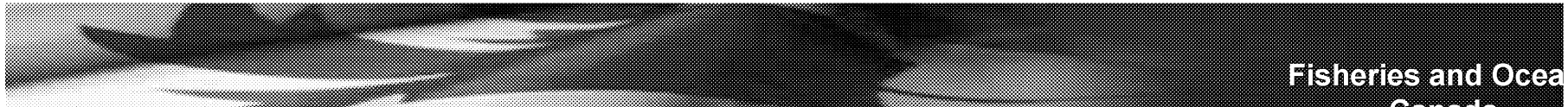
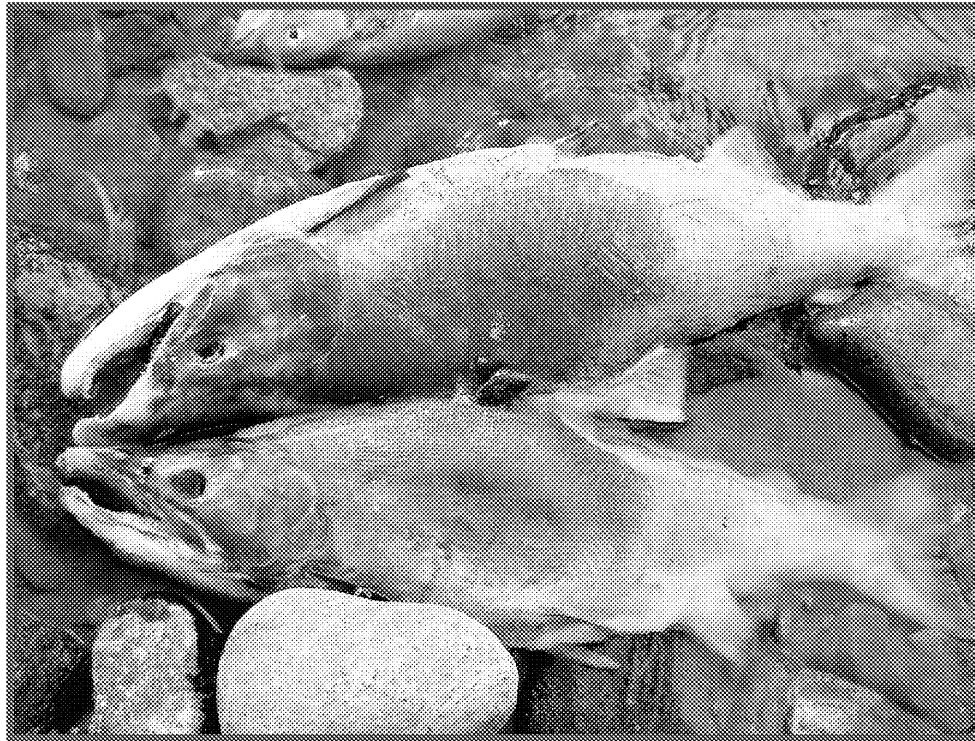


Physiological control of entry timing and fate



DFO-05239

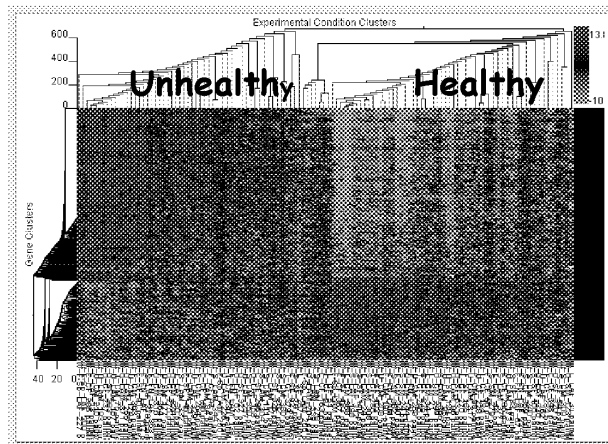
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risti Miller\Electronic Documents - Search 001\Pub
\Genome BC Project--2008\SEF_Bug Hunt\BugHunt_Key
Slides.ppt

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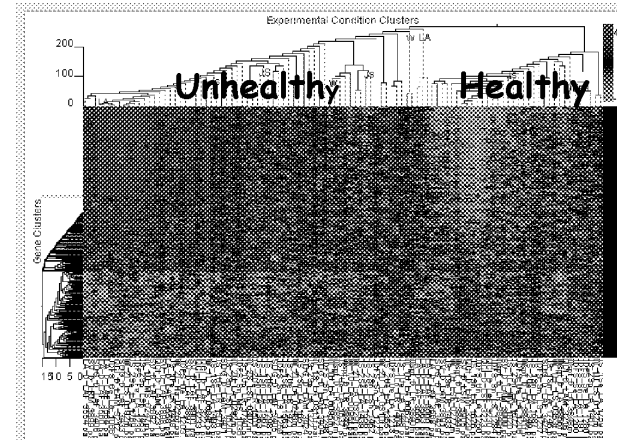
Genes expressed in FW (W) associated with Fate

Original two-class t-tests
Revealed 1,744 genes at
 $p < 0.001$

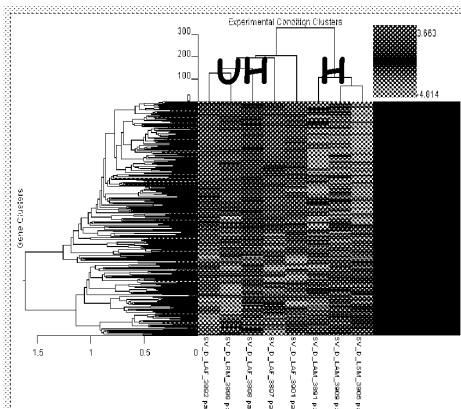
1,744 W 2006 gene list applied
To ALL LOCATIONS sampled
In 2006: 2 groups



Savona 2006

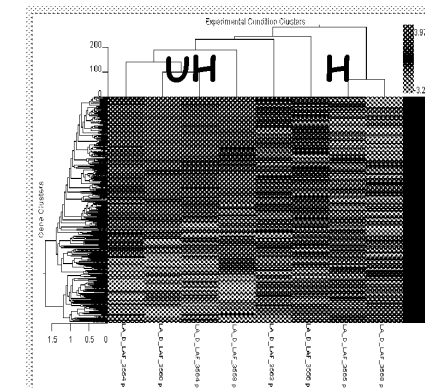


LA Spawning 2006



77 genes overlap with SW
“fate” list -- same direction
of fold change

Same relationships among
indiv. depicted with either
gene list – same underlying
mechanism related to fate

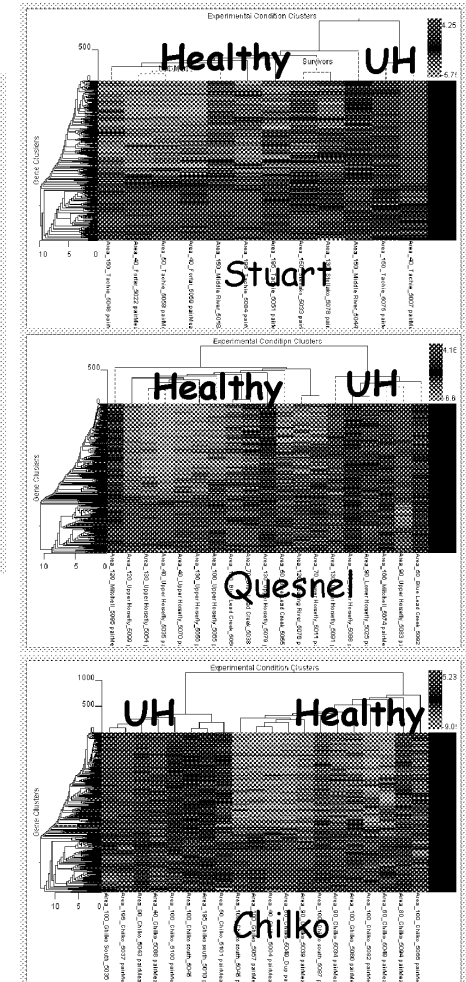
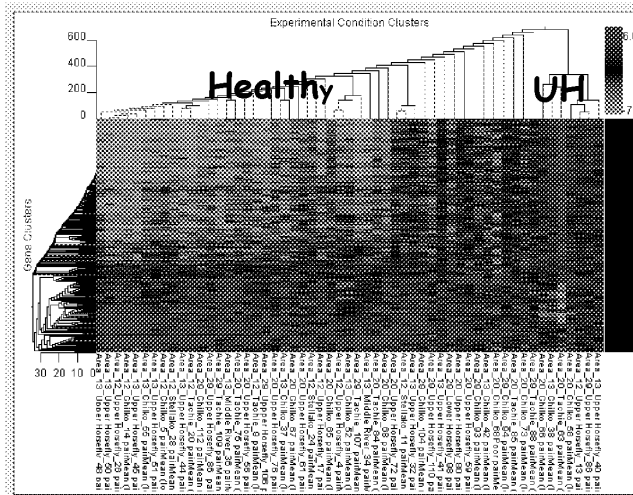
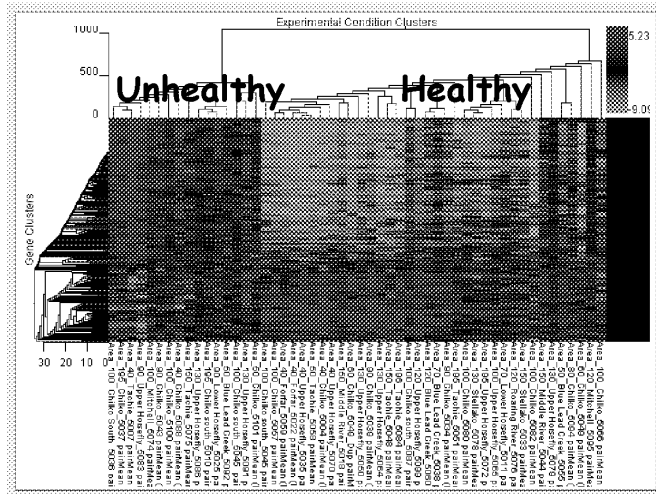


