



Genomic studies suggest that a novel disease is affecting sockeye and may be an important contributor to the Fraser River sockeye situation

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Functional Genomics: Assess Salmon Condition and Predict Fitness

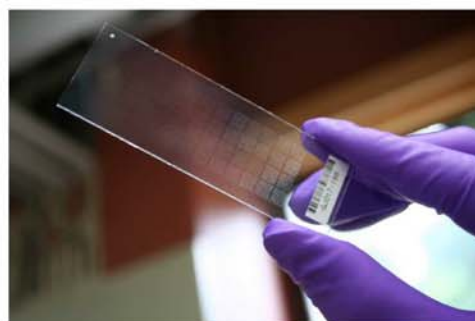
Genome-wide scan of physiology

3 Salmonid Arrays

16K cDNA array

32K cDNA array

4x44K oligo array



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



Discovery of a Mortality-Related Signature (MRS) in Adult salmon

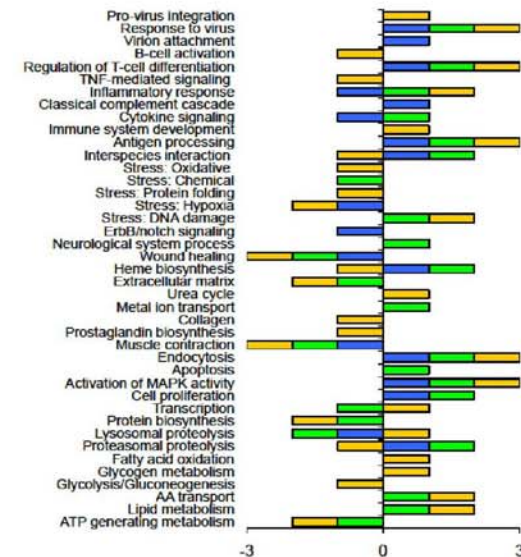
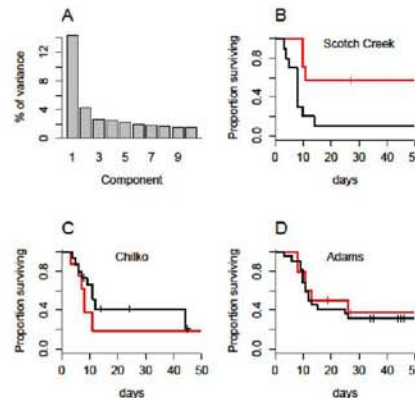
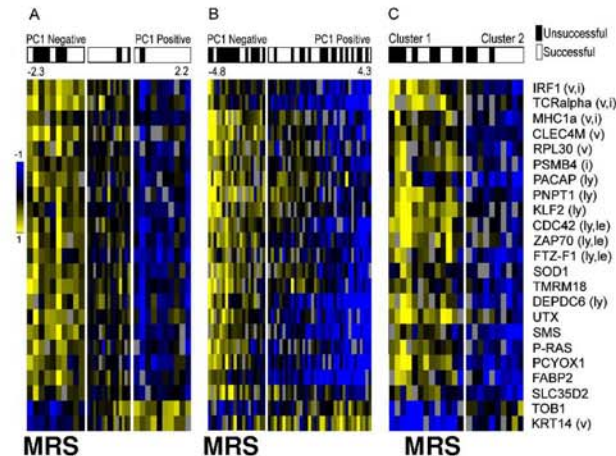
16K cDNA array



Published in Science
Jan. 14, 2011

This study identifies the same mortality-related signature (MRS) in gill tissue from 3 independent tagging studies

Adult migrants were physiologically compromised before entering the river



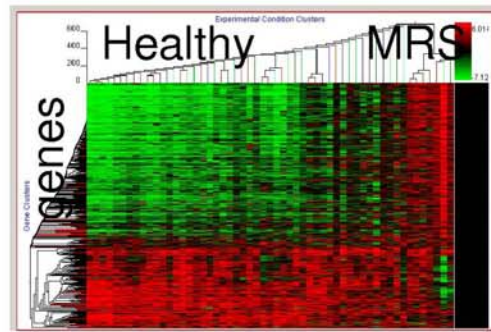
We hypothesized that the MRS may be a response to a viral infection



