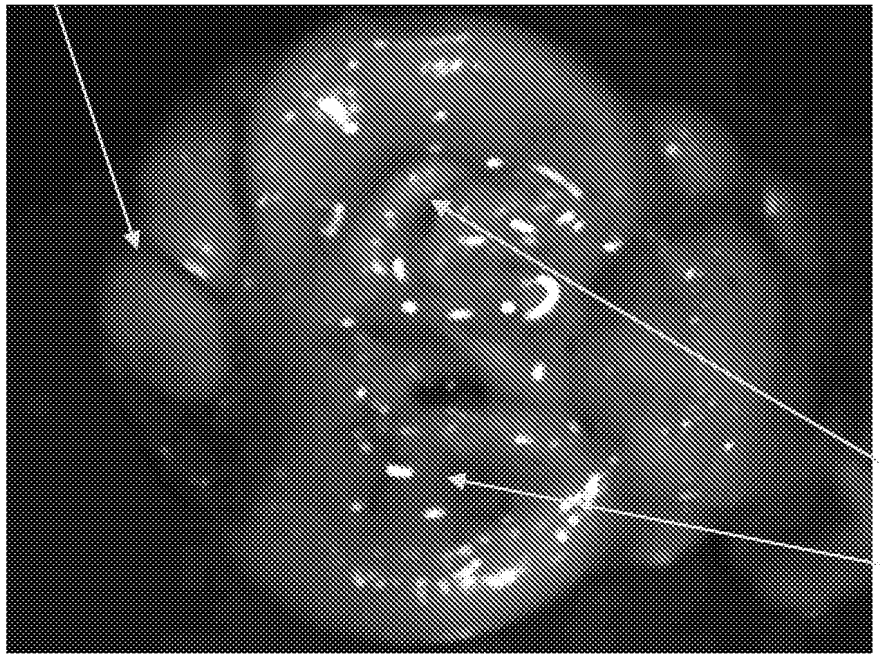
Gene ID	Viral Relationships	Functional Role	FOLD CHANGE
PCSK5	Viral--viral assem	Anti-viral state	3.28
STAT1	Viral--anti-viral sta	Anti-viral state	1.76
CREBZF	virus--neg reg tran	transcription (virus -)	1.87
CREBZF	virus--neg reg tran	transcription (virus -)	1.57
IFI44	virus--anti-viral sta	transcription (virus -)	1.7
GTF2B	viral--host-virus in	transcription (host +)	2
GTF2B	viral--host-virus in	transcription (host +)	0.99
Cd209e	Viral: Retrovirus;	Viral Entry	2.59
	viral--host-virus in	viral induced stress	1.23
	viral--retrovirus	Endogenous Retrovirus	2.05
MAK3	Viral--L-A virus GA	viral reproduction (+)	1.31
HNRPA3	Viral--retrovirus; le	mRNA splcing (viral)	1.54
Hnrpa1	Viral--retrovirus; le	mRNA splcing (viral)	-1.4
SFPQ	Viral--retrovirus; P	mRNA splcing (viral)	1.31
Eif4g2	Viral--cleaved by s	Translation (host -)	-1.8
ABCE1	Viral--retrovirus; in	Translation (host -)	2.68
Eef1d	Viral--retrovirus; hi	Translation (host -)	1.5
EIF4G1	Viral--cleaved by p	Translation (host -)	2.52
Eif2b3	Viral--response to	Translation (viral -)	-1.2
SKIV2L	Viral--Antivirus He	Translation (viral -)	2.18
Sars	Viral--retrovirus; ti	Translation (viral +)	1.65
IARS2	Viral--retrovirus; ti	Translation (viral +)	2.54
WARS	Viral--retrovirus; ti	Translation (viral +)	2.32
HARS	Viral--retrovirus; ti	Translation (viral +)	1.16
DARS	Viral--retrovirus; ti	Translation (viral +)	1.08
PABPC4	Viral--retrovirus; P	Translation (viral +)	2.17
Pvrl3	virus--pollovirus re	Receptor	1.6
HYAL2	Viral--retrovirus re	Receptor	2.37
RRAGA	virus--adenovirus	anti-apoptosis (viral mediated)	1.68
sumo3b	Viral?: somulation	Lytic Activity (viral mediated)?	2.02
BAT1	Viral--retrovirus; s	Transport (viral)	2.29
Ddx5	Viral--retrovirus; D	Transport (RNA)	-1.2
EIF5	Viral--retrovirus; EI	Transport (RNA)	1.37
STAU1	Viral--retrovirus; D	Transport (RNA)	1.77
DDX23	Viral--retrovirus; D	Transport (RNA)	1.91
	Viral--retrovirus; A	Transport (RNA)	1.65
	Viral--retrovirus; A	Transport (RNA)	1.43
NCL	Viral--retrovirus; R	Transport (RNA)	2.02
	Viral--viral reprod	Viral replication	-1.3
Mgll	Viral--hMGL, like	Viral replication	1.56
Ppia	Viral--viral reprod	Viral replication (+)	-1.2
Ppia	Viral--viral reprod	Viral replication (+)	-1.3
TOP2A	viral--retroviral ge	Viral replication (+)	1.16
ef1a	Viral--retrovirus--E	Viral encapsidation	-1.2
eef1ao	Viral--retrovirus--E	Viral encapsidation	2.05
SGTA	Viral--retrovirus; V	Virion release	1.61
SGTA	Viral--retrovirus; V	Virion release	1.37
FLI1	Viral--retrovirus; le	Integration	1.88
banf1	viral-retrovirus	Integration	1.38
ATP6V0C	Viral--host-virus in	Anti-cancer	1.84
ATP6V0C	Viral--host-virus in	Anti-cancer	1.06
ATP6V0C	Viral--host-virus in	Anti-cancer	-1.4
IL13RA2	pro-inflam matory	Cancer marker (brain)	2.11
KRT18	Viral--host-virus in	pro-cancer	1.35
KRT18	Viral--host-virus in	pro-cancer	-1
RALB	Viral--retrovirus; V	lam mation/cancer (viral mediate	2.11
F10	Viral--coagulation	Coagulation (viral mediated)	1.23