1,744 W 2006 gene list applied to 2005

To EACH stock
sampled in 2005 at
W: Again, 2 groups
of fish

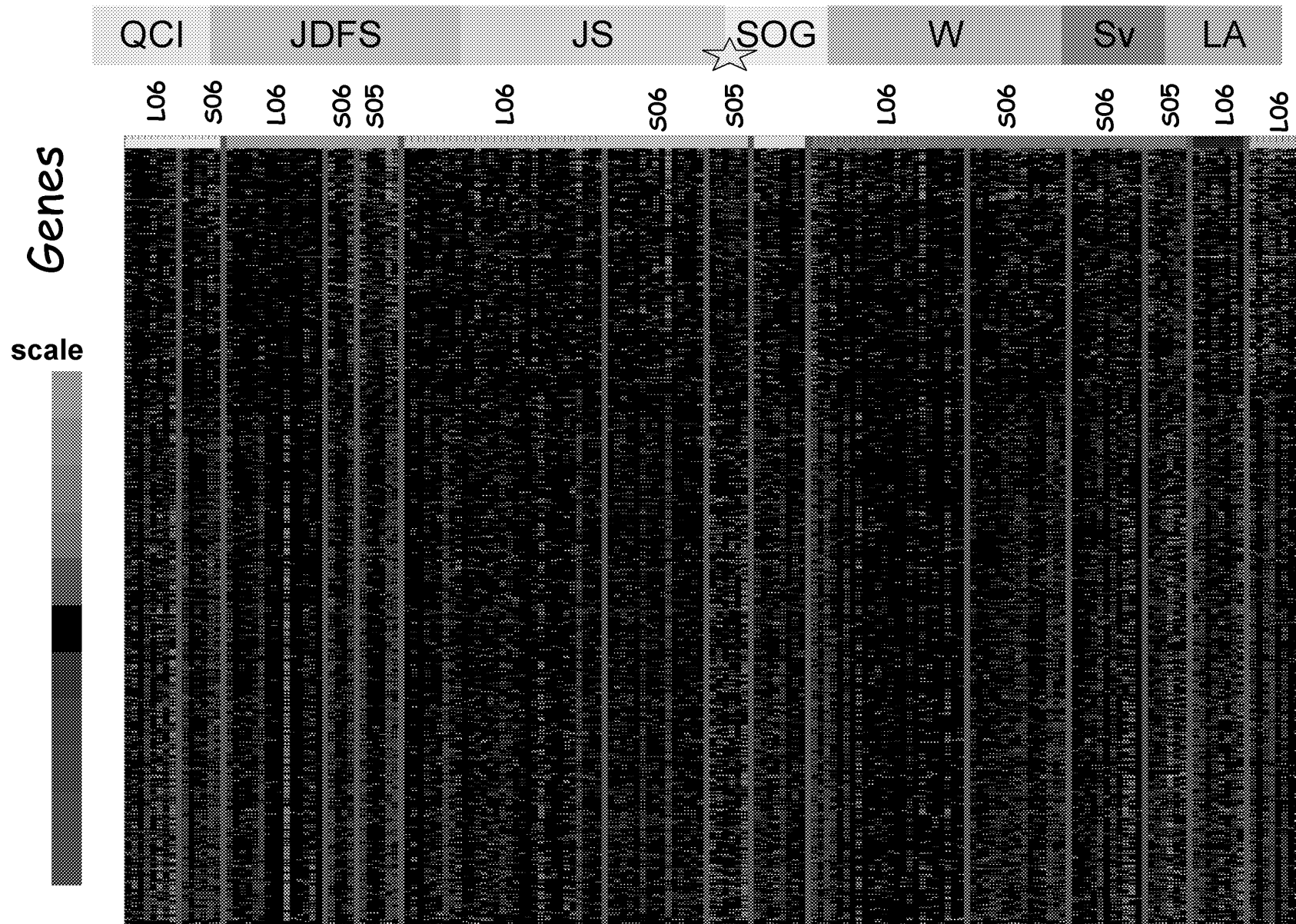
To W 2005 summer-run fish:
Again, 2 groups of fish

To ALL LOCATIONS
sampled in 2005:
Again, 2 groups of fish



Migrating "unhealthy" fish were also present in the SW and FW environments in 2005 and 2007 (data not shown), but proportions varied significantly by year

Spatial patterns associated with condition and fate



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risti Miller\Electronic Documents - Search 001\Pub
\Genome BC Project--2008\SEF_Bug Hunt\BugHunt_Key
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What are Healthy and Unhealthy profiles associated with?

Fate

URM-Surv p-value N Odds Ratio

Ocean Health

H vs. UH (no intermed)

JS 0.0081 24 16

JS/JDFS 0.0078 28 11

Freshwater Health

Chilko, Scotch Creek, Lower Adams Stocks Combined

UH vs. H-I 0.0233 52 2.6

mostUH vs. H 0.0135 32 3

mostUH vs. H-I 0.0035 46 6

mostUH vs all others 0.00227 52 7.3

Scotch Creek: NO UH fish made it to spawning grounds

Chilko: NO mostUH fish made it to spawning grounds

LA: Two mostUH fish made it to spawning grounds

Both present in late Oct/Nov during high pre-spawn mort

Entry Timing

Early vs Normal

UH vs. H (no Intermed) 0.007 31 5.25

Unhealthy fish in SW 16x less likely than healthy fish to make past HWT region to reach spawning grounds

The "most" unhealthy fish 7.3x less likely than healthy fish to make past HWT region to reach spawning grounds

Weaker effect on only moderately UH fish

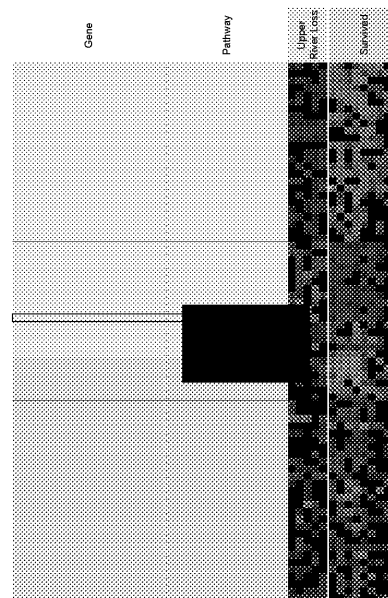
Unhealthy fish 5.25x more likely than healthy fish to enter the river early

GO Processes in SW associated with Fate In River

Rank	Biological/Molecular Process	Profile	Explanation
Blood Processes			
1	von Willebrand factor containing domain/EGF-like region	Down in URM	involved in cell adhesion/coagulation
2	oxygen transport/globin/metalloprotein	Up in URM	Hemaglobin is up, but iron binding down
7	Iron binding	Down in URM	Hemaglobin is up, but iron binding down
Metabolism			
3	fatty acid metabolism	Down in URM	
4	creatine kinase activity	Down URM	Metabolic processes down-regulated
8	aerobic respiration	Down in URM	
Disease/stress/toxicant response			
5	response to abiotic stimulus (includes many immune/stress signals)	24 down, 15 up in URM	
6	bZip transcription factor	Down in URM	often in response to pathogen exposure

DAVID analysis
<http://nida.abcc.ncifcrf.gov/>

Pathway	Count	URM	Stress
osmoregulation	0.375		
iron ion transport	0.250		
ion transport	0.250		
oxygen transport	0.345		
electron transport	0.307		
Stress response, positive regulation of catabolic process	0.354		
Defense Response	0.345		
Detoxification	0.345		
reproduction	0.345		
proteolysis and peptidolysis	0.345		
Proteasomal proteolysis	0.345		
protein transport	0.345		
protein metabolism	0.345		
protein lipidation	0.345		
protein folding	0.345		
protein biosynthesis	0.345		
protein binding/mRNA export from nucleus	0.345		
protein amino acid acetylation	0.345		

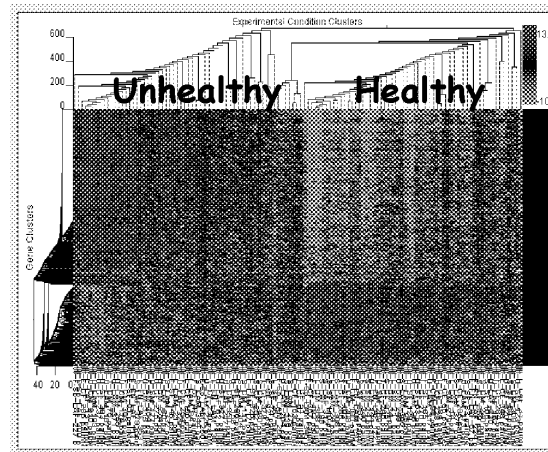


ALSO

Proliferative, anti-apoptotic profile in Unhealthy Fish

Osmotic genes:
Na-K ATPase alpha 1c (up UH)

