

Immune suppression in Atlantic salmon: smoltification, sea water transfer and breeding

Aleksei Krasnov, Nofima
FHF's fiskehelsesamling
1.-2. september 2015

Smoltification and breeding suppress
immunity in Atlantic salmon

There is space for improvement

Projects & methods

Multifactorial disease

Smolt brain

Fit Smolt

Collaboration with VI & NVH

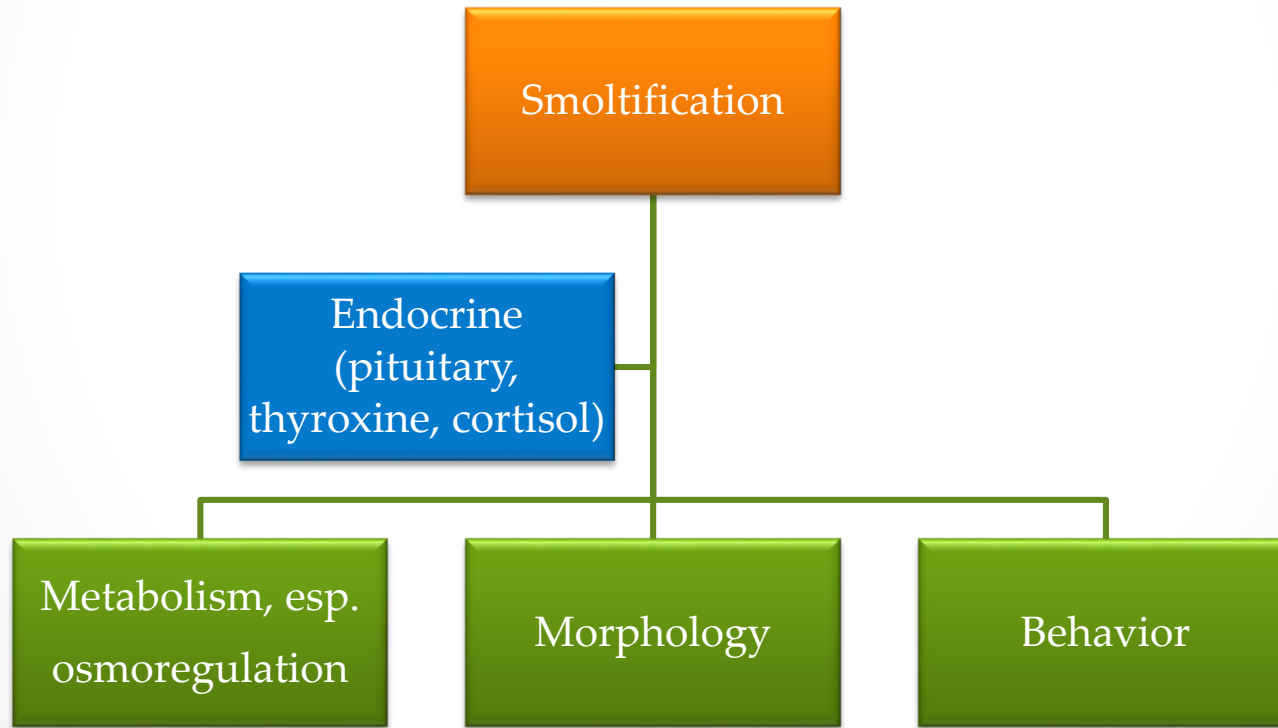
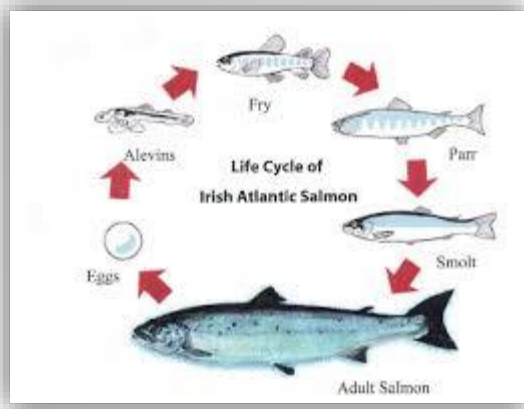
Transcriptomics – multiple gene expression profiling:

- Oligonucleotide microarray
- RNA-seq

Disease occurrence increases after SWT

Sea water transfer
IPN, PD, ISA, HSMI, CMS

Stress?
Pathogen pressure?
Management?
Immunity?



Smoltification and breeding suppress immunity in Atlantic salmon

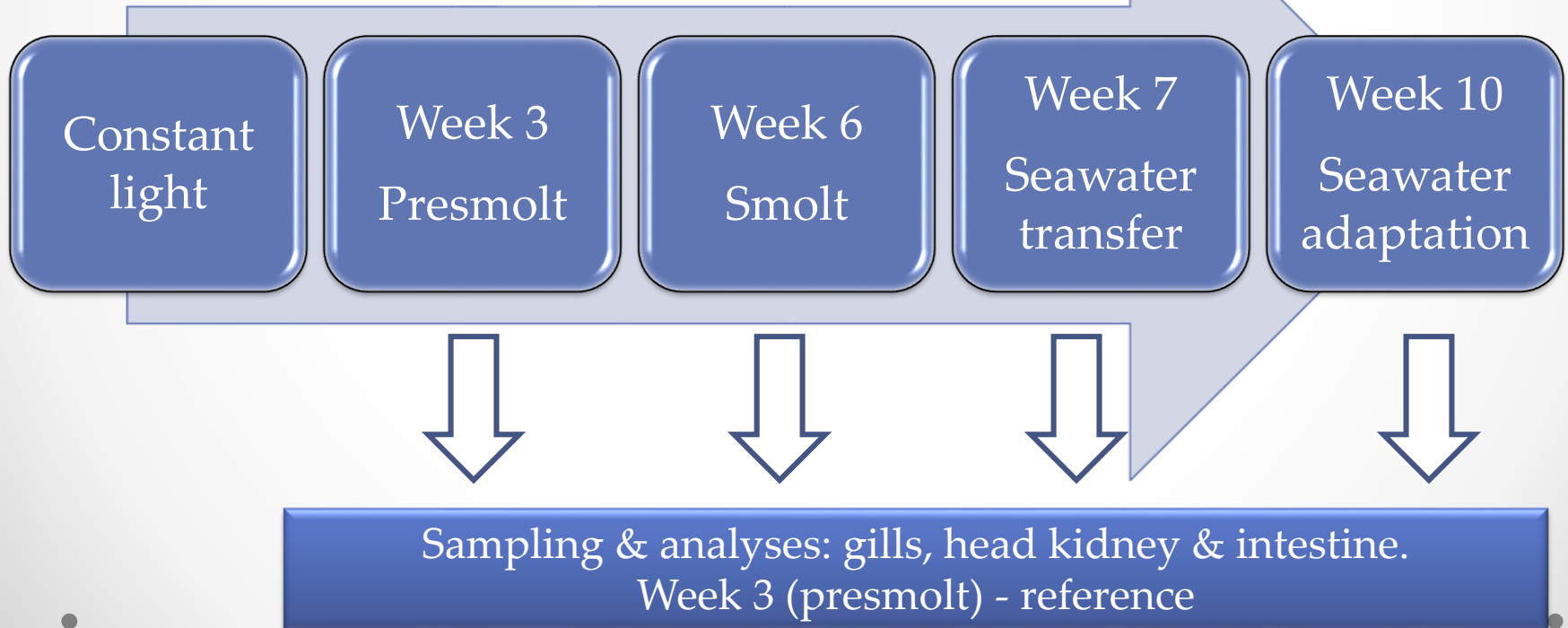
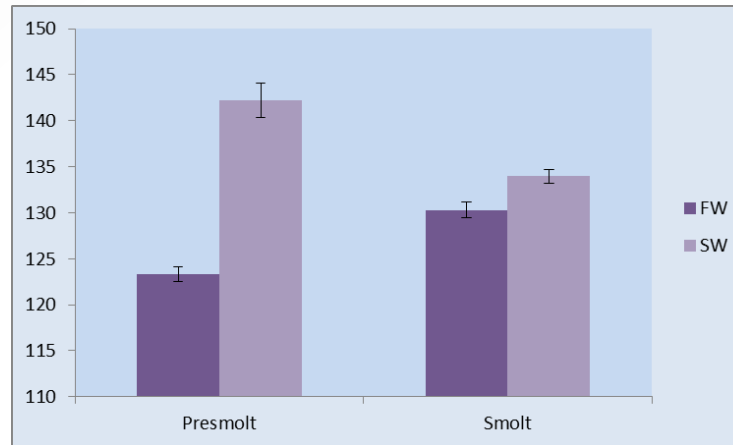
- Endocrine changes, osmoregulation & immunity need much resources
- Breeding allocates resources to growth
- Competition is not in favour of defence
- Immune suppression remained unknown