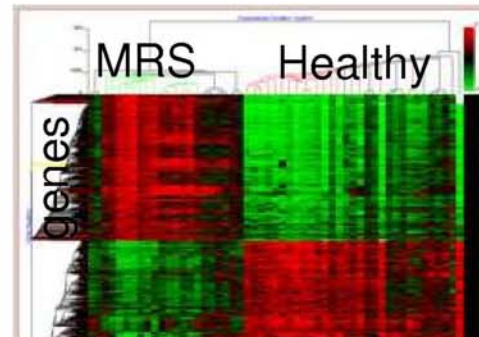
MRS profile present in at least three tissues (liver, brain, gill), with high overall prevalence rates

Scierocinski et al. in prep

Profiles in Adult salmon returning to the Fraser River in 2005



individuals



individuals



individuals

Gill: 13% prevalence
Early Stage

Liver: 40% prevalence
Advanced Stage

Brain: 30% prevalence
Advanced Stage

Note: The MRS is generally resolved as the first principle component in PCA, explaining the largest source of gene expression variance in these tissues

A fish can contain MRS in one tissue and not in another

Overall (three tissues combined) prevalence of MRS in 2005 was 75%

16K cDNA array

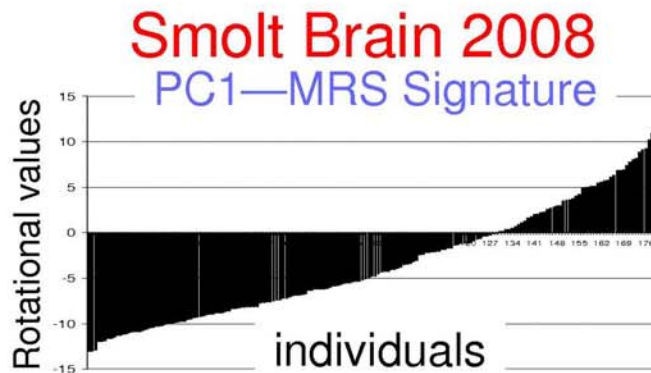
Red up-regulated Green down-regulated

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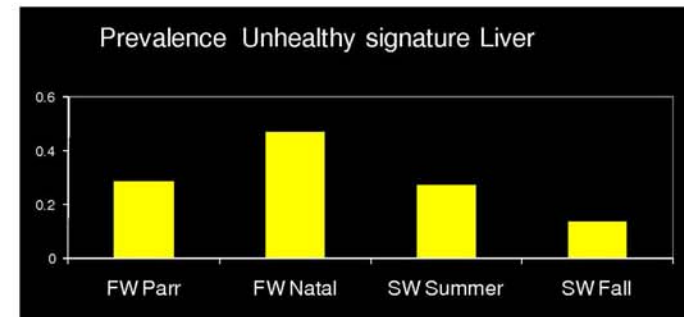
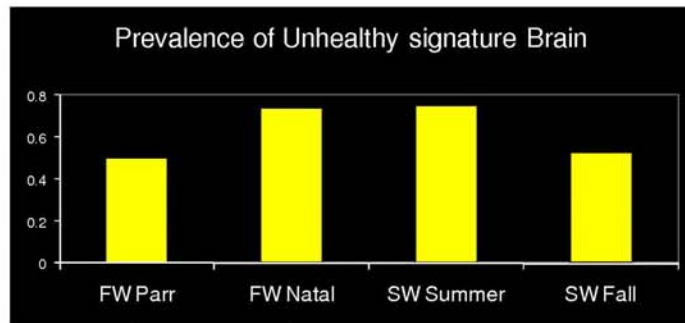
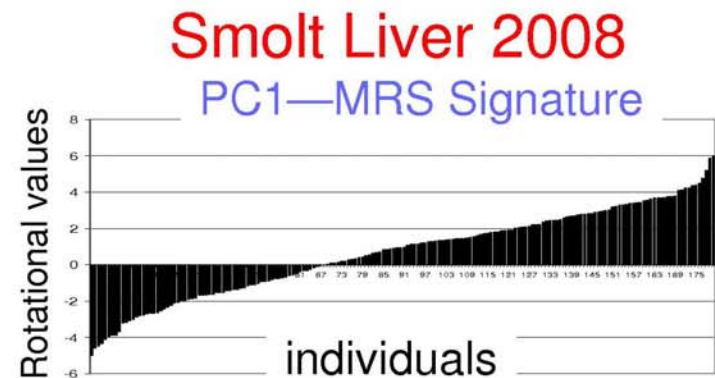
The same powerful MRS profile is present in smolts