GO Processes in FW associated with Fate In River



"Unhealthy Signature"

Induction of defence response

antigen presentation

membrane attack

basal transcription

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

Viruses evade host,
aid replication

"Healthy Signature"

Protein Biosynthesis

Oxidative Phosphorylation

**"Unhealthy" Signature
is a "disease" signature**

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"Unhealthy" Signature May be a Virally-induced signature

Gene ID	Viral Relationships	Functional Role	FOLD CHANGE	Most UH	Mod 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 tra	transcription (virus -)	1.87				
CREBZF	virus-neg reg tra	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; t	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; li	mRNA splicing (viral)	1.54				
Hnrpa1	Viral-retrovirus; li	mRNA splicing (viral)	-1.4				
SFPQ	Viral-retrovirus; F	mRNA splicing (viral)	1.31				
Eif4g2	Viral-cleaved by :	Translation (host -)	-1.8				
ABCE1	Viral-retrovirus; i	Translation (host -)	2.68				
Eef1d	Viral-retrovirus; h	Translation (host -)	1.5				
EIF4G1	Viral-cleaved by t	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; F	Translation (viral +)	2.17				
Pvrl3	virus-poliovirus 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; E	Transport (RNA)	-1.2				
EIF5	Viral-retrovirus; E	Transport (RNA)	1.37				
STAU1	Viral-retrovirus; E	Transport (RNA)	1.77				
DDX23	Viral-retrovirus; E	Transport (RNA)	1.91				
	Viral-retrovirus; A	Transport (RNA)	1.65				
	Viral-retrovirus; A	Transport (RNA)	1.43				
NCL	Viral-retrovirus; F	Transport (RNA)	2.02				
	Viral-viral reprod	Viral replication	-1.3				
Mgll	Viral-hMGL, like i	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; k	Integration	1.88				
banf1	viral-retrovirus	Integration	1.38				
ATP6V0C	Viral-host-virus ir	Anti-cancer	1.84				
ATP6V0C	Viral-host-virus ir	Anti-cancer	1.06				
ATP6V0C	Viral-host-virus ir	Anti-cancer	-1.4				
IL13RA2	pro-inflammatory	Cancer marker (brain)	2.11				
KRT18	Viral-host-virus ir	pro-cancer	1.35				
KRT18	Viral-host-virus ir	pro-cancer	-1				
RALB	Viral-retrovirus; v	Inflammation/cancer (viral mediate	2.11				
F10	Viral-coagulation	Coagulation (viral mediated)	1.23				

Molecular Pathogen Screening

SCREENED AND NEGATIVE

RNA Viruses

ISAV

IHNV

VHSV

IPNV

Picornavirus

DNA Viruses

Herpes

SPDV

Retroviruses

Lentivirus deg primers
pos endogenous

NOT YET SCREENED

Viruses

PSPV

Aquareovirus

Parasites

Myxobolus cerebralis

Microsporidium Nucleospora

Bacterial Pathogens

General 16S primers-DGGE

Retroviruses

Many other degen. Primers to try

Salmon Leukemia Virus?

Parasites

Ciliates—20/96 in river, most PSM

Myxosporidia

LOMA—9/96 samples pos

Some microsporidia

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Known Exogenous Retroviruses in Fish

Confirmed Retroviruses

Zebrafish -full length expressed endogenous retrovirus sequenced (ZFERV)—suggested that it may produce intact virus particles

Snakehead Fish Retrovirus—completely sequenced
similar to bovine leukemia and human T-cell leukemia virus group
enhances cytopathic effects of other viruses (e.g. nodavirus)

Walleye dermal sarcoma virus (WDSV)—type I and II completely sequenced, neoplasms seasonal (temperature related?)

phylogenetically closest to murine leukemia virus

Walleye epidermal hyperplasia virus (WEHV)—completely sequenced, neoplasms seasonal

Atlantic Salmon Swim Bladder Sarcoma Virus (SSSV)—completely sequenced
only sequenced salmon retrovirus, first observed in 1975 on Scottish salmon farms with neoplastic disease, farms in Maine in 1996
Endogenous sequence annotating to SSSV observed and up-regulated in UH fish

Suspected retroviruses (infectious neoplasms--oncogenic)

Lyphnosarcoma of pike

Plasma cell leukemia of brown bullhead

Neurofibromatosis of damsel fish

Salmon Leukemia Virus

Plasmacytoid Leukemia—Marine Salmon Anemia—Salmon Leukemia Virus

First diagnosed a leukemia-like disease in farmed chinook salmon in BC in 1988

associated with marine mortalities (rates 2.5-20% in marine environment, not tested in FW)

Also identified in wild salmon in the SOG

Transmission studies using homogenates show **susceptibility of chinook, sockeye and Atlantics** in farms, peak diagnoses in Sept, coincide with highest proportion of associated deaths
failure to adapt to seawater noted soon after smolts moved to SW

Inflammatory disease—shifts in specific and non-specific inflammation

higher incidence in 1992 than 1993 (coincides with sockeye 2000, 2004, 2008)

Diagnosed by grossly pale gills, massive numbers of plasmablasts (large immature cells) in posterior Kidney, immature blood cells in stained liver smears and renal interstitial hyperplasia of proliferating plasmoblasts, proliferation and infiltration of plasmablasts into the visceral organs and retrobulbar tissue of the eyes, **difficult to diagnose histologically (specificity 100%, sensitivity 32%)**

Eaton et al. (1993) suggested that it was caused by a **retrovirus**, named **salmon leukaemia virus** or SLV

Evidence: can infect fish from .2 micron filtered serum, presence of Mn^{2+} -dependent poly(rA)-directed Reverse Transcriptase, electrophoretic pattern of polypeptides from purified virions, RNA band at density of 1.16 to 1.18g/ml in sucrose, cytolytic cell line developed

The **virus could not be "cultured"** and **no sequence** was obtained

Potential role of TEMPERATURE of SLV—associated with high water temperatures—
induced cytolytic infection at 15-20°C

However: SLV infection histologically indistinguishable from an intranuclear microsporidian infection caused by **Nucleosporia salmonis** (previously Enterocytozoan salmonis), and the presumed virus is often found in association with this parasite, however the parasite alone infections (in California RT) can be cleared by Fumagillin DCH, but not the BC chinook infections

Microsporidia are intracellular unicellular (very small) parasites

Nucleosporia infects the nuclei of lymphopoietic-like cells, inducing proliferation

Found globally and assumed that all salmonid species are potential hosts

Has caused extensive mortality in FW and SW—considered threat to cultured and wild salmon
threatening the supplementation programs for endangered salmon in France

May also fit well with the transcriptional profile observed

Why focus on Plasmacytoid Leukemia ?

- Transcriptional data indicate the salmon may be responding to a retrovirus, and specifically a leukemia-type retrovirus
 - SLV is the **only suspected retrovirus in BC Salmon**, but has yet to be isolated or sequenced
- Screened for other common viruses, including ISAV, IHNV, VHSV, Herpes, IPNV, Picornavirus all negative. Also screened for bacterial pathogens and myxosporidian parasites, and Loma, all negative.
- Fish with plamacytoid leukemia are **anemic**, with diseased Chinook salmon have **pale gills**
 - pale gills often observed in dying sockeye salmon in the FR
 - Anemia generally involves iron deficiency—very low transcription of ferritin in our “unhealthy” fish indicates low iron
- Our fish generally **“look” healthy**
 - this disease does not have easily distinguishable features and is difficult to diagnose even with histology unless you know what you are looking for, and then, histology is only 32% sensitive (i.e. miss 68% of positives)
- Temperature sensitivity of SLV established
 - Our data indicate that temperature and potentially stress are a factor in poor survivorship of “unhealthy” profile fish
- Salinity sensitivity of SLV hypothesized
 - Highest mortality of SLV infected chinook upon transfer from FW to SW
 - Transcriptional data indicate osmoregulatory shifts in unhealthy fish towards FW state
- Retroviruses, including those that cause leukemia, well known for inducing **immunosuppression**—observed in marine sampled fish
- Organisms with leukemia often succumb to secondary bacterial infections—expected in river
 - Chinook infected with Plasmacytoid leukemia also carry higher incidence of other infections, especially BKD
- Leukemia is associated with coagulation disorders—observed in 2003 sockeye salmon
- Retroviruses are neoplastic viruses, and hence associated with cancer,
 - Numerous cancer biomarkers up-regulated in the brain of unhealthy fish

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Approach

A. USE MOLECULAR APPROACHES TO IDENTIFY THE DISEASE AGENT

I. Apply molecular markers to identify well characterized pathogens

Already screened for viruses: ISAV, IHNV, VHSV, Herpes, IPNV, Picornavirus
all bacterial pathogens (e.g. vibrios, flavobacterium, aeromonas, Yersinia, ...), and some myxo and microsporidian viruses (e.g. Loma)

Still to screen: Pacific Salmon Paramyxovirus (proliferative gill disease; PSPV),
aquariovirus, reovirus, *Oncorhynchus masou* virus (OMV), *Myxobolus cerebralis*,
microsporidian *Nucleospora salmonis* (often found associated with SLV in BC)

II. Develop and apply generalized primers to additional viral families

A. Primers available for:

Retroviruses: to look for SLV (suspected) or other retroviruses

Paramyxoviruses

More...Kyle

B. Viral families for which generalized screening needs to be developed

Kyle...

