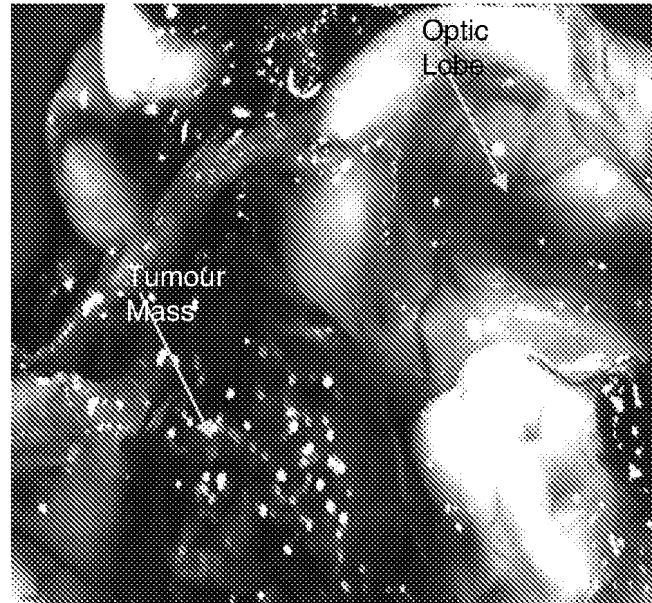


# Epidemic of a novel, cancer-causing viral disease may be associated with wild salmon declines in BC



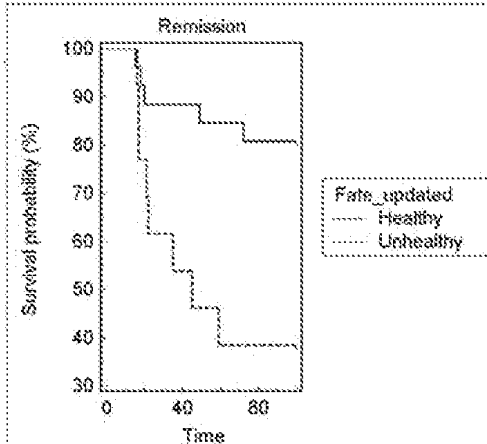
Kristi Miller  
Molecular Genetics Lab  
Karia Kaukinen, Shaorong  
Li, Tobi Ming, Norma Ginther,  
Angela Schulze

David Patterson, Jayme Hillis,  
and others....

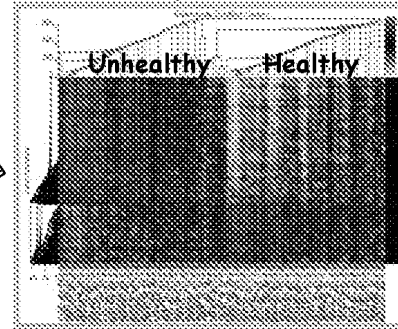
- Genomic Evidence
- Tumour Evidence
- Adults
- Smolts
- Sockeye, Coho and Chinook salmon

Sept 27, 2008

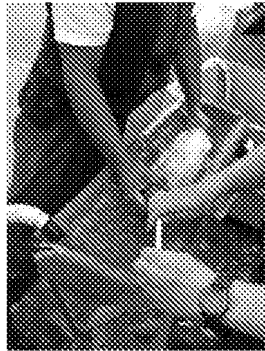
# Genomics Research uncovers a viral-induced signature correlated with adult sockeye salmon mortalities in the river