Miller et al in prep



Healthy

MRS



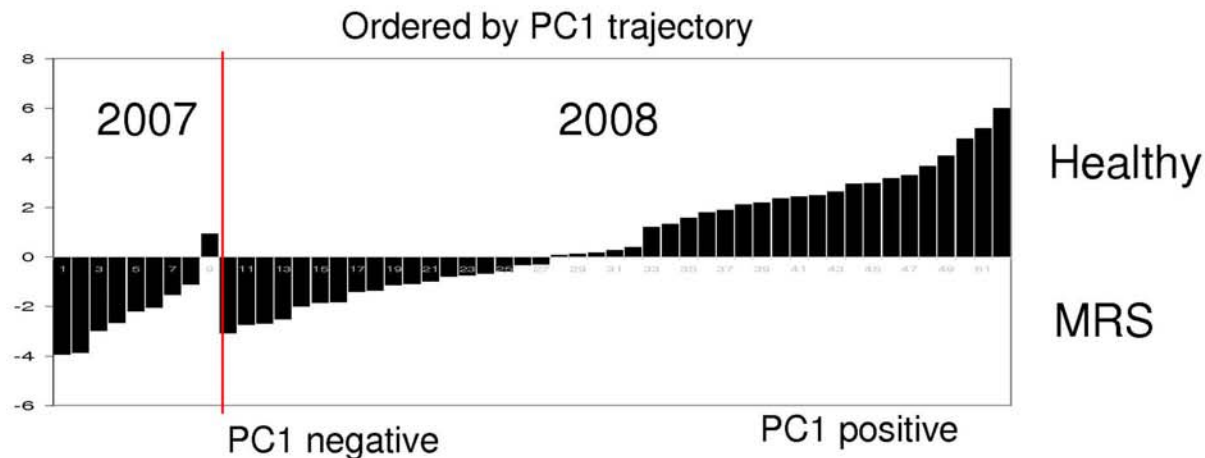
32K cDNA array

Smolts show signs of MRS signature well before they leave the river with prevalence rates that decrease during ocean residence



Contrast 2007 and 2008 sockeye smolts sampled in the ocean in June

Liver tissue



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

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

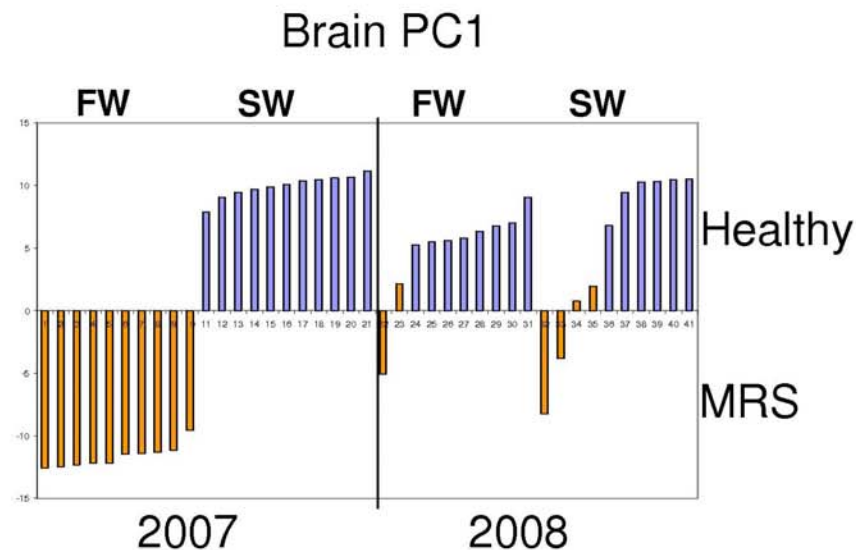
32K cDNA array



Contrast 2007 and 2008 sockeye smolts sampled in FW and in the ocean in June

Brain tissue

NEW DATA



All 2007 out-migrants carried a powerful MRS signature in FW, but none in SW in June—
Mortality-related shift in prevalence?