Utilization of degenerate primers for generalized viral screening may require that the virus be concentrated

III. Apply viral microarray

There are DNA arrays developed that contain conserved sequences from all known viral families that can be used to identify an unknown virus to family level—

e.g. SARS was identified and sequenced in one week using this approach

We have contacted the lab that performed this SARS analysis and they are willing to collaborate.

This technology could be applied in our labs to identify unknown disease agents in future

VI. Develop specific PCR primers to the pathogen, if not already done

These can be used in screening for the disease in other life stages, drainages and species

Approach

B. USE HISTOLOGY TO IDENTIFY THE DISEASE AGENT

Over 300 histology samples were taken from sockeye salmon returning to the Fraser River in 2008. Many Of these samples were taken from moribund salmon. While associations with genomically characterized "Unhealthy" status are not yet established, histological examination specifically geared toward detecting plamacytoid leukemia will be conducted. A full set of tissue samples for RNA analysis are also available From these same fish, and we will conduct genomic characterization of any fish positive for plamacytoid leukemia to determine if associations with previous "unhealthy" profiles exist

C. ISOLATE THE DISEASE AGENT AND ESTABLISH A CHALLENGE MODEL

I. Concentrate and Isolate the virus from various tissues

Previous microarray studies have characterized individual sockeye salmon as "unhealthy", and we would concentrate initially on kidney, brain, gill and liver tissues from these individuals

II. Establish an infected cell line

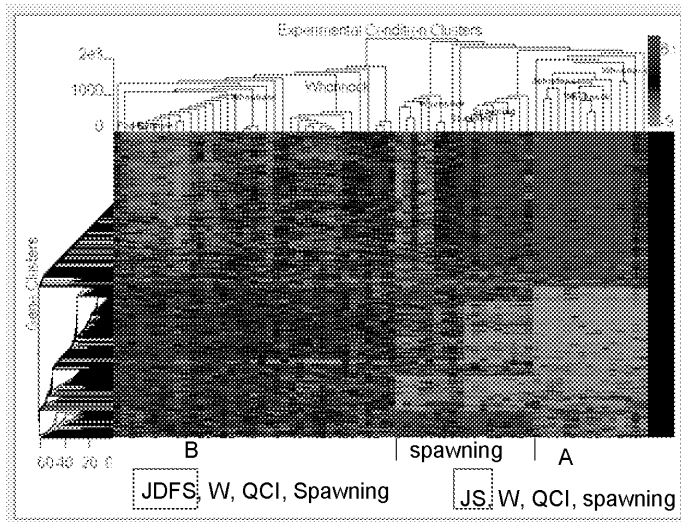
III. Begin development of in vitro and/or in vivo challenge model

D. ESTABLISH THE RANGE OF SALMONID SPECIES, STOCKS, AND LIFE STAGES POTENTIALLY AFFECTED BY THE PATHOGEN

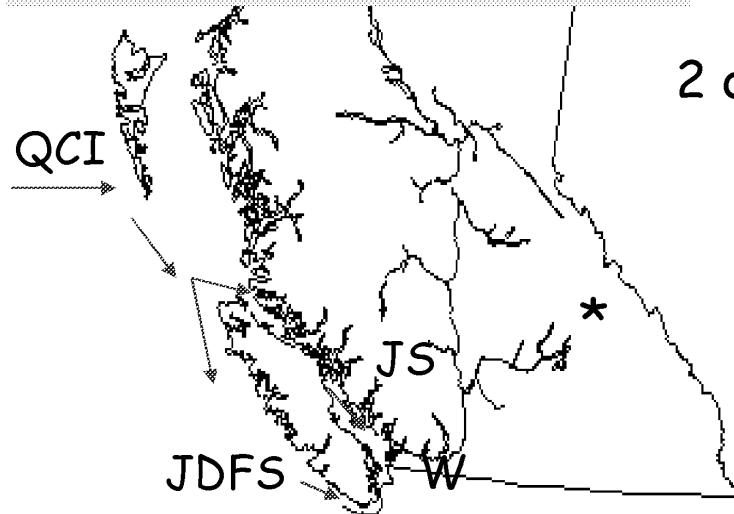
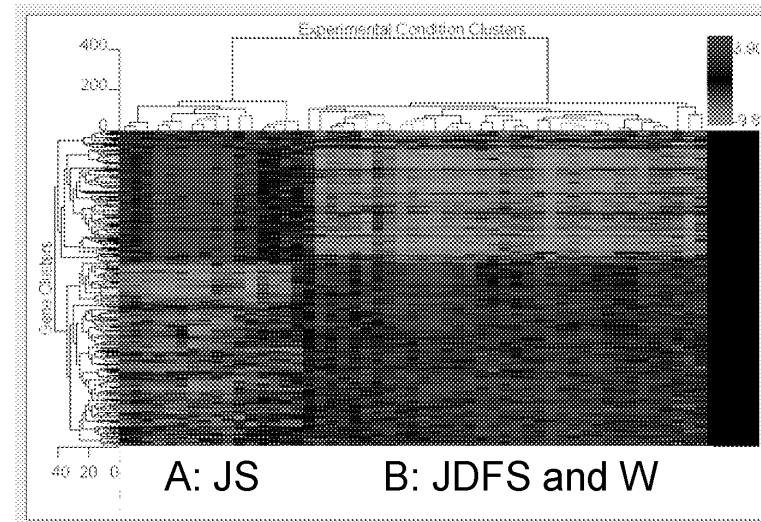
We have amassed tissue samples from both outmigrating smolts (>2000 fish) and return migrating adults (>1000 fish) that include multiple stocks (determined already through stock ID) of sockeye, chinook and coho salmon from BC and Washington State. We will screen a subset of these using the primers developed for the pathogen, once determined.

Brain: migratory route--outside or inside?

2003 migration



2005 migration



2 distinct profiles

correlate with ocean route taken
to reach river
>1000 km from the river--spawning

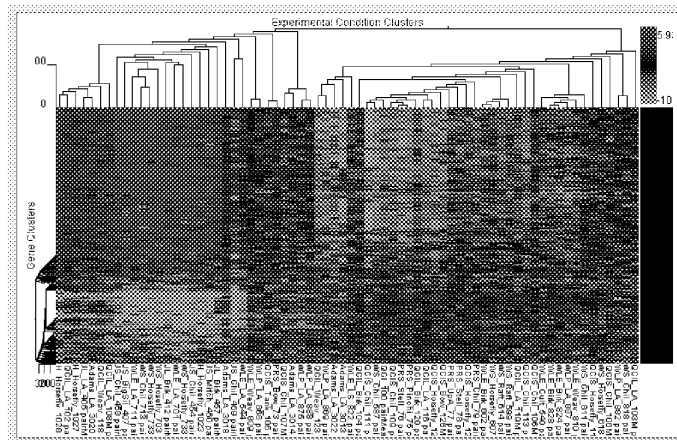
Coastal versus offshore routes?

15

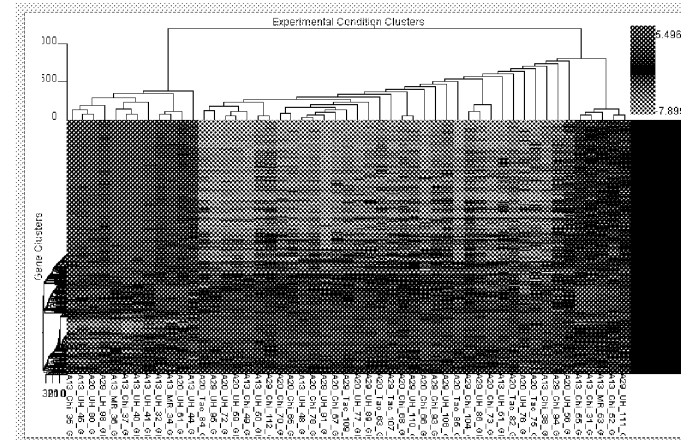
Brain Diversion

What if we import the 1,744 W Fate list to other tissues?