Physiology in saltwater predictive of Fate In River



1881 genes



## "Unhealthy Signature"

Induction of defence response

antigen presentation

membrane transport

Intracellular Pathogen Response

cytokine 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

## "Healthy Signature"

Protein Biosynthesis

Oxidative Phosphorylation

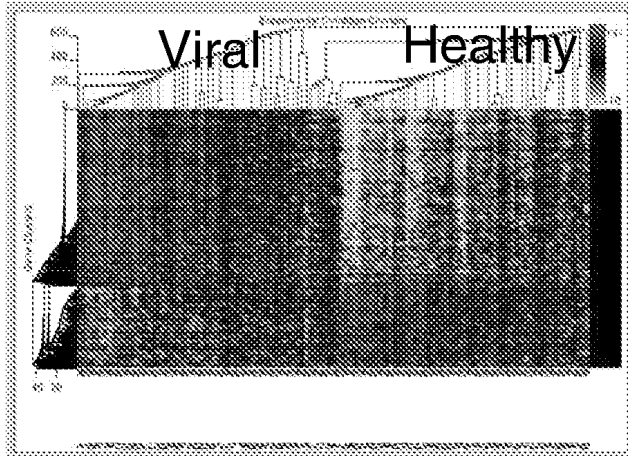
## "Unhealthy" Signature

Is a Viral signature

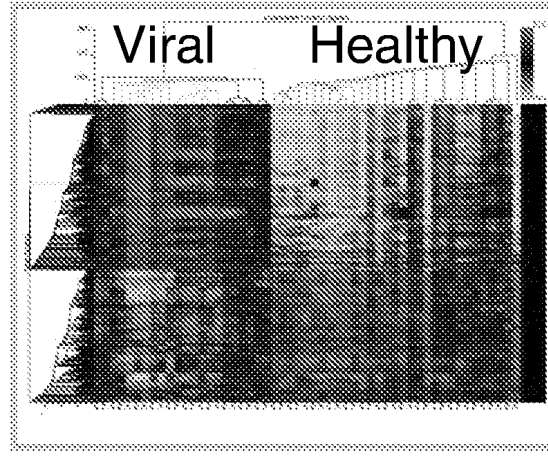
Viruses evade host  
aid replication

"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)

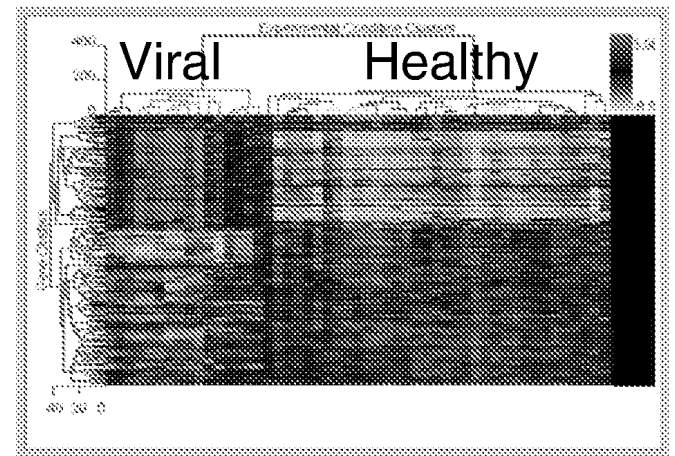
# Viral Signature Observed in Multiple Tissues



