induction, the GS2 and GS1A cell lines still have a remarkable ability to inhibit viral replication. Therefore, other STAT1/2-independent pathways may be induced by the viral infection, potentially illustrating the robustness and redundancy of the innate antiviral defences in fish.
Keywords: Chinook salmon, CRISPR/Cas9, STAT2, STAT1, interferon signalling

Corresponding author.
E-mail address: Bertrand.collet@inra.fr (B. Collet).

O-032.

Phylogeny and expression of the tetraspanin CD9 in salmonid cell lines in response to interferon stimulation

C.E. Dehler [1], P. Boudinot [2], B. Collet [2], S.A.M. Martin [1,#].

[1] School of Biological Sciences, University of Aberdeen, Tillydrone Avenue, AB24 2TZ, UK
[2] INRA Jouy-en-Josas, Domaine de Vilvert, 78352, Jouy-en-Josas Cedex, France

Abstract
CD9 is a member of the cell membrane associated tetraspanin family and has been shown to have a wide array of functions, including promotion of MHC clustering, antigen presentation, T cell activation, cell adhesion, motility, growth and differentiation, signal transduction, tumor formation and egg/sperm fusion. CD9 is ubiquitously expressed in mammalian tissues and its roles are cell type dependent. CD9 is a typical interferon stimulated gene and further associated with MHC II and the immune system and inflammation in general, as has been shown in mammals and to a lesser extent in fish. In mammals, some viruses, such as influenza, coronavirus and hepatitis C, exploit CD9 for exit of new virus particles from host cells. In contrast, increased expression of CD9 can limit HIV-I virus budding.
Due the limited knowledge of the involvement of CD9 in immune system responses in fish, we explored the phylogeny and expression of this gene in salmonids. We found 6 paralogues, which can be further organized into three distinct clades. We termed these clades CD9a, CD9b and CD9c, each of which include two paralogues reflecting the salmonid specific whole genome duplication. CD9a and CD9b are closely related and have the greatest sequence homology with the mammalian single copy gene of CD9, indicative of the teleost specific whole genome duplication. The CD9c clade is very distinct to CD9a and CD9b in sequence identity and further shows little sequence homology with the mammalian CD9, therefore could be an ancestral form of CD9 that was subsequently lost in all other vertebrate classes.
We investigated the expression of the different paralogues in embryonic chinook salmon cells (CHSE) stimulated with interferon type I, an inducer of the antiviral pathways in fish.
The paralogues of clade CD9c were highly inducible by interferon stimulation, whilst CD9a and CD9b appeared to be non-responsive. The specific inducibility of the ancestral CD9c clade to interferon type I highlights the unique immune responses in teleost. The presence of 6 paralogues organized in three clades may also reflect the diversity of roles this gene has been implicated in. In future, we aim to explore the expression of CD9, especially the putatively immune system relevant clade CD9c, in different cell types at baseline and in response to virus stimulations.
This study contributes to a better understanding of CD9 involvement in immune system responses and how the gene is related to the antiviral interferon type I response. As CD9 has been shown to be important for the replication of certain viruses in mammals, this could be explored for fish viruses and potentially used as an anti-viral target.
Keywords: Tetraspanins, Salmonid, Interferon signaling, Antiviral immune response, Phylogeny

Corresponding author.
E-mail address: sam.martin@abdn.ac.uk (S.A.M. Martin).

O-033.

Genomics for the understanding of the host-pathogen interaction: the case of the Atlantic salmon and *Piscirickettsia salmonis*

D. Valenzuela-Miranda[#], C. Gallardo-Escárate.

Interdisciplinary Center for Aquaculture Research, Laboratory of Biotechnology and Aquatic Genomics, Universidad de Concepción, Concepción, Chile

Abstract
During an infection both host and pathogen undergo a deep transcriptomic remodeling that will orchestrate either the pathogen clearance or host infection. These changes involve both the regulation of protein coding genes (mRNA) and non-coding RNAs (ncRNAs) elements, such as lncRNAs and miRNAs. Thus, knowing how these elements are modulated can reveal key aspects about hostpathogen interaction. Through RNA-seq, miRNA-seq and dual RNA-seq, we explored the coding and non-coding transcriptional response in Atlantic salmon infected with the intracellular bacterium Piscirickettsia salmonis. Differential expression analysis revealed that fish respond to P. *Salmonis* infection through modulation of different coding genes associated with immunity, clathrin mediated endocytosis and iron metabolism responses. In addition, a strong response associated with ncRNAs was also evidenced. Our results suggested that these ncRNAs might fulfilling key regulatory roles in the response of the Atlantic salmon to *P. salmonis* infection. On the other hand, bacteria transcriptomic response was associated with a large number of genes involved in amino acid metabolism. Genome wide comparison and in vitro studies evidenced a metabolic dependency of *P. salmonis* on salmon amino acids. Based in our results, we propose that amino acids might be an important component of the nutritional immunity triggered by the Atlantic salmon to cope with *P. salmonis* infection. Overall, our results evidence how genomics can lead us to the understanding of novel means of interaction between host and pathogens in marine models.
Keywords: Dual RNA-Seq, Piscirickettsia salmonis, Atlantic salmon, Nutritional immunity, metabolic dependency, amino acids