Study 1

Smoltification / SWT

FHF Multifactorial disease

FHF Multifactorial disease. Spring 2013

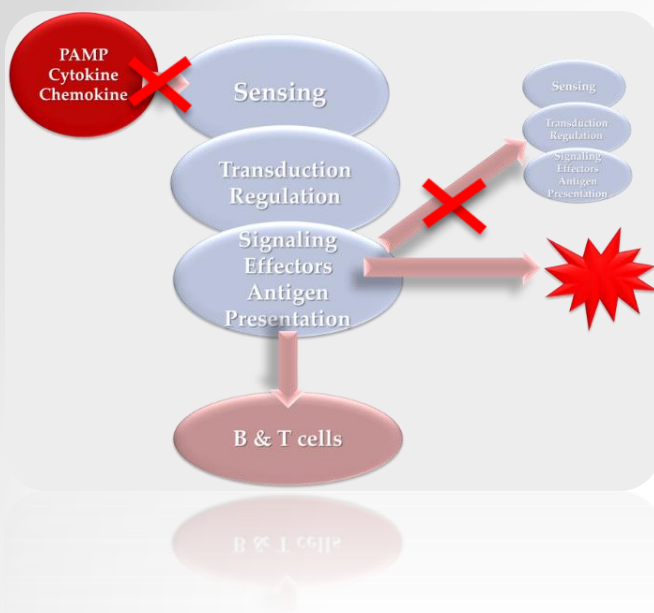


A red, three-dimensional oval with a slight shadow, containing white text.

56 genes up-
regulated

A green, three-dimensional oval with a slight shadow, containing white text.

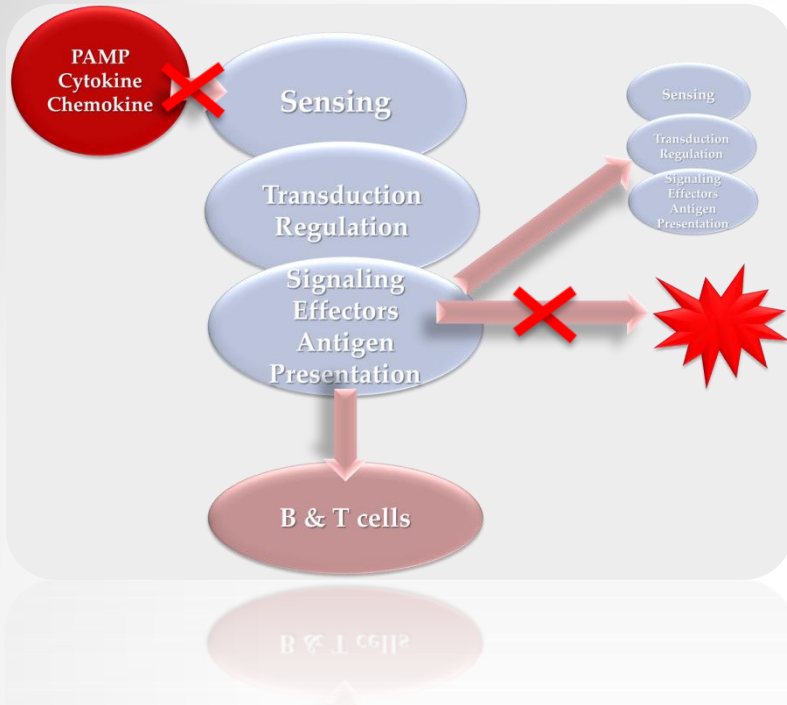
309 genes down-
regulated



Cell signaling
 Cytokines & receptors
 Chemokines and receptors
 Eicosanoid metabolism &
 signaling

Organ	Gene	Smolt	SWT1	SWT2
Gill	interleukin 17 isoform D-1	-0.78	-0.92	-1.06
Gill	interleukin-1 receptor antagonist	-0.89	-1.50	-1.68
Gill	interleukin-11	-1.45	-0.17	-0.08
Gill	Small inducible cytokine A13	-0.61	-2.09	-2.63
Head kidney	C-C motif chemokine 19 precursor	-0.02	-1.71	-2.50
Head kidney	C-C motif chemokine 20 precursor	-1.45	-2.39	-2.02
Head kidney	C-C motif chemokine 21 precursor	-0.12	-1.18	-2.16
Head kidney	C-X-C motif chemokine 10 precursor	-1.64	-2.40	-1.38
Head kidney	Interleukin-12 subunit beta precursor	0.01	-0.08	-1.73
intestine	C-C motif chemokine 20 precursor	-2.57	-1.72	-1.49
intestine	interleukin-1 receptor antagonist	-1.28	-1.01	-1.07
intestine	interleukin-1 receptor type II	-0.69	-1.11	-1.60
intestine	Interleukin-17 receptor A precursor	-1.05	-0.81	-0.38
intestine	Small inducible cytokine A13	-1.35	-0.49	-1.69
intestine	Cytochrome P450 4F3	-1.36	-1.36	-0.51
intestine	Cytochrome P450 2K5	-1.06	-1.12	-1.20
Head kidney	Microsomal prostaglandin E synthase 1	-0.69	-1.12	-0.30
Gill	Cytochrome P450 4F3	-1.08	0.03	-0.40
Head kidney	Arachidonate 5-lipoxygenase	-1.00	-0.88	-0.74