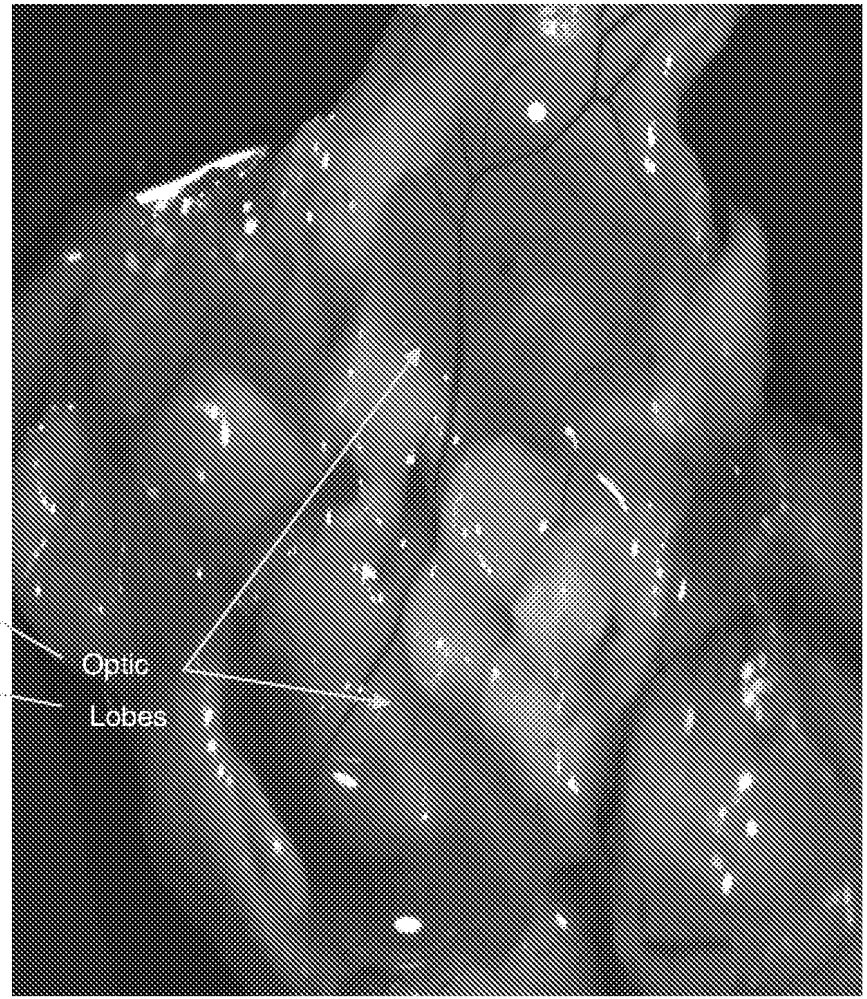
Retroviruses are often neoplastic (tumour-causing) and have been associated with wide range of cancers