Could potentially explain a very large mortality event early in the ocean

Ho: Mortality associated with the MRS may depend, in part, on ocean conditions

4x44 Oligo Array

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

1. MRS fish show escalating immune response from SW to FW in gill

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

2. Effects on multiple tissues, but not necessarily all at once

Other kinds of stressors, e.g. toxins, high temperature, low oxygen, will consistently affect specific tissues whereas pathogens can affect a broad range of tissues, but not necessarily all at once

3. Functional signature

“Intracellular Immune Response” with 65% of affected biological processes consistent with viral activity

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

STAT1, MX, IFN, PRF1, TCR α , TAP2, MHCI classically associated 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

4. Viral Array

“MRS” tissue gave 6x higher intensity binding than “healthy” tissue

5. Temperature Holding Study--infectivity

Initial sampling indicated 35% of fish contained the “MRS” signature, after 1 week of holding, 69% MRS

All mortalities were MRS-positive, no surviving Adams fish were MRS positive, MRS Chilko survivors were mostly from lower temperature holding



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

4. We have already identified a potential *Parvicapsula* “signature”

Response to wounding, inflammatory response, stress response, cytokine production, blood coagulation, and homeostasis all up-regulated with *Parvicapsula*, not the viral-signature

Identification of a Novel Viral Sequence from MRS Positive Liver Tissue



NEW DATA

1. Sucrose Gradients on MRS positive tissues to concentrate enveloped viruses

Applied to liver and gill, with best results from liver tissue

2. 454 sequencing

- Sent RNA/DNA isolates from one smolt liver (river-sampled) and one adult liver (ocean-sampled)
- 300K reads per DNA and RNA isolates (combined individuals)
 - ~600K reads total with average length <300 bases
 - Aligned sequences into contigs and blasted sequencing databases to look for hits to viruses and to salmon (to remove known salmon sequences)

3. Strong hit to Parvovirus in DNA blast

One 2.2 kb DNA contig that contained 76 individual sequencing reads hit a parvovirus with a p-value of 6×10^{-63} ; no hit to salmon RNA or DNA databases

4. PCR primers developed to novel Salmon Parvovirus

Applied multiple PCR primer sets to determine whether the sequence was endogenous (within all salmon DNA) or only found in a subset of individuals and tissues (i.e. a virus)

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Of the 2 open reading frames of this virus, we have the full sequence of the first (REP) and partial sequence to the second (VP1)

[illegible]



What do we know about Parvoviruses?

- Small (5 kb), linear single-stranded DNA virus with base-paired ends
- Not previously described in fish, but present in mammals, lower vertebrates (birds and reptiles), shrimp and insects
- Not all family members are known to cause disease, but most affecting lower vertebrates have been associated disease and mortality. Most cause both acute and chronic disease.
- Highly virulent pandemics in dogs ongoing since the 1970's
- Some family members require co-infection with helper viruses to replicate (e.g. AAV)
- Replication often stimulated under conditions of cellular stress—including heat, exposure to toxins, UV irradiation, others...

NEW DATA

Potentially important role of environmental conditions on viral virulence?

