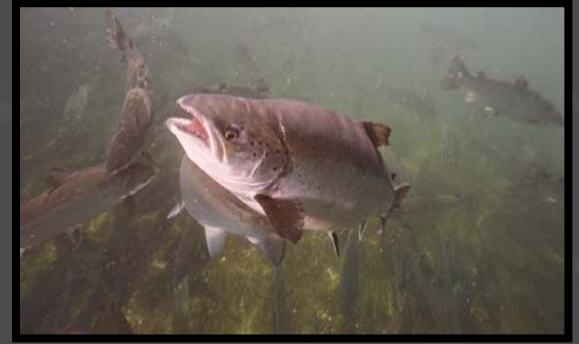




Atlantic salmon disease QTL: Application of RAD Tag Sequencing



Ross Houston



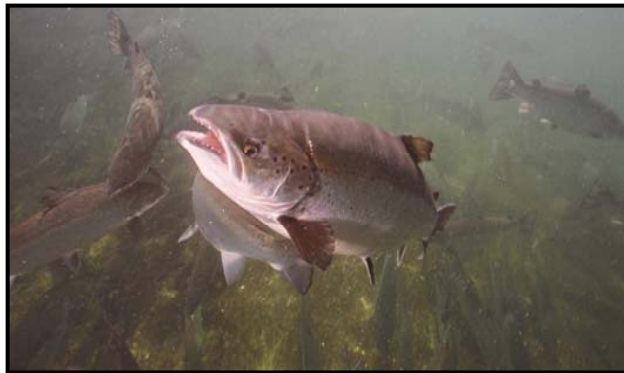
Outline

- **Introduction to Infectious Pancreatic Necrosis (IPN) in salmon**
- **Results to date:**
 - **Major QTL affecting host resistance**
 - freshwater lifecycle stage
 - seawater lifecycle stage
 - gene expression comparison alternative genotypes
- **Limitations in Atlantic salmon**
- **Plan for applying RAD Tag sequencing**



Atlantic salmon

- **Important aquaculture species (European production value > \$2billion)**
- **Hatched in freshwater**
- **Transferred to seawater ~ 1year**

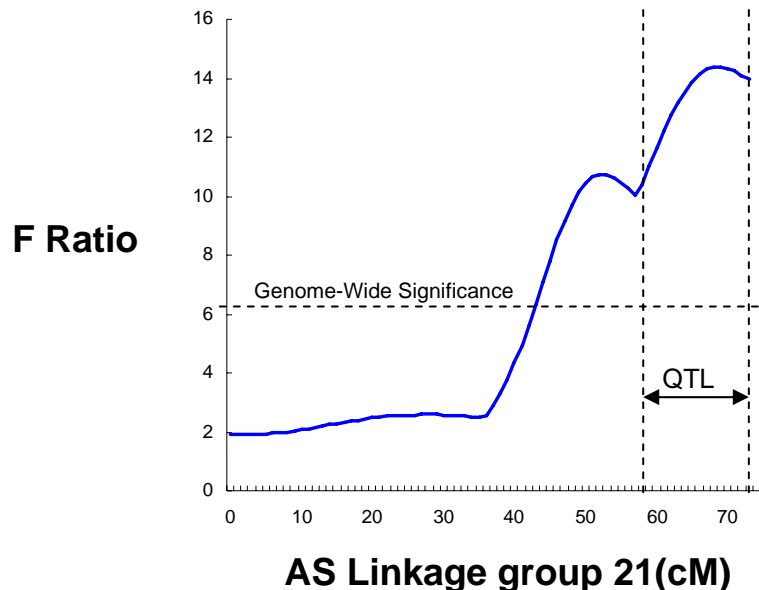


IPN in Salmon

- **Infectious viral disease**
- **Two specific windows of salmon lifecycle:**
- **Fry IPN**
 - salmon susceptible soon after hatching
 - mortalities up to 70%
- **Post-smolt IPN**
 - salmon susceptible soon after seawater transfer
 - mortalities up to 40%