Healthy Brain—No Tumours

Cerebrum
and Olfactory
Lobe



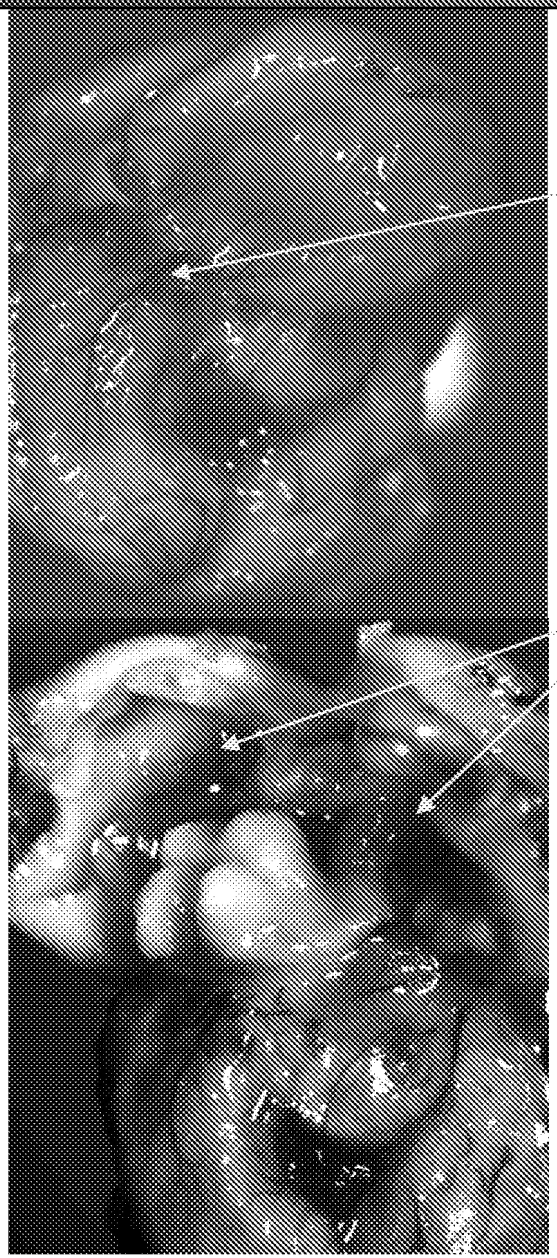
External View
Clear and white,
No heavy vascularization
or bleeding



Internal View Optic Lobe
clear and white

Onne_ad_2009_tumour_3239

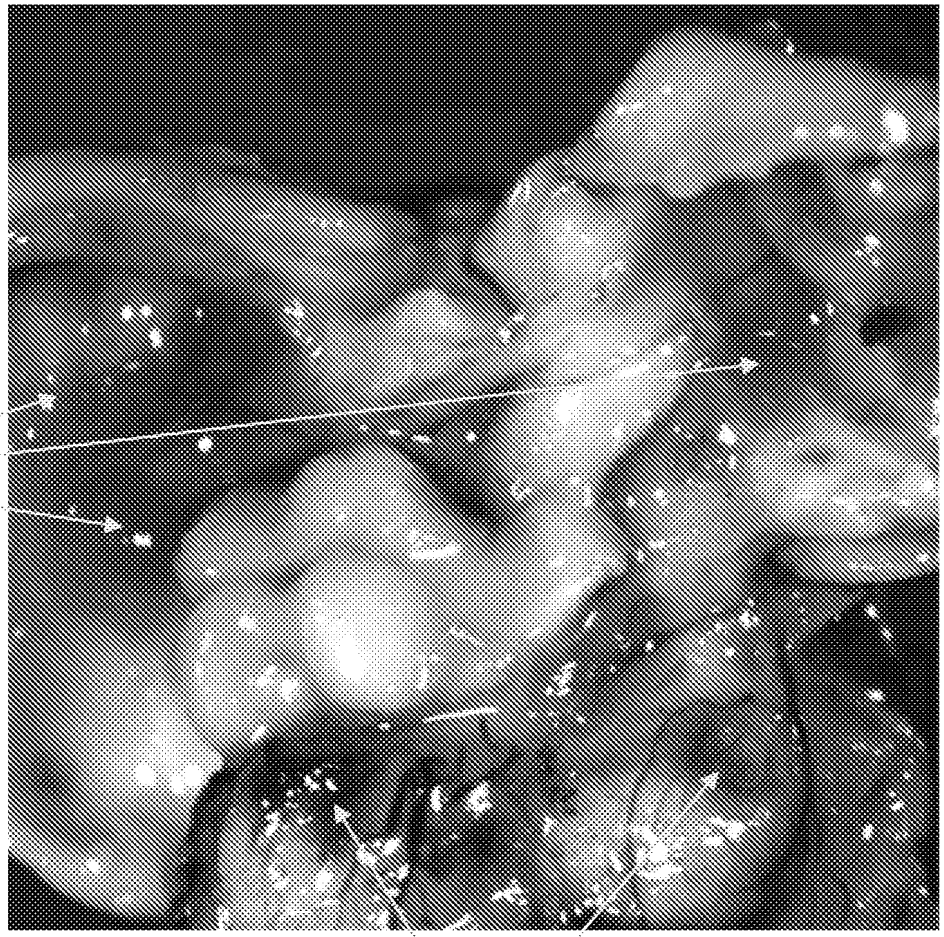
Stuart Fish (Middle River) Collected at Gill Road (Area29)