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

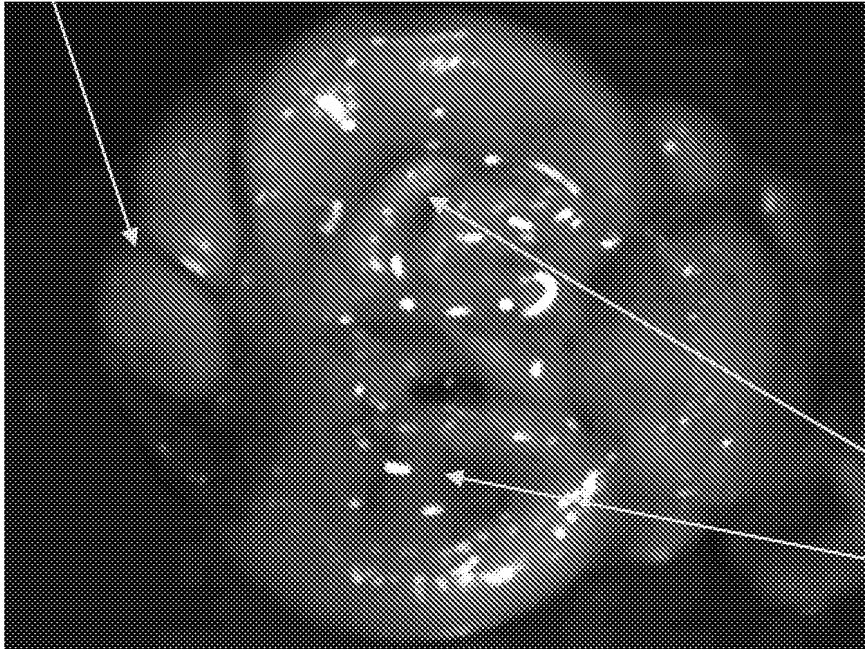
Gene ID	Viral Relationships	Functional Role	FOLD CHANGE	Most UH	Med UH	Intermediate	Healthy
PCSK5	Viral-viral assem	Anti-viral state	3.28				
STAT1	Viral-anti-viral sta	Anti-viral state	1.76				
CREBZF	virus--neg reg trac	transcription (virus -)	1.87				
CREBZF	virus--neg reg trac	transcription (virus -)	1.57				
IF144	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: E	Viral Entry	2.59				
	viral--host-virus in	viral induced stress	1.23				
	viral--retrovirus	Endogenous Retrovirus	2.05				
MAK3	Viral--L-A virus: G	viral reproduction (+)	1.31				
HNRPA3	Viral--retrovirus: I	mRNA splicing (viral)	1.54				
Hnrpa1	Viral--retrovirus: I	mRNA splicing (viral)	1.4				
SFPQ	Viral--retrovirus: P	mRNA splicing (viral)	1.31				
Eif4g2	Viral--cleaved by S	Translation (host -)	1.8				
ABCE1	Viral--retrovirus: I	Translation (host -)	2.68				
Eef1a	Viral--retrovirus: h	Translation (host -)	1.5				
EIF4G1	Viral--cleaved by F	Translation (host -)	2.52				
EIf2b3	Viral--response to	Translation (viral -)	1.2				
SKIV2L	Viral--Antivirus He	Translation (viral -)	2.18				
Sars	Viral--retrovirus: t	Translation (viral +)	1.65				
IARS2	Viral--retrovirus: t	Translation (viral +)	2.54				
WARS	Viral--retrovirus: t	Translation (viral +)	2.32				
IARS	Viral--retrovirus: t	Translation (viral +)	1.16				
IARS	Viral--retrovirus: t	Translation (viral +)	1.08				
PABPC4	Viral--retrovirus: P	Translation (viral +)	2.17				
Pvrb3	virus--poliovirus re	Receptor	1.6				
HYAL2	Viral--retrovirus re	Receptor	2.37				
HRAGA	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: E	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 S	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				
eif1a	Viral--retrovirus: E	Viral encapsidation	1.2				
eef1a	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: I	Integration	1.88				
bant1	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-inflammatory	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: W	Inflammation/cancer (viral mediated)	2.11				
F10	Viral--coagulation:	Coagulation (viral mediated)	1.23				

Retroviruses?

