and vaccine model. The further use of the MVs as vaccines in their natural hosts such as strain-specificity and cross-immunity will be discussed.

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**O-010.**

**Potential role of rainbow trout red blood cells as mediators in the immune response induced by DNA vaccines**

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Abstract
Fish red blood cells (RBCs), unlike mammals, possess nucleus and organelles in their cytoplasm that give them the necessary machinery to generate an immune response at transcriptional and at proteomic level. In the last years nucleated RBCs have demonstrated to act as phagocytic cells, release cytokine-like factors and modulate leukocyte activity upon different stimulus. Also, they have been implicated in the response against viral infections. And recently, rainbow trout RBCs have been also implicated in the immune response to a DNA vaccine. So far, DNA vaccination is the best strategy to prevent and control viral infections, and for fish rhabdoviruses, only the DNA vaccine based on glycoprotein G (gpG) have resulted effective. However, the whole mechanisms involved in this protection and the immune response triggered by the DNA vaccine remain to be fully understood. In order to investigate the role of nucleated RBCs in DNA vaccination, we evaluated the immune response triggered by a DNA vaccine encoding the gpG of viral hemorrhagic septicaemia virus (VHSV) (GVHSV) in rainbow trout RBCs and explored RBCs as future targets or carriers for DNA vaccination. Upon fish vaccination of rainbow trout with GVHSV DNA vaccine, RBCs upregulated antigen presentation pathways at transcriptome and proteome level. In addition, rainbow trout RBCs responded to the DNA vaccine upregulating interferon type 1 (IFN-1) pathway. Also, RBCs transfected *in vitro* with GVHSV DNA vaccine protected RTG-2 cell line against subsequent viral infection. Besides, RBCs carrying the GVHSV DNA vaccine were able to induce specific antibody against VHSV *in vivo*. Also, RBCs transfected *in vitro* with GVHSV were able to modulate leukocyte activity *in vitro*. In summary, we suggest nucleated RBCs as cell mediators of the immune response playing an active role in DNA vaccination and propose nucleated RBCs as potential cell targets or carriers of antiviral prophylactics.
**Keywords:** Red blood cells, DNA vaccine, GVHSV, transcriptome, proteome

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**O-011.**

**Characterization of immune responses in different environmental temperature through linear array epitope vaccine induction**

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Abstract
Grouper fish have a very high value economically and also nutritionally; these make them a very important fish species across the world. However, this fish often suffers a very high mortality rate, up to 90%-100% whenever they are infected with a virus and always bring about a tremendous lost to the farmers of the relative fisheries. As fishes are ectothermic animals, where their bodily temperature and the immune system are regulated by the constant change of environmental temperature, we would like to characterize the vaccine in immune response induction upon virus infection in fish under various modulation of temperatures. Previously, our previous study has developed a vaccine NNVCP-S5E that was developed by a PCR based technique, linear array epitope (LAE) where the immunogen is having multiple linear epitope copies, which were predicted and chosen from NNV and the epitopes are amplified by template-repeat polymerase chain reaction (TR-PCR). Efficacy tests of the vaccine have been done on NNV challenged fishes previously and the relative percent survival (RPS) results were 72%, which indicates that NNVCP-S5E provides an effective prevention against NNV infection. However, the immune response characterized, whether TH1 or TH2 immune pathway is induced by vaccination of LAE vaccine in different temperatures are still unknown. Thus, juvenile giant grouper (*Epinephelus lanceolatus*) is used as a test subject in this project and the fishes were first acclimatized under three temperatures, 20°C, 28°C and 36°C for 1 week, and vaccinated through intraperitoneal injection (IP). Then, the fishes are challenged with purified NNV with a median lethal dosage, LD50 of 3.16.105copies/mL, and sacrificed to obtain immune related organs such. Real-time PCR (RT-PCR) is done in order to investigate the mRNA expressions of the TH1 or TH2 immune pathway markers, *T-bet* for TH1 pathway; *Gata-3* and *c-Maf* for TH2 pathway. Based on the RT-PCR analysis from all three groups, the gene expression results indicate that the vaccination tends to direct the fish immune response towards TH2 pathway of the adaptive immune response, which is a pathway towards the proliferation of B cells and a stronger antibody production. Furthermore, the study also shows a higher survival rate for the fishes that were acclimatized and vaccinated under 35°C compared to other temperatures after challenged with NNV for 14 days. This study contributed different insights which would help in a better protection against virus infection.
**Keywords:** Linear Array Epitope Vaccine, Grouper, Nervous Necrosis Virus, Temperature Difference, Immune Pathway

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**O-012.**

**Stress hormones modulate early immune activities inthe head kidney of *Coregonus maraena***

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Abstract
Under challenging conditions including threat and discomfort, the vertebrate stress response triggers endocrine and neurologic networks. They release stress hormones such as cortisol and catecholamines in the head kidney. This endocrine and hematopoietic tissue is thus of central

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 α and β 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 IFNphil4 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 langsdorfii***

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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 leucocytes, contacted *I. multifilii*. The cytotoxic activity of CD8+ T cells was significantly higher than that of other leucocytes, 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☆**

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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 contaminates 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