in rivers. Particularly, the change of dietary lipid composition from around 20% to 10% drastically impacts fry physiology. Indeed, lipids play a key role in fry development and some polyunsaturated fatty acids (PUFA) such as eicosapentanoid acid (EPA) are involved in immune defenses. In addition, the use of dry “germ-limited” food in a controlled environment probably reduces the exposure of fry to environmental bacteria known to shape the immune system and that could later lead to weak immune responses to fight pathogens present in the environment.

In this study, we aimed to optimize the diets of fry to improve their immune defenses and thus their ability to survive in the wild. For this, fry were fed six experimental or commercial diets: experimental diets with 12.7% lipids enriched either with EPA (1) or ALA (2), experimental diets with 20% lipids enriched either with EPA (3) or ALA (4), commercial diet (5), commercial diet (70% of energy supply) supplemented by living chironomids (30% of energy supply) containing complex microbiota (6). After 6 weeks of nutritional conditioning, fry were challenged with the pathogen *Aeromonas salmonicida salmonicida* by bathing. The body part of fry was sampled before and 24h after infection and was used for immune gene expression analyses involved in innate immune responses (*mpo, mcsfra, lysozyme*). Before bacterial infection, the expression of *mpo* and *igm* genes was lower in fry fed chironomids (F) diets compared to all other diets. This suggested that the supplementation of chironomids in diet could differentially modulate the immune system compared to dry diet. The results of immune gene expression after bacterial challenge, still under analysis, should provide information on how this living preys influence immune defense when fry are exposed to a pathogen. In addition, despite no differences were observed before infection between fry fed EPA- or ALA-enriched diets at different lipid percentage, we could expect some differences in immune responses after bacterial challenge as EPA is a direct precursor of leukotriene and prostaglandins production.

Keywords: Living prey diet; PUFA; bacterial challenge; restocking program; *Salmo salar*

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O-100.

Meta analyses of transcriptome responses to infections and stress in Atlantic salmon

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Abstract

Transcriptomics provides comprehensive information on host responses to pathogens, PAMP, antigens and treatments affecting the immune system. The main goal and challenge in data analyses is finding of consistent trends. Identification of transcription signatures (TS) – gene sets with reproducible expression profiles – is of high value. TS are instrumental for functional annotations of genes, interpretation and classification of transcriptome profiles. We have accumulated a large volume of data for Atlantic salmon produced with DNA microarrays, studies included multiple challenges with viruses, bacteria and parasites and various treatments stimulating immune and stress responses, totally 125 experiments and 4464 microarrays. Meta analyses started with selection of representative controlled experiments with large scale transcriptome responses, finding and ranking of genes with expression changes in at least two related experimental series followed with analyses of expression profiles in the entire database. Three large functional groups with several TS in each are presented, gene numbers and five genes with top ranks are indicated. **Virus responsive genes – VRG** (123 genes, *receptor transporting protein 3, viperin, isg15, ifit5, sacsin*) were identified in multiple trials with four viruses (IPNV, PRV, SAV and salmon poxvirus). These genes are equally activated with viruses and PAMPs (poly-IC, CpG, gardiquimod and bacterial DNA) *in vitro* and *in vivo*. In addition to specialized immune genes, VRG include many members with versatile or unknown functions and genes that most likely have changed their roles in higher vertebrates. While viral infection and exposure to PAMP induce the entire group, VRG fall into subgroups under different conditions suggesting a complex regulatory network. Systemic suppression of VRG was observed in fish infected with *Moritella viscosa* and sea lice. **Markers of inflammation** (105 genes, *C-C motif chemokine 4, serum amyloid, mmp 9, neutrophil cytosolic factor 1 and cathelicidin*) were selected by responses to PAMP and wounds. Multiple functional groups and pathways represent different aspects of immune responses, while several transcription signatures correspond to different scenarios of inflammation. **Stress markers** (31 genes, *mmp 9*, *immediate early response 2, junb, c/ebp-b* and *natterin*) were identified in studies of wound healing and exhausting physical load. These genes respond to various stressors in different tissues of Atlantic salmon. Stress component is manifested in various diseases with different magnitude.

Keywords: Atlantic salmon, transcriptome, antiviral response, inflammation, stress

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O-101.

Genomic and biological characterization of inhibitors and activators of the NF-kB pathway in rainbow trout (*Oncorhynchus mykiss*)

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Abstract

Bacterial infection on aquaculture facilities are a major problem for productivity and quality of fish production. One of the most common and important pathogens is Aeromonas salmonicida. This Gram-negative bacterium is the causative agent of the disease furunculosis, which had a devastating impact on salmonid aquaculture up until the late 1980’s. An efficient vaccination against this pathogen would represent a decisive improvement of healthcare concepts in aquaculture systems. The NF-kB pathway is considered an important target of vaccinations as it is the responsible for the expression of cytokines and other pro-inflammatory molecules. We profiled the expression of several genes involved in the NF-kB pathway and NF-kB-dependent effector genes in rainbow trout after immunization. using multiplex RT-qPCR. NKIRAS1/NKIRAS2, RelA/NFKB1 and PIAS1 were selected as interesting indicator genes. Using CHSE-214 cell line derived from a Chinook salmon (*Oncorhynchus tshawytscha*) embryo, as in vitro model, we transfected these three factors in order to analyze the subcellular localization

of these proteins. Additional luciferase reporter assays showed that NKIRAS1 inhibits the activation of the NFkappa-β pathway following stimulation with different PAMPs. Surprisingly, NKIRAS2 showed the opposite effect as reported in literature for mammals. On trout cells it rather acts as pro-inflammatory molecule which drives the overexpression of cytokines. Furthermore, a panel of genes, related to the immune response, was selected to determine the modulation of gene expression during the immunization and transfections and analyzed with

Fluidigm technology. Results will contribute to better understanding of the role of inhibitors during inflammatory stimuli.