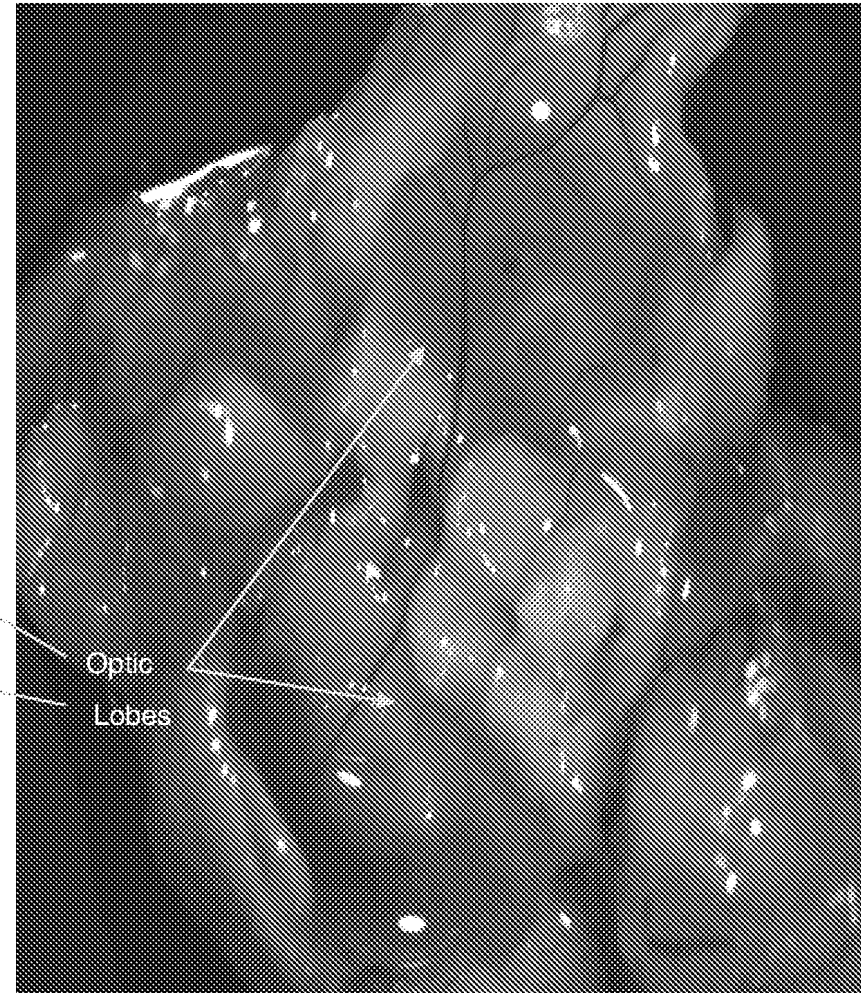
Retroviruses are often neoplastic (tumour-causing) and have been associated with wide range of cancers

# 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



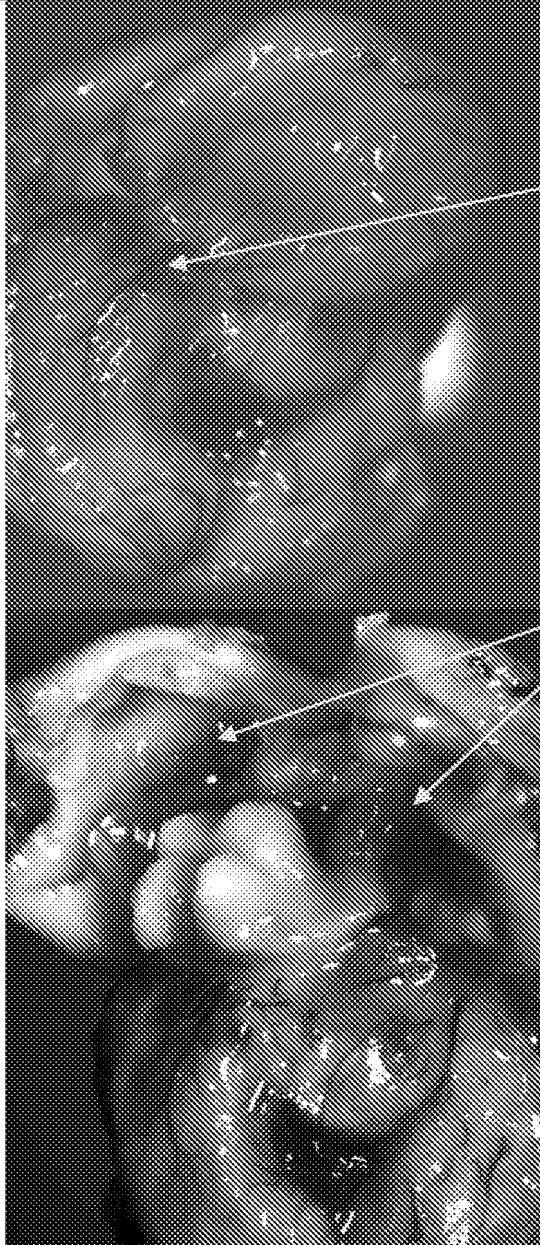
# Onne\_ad\_2009\_tumour\_3239

## Stuart Fish

(Middle River)