2003 BRAIN



2005 BRAIN



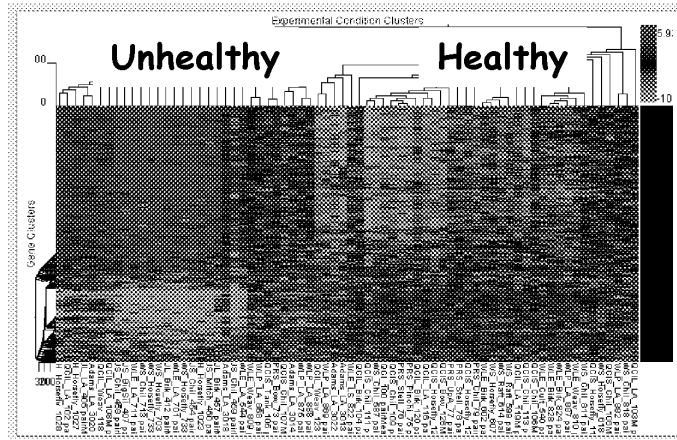
Two highly distinct profiles of fish in both years
>1,000 genes overlap with "diversion" gene list

100% correspondence between fate and diversion-related
physiological relationships (previous "A" profile = unhealthy)

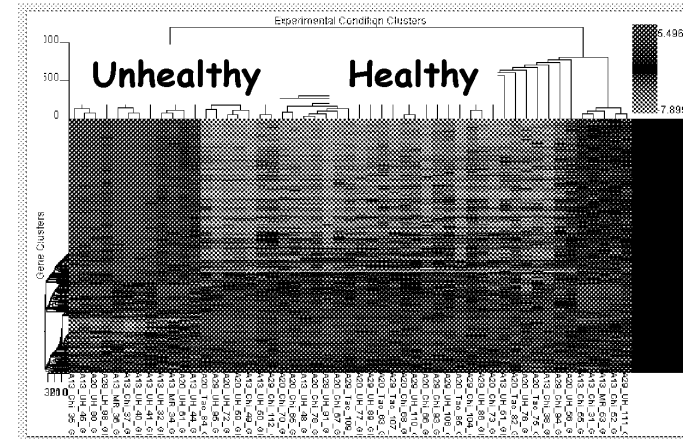
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2003 BRAIN



2005 BRAIN



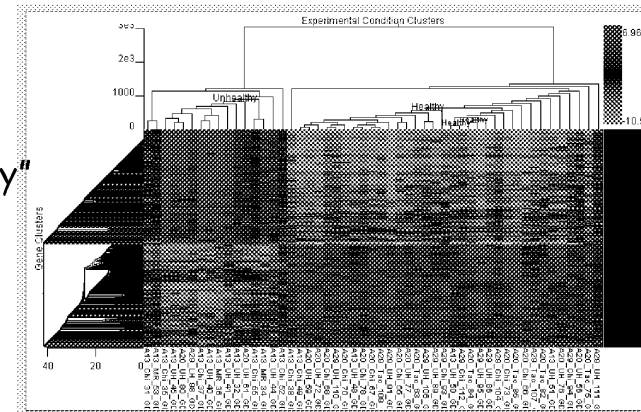
Signal even greater when two physiologies directly compared



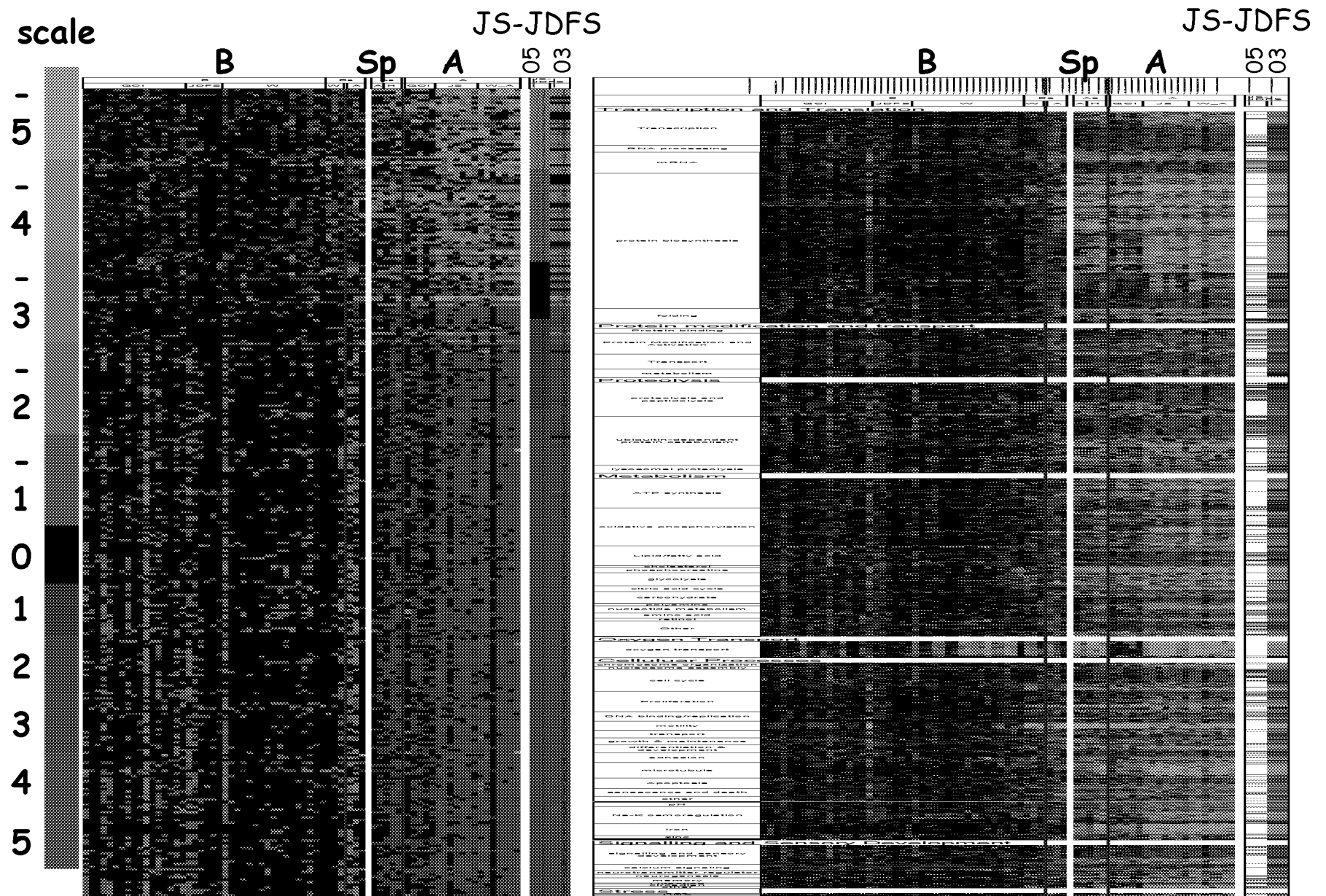
4608 genes sig at Holms 0.001
3468 at 10^{-10}

Unlike gill, fish with "unhealthy" brain profile are not changing transcriptionally as much as those with "healthy" Profile—635 genes QCI-spawning vs. 1401 at $P < 0.001$
Most sig change of Healthy W-Spawning—maturation

Unhealthy Whonnock Fish in 2003 were the bleeders!

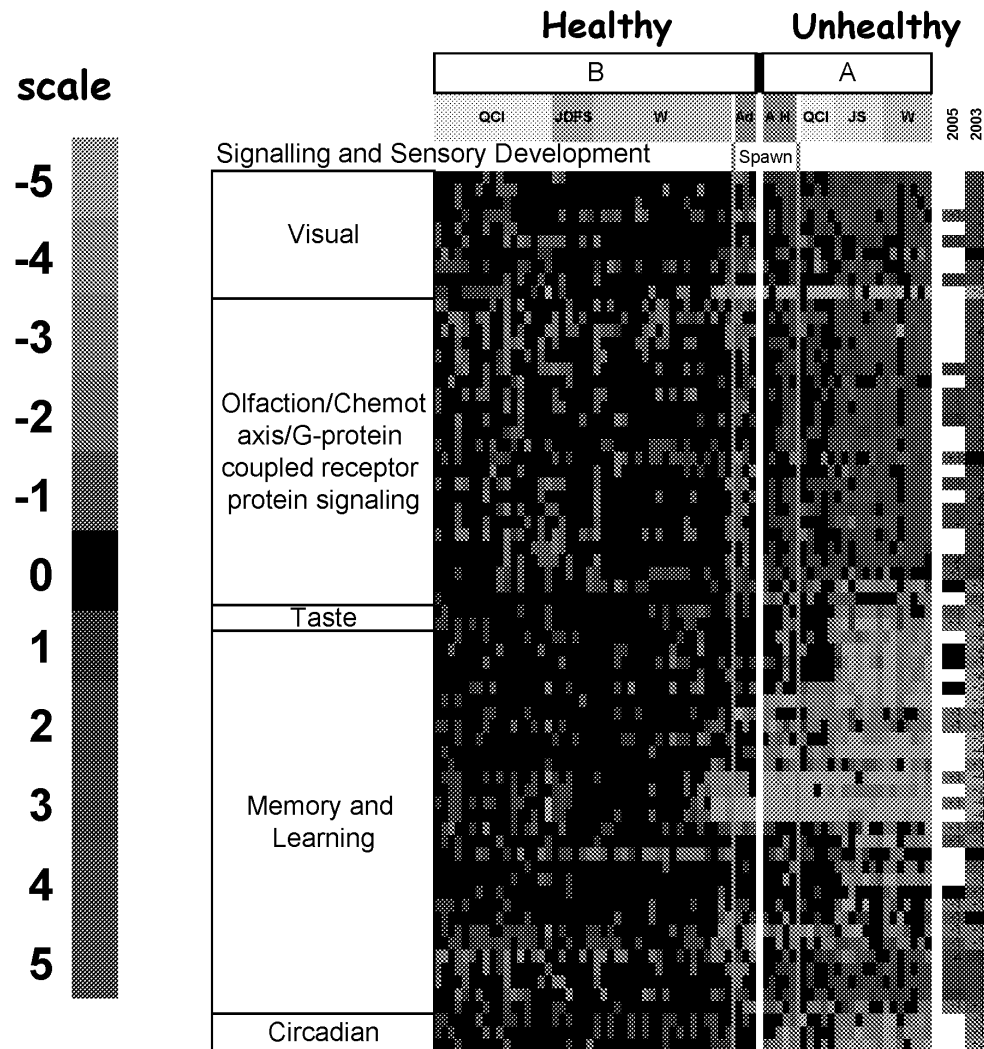


High degree of overlap between years



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Inside vs outside migration routes: Different navigational strategies