Right Optic Lobe: Outside and inside view

Vascularization outside optic lobe

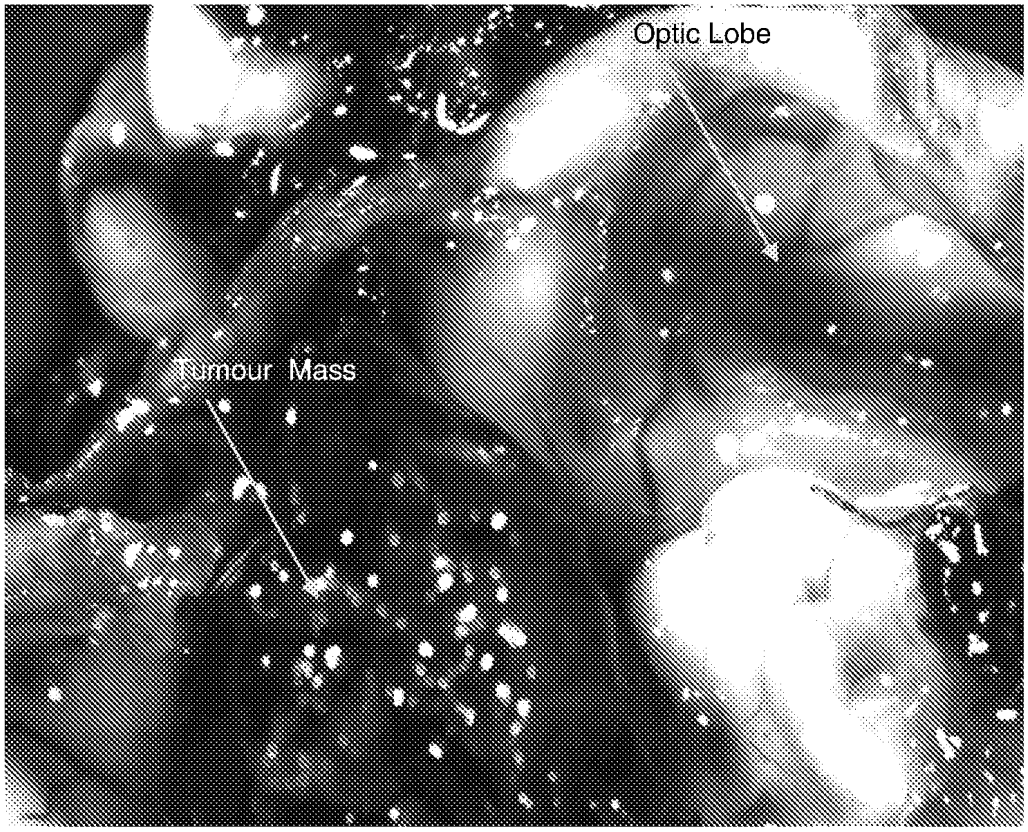
Pink Tumour Masses within the Optic Lobes