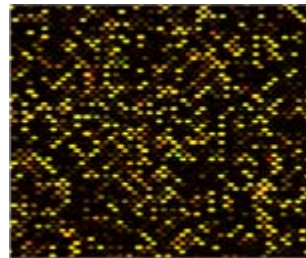
IPN in Salmon

- **Genetics of resistance in seawater**
 - moderate to high genetic component (30-50%)
 - single major locus on LG 21 overwhelmingly important



Freshwater IPN

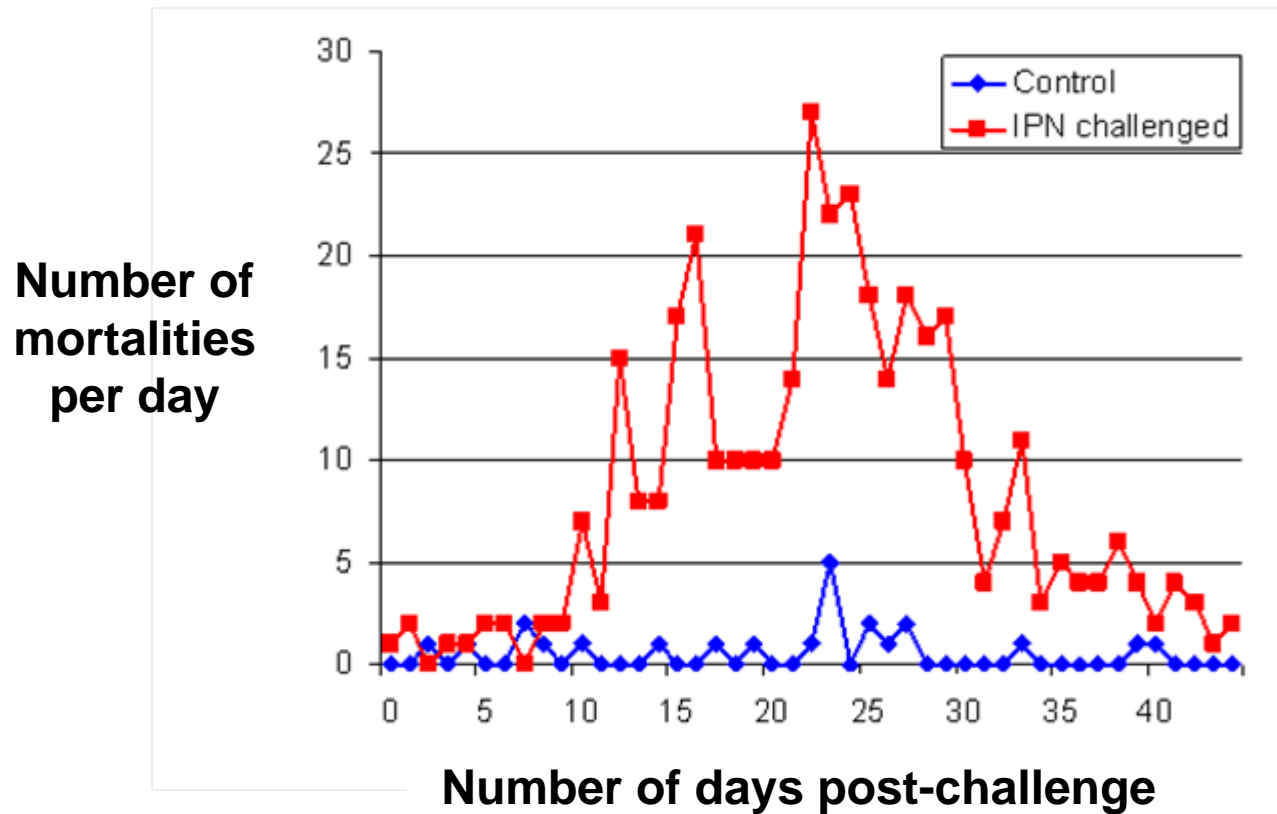
- **Ongoing project 2007-2010**
- **Aims:**
 - **Investigate genetic control of fry IPN resistance**
 - **Compare gene expression profiles of alternative genotypes**