Gill – 33
 Head kidney – 19
 Intestine - 30



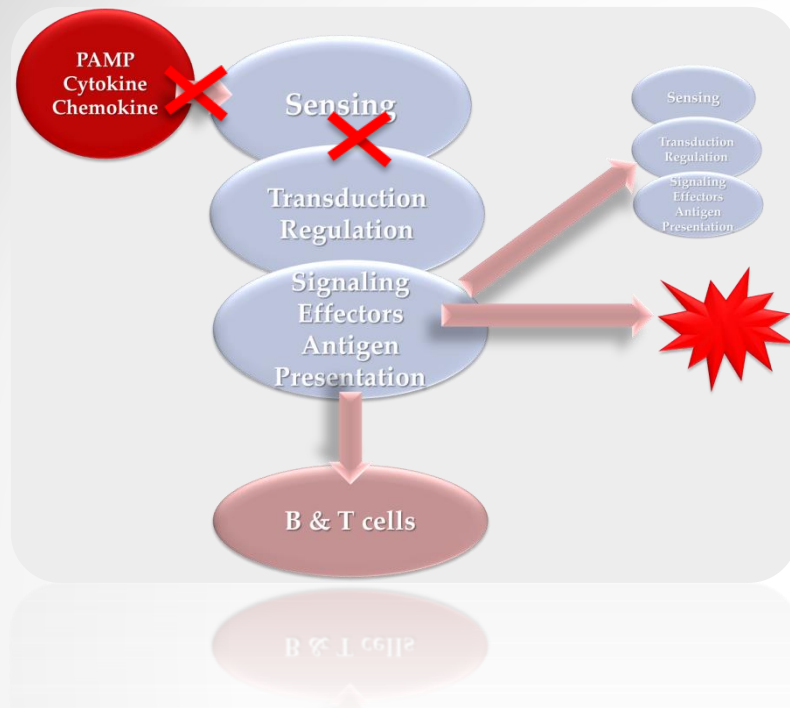
Organ	Gene	Smolt	SWT1	SWT2
Head kidney	Barrier-to-autointegration factor	-1.82	-2.48	-2.05
Head kidney	Gig2-3	-1.83	-0.77	-2.34
Head kidney	Gig2-4	-1.54	-2.24	-1.50
Head kidney	Radical S-adenosyl methionine domain	-1.05	-1.92	-1.10
Head kidney	VHSV-inducible protein-4	-1.79	-1.72	-1.12
Intestine	Barrier-to-autointegration factor	-1.23	-1.61	-1.76
Intestine	interferon-inducible protein Gig2-like	-1.65	-1.58	-1.04
Intestine	VHSV-induced protein-1	-1.06	-1.55	-0.97
Intestine	Tyrosine-protein kinase Jak1	-1.47	-0.62	-1.51
Intestine	Myxovirus resistance 1	-0.66	-1.06	-1.45
Gill	Gig2-3	-1.63	-1.10	-1.70
Gill	Barrier-to-autointegration factor	-0.81	-1.51	-1.62
Gill	STAT1	-0.89	-1.54	-1.08
Gill	Barrier-to-autointegration factor	-0.50	-0.80	-1.12
Gill	Fish virus induced TRIM-2	-0.92	-0.30	-0.97

Innate antiviral responses – VRG

Receptors, sensors
Transducers
Effectors

Genes with unknown roles

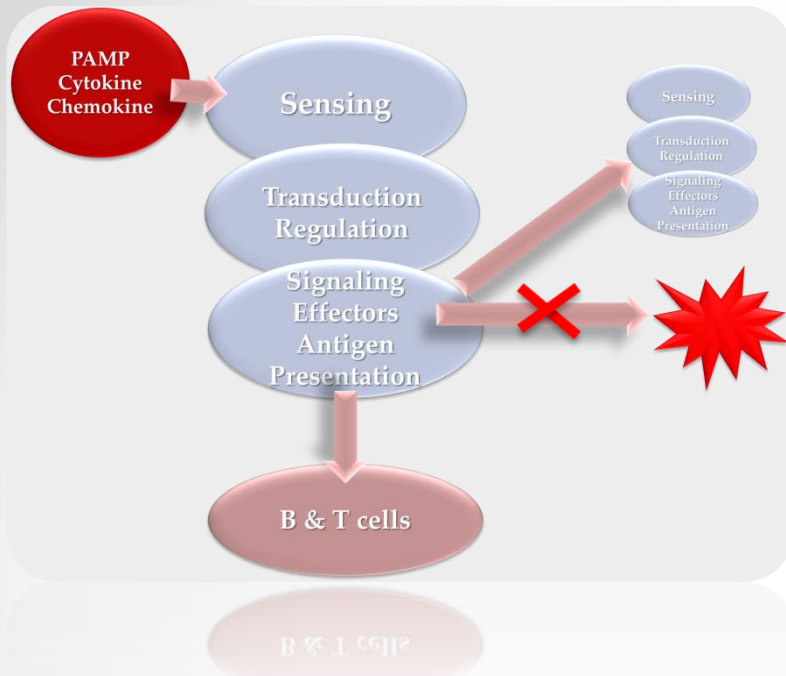
Gill – 27
Head kidney – 44
Intestine – 27



Organ	Gene	Smolt	SWT1	SWT2
Intestine	TNF receptor member 11B	-0.41	-2.11	-0.15
Intestine	TNF alpha-induced protein 8-like	0.00	-1.59	0.22
Intestine	Mitogen-activated protein kinase 13	-0.83	-1.51	-0.71
Intestine	SAPS domain family member 3	0.05	-1.40	0.64
Intestine	Sequestosome-1	-0.92	-1.29	-0.14
Intestine	TNFAIP3-interacting protein 2	-1.12	-1.08	-0.83
Intestine	CD265 Tumor necrosis factor receptor super	-0.92	-0.67	-0.54
Intestine	TNF superfamily member 13b	-0.88	-0.46	-0.92
Head kidney	SAPS domain family member 3	-0.78	-1.49	-2.02
Head kidney	CD265	-0.86	-1.25	-1.00
Head kidney	TNFR superfamily member 5	-0.53	-1.04	-0.14
Gill	Sequestosome-1	-0.68	-2.00	-1.09
Gill	TNF superfamily member 13b	-0.83	-0.32	-1.17
Gill	TNF receptor member 11B	-0.97	-0.48	-1.14
Gill	TNFR associated factor 3	-1.03	-0.11	-0.32
Gill	CD40 ligand	-0.99	-0.40	-0.80
Gill	TNF decoy receptor	-0.66	0.03	-0.94

TNF α -pathway
Acute inflammation
 TNF receptors
 Signal transduction



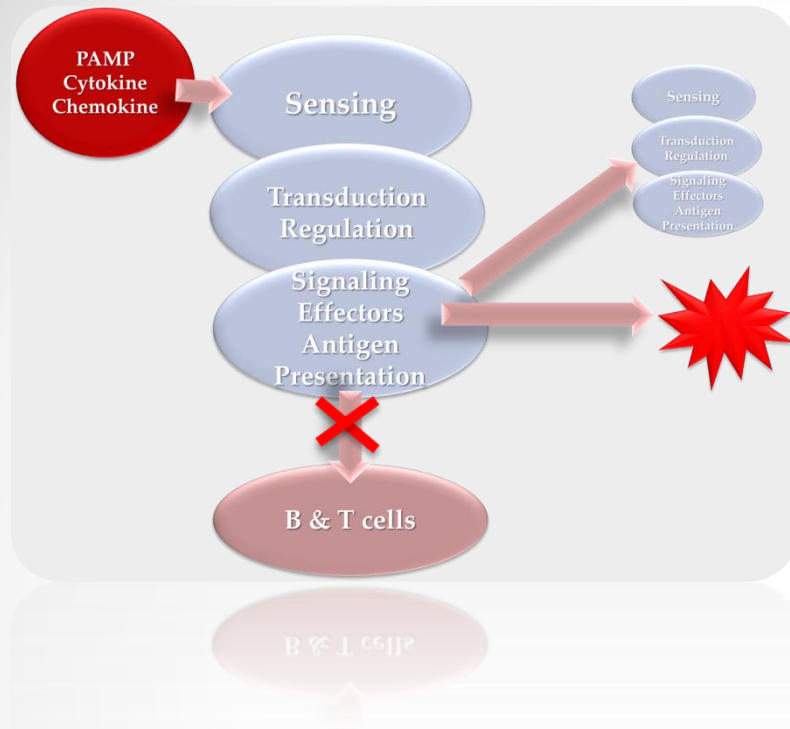


Humoral & cellular effectors
 Acute phase proteins
 Antibacterial proteins
 Lectins
 Complement
 Oxidative burst