Unhealthy Profile:

Up-regulates sensory cueing system
visual and olfaction

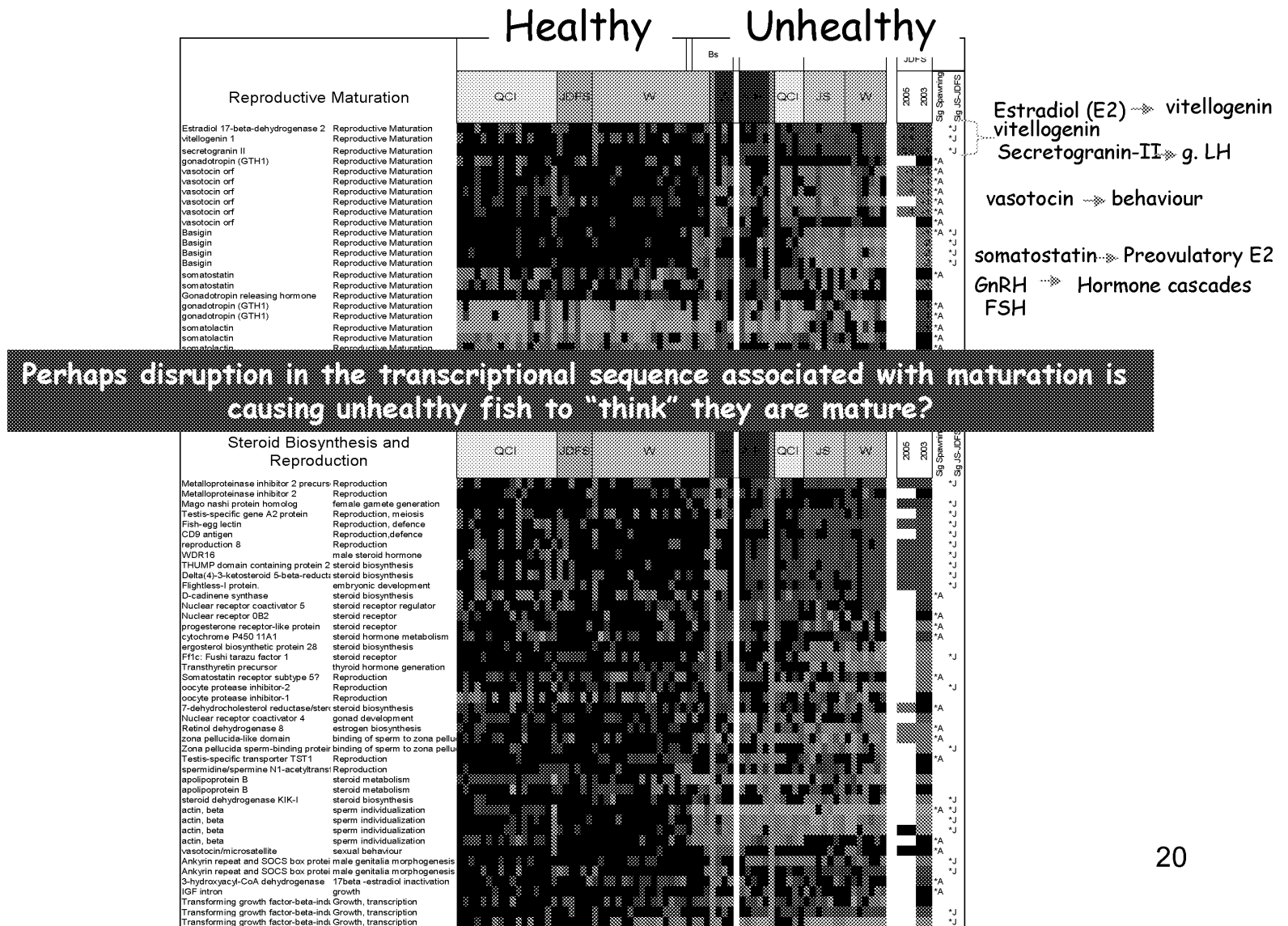
Healthy Profile:

Utilizes long term memory
and circadian rhythm (sun-compass
system?)

**Could these shifts be
the effects of SLV and
Brain neoplasms?**

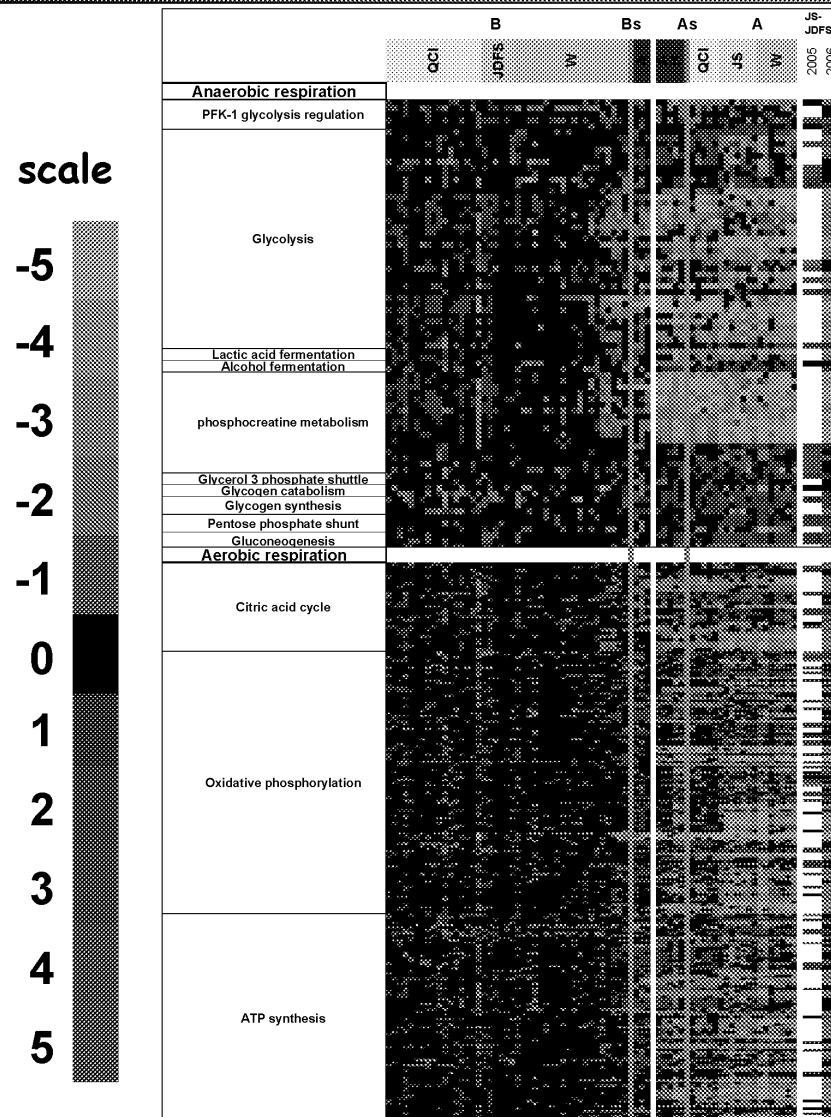
Reproductive Maturation

Unhealthy assoc with mature profile

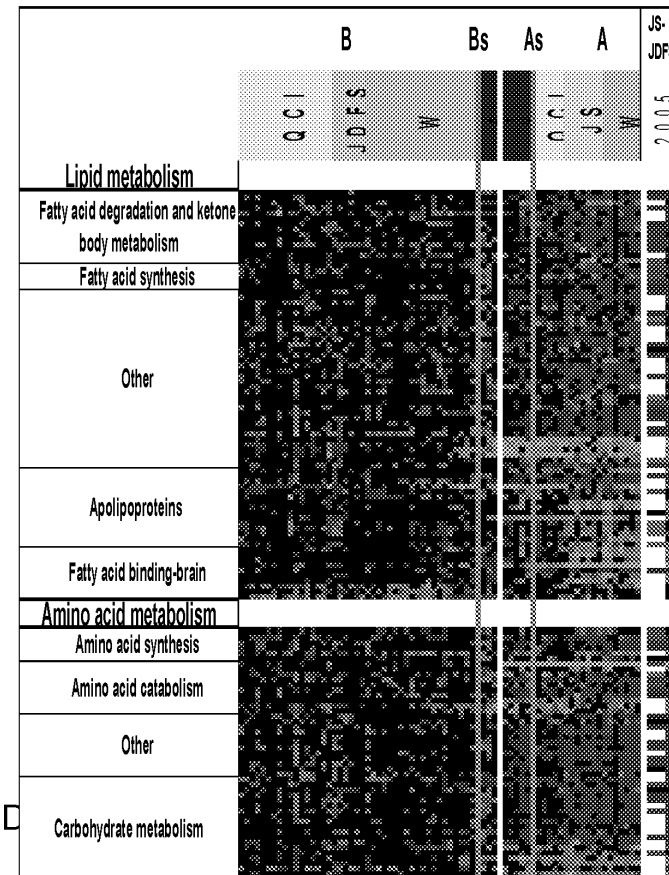


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\Genome BC Project--2008\SEF_Bug Hunt\BugHunt_Key
Slides.ppt