IPNV Challenge Experiments

- 1. Genetic architecture of fry IPN resistance**
 - Heritability estimation and QTL mapping
- 2. Gene expression comparison**
 - resistant and susceptible families
- 3. Gene expression comparison**
 - alternative QTL genotypes within family

Daily Mortalities



Genetics Results

- **Polygenic heritability**
 - observed scale 0.26 (\pm 0.09)
 - underlying scale 0.55 (\pm 0.05)
- **QTL heritability (genotyped families only)**
 - h² QTL 0.18 (\pm 0.03)
 - h² Poly negligible
- **All genetic variation in ten genotyped families due to LG21 QTL!**

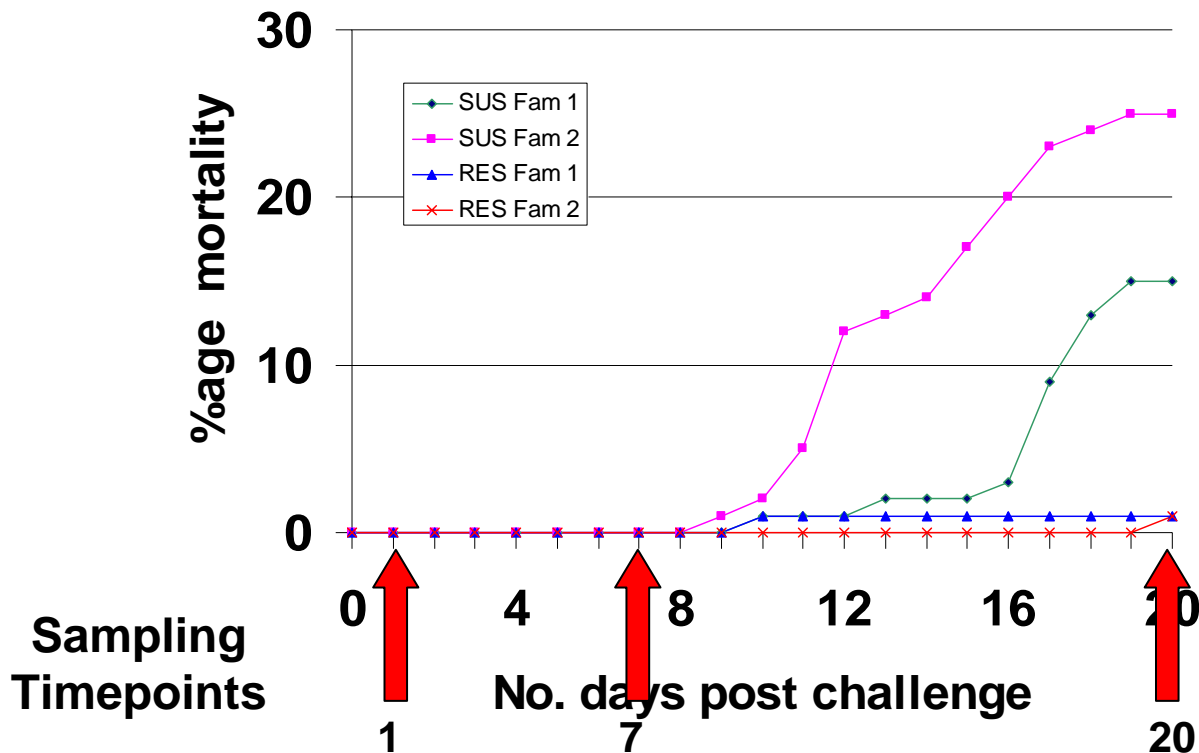
Mortality levels in fry with alternative QTL haplotypes

