a mean infection load of 1.25 . 106 ± 3.12 . 105 GAV copies μg-1 TNA. After 14 days of feeding the experimental diets, eight shrimp per diet were sampled and their GAV infection load was quantified to determine the relative change. GAV infection loads increased over the 14 days, however, shrimp fed the three functional feeds, B-glucan, PHB or microbial floc, on average had 10-fold lower GAV infection compared to shrimp fed the basal diet. Although the functional feeds did not clear pre-existing infections, the results suggests they may have increased the immune capacity of shrimp to better control GAV proliferation compared with the standard diet. The impact of these functional feeds was further explored by measuring several immune parameters from haemolymph samples collected from the same eight prawns that GAV titre was quantified, including total haemocyte counts, phenoloxidase activity and the production of reactive oxidative species and antioxidants.

Keywords: Functional feeds, immunostimulant, GAV, immune response, shrimp

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O-068.

Antibody repertoire and kinetics in atlantic salmon following vaccination and challenge with Salmonid alpha virus

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Abstract
Immunoglobulin M (IgM) is important in protection against disease in Atlantic salmon (*Salmo salar*). For many diseases, specific antibody response may be good despite poor disease protection, and vice versa. Non-specific antibodies (NAB), broadly cross-reactive antibodies with low affinity, are typically abundant in the blood of Atlantic salmon, but their role in disease protection is poorly understood.
In this study, we have examined the antibody repertoire and the immune gene expression of Atlantic salmon following vaccination and experimental infection. Parr was vaccinated with an commercial multivalent vaccine that included inactivated Salmonid alpha virus (SAV), and three weeks after vaccination the fish was transferred to sea water and infected with SAV subtype 3 in a shedder-cohabitant model. Blood plasma and samples from lymphoid tissue and heart were collected at several timepoints pre -and post-vaccination and post-infection.
Multiplex antibody assays of blood plasma from vaccinated fish showed an increase of specific antibodies to antigenic components of the vaccine after 6 - 9 weeks. In parallel, titers of non-specific antibodies increased in plasma of vaccinated fish, showing an earlier onset of increase than specific antibodies. The presence of non-specific antibodies is detected by the recognition of a synthetic hapten-carrier complex. In contrast to vaccinated fish, titers of non-specific antibodies in plasma of control (saline-injected) fish first increased after challenge with SAV. Based on these findings, high-throughput immunoglobulin sequencing (IgSeq) of the variable (antigen-binding) region of the B cell antibody receptor will be performed. With this technology, we can study the presence of shared (present in multiple individuals) and unique (present only in one individual) B-cell clonotypes in vaccinated and control fish before and after challenge. Results from microarray transcriptome analysis and RT-qPCR of selected immune genes will also be presented.
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Keywords: Vaccination, Atlantic salmon, Salmonid alpha virus, Antibody-repertoire

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O-069.

Characterization of ten CCL20-like cc chemokines in rainbow trout (*Oncorhynchus mykiss*): Sequence and expression analysis

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Abstract
Mammalian CCL20, or macrophage inflammatory protein-3α, can function as a homeostatic and inflammatory chemokine. In relation to the latter, it is responsible for the chemoattraction of lymphocytes and dendritic cells to mucosal immune sites under inflammatory and pathological conditions. CK1, CK8A and CK8B are rainbow trout (*Oncorhynchus mykiss*) CC chemokines that were reported previously to be phylogenetically related to mammalian CCL20. In the current study, an additional seven CCL20-like paralogs in rainbow trout are reported, that are divided into three subgroups (by phylogenetic tree, homology and synteny analysis) in agreement with past studies in fish. They have been designated here as: CCL20L1a (also referred to as CK1), CCL20L1b1, CCL20L1b2, CCL20L2a (CK8A), CCL20L2b (CK8B), CCL20L3a, CCL20L3b1, CCL20L3b2, CCL20L3b3 and CCL20L3b4. Like mammalian CCL20, rainbow trout CCL20-like molecules possess a high positive net charge with a pI of 9.34-10.16, that is reported to be important for antimicrobial activity. Rainbow trout CCL20-like paralogs are differentially expressed and in general highly expressed in mucosal tissues, such as gills, intestine and thymus. The expression levels of rainbow trout CCL20-like paralogs are increased during development and following PAMP/cytokine stimulation. For example, in RTS-11 cells CCL20L3b1 and CCL20L3b2 are highly up-regulated by LPS, poly I:C, recombinant(r) IFNa and rIL-1β. Trout CCL20-like paralogs are also increased after *Yersinia ruckeri* infection or poly I:C stimulation *in vivo*, with CCL20L3b1 and CCL20L3b2 again highly up-regulated. Overall, this is the first report of the complete CCL20 chemokine subfamily in rainbow trout, and the analysis of their expression and modulation *in vitro* and *in vivo*. These results suggest that teleosts possess divergent CCL20-like molecules that may have important roles in mucosal immunity.
Keywords: Rainbow trout, CCL20-like chemokine, characterisation, expression, mucosal immune response

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O-070.