- Often associated with inflammatory autoimmune diseases, anaemia and hematological abnormalities, hepatitis, and leukemia. Feeding, growth, and coordination also impacted.
- Known to target and replicate in the brain; associated with brain pathologies, behaviour
- Target rapidly dividing cells, which it can infect and kill, with generally most profound effects on the young



Similarities between Retroviral and Parvovirus Disease

NEW DATA

We had previously speculated that the purported virus associated with the MRS could be a retrovirus

- **Some parvoviruses can insert their genetic material into the host genome (i.e. become endogenous), a feature common to retroviruses.**
- **Both viral families require stimulation of the host DNA damage response for replication**
- **Members of both families are associated with strong immunosuppression of the host**
- **Members of both families can cause leukemia-like disease**
- **Both families have linkages with cancer, but while retroviruses can cause cancer, some parvoviruses have anti-cancer activities (as they target and kill rapidly dividing cells for their own replication)**
- **Both involved in latent infections; can remain inactive for years waiting for the right conditions to replicate**



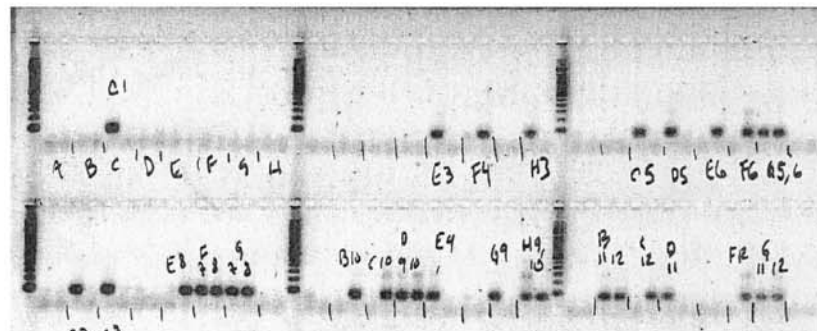
PCR of Parvovirus In Smolt and Adult Livers

NEW DATA

Year/Stage of Sampling	product size Amplification date	100 Feb 24-11		100 Feb 24-11		100 Mar 29-11		250 Mar 29-11		600 Mar 29-11		600 to 100 Mar 30-11		600 to 100 Mar 30-11		250 to 100 April 1-11	
		Rep60-2	Rep60-6	Rep60-6	Rep60-6	Rep60-6	Rep60-6	Rep60-6	Rep60-6	Rep60-6	Rep60-6	Rep60-6	Rep60-6	Rep60-6	Rep60-6	Rep60-6	Rep60-6
Smolt 2010	4736	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	4732	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	4740	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	4730	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	4733	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	4735	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	4746	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	4748	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	4754	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	4820	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	4860	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	4712	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	4870	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	4744	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	4738	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	4697	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	4713	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	4701	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	4737	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	4737	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	4502	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Adult 2009	3012	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3065	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3035	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3094	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3013	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3096	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3066	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3064	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3034	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3033	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Adult 2010	3124	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3136	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3120	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3047	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3135	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3090	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3126	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3127	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3048	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3043	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3121	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3130	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3093	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3042	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3045	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3046	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3009	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3005	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3084	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3091	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3040	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3092	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3094	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3007	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3041	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3049	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3006	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3088	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3122	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3123	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3125	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3126	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3127	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3129	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3131	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3132	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3133	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3134	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	3138	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P