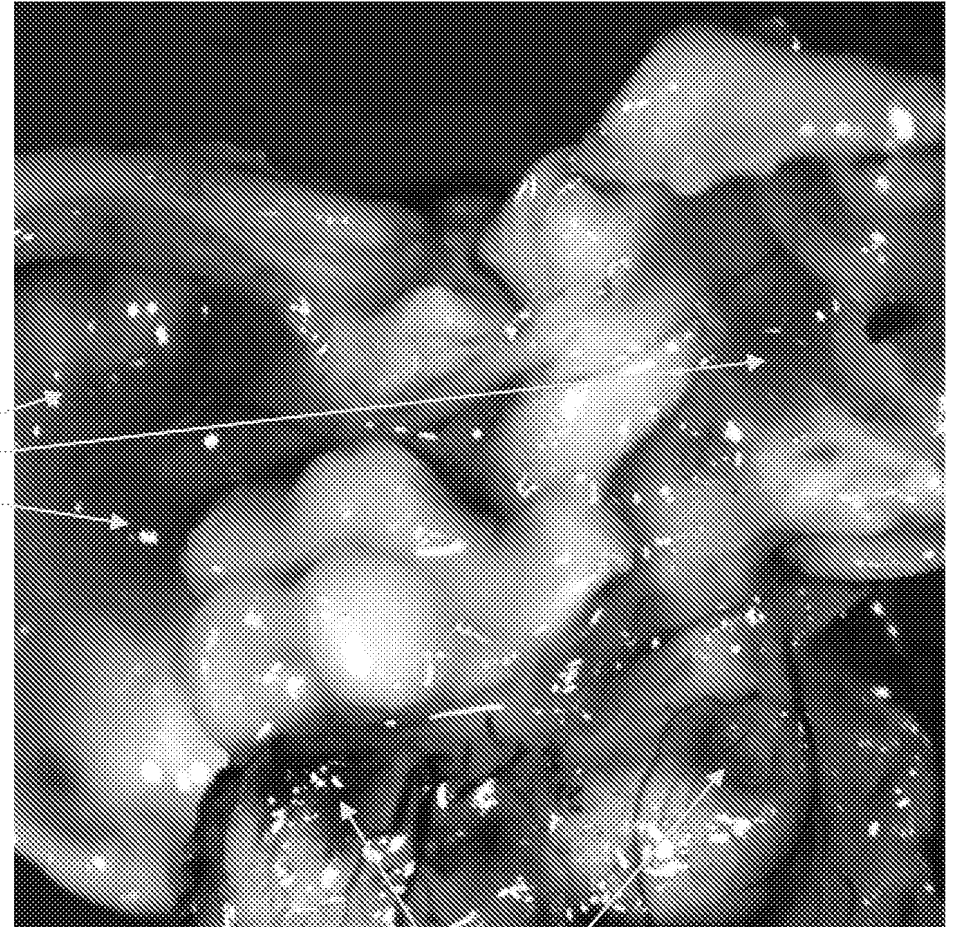
Collected at Gill Road (Area29)