Bloody, necrotic appearance on outside of cerebellum

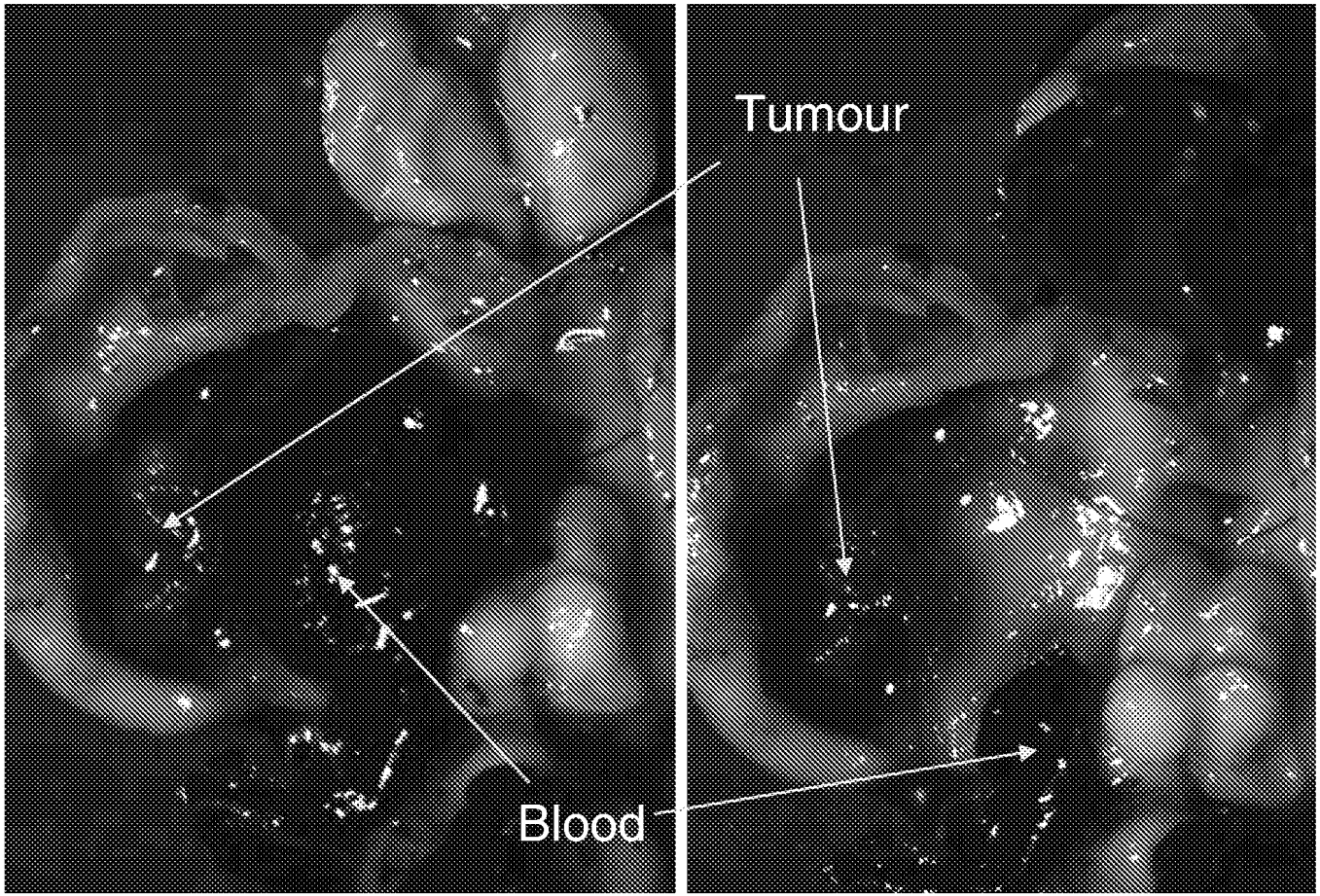
CAN185457_0006

Large dark attached tumour mass

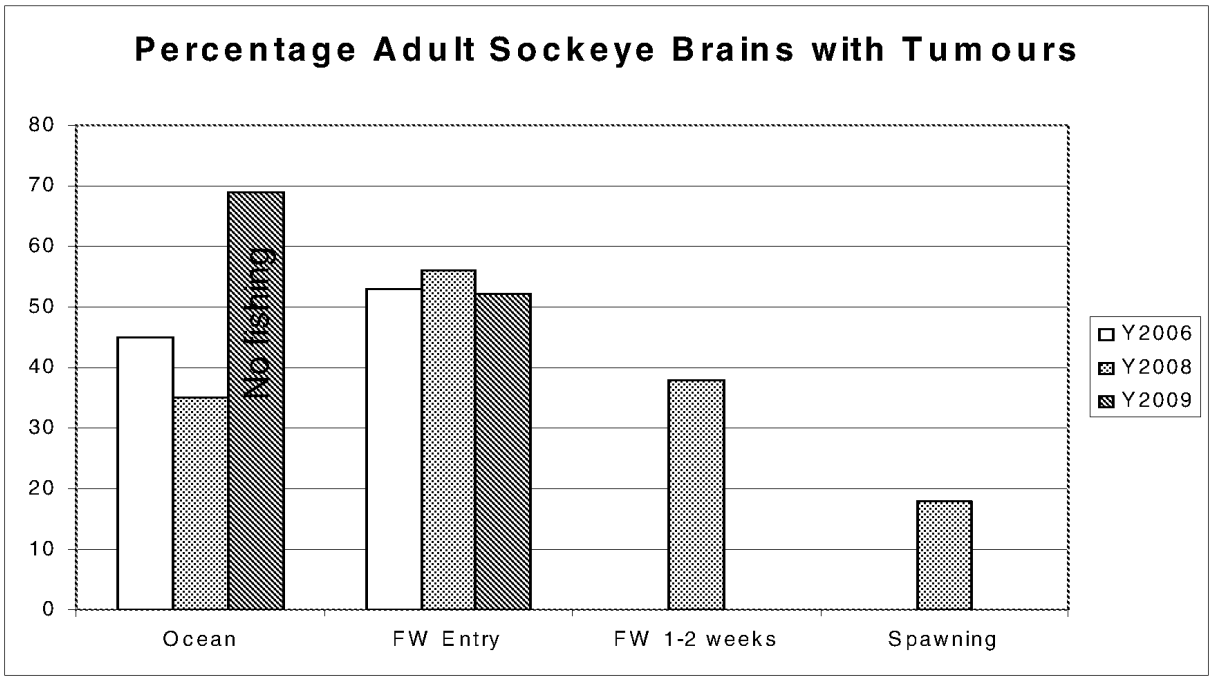


3201 Close up: Inside and outside of the brain (note that inside the optic lobe with mass flipped out there remains pink tissue attached and vascularised)

Optic lobe is has large tumour mass and is hemorrhagic (tumours are attached, blood is a different consistency)