Unique metabolic processes associated with stimulation of different regions of the brain



Unhealthy: stimulation of biosynthetic processes
(reproductive hormones, olfactory and visual proteins, all require lipids)
Healthy: fuel for neural processing (Memory)



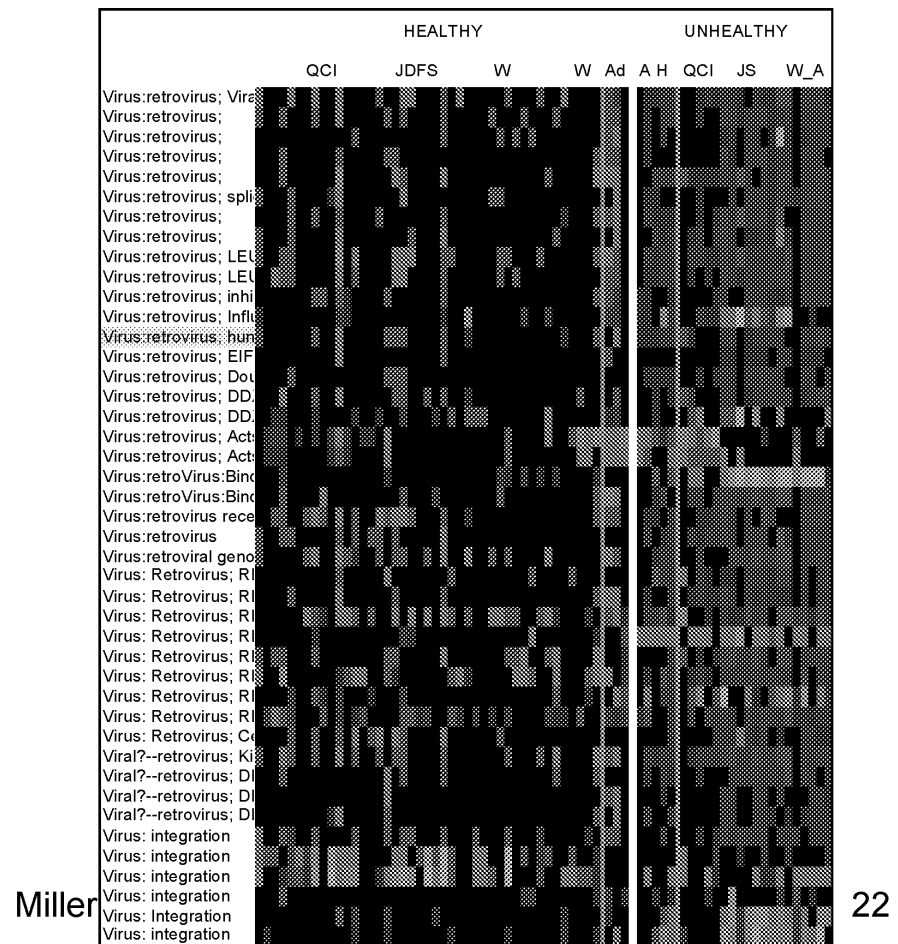
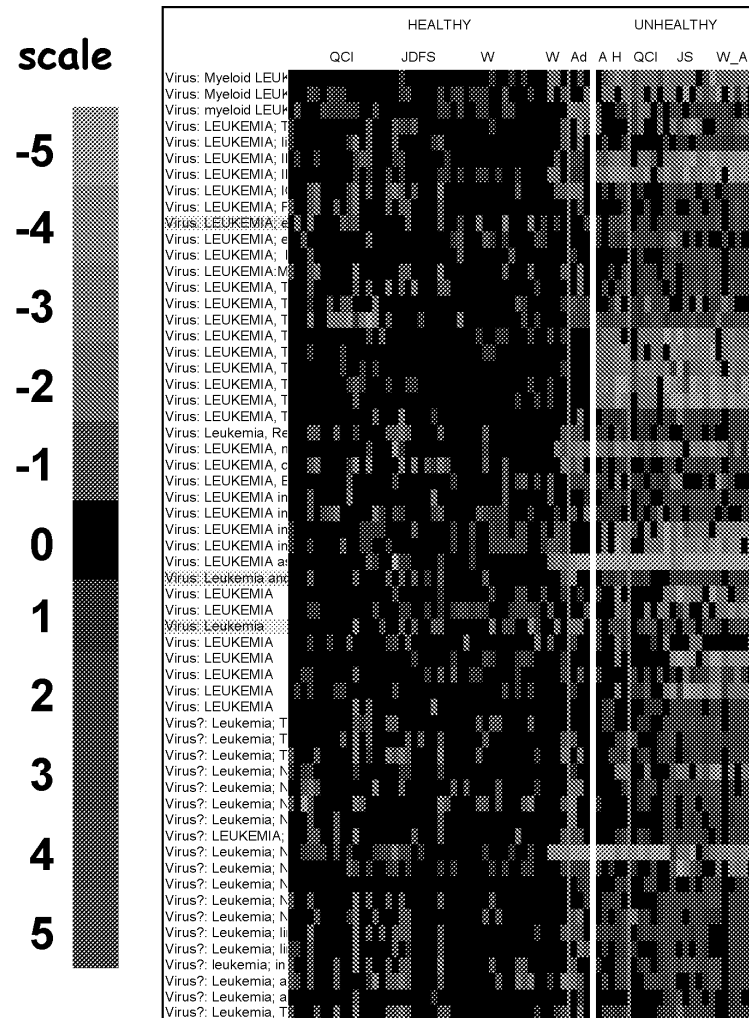
Miller-D

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The Unhealthy Brains—response to retrovirus? Leukemia virus?

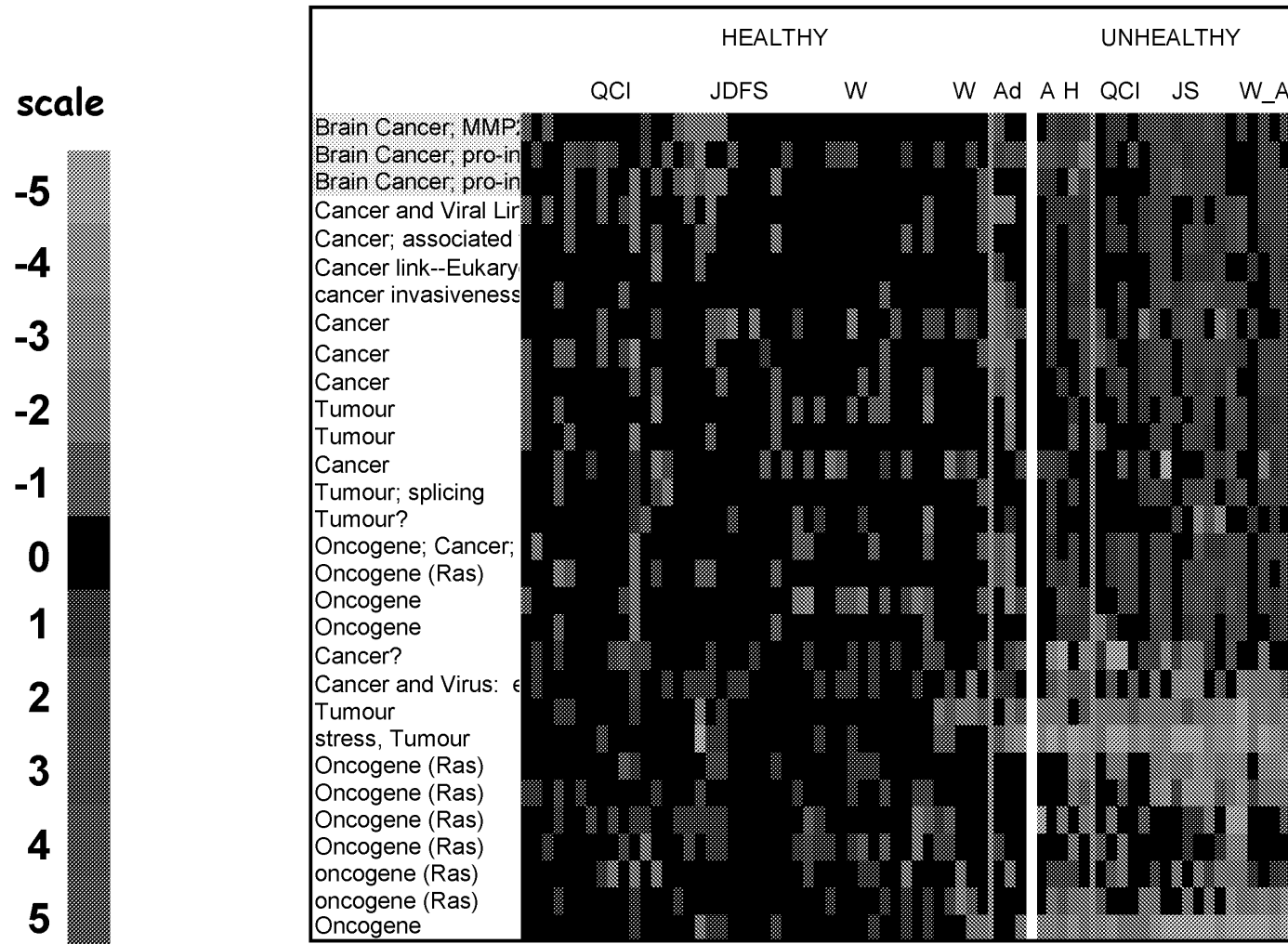
40 Genes co-opted by Leukemia viruses
20 Genes within co-opted pathways