(four families with both parents heterozygous)

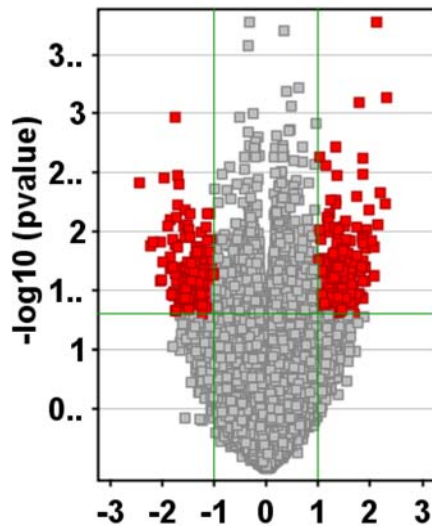
		Dam Haplotype	
		R	S
Sire Haplotype	R	0 / 73 (0 %)	1 / 74 (1 %)
	S	2 / 85 (2 %)	69 / 109 (63 %)

Experiment 2

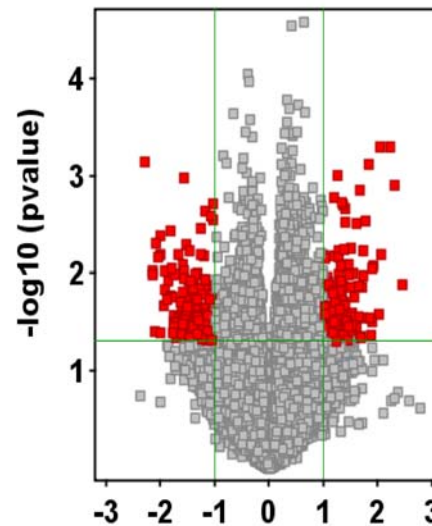
Cumulative Mortality



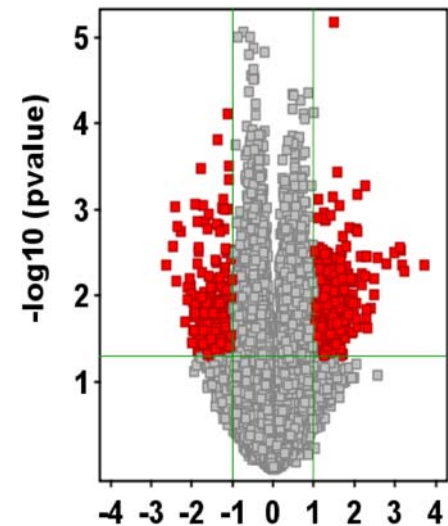
Transcriptomics Results: Resistant Families



1 day p.c.



7 day p.c.

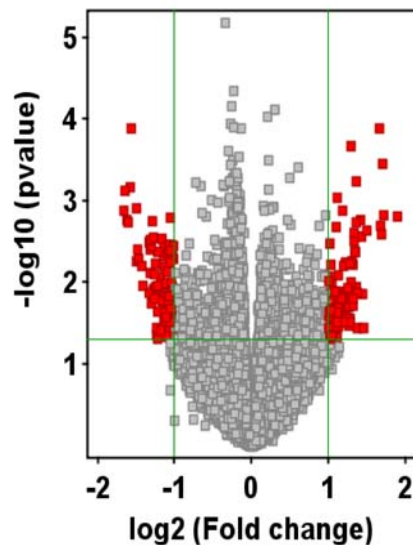


20 day p.c.