Organ	Gene	Smolt	SWT1	SWT2
Gill	Amyloid protein beta A4	-0.08	-2.27	-3.36
Gill	Complement component C6	-0.97	-0.54	-1.88
Gill	Pentraxin	-0.74	-0.44	-1.20
Gill	Rhamnose-binding lectin WCL1 [Salvelinus]	-0.13	-0.63	-1.12
Gill	Complement C1q C	-0.26	-1.04	-0.99
Head kidney	Antimicrobial peptide 2 (LEAP2)	-0.42	-0.35	-2.18
Head kidney	Complement C7-1	-0.02	-0.31	-1.69
Head kidney	P-selectin	-1.01	-1.25	-1.67
Head kidney	Complement C4	-0.69	-1.13	-1.53
Head kidney	Complement component C6	-0.73	-1.52	-1.13
Head kidney	Mannose-binding protein C	-0.90	-1.21	-0.42
Intestine	Serum amyloid P component	-1.33	-2.33	1.30
Intestine	Complement C1qC	-0.90	-2.16	-0.13
Intestine	C1q-like adipose specific protein	-2.09	-2.13	0.80
Intestine	Rhamnose-binding lectin	0.00	-0.89	-2.09
Intestine	Complement component C8 beta	-1.37	-1.16	-0.70

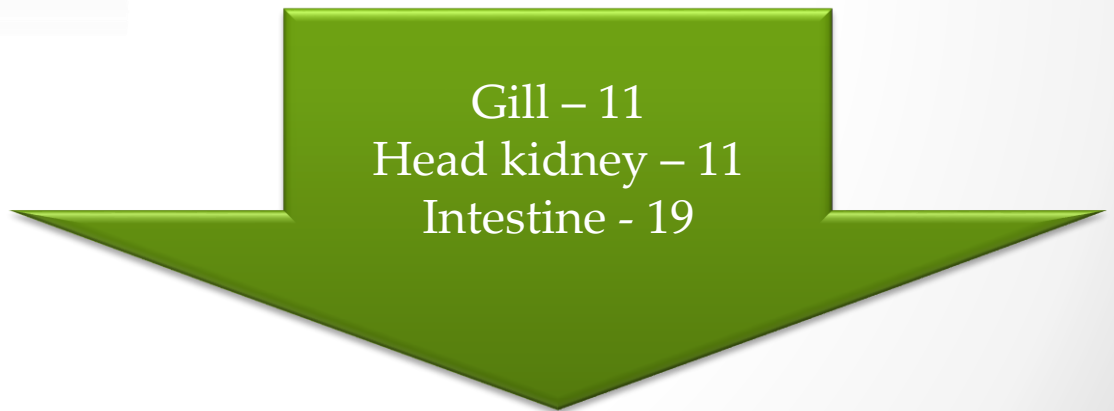
Organ	Gene	Smolt	SWT1	SWT2
Gill	Myeloperoxidase	0.58	-2.34	-3.82
Gill	Natterin-like protein	0.46	-1.97	-2.67
Gill	Inducible nitric oxide synthase	0.18	-1.63	-2.45
Gill	Neutrophil cytosolic factor 1	0.00	-0.95	-0.43
Gill	Perforin-1	0.00	-0.18	-0.47
Head kidney	Inducible nitric oxide synthase	0.00	-0.01	-1.89
Head kidney	Defensin	0.00	-0.59	-1.79
Head kidney	Myeloperoxidase	0.00	-0.28	-0.85
Head kidney	Neutrophil cytosolic factor 1	0.00	-0.43	-0.43
Intestine	Myeloperoxidase	0.00	-0.02	-1.17
Intestine	Inducible nitric oxide synthase	0.00	0.01	-1.51
Intestine	Cytochrome b-245, beta polypeptide	-1.05	-1.47	-1.10
Intestine	Perforin-1	-1.43	-1.09	-1.40
Intestine	Neutrophil cytosolic factor 1	-0.95	-0.56	-0.36

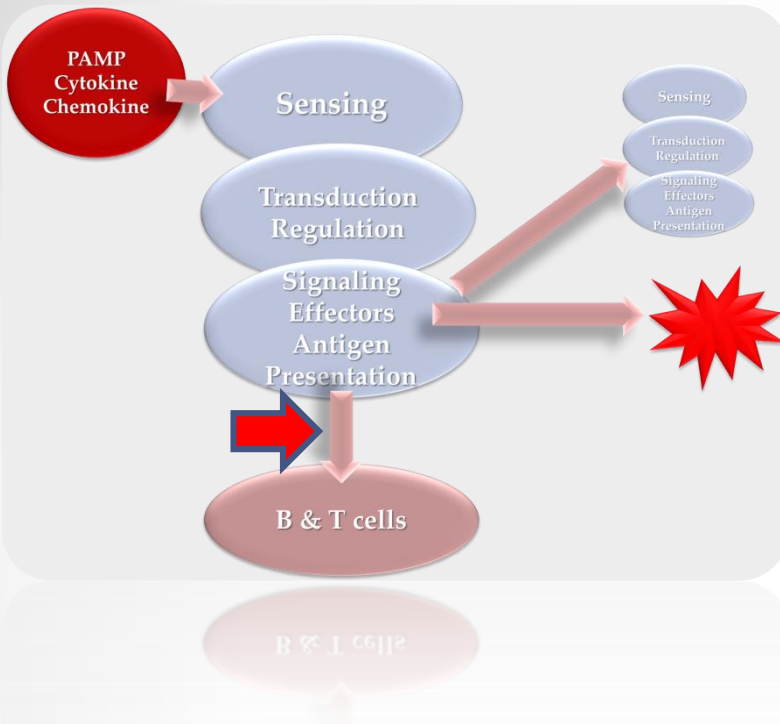
Gill – 31
 Head kidney – 26
 Intestine – 29



Organ	Gene	Smolt	SWT1	SWT2
Gill	MHC class I antigen	-0.36	-1.51	-3.39
Gill	MHC class II antigen	-0.50	-0.94	-2.05
Gill	Tapasin	-0.28	-1.20	-0.98
Gill	TAP2b	-1.01	-1.04	-0.99
Gill	MHC class I antigen	0.01	-0.40	-1.02
Head kidney	MHC class I antigen	-0.66	-2.81	-4.22
Head kidney	MHC class I heavy chain	-1.51	-1.23	-3.41
Head kidney	Hmha1 protein	-1.35	-1.27	-1.42
Head kidney	H-2 class II histocompatibility antigen gamma	-0.61	-0.54	-1.11
Head kidney	CD86 molecule	-0.33	-1.01	0.47
Intestine	MHC class I antigen	-0.90	-3.97	-3.56
Intestine	MHC class I MHC class I	-1.40	-2.94	-2.56
Intestine	MHC class I antigen	-1.43	-1.88	-1.76
Intestine	CD40	-1.47	-1.53	-1.14
Intestine	H-2 class II histocompatibility antigen gamma	-1.31	-1.15	-1.48

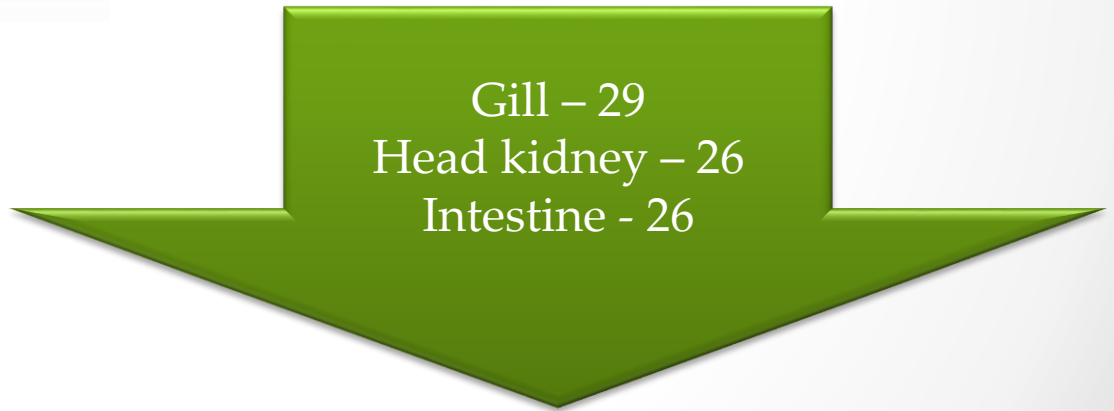
Antigen presentation
 MHCI
 MHCII
 CD40, CD86

