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

this work a recombinant VNAR antibody library was generated from a nurse shark (*Ginglymostoma cirratum*) host immunised with a combination of inactive SAV and recombinant SAV E2 protein. This library was panned using phage display technology to identify SAV-specific VNARs for use as immunological detection reagents. Of the novel VNAR clones identified three bound viable SAV with a high degree of sensitivity. These VNARs were shown to detect SAV subtypes 1, 2, 3, and 5 by ELISA. Two of the VNARs have specificity to SAV E2 glycoprotein. All of the VNARs showed characteristically high resistance to irreversible thermal denaturation. The subtype cross- reactivity and demonstrable robustness of these VNAR domains should enhance their utility as diagnostic reagents in the field.

**Keywords:** Shark IgNAR; VNAR antibody; Pancreas disease; Salmonid Alphavirus; Phage display

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## O-016.

### Influence of hypoxia stress on the immune response of pikeperch (*Sander lucioperca* L., 1758)

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Abstract
Animal welfare is a main issue in today's aquaculture. Pikeperch (*Sander lucioperca* L., 1758) is a native food fish of the northern hemisphere and very attractive for European aquaculture. It is susceptible to typical stress parameters of ongoing domestication. Unsuitable water temperatures and the lack of oxygen influence the well-being of fish and evoke to stress responses. Oxygen saturations lower than 40% are considered unfavourable for aquaculture facilities. These can be caused by insufficient water circulation, high stocking densities or high water temperatures. Temperatures above 20°C are optimal for pikeperch farming. Above 25°C, the oxygen concentration in water and the growth rate in pikeperch are reduced. These conditions are thus considered almost critical. Acute stress in fish modulates the immune system, including the release of a distinct set of cytokines and acute phase proteins. Chronic stress suppresses the immune system impairing the defense against pathogens. Hypoxia inducible factor (HIF)-1α is the most prominent regulator of hypoxic conditions in vertebrates, including Teleostei. It is expressed in nearly all immune cells and an interesting candidate as possible biomarker for hypoxic stress conditions. Little information has been published on the stress physiology of pikeperch. For successful breeding in aquaculture, details on effects of the stress parameters temperature and hypoxia will be important. We demonstrated recently, that a rise from 15°C to 25°C not only changes the expression pattern of heat shock induced genes (e.g. *HSP90AA1* and *SERPINH1*), but also of the hypoxia induced gene *HMOX1*, in liver and gills. The present study investigates the influence of oxygen deficiency on the early immune response of pikeperch. After peritoneal stimulation with inactivated *Aeromonas hydrophila* cells, we evaluated the transcript levels of possible biomarker genes, cell composition in certain tissues and immune cell activity. Preliminary data show that persistent lymphocytes of the peritoneum decrease in number after stimulation. Myeloid cells invade the site of infection to clear the pathogens. We suggest that hypoxia stress decreases the number of myeloid cells entering the peritoneum and these cells seem to originate from the head kidney. This work of the Campus bioFISH M-V was financed by the European Maritime and Fisheries Fund (EMFF) and the Ministry of Agriculture and the Environment Mecklenburg-Western Pomerania, Germany (Grant #: MV-II.1-RM-001).

**Keywords:** Pikeperch, hipoxia, welfare, immune System, stress Physiology

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## O-017.

### Anti-inflammatory and antioxidant properties of ethanol extract from Verbenaceae plant *Clerodendrum cyrthophyllum* turz in copper sulfate induced inflammation in zebrafish

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Abstract
Oxidative stress and inflammation are commonly present in several chronic diseases. Interestingly, these responses are closely related to pathophysiological processes. The inflammatory process can induce oxidative stress and *vice-versa* through activation of multiple pathways. Therefore, agents with antioxidant and/or anti-inflammatory activities are very useful in the treatment of many pathologies. *Clerodendrum cyrthophyllum* Turcz, a plant belonging to the Verbenaceae family, is used in Vietnamese traditional medicine for treating migraine, hypertension, inflammation of the throat, rheumatic arthritis. Despite its usefulness, studies on its biological properties are still limited. In this study, antioxidant and anti-inflammatory properties of an ethanol extract from leaves of *C.cyrtophyllum* (CCEE) were evaluated. In an *in vivo* anti-antioxidant test, 3 day-post fertilization (dpf) zebrafish larvae were treated with CCEE at 5, 20 and 40 μg mL-1 for 1 h and then exposed to 10 μM CuSO4 during 20 min to induce oxidative stress. Fluorescent probes were used to detect and quantify oxidative stress by measuring the fluorescent intensity (FI) in larvae. At 5 and 20 μg mL-1, the CCEE displayed a significant reduction of FI when compared with control group, indicating that it had profound antioxidant effects, reducing or preventing oxidative stress from CuSO4. Moreover, an experiment on 3 dpf zebrafish larvae treated with CCEE at 5, 20 and 40 μg mL-1 for 1 h and then exposed to 10 μM CuSO4 for 4 h showed that CuSO4 elicited a general stress response by the upregulation of *hsp70* and *gadd45bb,* involved in inducible DNA damage repair. But, the co-administration of CCEE protected zebrafish larvae against oxidative damage of CuSO4 through a down-regulation of *hsp70* expression and the upregulation of glutathione S-transferase genes *gstp1* and *gstp2*. To evaluate the anti-inflammatory properties of CCEE, a similar experiment was designed, using 10 μM CuSO4 to stimulate inflammation reaction. After 4 and 24 h of CuSO4 exposure, the expression of genes related to inflammatory process was analyzed in zebrafish larvae. Due to the copper accumulation in zebrafish tissues, the damage and oxidative stress were exacerbated overtime, resulting in the upregulation of genes related to inflammatory process such as *COX-2, PLA2*, *C3a*, pro- and anti-inflammatory cytokines (*IL-1, TNF-α; IL-10* respectively). However, the association of CuSO4 with CCEE reduced significantly *COX-2, PLA2, C3a, IL-1*. Taken together, the results suggested that CCEE has potent anti-oxidant and anti-inflammatory activities and may be useful in the treatment of various inflammatory diseases.

**Keywords:** Anti-inflammation, oxidative stress, anti-oxidant, CUSO4, zebrafish larvae, *Clerodendrum cyrthophyllum*

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