Right  
Optic  
Lobe:  
Outside  
and  
inside  
view



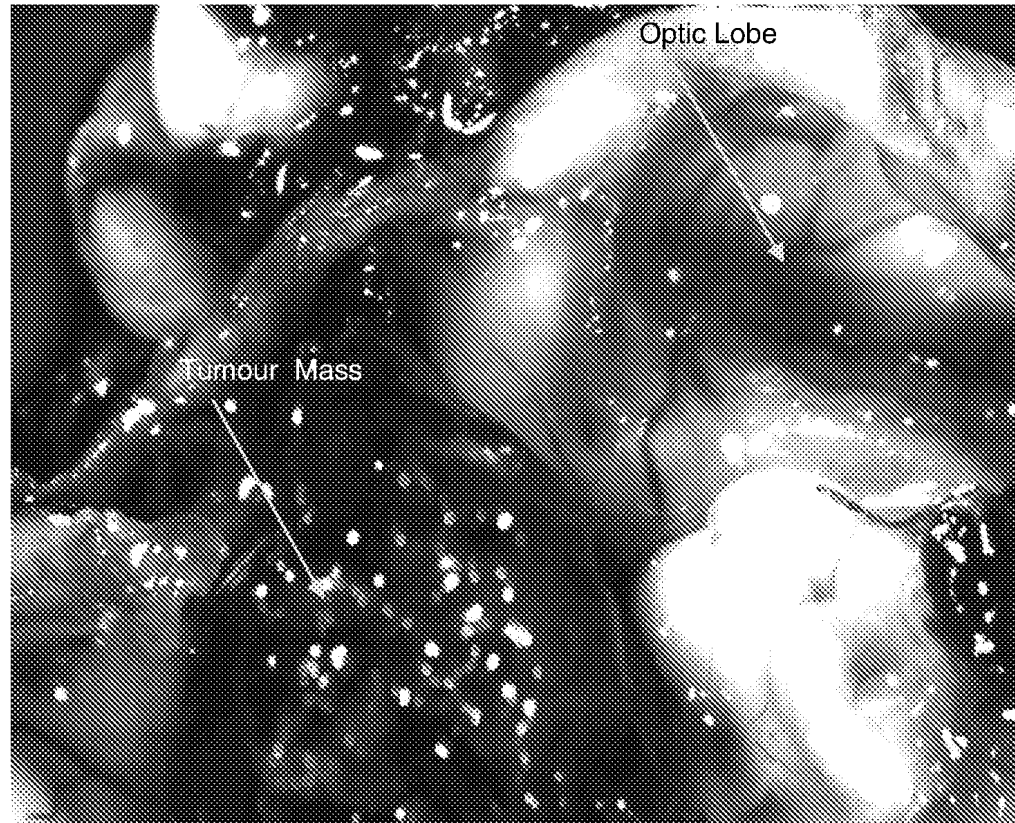
Vascularization  
outside optic lobe

Pink Tumour  
Masses  
within the  
Optic Lobes



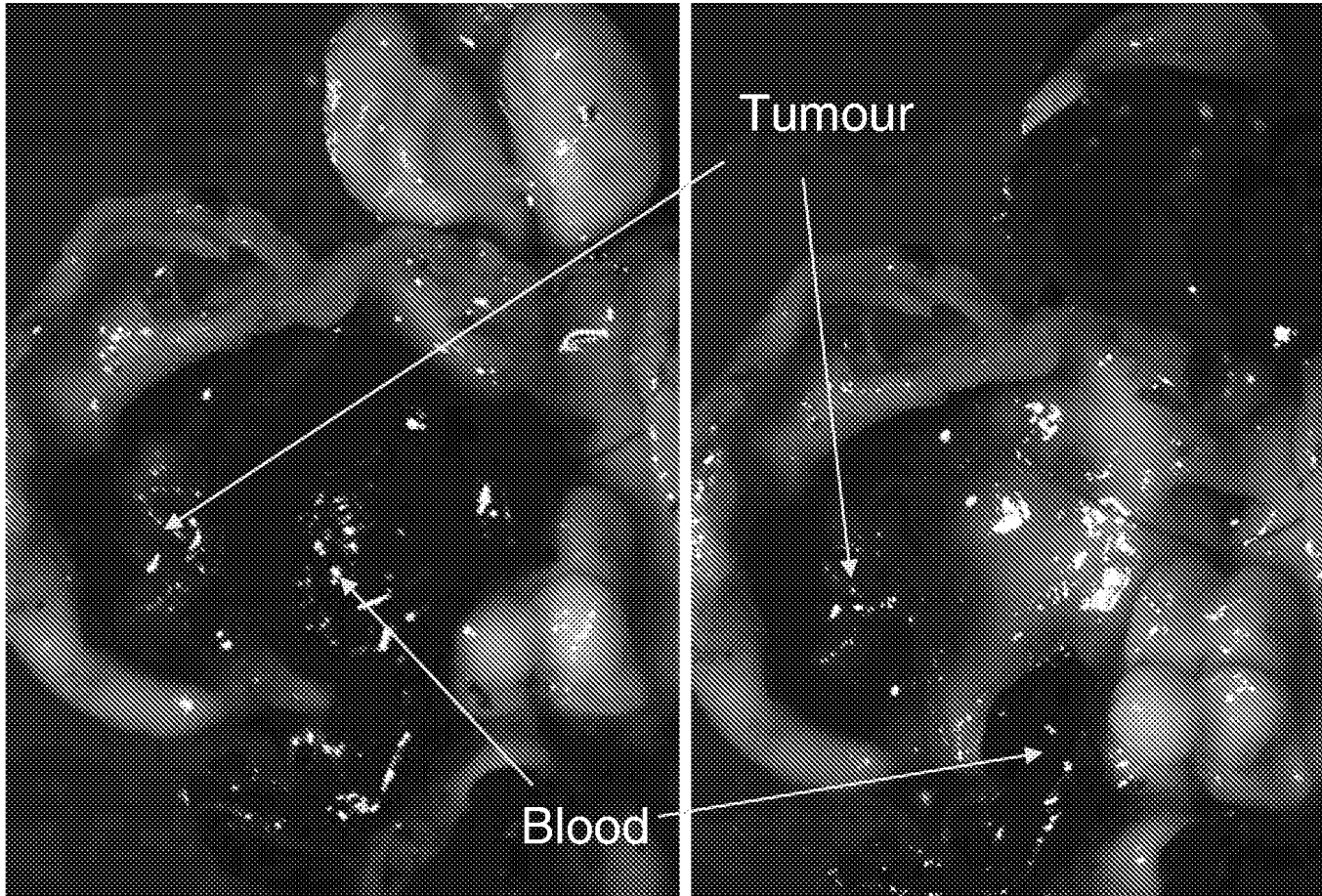
Bloody, necrotic appearance  
on outside of cerebellum

