

P-054.**NKEF, an antiviral protein involved in the immune response of rainbow trout red blood cells**

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Abstract

Nucleated red blood cells (RBCs) of rainbow trout (*Oncorhynchus mykiss*) possess, in addition to the transport of oxygen and carbon dioxide functions, other relevant roles in the immune response. Its participation in the antiviral response against the VHSV (viral hemorrhagic septicaemia virus) is being studied. To establish the proteins of rainbow trout RBCs that interact directly with VHSV, an immunoprecipitate (IP) of the RBCs exposed to VHSV was performed using an antibody against the VHSV N protein. Thirty-one proteins were identified by mass spectrometry analysis and among them the natural killer-enhancing factor (NKEF) was selected. This protein belongs to the family of peroxiredoxins that have an antioxidant function and improve the cytotoxic cells activity. In addition, NKEF has been identified in several species of fish, including the rainbow trout, where its role has been related to oxidative stress and immunity. In order to establish the antiviral role of NKEF in rainbow trout RBCs, the expression profile of NKEF has been studied in RBCs exposed to VHSV. After VHSV-exposure, NKEF was up-regulated at transcriptional and protein levels which correlated with lower levels of VHSV replication. Moreover, the implication of NKEF in the antiviral response of RBCs against VHSV are being evaluated.

keywords: NKEF, peroxiredoxins, immunoprecipitate, VHSV, red blood cells.

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P-055.**The perfect balance: Trade-offs between reproduction and the immune system in reproducing female rainbow trout (*Oncorhynchus mykiss*)**

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Abstract

Optimal allocation of available resources between different physiological functions is fundamental for survival of any organism. Phenotypic plasticity allows, especially in limited resource situations, trade-off between physiological systems. Since both reproduction and immunity are resource-intensive, trade-offs have been studied in different species. Still, there is lack of knowledge on the relation between reproductive and immune systems during the reproductive cycle of a seasonally reproducing fish.

Here we aim to study the reproductive and immune changes during the seasonal reproductive cycle of female rainbow trout (*Oncorhynchus*

mykiss), in order to identify potential trade-offs. To gain insights of the activation of the immune system, fish were immunostimulated with an intraperitoneal injection of LPS.

Our results show a main upregulation of reproductive parameters during the cycle. Immune parameters, in contrary, displayed a general down-regulation during reproduction. Recovery of them 3 months after the spawning moment, with exception of the phagocytic activity, supports the trade-off theory. Moreover, 17 β -estradiol (E2) concentration in plasma was the only parameter significantly correlated with all immune parameters. We had no evidence for an effect of the immunomodulation on the reproductive parameters.

This suggests that reproduction is able to modify immunocompetence, and potentially infection resistance in rainbow trout, and that E2 seems to participate in those immuno-neuro-endocrine interactions.

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P-056.**In vitro study of the serum effect on the supplementation of sea bass (*Dicentrarchus labrax*) head-kidney macrophage culture medium: Effect on the immune response**

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Abstract

Macrophages are the main phagocytic cell in fish and their optimum function is required to protect them against pathogens, playing nutrition an important role in fish resistance to diseases and therefore being an important factor, which affects macrophage function. Aquaculture is a field in continuous growth, which is acquiring high importance due to the increase in the demand of high-quality protein from the rapidly growing world population. Being fish diseases the main cause of economic loss in Aquaculture production, and due to the importance in macrophage activity in the protection against them, fish macrophage function is a key element which has a direct effect on fish welfare and the response of the immune system. In order to evaluate macrophage function, mainly in vivo studies are being performed, while in vitro macrophage culture, with the potential to study and enhance macrophage function, is an attractive field that is yet to be developed and exploited.

The present study was intended to analyze the impacts of the serum in the culture medium enrichment for sea bass head-kidney macrophages, evaluated through the cytokine expression of the macrophages previously stimulated with Poly IC and LPS obtained from *L. angillarum*. Furthermore, a comparative study between three different diets (Diet 1, fish oil based; diet 2, cameline oil based; diet 3, rapeseed oil based) was performed in order to survey their effect on the immune response expressed by the macrophages cultured in vitro for a period of 72h.