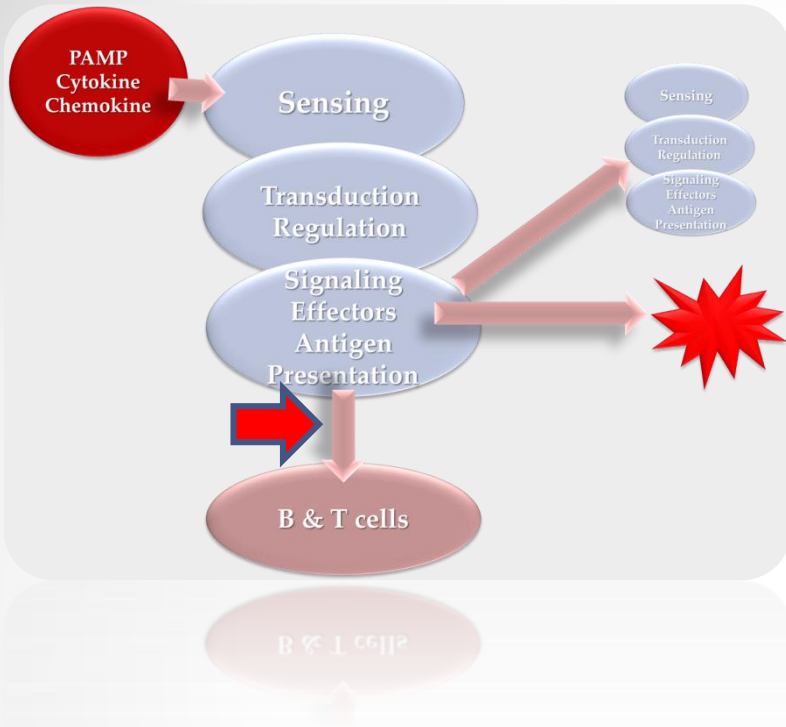




Organ	Gene	Smolt	SWT1	SWT2
Gill	CD8 beta	-1.60	-0.76	-1.04
Gill	CD3epsilon	-0.85	-1.31	-1.41
Gill	CD8 alpha	-0.78	-1.04	-1.39
Gill	CD4-like protein	-1.27	-0.25	-0.50
Gill	T cell receptor alpha	-0.49	-1.22	0.33
Head kidney	Transcription factor SOX-4	-0.56	-1.39	-0.61
Head kidney	CD8 alpha	-0.52	-1.25	-1.32
Head kidney	T-cell activation Rho GTPase	-1.07	-1.32	-1.03
Head kidney	Drebrin-like protein	-0.58	-1.27	-2.10
Head kidney	CD3epsilon	-1.12	-1.15	-1.05
Intestine	T-cell activation Rho GTPase	-1.08	-1.88	-1.34
Intestine	L-plastin	-1.43	-1.11	-0.59
Intestine	Modified T cell receptor alpha	0.22	-1.39	0.63
Intestine	CD28 T-cell-specific surface glycoprotein	-1.34	-0.95	-0.55
Intestine	T-cell surface antigen CD2	-0.46	-1.10	0.81

T cells, lymphocytes
 CD4, CD8
 Receptor – CDR3
 Transducers
 Transcription factors
 Effectors





Organ	Gene	Smolt	SWT1	SWT2
Gill	Ig kappa chain	-0.10	1.41	-0.50
Gill	AF297518_1 immunoglobulin light chain	-0.42	1.39	0.63
Gill	Ig kappa chain V region K29-213	-0.02	1.37	0.08
Gill	Ig kappa chain V-III region CLL	0.00	1.27	-0.12
Gill	Ig kappa chain V-III region MOPC 63	-0.71	0.83	-0.47
Head kidney	Ig kappa chain V-IV region Len	2.44	3.05	-0.67
Head kidney	Ig kappa chain V-III region MOPC 63	0.79	1.43	0.71
Head kidney	Ig kappa chain V-III region CLL	0.40	1.27	0.28
Head kidney	Transcription factor PU.1	-1.23	-1.06	-0.60
Head kidney	Immunoglobulin heavy chain AF141606_1	-0.23	-1.41	-1.09
Intestine	Ig heavy chain membrane bound	-1.38	1.28	-1.15
Intestine	Ig kappa chain V-III region CLL	-0.23	-0.45	-1.08
Intestine	Ig mu chain C region	-0.63	-0.94	-1.27
Intestine	Immunoglobulin lambda-like polypeptide 1	-0.79	-1.21	-0.49
Intestine	Zinc finger_AN1-type domain 1	-0.98	-0.25	-0.55

IgM

HK: increase in smolt and shortly after SWT

Gill – transient increase shortly after SWT

Intestine – stable decrease

Smoltification and SWT stimulate proliferation and redistribution of B cells

- Smoltification suppressed major immune pathways and functional groups of immune system
- Exclusion: B cells & Ig
- No recovery was observed at 3 weeks after SWT, further unknown
- Changes were similar by character and magnitude in gill, head kidney and intestine, but different genes were affected
- Results are relevant for commercial aquaculture

Happens always, commonly or in rare cases?

Consequences for disease resistance?

Study 2

HSMI in parr & smolt

NVI / NVH

(Espen Rimstad, Maria Dahle)

Challenge by co-habitation

Parr, FW

- No heart pathology,
- Effective clearance of PRV

Smolt, SW

- Heart pathology – HSMI
- Persistent infection with PRV

Differences in immunity?
Before and / or after infection?

Innate antiviral responses, antigen presentation (97 genes)

Head kidney



FW-HK SW-HK

Spleen



FW-spleen SW-spleen

	Head kidney			
	week 0	week 4	week 6	week 8
IFN-induced 44	3.11	2.04	1.23	-2.38
Stat 1	2.59	1.26	3.05	-1.04
Stat 1	2.47	2.12	2.83	-1.05
IFN-induced 44	2.22	2.43	2.55	-1.05
Stat 1	1.95	1.66	2.01	1.22
IFN-induced 44	1.93	2.48	2.14	1.14
VLIG1-1	1.84	2.22	2.44	1.28
Cholesterol 25-hydroxylase	1.84	2.67	2.25	1.00
Ubiquitin-like protein-2	1.80	2.11	1.82	1.11
VLIG1-3	2.03	3.06	3.58	1.15

	week 0	week 4	week 6	week 8
Receptor transporting protein 3	3.33	1.89	4.42	-1.81
VLIG1-2	1.76	1.48	2.76	-1.25
VLIG1-3	1.70	1.50	3.42	-1.27
Fish virus induced TRIM-10	-1.03	1.73	2.77	1.14
Viperin	-1.04	4.03	3.46	-1.17
IFIT	-1.06	2.31	2.99	-1.12
Viperin	-1.10	3.97	2.94	-1.13
Viperin	-1.11	3.70	3.76	-1.23
Ubiquitin-like protein-1	-1.15	2.30	2.81	-1.10
52 kDa Ro protein	-1.39	2.02	2.87	4.07
Thymidylate kinase	-1.49	1.42	2.83	1.00

T cells (72 genes)