# Large dark attached tumour mass



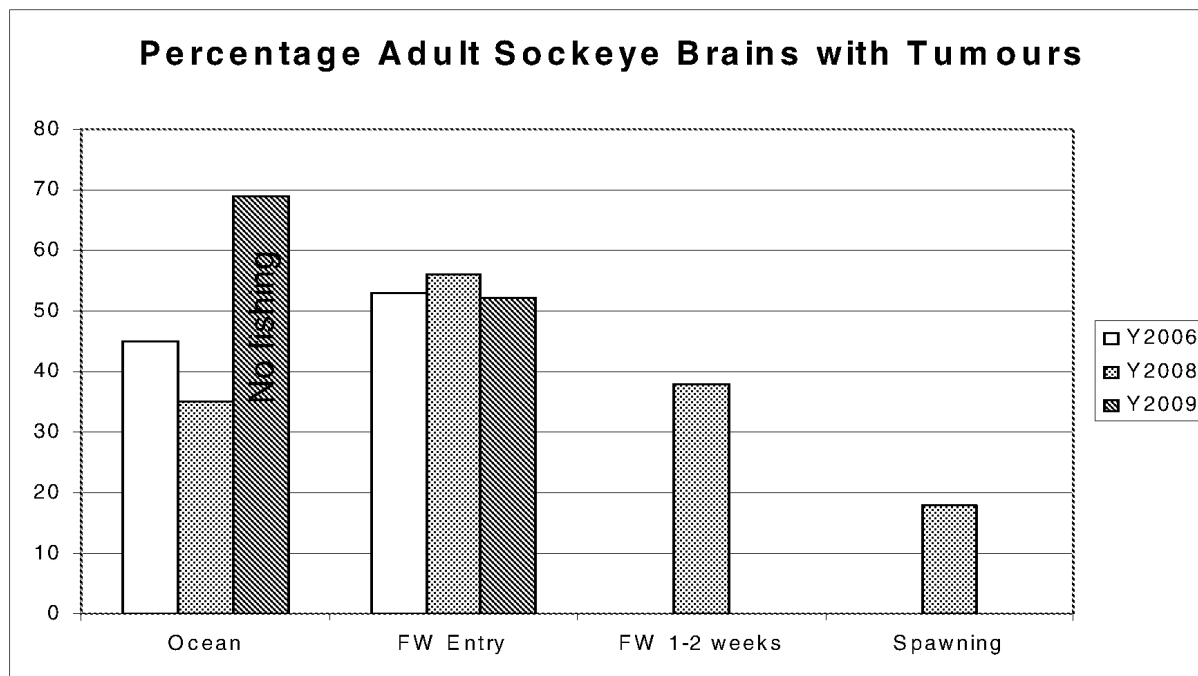
3201 Close up: Inside and outside of the brain (note that inside the optic lobe with mass flipped out there remains pink tissue attached and vascularised)