Smolt amplifications

Early June in SOG



Adult amplifications

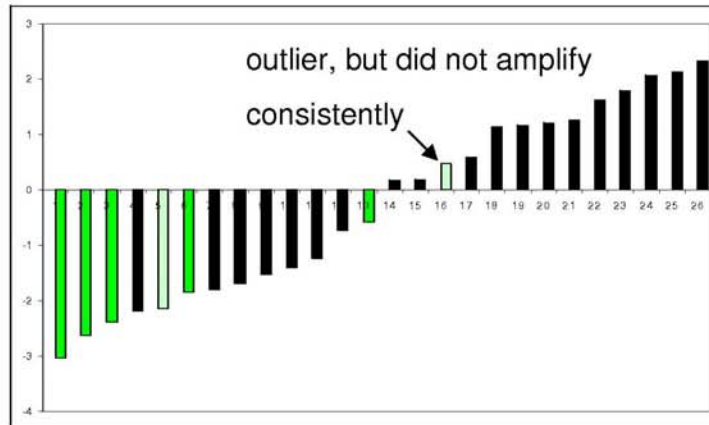
Marine and FW

Positives in both environments



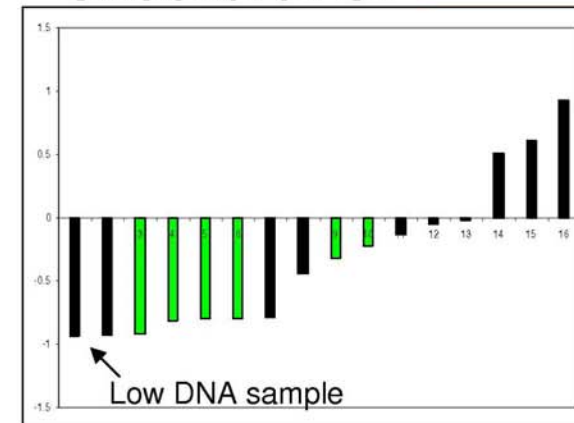
PCR of Parvovirus correlates with the PC1 MRS In Smolt and Adult Livers

Adult PC1



Smolt 2010 PC1

NEW DATA



Healthy

MRS

All but potentially one liver parvovirus positive was classified with genomics as containing the mortality-related signature (MRS)

If Parvovirus is found to be causative of the MRS signature, amplifications of the virus are not as sensitive as the genomic response of the host

This was also found to be the case with IHNV

Preliminary data indicate that intensity of infection (number of viruses/mg tissue) may be greater in smolts

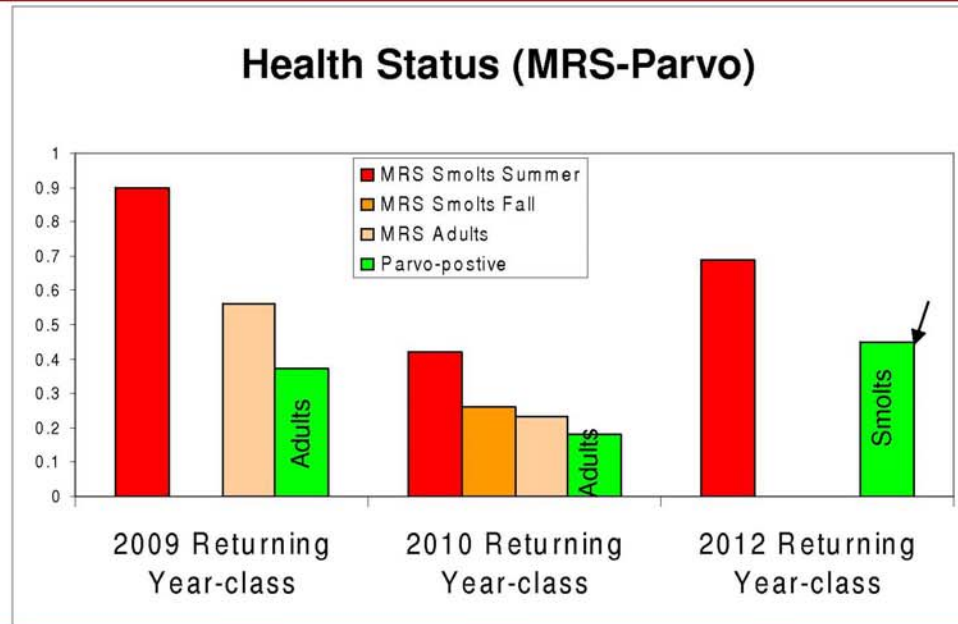
We have also amplified this virus in brain and gill tissue, but at a lower level and intensity

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Proportion of MRS-positive Salmon Livers Decreases During Ocean Residence

NEW DATA



2009 returning year-class smolts contained the highest proportion of MRS Livers

Decrease in prevalence of MRS livers during ocean residence—"Missing" MRS fish

Smolt to Adult Percentage "Missing" Fish: $(1 - \text{adult}^p / \text{smolt}^p) * \text{smolt}^p$

2009 returning Year-class 33.75% fish went "missing"

2010 returning Year-class 18.78% fish went "missing"

And these fish had not yet made it to spawning grounds...