Head kidney



	Head kidney			
	week 0	week 4	week 6	week 8
TCR-gamma constant region 2	3.03	1.38	2.13	1.55
TCR-gamma constant region 5	3.01	2.70	1.82	1.31
TCR-gamma constant region 2	2.76	1.58	2.03	-1.12
TCR-gamma constant region 2	2.59	1.91	1.32	1.15
TCR-gamma constant region 2	2.59	1.91	1.32	1.15
TCR-gamma constant region 2	2.35	1.33	2.02	1.34
TCR-gamma constant region 4	2.22	1.01	1.40	1.04
TCR-gamma constant region 2	2.19	1.66	1.76	1.45
T-cell receptor V-alpha	2.18	1.39	1.49	-1.17
TCR-gamma constant region 2	2.14	1.43	1.10	1.42
T-cell receptor alpha chain VR	2.10	1.36	1.76	1.10

Spleen



	Spleen			
	week 0	week 4	week 6	week 8
TCR-gamma constant region 5	3.85	1.64	2.60	1.30
TCR-gamma constant region 2	2.91	1.38	1.29	-1.10
TCR-gamma constant region 2	3.58	1.25	2.19	1.25
TCR-gamma constant region 2	3.58	1.25	2.19	1.25
TCR-gamma constant region 2	2.76	1.18	2.14	1.31
TCR-gamma constant region 4	3.80	1.52	2.42	1.11
TCR-gamma constant region 2	2.71	1.35	2.00	1.07
TCR-gamma constant region 2	2.86	1.47	2.15	1.14
TCR-gamma constant region 2	3.56	1.05	1.50	-1.04
TCR-gamma constant region 1	2.54	1.19	1.62	1.18

B cells (72 genes)

Head kidney



	Head kidney			
	week 0	week 4	week 6	week 8
Ig kappa chain V-IV	-9.07	10.88	-1.17	11.64
Ig tau heavy chain	1.54	2.80	-1.84	5.09
Ig kappa chain V-IV	-1.33	3.12	2.65	3.68
Ig heavy chain	-1.02	1.09	-1.45	3.16
Ig kappa-b4 chain C region	1.05	1.36	1.29	2.98
Ig kappa chain V-IV	1.62	1.19	1.75	2.96
Ig kappa chain V	3.60	-1.13	1.47	2.96
Ig heavy chain	-1.02	-1.37	-1.54	2.82
Ig tau-2 heavy chain C-region	1.59	-1.03	1.08	2.70
IG heavy HC V-region	-1.49	-1.10	1.00	2.65
Ig lambda-like	-1.02	1.11	1.18	2.65

Spleen



	Spleen			
	week 0	week 4	week 6	week 8
Ig kappa chain V-IV	-5.85	1.84	1.21	7.55
Ig tau-2 heavy chain C-region	-1.15	-1.59	-1.30	5.56
Ig kappa chain V-IV	-4.80	2.09	1.70	4.31
Ig tau heavy chain	1.19	1.93	-1.43	4.02
Ig kappa chain V	1.47	1.31	-1.01	2.87
Ig heavy chain	-1.57	-1.61	-2.44	2.77
Ig heavy chain	-1.22	-1.39	-1.58	3.19
Ig heavy chain V-I	-1.17	1.12	-1.22	2.95
Ig heavy chain	1.00	-1.46	-2.24	2.61
Ig heavy chain	1.21	1.83	1.08	2.53
IG heavy HC V-region	-6.48	-1.66	-2.44	2.50

HSMI in parr and smolts

Smoltification slightly suppressed immunity in head kidney and spleen under basal conditions

Immune responses to HSMI were earlier in parr

- Innate antiviral immunity: both tissues at 4 & 6 wpi
- B cells: head kidney (strong) and spleen (weak) at 8 wpi

Smoltification might reduce immune protection against HSMI

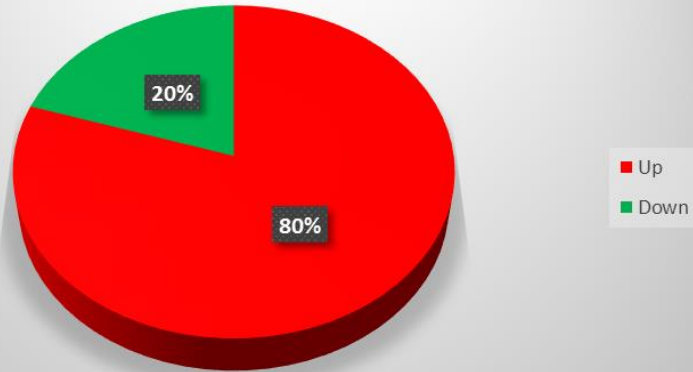
Study 3

Parr & smolt, wild salmon

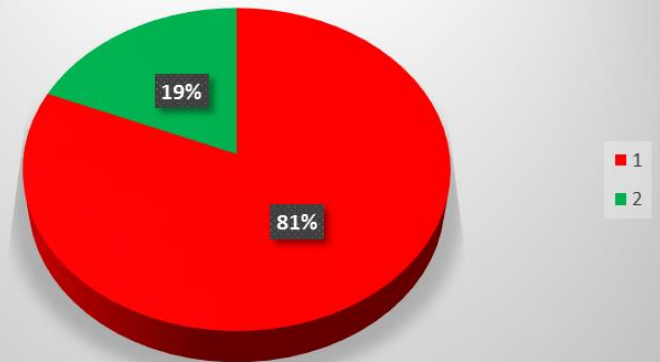
FreeBio Smolt Brain

(Lars Ebbesson, Simon MacKenzie)