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

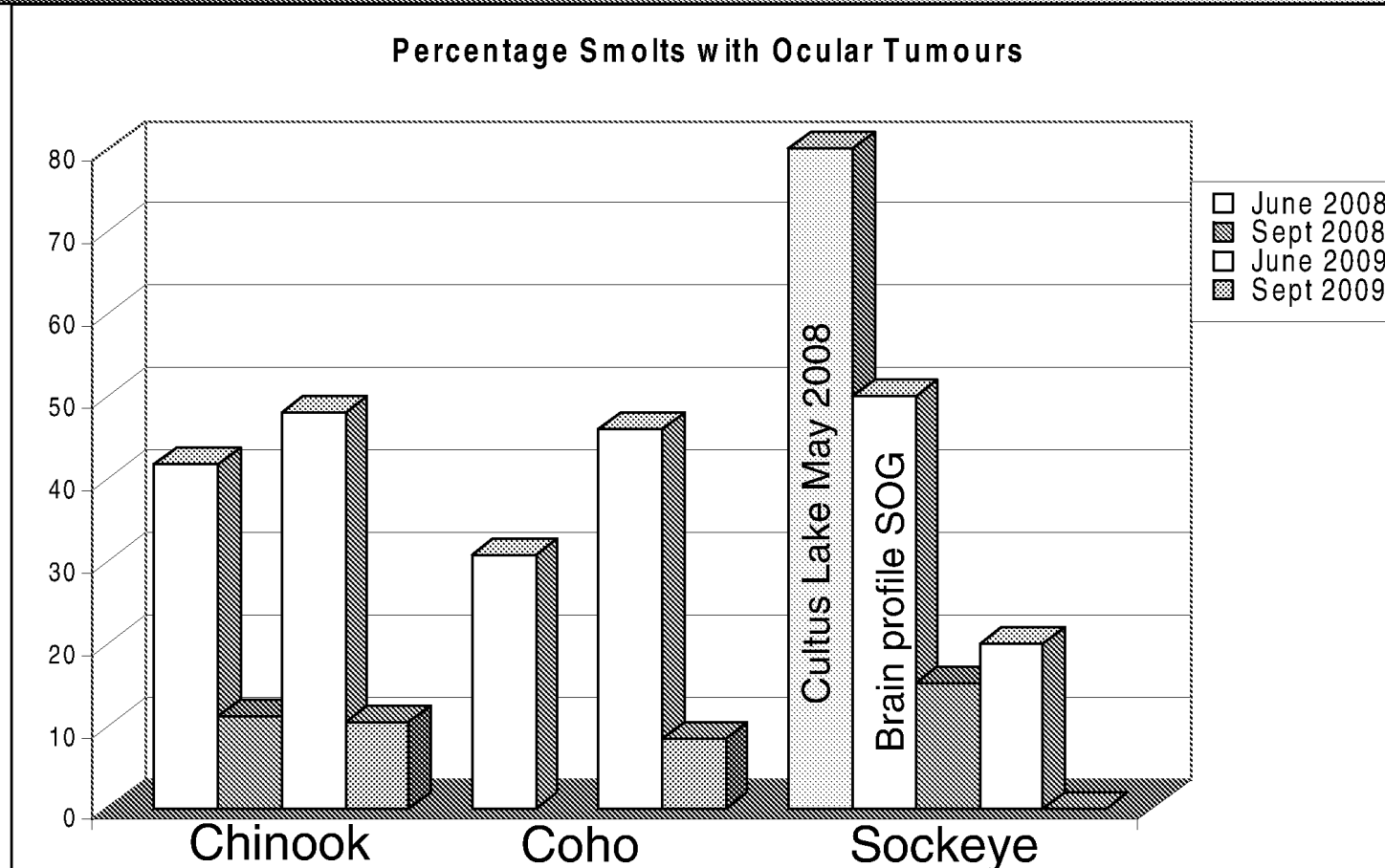
20% decline in Tumours between SW-FW in 2009 indicates enhanced SW mortality in the SOG  
could account for 0.9 million tumour-assoc fish going missing in the SOG (N=62)

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

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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

How 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

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## 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