Tumour-Associated in-River Mortality of Adult Sockeye Salmon



2008 unprecedented levels of mortality of a wide range of stocks in the river

Declines in Tumours in FW in 2008 indicate enhanced river mortality (N=250)

2009 sockeye salmon did not show up

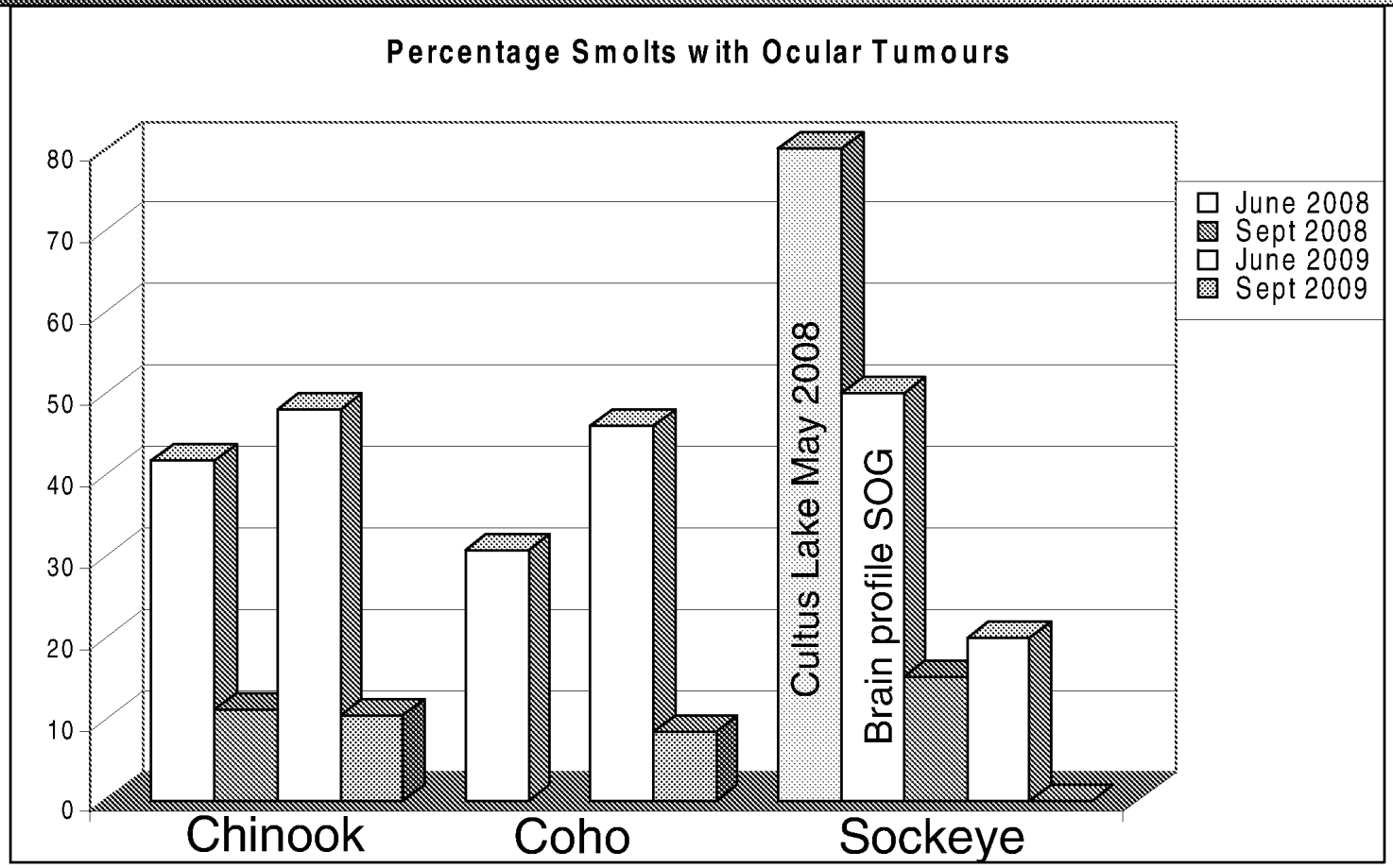
20% decline in Tumours between SW-FW in 2009 indicates enhanced SW mortality in the SOG could account for 0.9 million tumour-assoc fish going missing in the SOG (N=62)