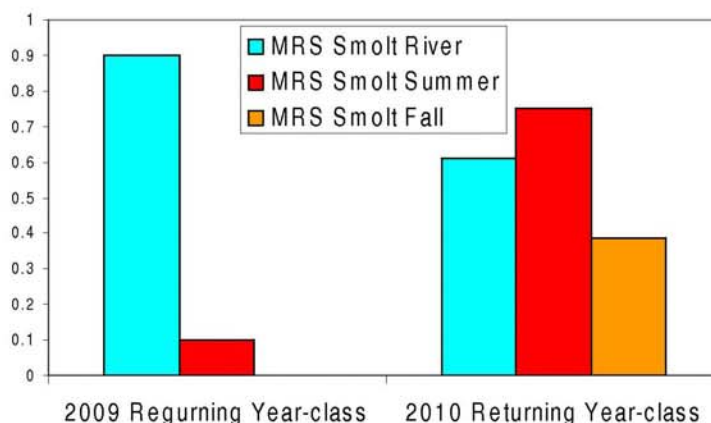
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Proportion of MRS-positive Salmon Brains Decreases During Ocean Residence, Faster in 2009 year-class smolts

NEW DATA

Health Status MRS



In 2009 year-class smolts, >90% of brains were MRS-positive before leaving the river
2 months later in the ocean (end of June), <10% were MRS-positive
If the disappearance of fish with MRS-positive brains was due to mortality (and not recovery or sampling artefact, which remains to be shown), this purported disease *could* account for the mortality of 80% of smolts by the end of June

Note in 2010 year-class, summer to fall decrease in MRS fish



Conclusions

Previous Report to PSC showed:

- 1) Discovery of a genomic signature correlated with fate of adult salmon in the river
(termed the Mortality-related signature or MRS, previously termed “unhealthy”) (Miller et al. 2011 Science)
- 2) Identification of MRS signature in multiple salmon tissues
Only a subset of tissues within an individual affected at once
Prevalence over all three of the tissues tested in 2005 returning adults was 75%
- 3) Discovery of MRS signature in smolts before they leave natal rearing areas
- 4) High prevalence of the MRS signature in smolts of 2009 returning year-class
- 5) **Hypothesized that this signature is associated with an intracellular pathogenic disease, most likely viral**

New Data show:

- 1) New data contrasting 2007-2008 outmigrating smolts indicates very high incidence of the MRS and severe immunosuppression in smolts of 2009 returning year-class
- 2) Discovery of a novel salmon virus in the Parvovirus Family
Amplifies in liver, brain and gill tissues (all tissues that are affected by our MRS genomic signature)
Preliminary data indicate potential correlation with MRS in liver, beginning analyses on other tissues

STILL REQUIRES FURTHER RESEARCH AND ANALYSIS



Next Steps....

- Identification and validation of viral origin of the MRS profile to satisfy Koch's revised 21st century postulates
- Full sequencing of novel parvovirus
- Molecular epidemiology of novel salmon parvovirus in the North Pacific to assess the evolutionary history of the virus
- Determine the etiology of the disease—routes of transmission, necessary conditions for viral replication, role of nutrition and impact of co-infections
- Determine the potential role of this disease in salmon declines
 - Range of stock/species affected
 - Establish relative role of mortality versus recovery and role of temperature and salinity on disease progression
 - Conduct further studies assessing in additional years the relative role of this disease versus other physiological factors in predicting year-class strength and spawning success
 - Determine the potential role of aquaculture and hatcheries



Matzinger's Damage Hypothesis

“While the immune response has several mechanisms to detect pathogens, immune response is not triggered unless there is also some evidence of tissue damage”

Our genomic IHNV data are consistent with this Ho: the genomic response of species that do not suffer significant disease (e.g. coho) or mortality (e.g. chum, chinook) from IHN virus is considerably diminished compared with those that do (e.g. Atlantics and sockeye).

Under this Ho, *if* the salmon parvovirus is causative of the MRS genomic signature, the strength of this signature, representing the largest source of physiological variation in many of our datasets, would imply that the virus is causing significant disease in salmon, and is not merely a benign endogenous virus.