Corresponding author.
E-mail address: divalenzuela@udec.cl (D. Valenzuela-Miranda).

O-034.

***In vitro* rainbow trout transcriptome reveals immune evasion associated with higher virulence of viral haemorrhagic septicaemia virus**

I. Cano[#,], R. van Aerle [*].*

Centre for Environment, Fisheries and Aquaculture Science, Barrack Road, The Nothe Weymouth, Dorset, DT4 8UB, United Kingdom

Abstract
Rainbow trout pathogenic freshwater viral haemorrhagic septicaemia virus (VHSV) emerged from an ancestral marine virus, however the pathogenic mechanism of the virulent freshwater VHSV remains unknown. In the present work, the transcriptome of RTG-2 cells inoculated with two pathogenic (J167 and DK-5131) and two non-pathogenic (96-43/8 and 1p49) isolates were analyzed at 3, 6, and 12 hours and compared to control samples using RNA-seq. Although VSHV isolates showed the same pattern of viral replication, the transcriptomic profiles in RTG-2 cells were dramatically different between pathogenic and non-pathogenic isolates, revealing a lack of sensing of the viral replication in cells inoculated with both pathogenic VHSVs at early stages of infection. Functional annotation analysis of differentially-expressed genes between non-pathogenic VHSV and controls revealed an enrichment of pathways involved in the defense to biotic stimulus and metabolic processes (strong up-regulation of genes), and lipid metabolism and cell cycle (down-regulation of genes) In contrast, cholesterol and cytoskeleton mobility pathways were enriched (up-regulation of genes) by both pathogenic VHSV. Furthermore, an increasingly

higher number of GRP78/BiP transcripts in cells inoculated with the pathogenic VHSVs suggests a role of the unfolded protein response in the VHSV immune evasion.

Keywords: Rainbow trout, VHSV, transcriptome, RNA-Seq, immune evasion, host-pathogen interaction

Corresponding author.
E-mail address: irene.canocejas@cefas.co.uk (R. van Aerle).
* These authors have contributed equally to this work

O-035.

Studies into B-glucan recognition in fish suggests a key role for the C-type lectin pathway

Jules Petit [1], Erin C. Bailey [2,3], Robert T. Wheeler [2,3], Carlos A. Ferreira de Oliveira [4], Maria Forlenza [1], Geert F. Wiegertjes [1,5,#].

[1] Cell Biology and Immunology Group, Wageningen University & Research, Wageningen, Netherlands
[2] Department of Molecular & Biomedical Sciences, University of Maine, Orono, ME, United States
[3] Graduate School of Biomedical Sciences and Engineering, University of Maine, Orono, ME, United States
[4] Department of Research and Development, Biorigin Company, Lençóis Paulista, Brazil
[5] Aquaculture and Fisheries Group, Wageningen University & Research, Wageningen, Netherlands

Abstract
Immune-modulatory effects of β-glucans are generally considered beneficial to fish health. Despite the frequent application of β-glucans in aquaculture practice, the exact receptors and downstream signalling remains to be described for fish. In mammals, Dectin-1 is a member of the C-type lectin receptor (CLR) family and the best-described receptor for β-glucans. In fish genomes, no clear homologue of Dectin-1 could be identified so far. Yet, in previous studies we could activate carp macrophages with curdlan, considered a Dectin-1-specific β-(1,3)-glucan ligand in mammals. It was therefore proposed that immune-modulatory effects of β-glucan in carp macrophages could be triggered by a member of the CLR family activating the classical CLR signalling pathway, different from Dectin-1. In the current study, we used primary macrophages of common carp to examine immune modulation by β-glucans using transcriptome analysis of RNA isolated 6 h after stimulation with two different β-glucan preparations. Pathway analysis of differentially expressed genes (DEGs) showed that both β-glucans regulate a comparable signalling pathway typical of CLR activation. Carp genome analysis identified 239 genes encoding for proteins with at least one C-type Lectin Domains (CTLD). Narrowing the search for candidate β-glucan receptors, based on the presence of a conserved glucan-binding motif, identified 13 genes encoding a WxH sugar-binding motif in their CTLD. These genes, however, were not expressed in macrophages. Instead, among the β-glucan-stimulated DEGs, a total of six CTLD-encoding genes were significantly regulated, all of which were down-regulated in carp macrophages. Several candidates had a protein architecture similar to Dectin-1, therefore potential conservation of synteny of the mammalian Dectin-1 region was investigated by mining the zebrafish genome. Partial conservation of synteny with a region on the zebrafish chromosome 16 highlighted two genes as candidate β-glucan receptor. Altogether, the regulation of a gene expression profile typical of a signalling pathway associated with CLR activation and, the identification of several candidate β-glucan receptors, suggest that immune-modulatory effects of β-glucan in carp macrophages.