Temperature drives the immune respose in atlantic salmon infected with sea lice: Novel insights through transcriptome sequencing analyses

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Abstract
The sea louse Caligus rogercresseyi is a copepod species responsible for the most relevant parasitic infections in the Chilean salmon industry. This ectoparasite causes immunosuppression and stressful conditions in farmed fishes, which led to the activation of key elements of host's immune system. However, how these host-parasite interactions are being impacted by environmental conditions such as temperature variations is

still poorly understood. The aim of this study was to describe the anti-parasitic immune response of the Atlantic salmon (*Salmo salar*) against C. rogercresseyi in contrasting temperature conditions. Fish were subjected to in vivo challenge against sea lice at two temperature regimes (8 and 16°C) with 35 copepodid/fish during 25 days. Samples were collected from hosts and parasites for transcriptome sequencing using Illumina HiSeq platform. Parasitic burden was highly divergent in the two temperaturas analyzed, being the double at 16°C (38.9% of the initial parasitic load versus 19.3% at 8°C).

Furthermore, many coding genes, immune-system pathways and long non-coding RNAs (lncRNAs) were significantly up-regulated in infected fishes at the highest temperature. Meanwhile, highly-relevant biological processes were also up-regulated in the sea lice at the highest temperature, such as genes related to the secretome system and homeostasis. This study contributes with novel knowledge regarding how temperature changes, that normally take place in salmon farms, could dramatically change key molecular elements involved in the hostparasite interactions between salmon and sea lice. The discovery of novel lncRNAs involved in these interactions are also discussed.

Keywords: Temperature changes, Atlantic salmon, *Caligus rogercresseyi*, immune response

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O-071.

Embryonic incubation temperature has a long-term effect on the spleen immune transcriptome and its response to lipopolysaccharide in adult zebrafish (*Danio rerio*)

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Abstract

Temperature has a profound effect on the immune system of fish. However, little is known about the effect of temperature during early embryonic development on the immune system of adult fish. We incubated zebrafish (*Danio rerio*) embryos at low (24 °C), high (32 °C), and reference temperature (28 °C) until hatching (3-5 days post-fertilization, dpf). Afterwards, all the three groups were maintained at the reference temperature until adulthood (100 dpf). At 12 h post intraperitoneal injection with lipopolysaccharide, spleens were sampled and RNA was extracted. RNA-seq was performed and the immune transcriptomes were compared between the temperature groups, as well as between LPS-challenged and control fish injected with phosphate buffered saline.

Both low- and high embryonic incubation temperatures resulted in decreased expression of some immune transcripts related to cytokines (*tnfa*, *cxcl8a*, *ccl20a.3*), neutrophil (*serpinb1l2*, *ncf1*, *ncf4*) and T cell functions (*sema4ab*, *crtam*, *alcama*) in the adult fish spleen. In addition, high incubation temperature also resulted in lower transcript levels of genes involved in neutrophil and respiratory burst (*cebp1*, *lsp1*, *cyba*), endocytosis (*rab5ab*, *rab7*, *pikfyve*), and lysosomal activity (*atp6ap1b*, *atp6ap2*, *atp6v1h*). In the same temperature group, the expression of various immunoglobulins (*rag1*, *ciita*, *cd74a*) and complement components (*c1qa*, *c1qb*, *c1qc*) was up-regulated.

Numerous immune transcripts, including antibacterial factors, and those involved in endocytosis, lysosome formation, respiratory burst, and inflammatory signaling were up-regulated with LPS challenge in fish from the low incubation temperature group. In contrast, fish from the high temperature group showed a limited immune response to LPS. In fish from the reference temperature group, expression of diverse apolipoprotein transcripts was up-regulated, while the level of cytokine transcripts was decreased.

Taken together, our data demonstrate that early developmental temperature has a long-term effect on the spleen immune transcriptome of adult zebrafish. This is relevant to understand the molecular basis of the temperature-induced immune developmental plasticity in fish and is particularly relevant in the context of climate change.

Keywords: Temperature, immune system, transcriptome, LPS, zebrafish

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O-072.

Turbot (*Scophthalmus maximus*) Nk-Lysin induces protection against the pathogenic parasite *Philasterides dicentrarchi* via membrane disruption☆

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Abstract

P. dicentrarchi is one of the most threatening pathogens for turbot aquaculture. This protozoan ciliate is a causative agent of scuticociliatosis, which is a disease with important economic consequences for the sector. Neither vaccines nor therapeutic treatments are commercially available to combat this infection. Numerous antimicrobial peptides (AMPs) have demonstrated broad-spectrum activity against bacteria, viruses, fungi, parasites and even tumor cells; an example is Nk-lysin (Nkl), which is an AMP belonging to the saposin-like protein (SAPLIP) family with an ability to interact with biological membranes. Following the recent characterization of turbot Nkl, an expression plasmid encoding Nkl was constructed and an anti-Nkl polyclonal antibody was successfully tested. Using these tools, we demonstrated that although infection did not clearly affect nkl mRNA expression, it induced changes at the protein level. Turbot Nkl had the ability to inhibit proliferation of the *P. dicentrarchi* parasite both *in vivo* and *in vitro*. Moreover, a shortened peptide containing the active core of turbot Nkl (Nkl71-100) was synthesized and showed high antiparasitic activity with a direct effect on parasite viability that probably occurred via membrane disruption. Therefore, the *nkl* gene may be a good candidate for genetic breeding selection of fish, and either the encoded peptide or its shortened analog is a promising antiparasitic treatment in aquaculture.

Keywords: *Philasterides dicentrarchi*, turbot, Nk-lysin, antimicrobial peptide, antiparasitic.

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O-073.

Lineage/species-specific expansion of the Mx gene family in teleosts: Differential expression and modulation of nine Mx genes *in vitro* and *in vivo* in rainbow trout *Oncorhynchus mykiss*

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