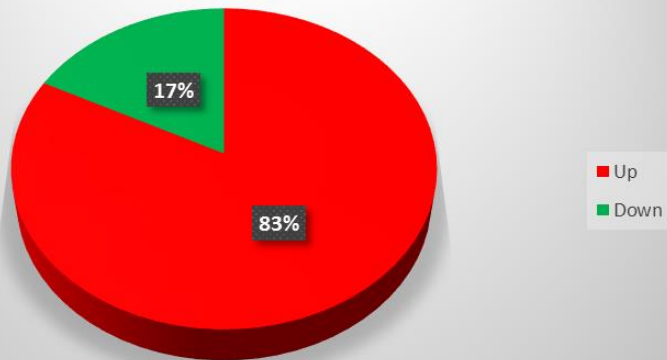
Gill - 100 genes

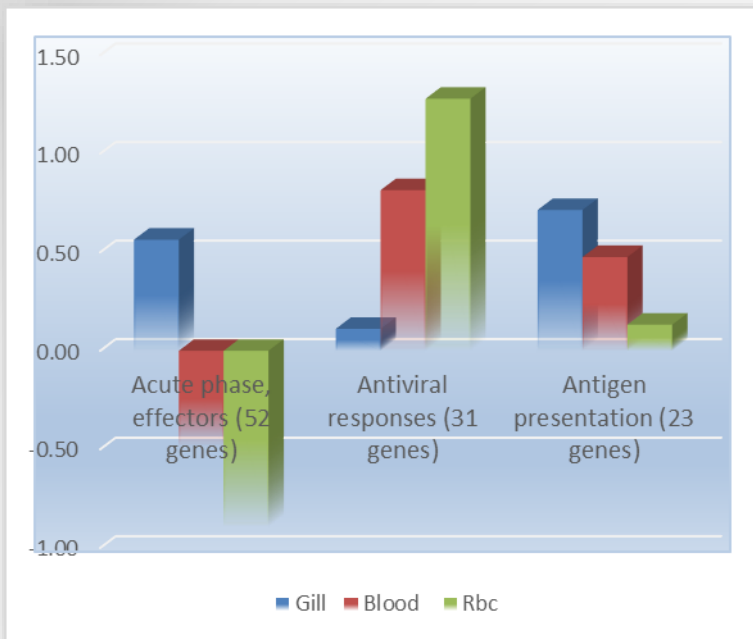


Blood - 145 genes



RBC - 167 genes

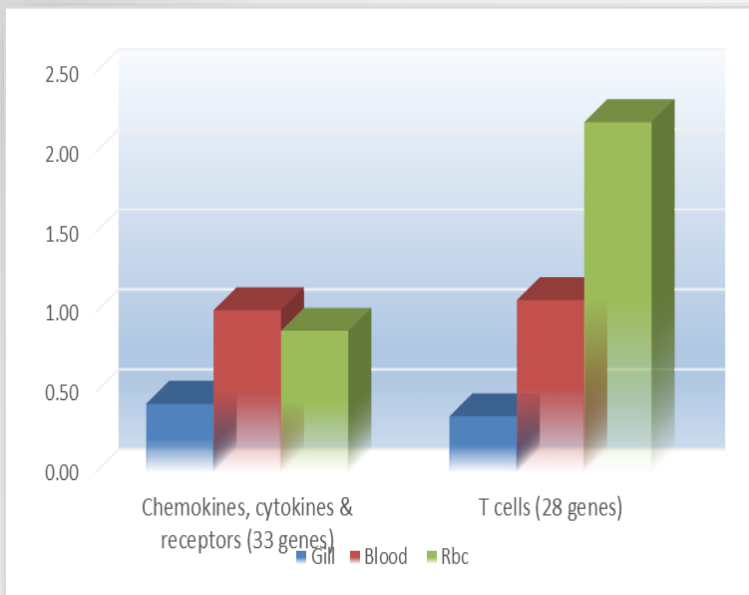




Name	Gill	Blood	RBC
Inducible nitric oxide synthase	-5.72	5.02	9.32
Myeloperoxidase precursor	-8.69	5.92	2.67
Complement C4	1.86	3.01	5.49
Lectin precursor	1.00	2.68	5.41
Complement C1q	1.35	2.99	4.81
Serine protease 1-like	1.02	1.82	4.45
Allograft inflammatory factor 1	1.92	1.00	4.43
Toxin-1	-1.64	4.32	3.01
Complement C1q-like protein 4	1.50	1.49	4.10
Collagenase 3 precursor	1.63	-1.14	4.01
Differentially regulated trout protein 1	-2.35	3.88	2.81
Apolipoprotein O precursor	-1.29	3.85	3.06
Serum amyloid A-5 protein	-2.96	3.67	-1.09
Matrix metalloproteinase-9	-1.02	-1.10	3.23
Serum amyloid A5	-2.43	3.28	1.75
Kidney injury molecule	1.43	1.60	3.28

Gene	Gill	Blood	RBC
Cholesterol 25-hydroxylase-like pro	-1.37	2.74	6.18
Gelsolin precursor	1.87	2.50	3.89
Hect domain and RLD3	2.75	-4.08	-4.66
Barrier-to-autointegration factor	2.85	-1.16	-1.05
VHSV-inducible protein-3	2.44	-1.96	-4.62
IFN-induced protein 44	2.43	-1.63	1.43
VHSV-induced protein-1	2.31	-1.74	-3.05
Gig1-2	1.75	-2.23	-2.01
VHSV-induced protein	1.97	-1.45	-2.60
RING finger protein 213	1.12	-1.23	-3.47
Fish virus induced TRIM protein	1.26	-3.20	-3.21
Vacuolar protein protein 52	2.02	-2.48	-4.15
Fish virus induced TRIM-3	1.43	-2.54	-1.68
VHSV-induced protein	1.84	-2.06	-2.39
Hect domain and RLD 3	2.12	-2.20	-2.26
Zinc finger_C3HC4	1.57	-1.34	-2.23

	Gill	Blood	RBC
MHC class I MHC class I	6.31	1.26	-1.11
MHC class I antigen	2.49	2.84	3.18
MHC class I antigen	2.38	-1.20	1.29
MHC class II antigen	2.03	1.74	4.36
HLA class II DP alpha	2.00	2.85	3.63
MHC class II alpha	2.00	2.29	1.55
MHC class II alpha	1.82	1.18	-1.57
MHC class II alpha	1.22	3.02	2.00
H-2 class I L-D alpha	1.09	2.88	3.38
H-2 class II Gamma	1.57	2.65	1.53
CD40	1.62	-2.06	-3.16
MHC class I antigen	1.92	-1.88	-2.16
MHC class I antigen	-1.09	-2.50	-1.81
MHC class I alpha 2	-1.06	-2.52	-1.77



Gene	Gill	Blood	RBC
C-X-C motif chemokine 10	6.44	1.08	6.43
C-C motif chemokine 19	-1.57	4.61	3.41
C-C motif chemokine 20	2.16	5.47	4.47
C-C motif chemokine 20	1.87	6.35	5.10
C-C motif chemokine 20	1.49	5.52	2.80
C-C motif chemokine 20	1.22	4.82	2.94
C-C motif chemokine 8	2.22	2.28	1.19
C-X-C motif chemokine 10	3.51	2.13	2.86
IFN-gamma receptor alpha	1.00	2.81	2.12
IL-12 subunit beta	2.28	4.68	3.33
IL-16	1.46	2.36	3.53
IL-17 receptor A	1.12	4.23	3.49
IL-18	-1.29	3.24	3.94
IL-20 receptor alpha	1.06	2.73	4.48
TNF receptor 11B	1.77	3.59	1.67

	Gill	Blood	RBC
CD4	1.47	5.78	1.41
CD4-like protein	2.01	1.75	2.73
CD53	-1.22	5.11	3.67
CD81 antigen	1.14	1.28	4.28
CD83	1.96	1.12	-1.52
Cytotoxic and regulatory T cell protein	2.10	2.19	1.80
Hmha1 protein	1.72	1.00	3.92
interleukin-16	1.46	2.36	3.53
SH3 and SAM domains 1	1.33	1.46	5.42
T cell receptor alpha chain	1.87	2.44	3.00
T-cell-originated protein kinase	-1.67	3.02	2.68
TCR-gamma constant region 2	-2.49	-1.01	8.25
TCR-gamma constant region 3	1.93	-1.23	-1.14
TCR-gamma constant region 4	1.97	1.63	2.06
Wiskott-Aldrich protein homolog	1.63	1.67	4.99

