

importance for stress effects on the immune response in teleosts. Individual aspects of the interference of stress hormones (mainly cortisol) with immune processes have already been reported in some bony fish. Although less studied, the catecholamines adrenaline and noradrenaline have also shown to modulate the immune response of teleost leukocytes via  $\alpha$  and  $\beta$  adrenergic receptors. This study aims to expand the actual knowledge on stress-induced immune modulation, in order to evaluate the effects of stress on the immune system of maraena whitefish (*Coregonus maraena*). This salmonid fish is highly sensitive to stress compared to other salmonid species long adapted to aquaculture. To this end, a large set of specific primers was designed for reverse-transcription quantitative real-time PCR (RT-qPCR) analyses. The primer panel included cell-specific marker genes characterizing the distinct cell populations in the head kidney of *C. maraena*, which had been sorted using flow cytometry. In addition, we analysed the expression of catecholamine and cortisol receptors in each population, in order to define the repertoire of stress-related modulators present in the cells. In the next step, we performed a series of in vitro stimulations of head kidney leukocytes to study the expression of genes involved in immune activation and acute phase together with catecholamine and cortisol receptors. The primary cells were cultured for defined periods of time with adrenaline, noradrenaline or cortisol. In addition, cells were stimulated with highly purified pathogen-associated molecular patterns (PAMPs), either alone or in combination with the above hormones. Our study characterises, on one hand, the cell populations of maraena-whitefish-head kidney and reveals potential stress-response targets. On the other hand, we recorded the impact of stress hormones and PAMPs on the immune activity in head-kidney cells giving insights in the regulatory mechanisms behind the interaction of cortisol and catecholamines with leukocytes during immunological challenges.

**Keywords:** Cortisol, catecholamines, immune system, gene expression, salmonids.

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#### O-013.

##### Divergent and overlapping functions of type I interferons in zebrafish

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#### Abstract

Teleost possess 2 subgroups of type I interferons (IFNs) (group I and II) which bind to distinct receptors to activate antiviral response. Multiple isoforms are common within the subgroups. However, the functional differences of individual IFNs are poorly understood. In zebrafish, IFNphi1 and IFNphi4 belong to the group I IFN subgroup containing 2 conserved cysteines in the mature peptide and share a common heterodimeric receptor consisting of CRFB1 and CRFB5. It has been shown that the IFNphi1 can elicit strong antiviral response and is able to enhance host resistance to viral infection. In contrast, the functions of IFNphi4 are largely unknown. In the present study, we found that the IFNphi1 and IFNphi4 were differentially modulated during bacterial and viral infection. RNA sequencing analyses indicate that the ZF4 cells stimulated with the recombinant IFNphi1 and IFNphi4 proteins showed considerable similarity of expression patterns of genes involved in antiviral responses but also displayed marked differences. The results provide insights into the divergence of type I IFN functions in teleost fish.

**Keywords:** Interferon, cytokine, function, antiviral response, zebrafish

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#### O-014.

##### Direct cytotoxic activity of CD8+ T cells against *Ichthyophthirius multifiliis* in ginbuna crucian carp, *Carassius auratus langsdorffii*

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#### Abstract

A line of studies has shown that several humoral immune factors including complement, lectins and antibodies are involved in protection from parasite infections. However, cell-mediated immunity against parasites has poorly been understood in teleost fish. In the present study, direct cytotoxic activity of leukocytes against *Ichthyophthirius multifiliis* has been demonstrated in ginbuna crucian carp. Leukocytes labeled by each monoclonal antibody (2C3: anti-CD8, 6D1:anti-CD4, GB20: anti-macrophages/neutrophils) were co-incubated with *I. multifiliis*. The fluorescent microscopic observation showed that CD8+ T cells from naïve ginbuna carp, but not other leukocytes, contacted *I. multifiliis*. The cytotoxic activity of CD8+ T cells was significantly higher than that of other leukocytes, indicating that CD8+ T cells are dominant effector cells against *I. multifiliis*. The cytotoxic assay using a trans-well insert suggested that CD8+ T cells require to contact the parasites for the direct killing. Furthermore, a serine protease inhibitor 3, 4-dichloroisocoumarin (DCI) inhibited the cytotoxic activity of CD8+ T cells, but a perforin inhibitor Concanamycin A (CMA) did not. These results indicate that teleost CD8+ T cells have natural cell-mediated cytotoxicity against extracellular parasite by utilizing serine proteases, such as granzyme, suggesting that CD8+ T cells play an important role in innate immunity against extracellular protozoan parasites.

**Keywords:** Ginbuna crucian carp, immune system, CD8+ T cells, cell-mediated cytotoxic activity, *Ichthyophthirius multifiliis*

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#### O-015.

##### Isolation and characterization of shark single domain antibodies capable of binding salmonid alphavirus<sup>\*</sup>

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#### Abstract

Salmonid alphavirus (SAV) causes pancreas disease and sleeping disease in farmed Atlantic salmon and rainbow trout, resulting in significant economic losses to the aquaculture industry. To enable the rapid detection of SAV, robust reagents, capable of providing sensitive and specific detection, are required. Purifying SAV free from cell contaminants is difficult and may explain why there are a lack of commercially available antibodies for SAV. In this study a different approach, utilizing the novel shark immunoglobulin IgNAR, was investigated as a strategy for the production of SAV-detection reagents. IgNAR is a heavy chain homodimer that binds to antigens via a pair of highly soluble, single domains, referred to as VNARs. In