2005 brood year—over 75% of adults positive for viral signature in at least one tissue

Increased SW-FW tumour prevalence in 2006/2008 may indicate

1) Sampling artefact (temporal/diversion variation),
2) Faster river entry assoc with tumour (some data support), enhanced fishing mortality with longer ocean residence (no fishing 2009)
3) Lower ocean mortality associated with tumour

Tumour-Associated Early Ocean Mortality of Smolts of Chinook, Coho and Sockeye Salmon



Declines in Tumours from June-Sept indicate tumour-associated early ocean mortality

2009 data based on SOG sampling only
2008 on Outer coast (June) (except sockeye) and SOG (Sept)—so reflect different stocks

Data are based on 289 brain dissections total—more to come (we have thousands)
Tumours observed in a wide range of stocks in all three species (not just Fraser River)

Strong Linkages of Genomic and Brain Tumour Data With Plasmacytoid Leukemia caused by the Salmon Leukemia Virus

Salmon Leukemia Virus (SLV)

- relatively unknown retrovirus associated with mortalities of cultured Chinook salmon in fresh and saltwater (1980's-1990's, not studied thereafter)
- May have been associated with Coho mortalities in Big Qualicum hatchery 1985-1986
- causes severe anaemia (also called Marine anaemia)
- primary infections in kidney/spleen, with plasmablasts invading secondary tissues (liver, pancreas, intestine, gill, brain) in advanced infections
- **Associated with ocular tumours from which viruses have been visualized by EM**
- Challenges show high susceptibility of Chinook, Sockeye and Coho, low susceptibility for Atlantics (no mortality), and resistance of rainbow trout
- SLV infected fish also show a high incidence of the nucleosporean parasite *Enterocytozoon salmonis* and bacterial kidney disease
- SLV-infected fish are slower growing, poorer feeders, generally less active-but with burst activity, often move lower or higher in the water column, adjust poorly to salinity transfer, may be more temperature sensitive
- Virus not well characterized (no sequence data, no cell lines)
- Studied by Bill Eaton/Mike Kent in the 1980s-1990s, but not significantly thereafter

Chinook Salmon

1974
PL discovered through histology at Washington State Hatchery
Released to ocean

1977
Chinook Declines Begin

1988
PL in BC Net Pens in S. BC
SLV involvement proposed
Ocular tumours with virus particles discovered

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 wild in the SOG
6% positive

1993
Vertical transmission demonstrated
Some evidence for horizontal transmission

1994
Experimental SLV challenge
100% infected

2009
Ocular tumours in 48% of smolts in June, 10% in Sept in the SOG

Coho Salmon

1983
Ramp up hatchery production in BC

1985
Similar lymphoblastic disease
Big Qualicum Hatchery
12% mortality
Released to ocean

1986
Similar lymphoblastic disease
Big Qualicum Hatchery
45% mortality
Released to ocean
Reduced activity/energy bursts

SW Challenges showed
Poor survivorship in SW
Osmoreg dysfunction

1986
Coho Declines Begin

1993
Large crash in coho Begins

1994
Experimental SLV challenge
70% infected

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

2009
Ocular tumours in 46% of smolts in June, 8% in Sept in the SOG

Sockeye Salmon

1991
PL/SLV wild in the SOG

1994
Experimental SLV challenge
100% infected

1996-present
Sockeye Behavioural Alterations
Altered River entry timing of Late-run FR sockeye salmon
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 suggestive of tumours

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 adults and 20% of smolts in June

Note: Linkages of PL/SLV with cultured fish may be an artefact, as there is no regular fish health/disease screening on wild salmon. 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), fish simply disappear.