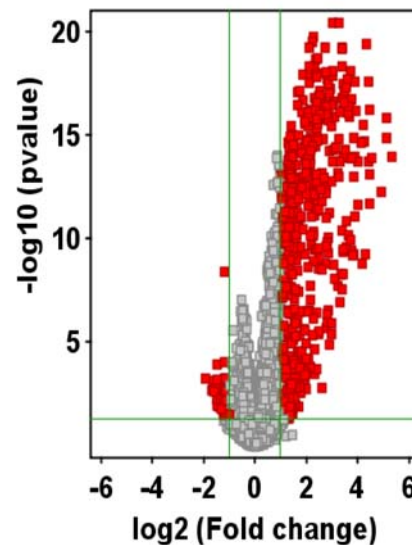
IPNV infected fish relative to same day controls

Significant expression ($P < 0.05$) with > 2 -fold change highlighted

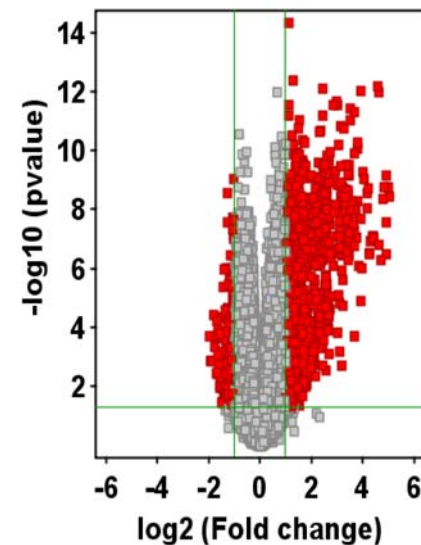
Transcriptomics Results: Susceptible Families



1 day p.c.



7 day p.c.



20 day p.c.

IPNV infected fish relative to same day controls

Significant expression ($P < 0.05$) with $>$ twofold change highlighted

Current Work

- **Gene Expression**
 - Microarray comparison of alternative QTL genotypes within-family
 - Pathway analysis of differentially regulated genes
- **Fine Mapping**
 - **Additional markers, especially SNPs**
 - Candidate genes from gene expression studies
- **Implementation**
 - Improved marker-assisted selection in breeding program



Limitations

- **Fine mapping QTL**
 - QTL in 3cM (~3MB) region
 - limited by lack of available markers and incomplete BAC contig coverage
- **Project to sequence salmon genome**
 - first draft due end 2010
- **Lack of closely related model species**

RAD Tag Application

- **Aims:**
 - **Generate SNPs with focus on QTL region**
 - linkage with QTL
 - linkage disequilibrium with QTL
 - **Further investigation of SNPs of interest with large scale genotyping**
 - need for SNP assay

RAD Tag Application

- QTL homozygous resistant and homozygous susceptible genotypes available across several families

		Dam Haplotype	
		R	S
Sire Haplotype	R	0 / 73 (0 %)	1 / 74 (1 %)
	S	2 / 85 (2 %)	69 / 109 (63 %)

RAD Tag Application

- **Atlantic salmon genome**
 - estimated $\sim 3 \times 10^9$ bp
 - RE with 8bp site
 - average distance between sites ~ 65 kb
 - expect ~ 92 K tags
 - **Partial genome duplication**

RAD Tag Application

- **Experimental design**
 - **LD markers**
 - pool ~6 unrelated RR individuals
 - pool ~6 unrelated SS individuals
 - look for SNPs matching QTL genotype across families
 - **Linkage markers**
 - several offspring of heterozygous parent
 - look for SNPs linked to QTL within family

RAD Tag Application

- **Questions/issues:**
 - **Obtaining sufficient sequence data to generate SNP assay for large scale genotyping?**
 - **Positioning markers on the genome**
 - **Duplicate regions**

Acknowledgments



**Steve Bishop
Chris Haley**



**Karim Gharbi
John Davey**



**John Taggart
James Bron
Brendan McAndrew
William Starkey**



**Almas Gheyas
Derrick Guy
Jose Mota-Velasco
Alastair Hamilton
Alan Tinch**



**David Verner-Jeffreys
Richard Paley
Georgina Rimmer
Ian Tew**