Keywords: β-glucan, primary macrophage, transcriptome analysis, C-type lectin-like domain, cyprinidae

Corresponding author.
E-mail address: geert.wiegertjes@wur.nl (G.F. Wiegertjes).

O-036.

The immune proteome of the zebra mussel deciphered by deep proteogenomics

M. Leprêtre [1,3,#], C. Almunia [2], J. Armengaud [2], A. Salvador [3], A. Geffard [1], M. Palos-Ladeiro [1].

[1] University of Reims Champagne-Ardenne, UMR-I 02 INERIS-URCA-ULH SEBIO, "Environmental Stress and Biomonitoring of Aquatic Environments", UFR Sciences Exactes et Naturelles, Campus du Moulin de la Housse, BP 1039 51687, Reims CEDEX, France
[2] CEA-Marcoule, DRF/IBITEC-S/SPI/Li2D, Laboratory "Innovative technologies for Detection and Diagnostics", BP 17171, F-30200, Bagnols-sur-Cèze, France
[3] University of Lyon, University of Claude Bernard Lyon 1, CNRS UMR 5280 Institute of Analytical Sciences (ISA), F-69100, Villeurbanne, France

Abstract
Bivalve immune system modulation appears to be a relevant strategy in environmental risk assessment. Indeed, immune system is known to be sensitive to different environmental and anthropogenic stresses. To date, the immune system of marine bivalves is well documented in comparison to continental bivalves. Among them, the freshwater mussel *Dreissena polymorpha*, a non-model organism, represents the counterpart of the marine mussel in ecotoxicological studies. While cellular responses of hemocytes are well characterized for *D. polymorpha*, the molecular immune mechanisms remain relatively scared. In order to get insights into the immune proteome of the zebra mussel, proteogenomics was conducted on both hemocytes and plasma compartment of this non-model species. This strategy, combining transcriptomic sequences with mass spectrometry data acquired on proteins was relevant since 3,227 proteins were identified, which represent the largest protein inventory for this sentinel organism. Functional annotation and gene ontology (GO) analysis performed on the identified proteins described the main molecular players of hemocytes and plasma in the immune response of *D. polymorpha*. The GO analysis carried out on immune proteins showed that these two hemolymphatic compartments perform closely related and complementary immune functions: in signal transduction, adhesion and cellular mobility but also related to the recognition and elimination of microorganisms. Functional annotation revealed new mechanisms into the immune defence of the zebra mussel. Proteins rarely observed in the hemolymph of bivalves were pinpointed such as natterin-like proteins and thaumatin-like proteins. Furthermore, the high abundance of complement-related proteins observed in plasma suggested a strong implication of the complement system in the immune defence of *D. polymorpha*. This study contributes to a better understanding of the molecular mechanisms involved in immunity of bivalves and paves the way for their use as biomarkers in aquatic ecotoxicology.

Keywords: Hemolymph, bivalve, immunity, non-model organism, proteogenomics

Corresponding author.
E-mail address: maxime.lepretre@univ-reims.fr (M. Leprêtre).

O-037.

Salmonid IGH genes: From genomics to repertoire sequencing

Susana Magadan [1,#], Aleksei Krasnov [2], Saida Hadi-Saljoki [3], Rosario Castro [4], Irene Salinas [5], Oriol Sunyer [6], John Hansen [7], Ben Koop [8], Marie-Paule Lefranc [3], Pierre Boudinot [4].

[1] Immunology Laboratory, Biomedical Research Center (CINBIO), University of Vigo, Campus Lagoas Marcosende, Vigo, Spain
[2] Nofima AS, Norwegian Institute of Food, Fisheries & Aquaculture Research, Ås, Norway