Study 4

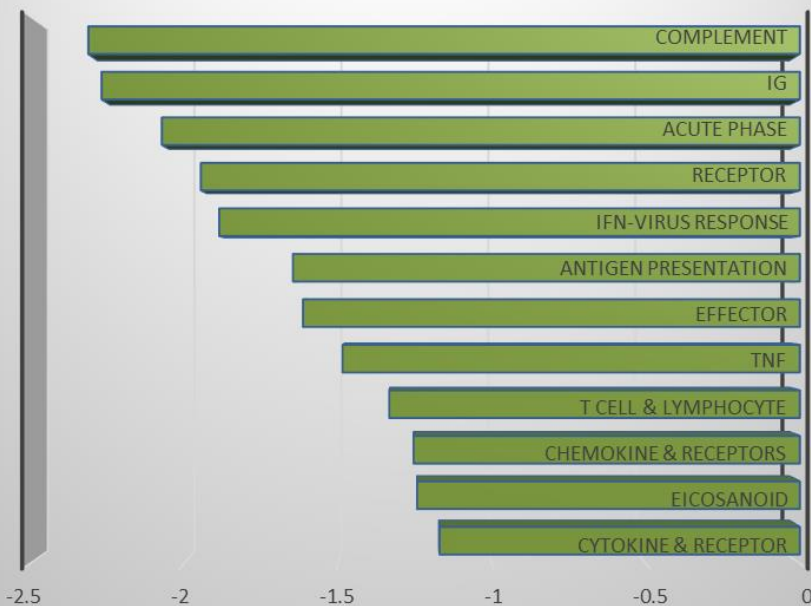
Lærdal & Bolaks salmon

FitSmolt

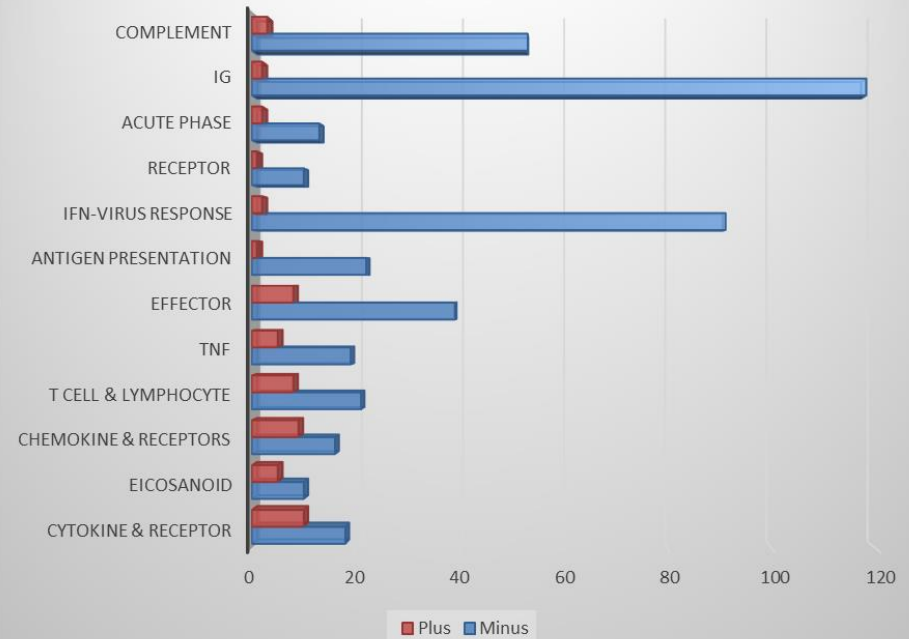
(Harald Takle, Sven Martin Jørgensen)

Heart RNA-seq: farmed vs wild

Mean Fold

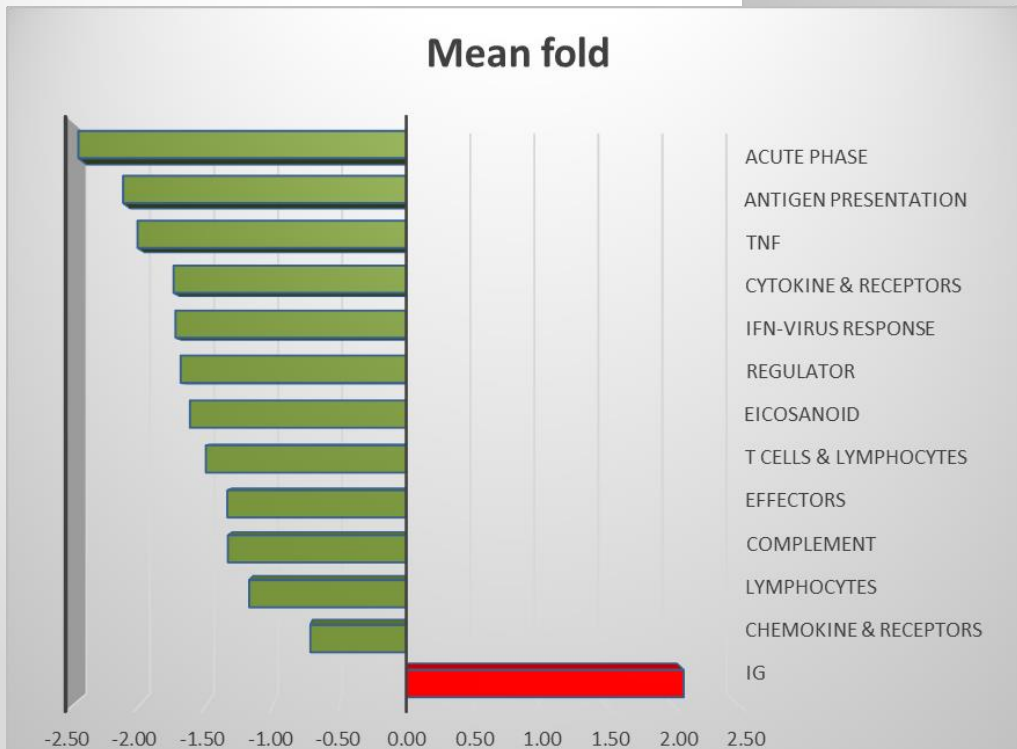
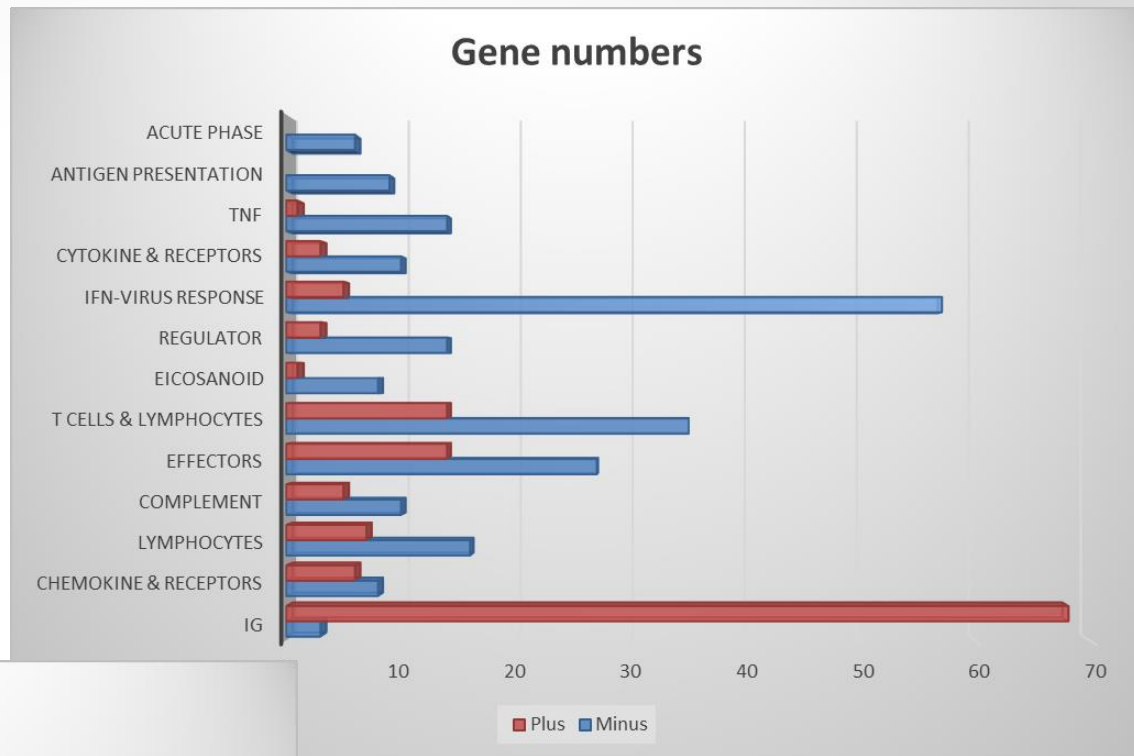


Number genes



Breeding for rapid
growth suppressed
immunity

Heart RNA-seq: smolt vs parr Bolaks



Moderate immune suppression

Stimulation of Ig

Trend to immune suppression was seen in different

- experiments & projects
 - fish & facilities
- transcriptomic methods

Advantage: confidence

Drawback:

- Large part of results obtained as a side product
 - Many questions without answer
 - Lack of system & stringent design

Questions:

- Immune suppression is different by magnitude – why?
 - Difference between strains & families?
 - Farming conditions & practice?
 - How long after SWT?
 - Impact on disease resistance?
 - Need to take measures and how?