Our Data Indicates 30-70% "Viral/Tumour" Positives in Brain, Gill, Liver

SLV infection levels may currently be >90% in Coho, Chinook and Sockeye salmon – well over the estimated 6% infection rates in wild Chinook salmon in the early 1990's

Ho that conditions in the external environment affect the virulence of SLV i.e. stressed fish will be more adversely affected

SLV infected fish could be easier prey

If SLV is a primary factor in the salmon declines in BC

Potential for Mitigation

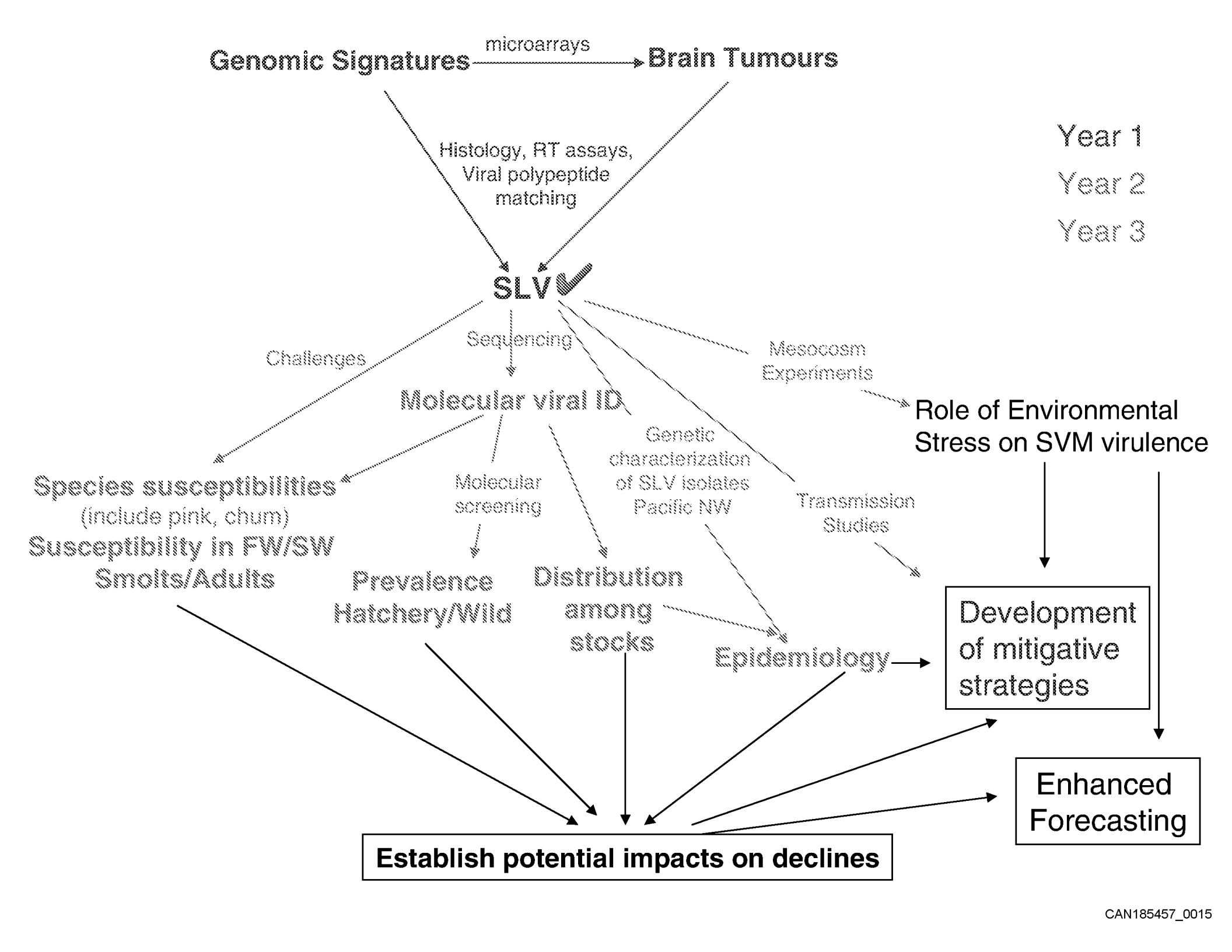
- rapid molecular-based broodstock screening in hatcheries to minimize vertical transmission
- improved husbandry to minimize horizontal transmission
- requires information on viral transmission

Potential for Enhanced Forecasting

- molecular screening to establish levels of infection smolts/adults
- requires better data on role of environmental stress (models)

New environmental playing field—not mismanagement or over-fishing

Not likely an effect of salmon aquaculture, but there could be linkages with the expansion of hatchery production


Genomic Signatures
microarrays
Brain Tumours
Histology, RT assays,
Viral polypeptide
matching
Year 1
Year 2
Year 3
SLV
Challenges
Sequencing
Mesocosm
Experiments
Molecular viral ID
Role of Environmental
Stress on SVM virulence
Genetic
characterization
of SLV isolates
Pacific NW
Species susceptibilities
(include pink, chum)
Susceptibility in FW/SW
Smolts/Adults
Molecular
screening
Transmission
Studies
Prevalence
Hatchery/Wild
Distribution
among
stocks
Epidemiology
Development
of mitigative
strategies
Enhanced
Forecasting
Establish potential impacts on declines
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