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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Abstract
Myxovirus resistance (Mx) proteins are interferon (IFN)-inducible Dynamin-like GTPases, with an important role in antiviral immunity. Three Mx genes (Mx1-3) are known in rainbow trout but nine have been reported recently in Atlantic salmon. In this study, an additional six Mx genes were cloned in rainbow trout, present in four chromosome loci. Further bioinformatic analysis suggests the presence of three teleost Mx groups (TMG) with distinct gene organisations. Whilst some teleost lineages possess all three TMGs, percomorphs have only one (TMG1). Salmonid Mx genes belong to TMG1 and TMG2. Synteny analysis suggests that teleost Mx genes may be evolved from three Mx genes present at two ancestral chromosomal loci, as seen in spotted gar, with the 3R duplicated Mx loci retained/lost in a lineage specific manner. The increased salmonid Mx gene copies result mainly from local gene duplications, that also show species specific differences. Trout Mx molecules have been diversified in loop 1 and 4, and in the nuclear localisation signal in loop 4.
The trout Mx genes are differentially expressed in tissues and cell lines. Whilst TMG1 (Mx1-4) were preferentially expressed in blood, TMG2 (Mx5-9) were highly expressed in the intestine. The expression of several Mx genes were increased in pre-feeding and post-feeding fry, perhaps to help fight potential food borne viruses. The expression of most of the trout Mx genes was induced by poly IC *in vitro* and *in vivo*, and by type I IFN. In addition, as seen in Atlantic salmon, notable induction of some isoforms was seen with IFNγ, and several genes showed some modulation with IL-1β. Overall, induction of trout Mx gene expression was gene-, cytokine- and cell line-dependent. These results suggest that salmonids not only possess a large number of Mx proteins but also possess diversified and complex regulatory pathways, which may help fight viral infection and contribute to their success in an anadromous life style.
Keywords: Rainbow trout Oncorhynchus mykiss, Mx gene family, Antiviral defense, Phylogenetic tree analysis, Interferons

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O-074.

The transforming growth factor (TGF)-B family in rainbow trout (*Oncorhynchus mykiss*): Characterization and expression analysis

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Abstract
TGF-β is an evolutionally conserved cytokine that belongs to a large family of morphogens and growth factors, with three members (TGF-β1-3) present in mammals. Mammalian TGF-β is widely expressed and modulates a large spectrum of biological processes including normal development, carcinogenesis, and immune responses. It is considered an immunosuppressive cytokine but can also promote the differentiation, function, and homeostasis of certain inflammatory populations of T cells, such as T-helper 17 (Th17) cells. TGF-β members are increased in teleosts, and this is assumed to be due to the teleost-wide third whole genome duplication (3R WGD) event. How exactly how many TGF-β members exist, and how many different paralogues are expressed and modulated is unclear in these fish species, including rainbow trout that experienced a 4R WGD. Analysis of the recently released rainbow trout genome (Acc. no. GCF_002163495.1) identified 10 chromosome loci that each encodes a TGF-β, including TGFβ1a and TGFβ1b cloned previously in our group. The cDNAs of the other TGF-β genes were cloned and reveal that rainbow trout possesses three TGF-β1, two TGF-β2, four TGF-β3 and one TGF-β6. This sequence analysis has been extended to other salmonids. Phylogenetic tree analysis suggests that WGD did play a major role in the expansion of the TGF-β family in teleosts. The expression of the ten TGF-β members in trout was comparative examined by real-time reverse-transcription quantitative PCR in tissues, cell lines and during early developmental stages. The TGF-β family members show differential expression in tissues and cell lines, suggesting functional diversification. The expression of TGF-β members was increased during early developmental stages from pre-feeding to post-feeding. Their expression could be modulated in cell lines by pro-inflammatory cytokines and PAMPs in a cell line- and stimulant-dependent manner. These results suggest that teleost TGF-β members may have undergone lineage-specific expansion and reveal further functional diversification in the teleost immune system.
Keywords: Rainbow trout, TGF-β gene family, phylogenetic tree, gene expression, cytokine.

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O-075.

Perforin-expressing IGT+ B cell with cytotoxic activity, a novel player in the innate immune response of teleost fish

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Abstract
We have previously reported that IgT plays a key role in teleost fish mucosal immune responses. This immunoglobulin is produced by a subset of B cells that uniquely express membrane IgT. We have also shown an involvement of these cells in innate immunity as they possess a high phagocytic and microbicidal capacity.
To provide further insight into the roles of B cells in immunity, we performed a comparative transcriptome analysis on FACS-sorted IgT+ and IgM+ B cells. To our surprise, the gene that showed the highest differential expression between these two B cell subsets was perforin, a molecule that is not traditionally associated with B cells. Further analysis confirmed that unlike IgM+ B cells, IgT+ B cells expressed high transcript numbers of several perforin isoforms, including, prf1-like-B, prf1-like-C and prf1-like-D. We further confirmed the unique expression of these perforin genes in IgT+ B cell by single cell transcriptome analysis. Besides the gene expression, perforin protein expression by IgT+ B cells was confirmed by immunostaining, using antibodies we generated against several of the trout perforin isoforms. Since perforin is a cytolytic protein that forms pores on the cell membrane of intracellular and extracellular targets, we hypothesized that IgT+ B cells could possess killing activity similar to that of other perforin-expressing cells, including CD8+ T cells and NK cells. To confirm this hypothesis, we tested the potential cytotoxic capacity of IgT+ B cells towards several mammalian cell lines, such as HL-60. Our results show that the killing activity of IgT+ B cells was significantly greater than that of IgM+ B cells. These data demonstrate a previously unrecognized new function for IgT+ B and vertebrate B cells in innate immunity.
Keywords: IgT+ B cell, Perforin, Innate immunity, cytotoxicity

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