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Keywords: Inhibitory factors; NF-kB; NKIRAS; Rainbow Trout; Furuncolosis

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O-102.

An improved genome assembly for *Larimichthys crocea* reveals hepcidin gene expansion with diversified regulation and function

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Abstract

Larimichthys crocea (large yellow croaker) is a type of perciform fish well known for its peculiar physiological properties and economic value. Here, we constructed an improved version of the L. crocea genome assembly, which contained 26,100 protein-coding genes. Twenty-four pseudochromosomes of L. crocea were also reconstructed, comprising 90% of the genome assembly. This improved assembly revealed several expansions in gene families associated with olfactory detection, detoxification, and innate immunity. Specifically, six hepcidin genes (LcHamps) were identified in L. crocea, possibly resulting from lineage-specific gene duplication. All LcHamps possessed similar genomic structures and functional domains, but varied substantially with respect to expression pattern, transcriptional regulation, and biological function. LcHamp1 was associated specifically with iron metabolism, while LcHamp2s were functionally diverse, involving in antibacterial activity, antiviral activity, and regulation of intracellular iron metabolism. This functional diversity among gene copies may have allowed L. crocea to adapt to diverse environmental conditions.

Keywords: *Larimichthys crocea*, Genome, Hepcidin

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O-103.

Role of sea lice secretome in host-parasite interaction: Immune modulation of SHK-1 cells exposed to *Caligus rogercresseyi* secretome

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Abstract

Caligus rogercresseyi is an ectoparasite that feeds on mucus, blood, and skin of its host. For a successful infestation, sea lice secrete proteins that allow avoiding host response. Among them, it has been described that trypsin and chymotrypsin have strong proteolytic activity in the peritrophic matrix of the intestinal parasite. In relation to the copepod *Lepeophtheirus salmonis*, it has been suggested that one of the strategies to successfully parasitize its host is given by the secretion of molecules such as proteases, prostaglandin synthetase E2 (PGE2) and cathepsin, causing immunodepression in fish. Moreover, from transcriptomic studies of *C. rogercresseyi* developmental stage has been identified secretome-related proteins as cathepsin, trypsin, and serpin highly regulated during the infective stage, copepodid. The aim of this study was to evaluate the effects of *C. rogercresseyi* secretome over salmon immune and stress response by an in vitro approach using SHK1 cell line. Proteins identification and characterization were performed using the transcriptome database of *C. rogercresseyi.* Characterized sequences were cloned into an expression vector, pET30a and expressed in *Escherichia coli* system. Recombinant proteins were purified by His-tag affinity chromatography. SHK1 cell line was stimulated with 25 ng/mL, 50 ng/mL and 100 ng/mL of recombinant proteins for 24 hours. After cells stimulation, cells were collected for RNA extraction for immune-related genes expression analysis by RT-qPCR. A total of two isoforms of cathepsin, serpin, and trypsin were characterized. With a molecular weight of 36.4, 36.3, 43.5, 49, 26.4 and 27 kDa, respectively. After 24 h of stimulation cell damage was observed in all groups exposed to secretome proteins. Furthermore, differences in immune-related genes expression levels were observed among cells exposed to secretome proteins and control group. This study provides novel information associated with host-parasite interactions associated with *C. rogercresseyi* secretome effects on salmon.

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Keywords: Interaction parasite-host, *Caligus rogercresseyi*; secretome, SHK1 cell line, RT-qPCR

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O-104.

TLR-mediated type-I interferon production and the regulatory mechanisms in carp thrombocytes

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Abstract

In early stage of viral infection, type-I interferons (IFNs) are produced by signaling from innate immune receptors such as Toll-like receptors (TLRs), which recognize virus-specific molecular patterns including nucleic acids. The type-I IFN transcriptions induced by TLRs are regulated via nuclear factor κB (NF-κB) complex and interferon regulatory factors (IRFs), but these details are still unclear in fish. In the present study, we show that thrombocytes in common carp (*Cyprinus carpio*) have a potent ability to produce large amount of IFNs in response to TLR signaling. Magnetic-sorted HB-8 mAb+ carp thrombocytes and negatively sorted other peripheral blood leukocytes (PBLs) were incubated with resiquimod (also called R848, a potent agonist of TLR7/8), followed by qPCR analysis. The expression levels of the common carp type-I IFNs (ccIFN1 and ccIFN2) in thrombocytes were considerably higher compared with that of in other PBLs. Whereas the ccIFN1 expression was relatively lower than the ccIFN2, the R848 stimulant highly upregulated the ccIFN1 expression than ccIFN2. Although typical inflammatory cytokines including interleukin-6 were also upregulated in thrombocytes, the expression levels were still lower than those in other PBLs. These results indicate that activation of carp thrombocytes by R848 inclines immune system toward antiviral response, rather than inflammation. Expression levels of IRF3 and IRF7 were also upregulated by R848, implying that the IFN transcriptions were activated by these IRFs. The expression of the IFNs and inflammatory cytokines were decreased by several NF-κB signaling inhibitors such as BAY11-7082 or phenethyl caffeate, however, sensitivities to each inhibitor were different between the IFNs and other cytokines. In the presence of those inhibitors, the ccIFN2 expression was correlated with the level of IRF3. In contrast, ccIFN1 expressions seem to be linked to IRF7, suggesting that these two IRFs regulates different IFN genes separately. Our finding suggests that fish thrombocytes are important components for antiviral immunity and can