>40 Genes involved in other retroviral infections



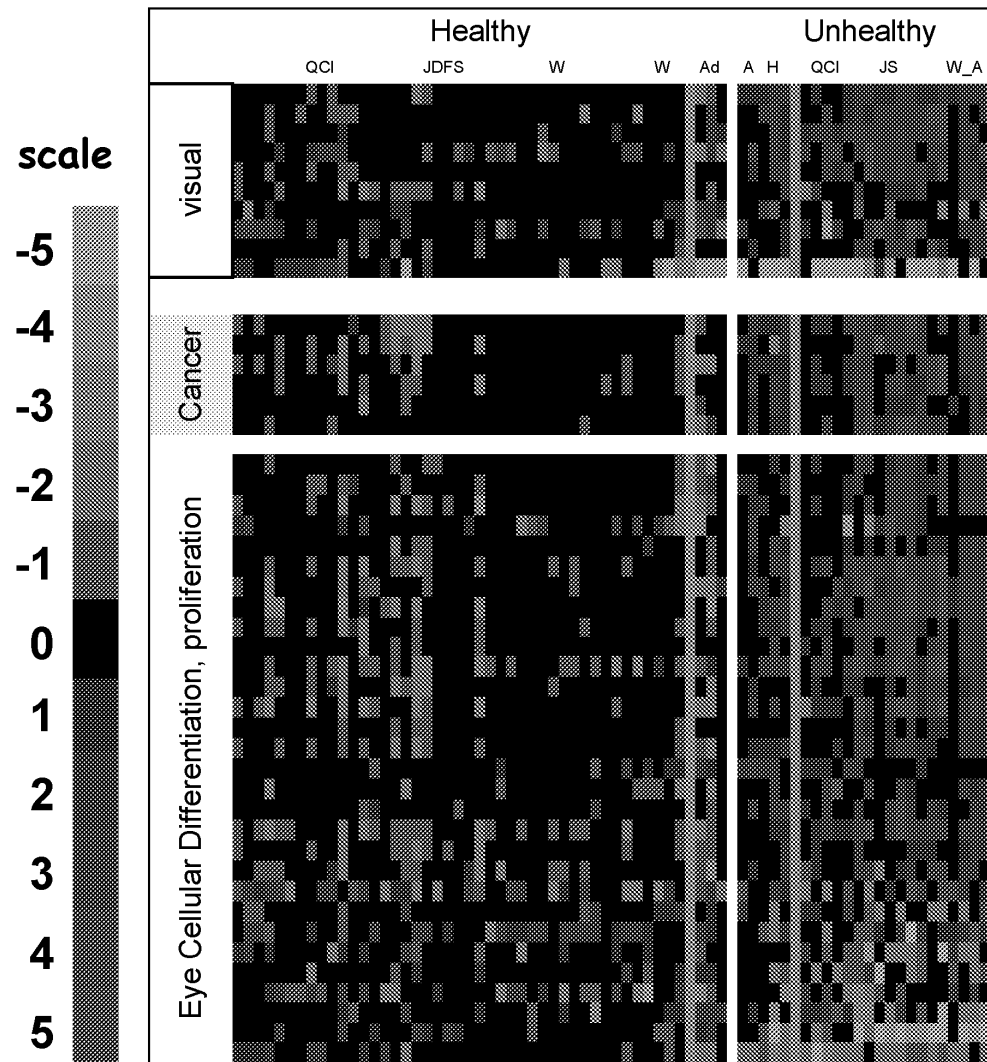
The Unhealthy Fish -- Links to Brain Cancer?

30 Genes linked with cancer, 3 BRAIN cancer



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SLV Isolated from eye tumours



Is the shift from memory to sensory homing mechanisms due to an ocular brain tumour?

Do these transcriptional shifts really result in differences in the way salmon navigate?

Does the virus kill memory cells, as in many viral infections of the brain resulting in reduced memory related transcription?

Are these profiles related to behavioural shifts?

Are the brain and gill profiles correlated within the same fish?

ONLY have data from 2005 where both tissues run (non-destructive sampling)

Only 14% (5) of gills were UH, 25% (13) of brains

Only 1 of the 5 individuals with unhealthy had unhealthy brain profile

Unhealthy brain profile almost exclusively observed in JS (1 W, 2 JDFS)

Did fish with unhealthy profile brains not make to the river?

If this is Marine Salmon Anemia, is the brain profile linked with the virus and the gill the microsporidian parasite?

if so, the virus may have been resident in the salmon for months or years (could be vertically transmitted), with exposure to the microsporidian upon arrival to the coast

Fits with “unchanging” brain profile and rapidly progressing gill response

As the two infections are histologically inseparable, perhaps the salmon respond transcriptionally in a very similar way

Screening with molecular markers for each would answer this question

**IF Brain profiles were linked with shifts in entry timing,
four potential Ho on why fish enter the river early**

1) Osmotic preparation

Unhealthy Fish enter FW faster because they can no longer tolerate SW

2) Maturation

Unhealthy Fish THINK that they are mature (increased transcription of estradiol, vitellogenin, Secretogranin-II - high even in QCI)

3) Senescent, Sick and Stressed

Enhanced senescence signals in unhealthy fish
Could be compounded by osmotic sensitivity

4) Shift in navigational system—memory to sensory causes enhanced sensitivity to FW Cues

Note: unhealthy fish migrate at the same speed as healthy fish in FW

If the virus is temperature sensitive, infected fish may move coastal as a result. Perhaps FW cues start the "senescence clock"?

Do Unhealthy Fish enter FW faster because they can no longer tolerate SW?

Osmotic Genes Gill			Most UH	Mod UH	Intermed	Healthy
Gene ID	Osmotic Function	Fold Change				
Na+/K+ ATPase subunit alpha-1c	salt pump--FW form	1.24				
Salmo salar hyperosmotic protein 21 (Shop21)	gill restructuring for SW and FW entry	1.73				
Na+/K+ ATPase beta 1a polypeptide	salt pump	-1.34				
Na+/K+ ATPase betaATPase beta-233	salt pump	-1.39				
Chloride intracellular channel protein 5	choride channel	-1.54				
hyperosmotic glycine rich protein [Salmo salar]	High Salinity responsive	-1.13				
hyperosmotic glycine rich protein [Salmo salar]	High Salinity responsive	-1.28				
Cold-inducible RNA-binding protein	High Salinity responsive	-1.06				
cold inducible RNA binding protein	High Salinity responsive	-1.5				
cold inducible RNA binding protein	High Salinity responsive	-1.51				
cold inducible RNA binding protein	High Salinity responsive	-1.58				
cold inducible RNA binding protein	High Salinity responsive	-1.78				

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While SLV or "marine salmon anemia" may not kill fish outright, it may reduce their fitness by causing:

- Increased sensitivity to high temperature
- Poor blood coagulation—observed in 2003 in fish with UH liver/brain profiles
 - Could reduce survivorship from catch release fisheries, increase susceptibility to fungal infection
 - Known to be associated with myeloblastic leukemia both in early stages and with solid tumours
- Disruption on osmoregulatory capacity in SW
 - Decreased survival in SW for smolts and adults
 - Highest mortalities with salmon anemia on farms when they first move smolts to SW
- Potential for Vertical Transmission—effects on Smolts
 - Vertical transmission common route of infection for retroviruses, gained either through exogenous means or through integration into the genome
 - Could be used as a means of control in hatcheries—testing broodstock first
- Potential for Horizontal Transmission—especially in high densities
 - Hatchery effects (for other Pacific salmon species)
- Increase susceptibility to other pathogens
 - Well supported literature on this, but not our data directly, yet...
- Tumour development could impact behaviour
 - increase in coastal migrations, earlier river entry?
- Increased straying due to loss of memory cells
- Disruption in circadian rhythm—wake/sleep cycle—tired fish?
- MULTIPLE SALMON SPECIES MAY BE AFFECTED!

Where to go from here: Upcoming "fate" study

- Is there enhanced Pre-spawning mortality of Unhealthy Fish?
 - Kim's fish: 2006 Egg retention and longevity in females study at Weaver (gill)
 - David's PSM and spawning samples in 2006, 2007, 2008? Multiple stocks-g/b
- Temperature response of Unhealthy fish (not treated with fungicide)
 - More fish from Savona? Or other High water temperature regions of the river
- Prevalence of Unhealthy profiles in additional years—2007 and 2008
 - 60 fish from 2007 radio-tagging could be used to link with fate in another year, but few morts and small numbers from range of stocks
 - Cooke's 2008 tagging study?
 - very high en route and PSM in 2008--Stuart, Nadina, Chilliwick from W, Weaver/Harrison from Ken's (David collected), multiple spawning grounds samples
- Association with Marine losses in 2006—run fish that did not make it to river
- Association between gill and brain profiles
 - Correlation non-existent for 2005 in part because brain signal exclusive of JS
 - Have brain and gill for adults collected in river in 2007 and 2008, and JDFS in 2008, but no JS.
- Smolts
- Other drainages? No samples that I know of—perhaps in marine mixed stocks?
- Other species—2007 David collected 70 Chinook/40 coho, QCI 2008—47 chinook, 30 coho
- Most of this could also be accomplished with molecular markers