Fish used in this experiment were juvenile sea bass with an average weight of 154g at the moment of kidney extraction, fed with three diets. Both head-kidneys were obtained and head-kidney macrophages were extracted and cultured in vitro both in sea bass serum and fetal bovine serum supplemented L-15 medium. Cytokine expression was measured through qPCR at 0h, 6h, 12h, 24h, 72h post stimulation with Poly IC and LPS. The results obtained through the study showed a stronger and earlier immune response to the Poly IC stimulation in the macrophages cultured in the medium supplemented with Seabass Serum in comparison with the medium supplemented with Fetal Bovine Serum, which is observed both in Diet 1 and Diet 2. Further studies must be performed in order to assess the effect of serum supplementation in macrophage culture medium,

measuring different cytokine expression and biochemical parameters, as well as phagocytic activity.

keywords: *Dicentrarchus labrax*, fish cytokines, head-kidney macrophages, cameline oil, sea bass serum

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P-057.

Characterization and expression analysis of *Salmo salar* heat shock proteins in response to *Piscirickettsia salmonis* infection under a cohabitation challenge

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Abstract

Heat shock proteins (HSPs) comprise several families of highly conserved proteins which function mainly as chaperonins that refold proteins in response to stressful stimuli (changes in pH, salinity, temperature, radiation, among others) and are players in the host immune system activation during infection. The different HSP families are roughly grouped according to their molecular weights (such as Hsp110, Hsp100, Hsp90, Hsp70, Hsp60 and small HSPs). HSPs have been widely characterized as being modulated by the aforementioned stimuli, and many have been linked to processes like signaling, apoptosis and regulation of homeostasis, in addition to their chaperonine functions. In previous studies exploring the modulation of gene expression for HSPs, results have been varying, mostly depending on the kind of stressing agent and host organisms. Infection by virus and bacteria show mostly an up-regulation of small HSPs and down-regulation of some constitutively expressed HSPs (cognates). The present study aims to characterize several HSPs (4 in the Hsp30 group, Hsp60, 2 Hsp70 proteins and 6 Hsp90-like proteins, for a total of 13 HSPs) in *Salmo salar* in terms of phylogenetic relationships, conserved synteny, basal gene expression in several tissues and modulation of gene expression at the transcript level in response to infection (via cohabitation challenge for 7 weeks) using two field strains of the bacterium *Piscirickettsia salmonis* (which is the most relevant pathogen in Chilean aquaculture and the etiological agent of *Piscirickettsiosis*) and under two different salinities (5 and 20%). The field strains used (AUS005 and AUS111) belong to two different genogroups (LF-like and EM-like) and were isolated from differing marine environments in terms of salinity (freshwater and estuary). Results show distinct patterns of gene expression for hsp in each tissue, with most of them expressed predominantly in liver and kidney. During infection, differential expression patterns were observed for most of the HSPs studied, generally showing a sharp up-regulation of *hsp30* genes in the initial phases of the challenge, up-regulation of *hsp90* genes and, interestingly, a slight down-regulation of *hsp60* and *hsp70* genes. These data demonstrate that *Salmo salar* HSPs possibly play a role in the immune response of fish against a bacterial infection and encourage further research in order to elucidate their concrete roles in those processes.

keywords: Heat shock proteins, *Salmo salar*, *Piscirickettsia salmonis*, cohabitation challenge, stress.

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P-058.

De novo assembly, characterization of tissue-specific transcriptomes and identification of immune related genes from the scallop *Argopecten purpuratus*

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Abstract

The scallop *Argopecten purpuratus* is one of the most economically important cultured mollusks on the coasts from Chile and Peru but its production has declined due, in part, to the emergence of mass mortality events of unknown origin. Driven by this scenario, increasing progress has been made on recent years in the comprehension of the immune response mechanisms in this species. However, it is still not entirely understood how different mucosal interfaces participate and cooperate with the immune competent cells, the hemocytes, in the immune defense. Thus, in this work we aimed to characterize the transcriptome of three tissues with immune relevance from *A. purpuratus* by next generation sequencing and de novo transcriptome assembly. For this, 18 cDNA libraries were constructed from hemocytes, gills and digestive gland tissues of scallops from different immune conditions, and sequenced by the Illumina HiSeq4000 platform. A total of 967.964.884 raw reads were obtained and 967.432.652 clean reads were generated. The clean reads were *de novo* assembled into 46.601 high quality contigs and 32.299 (69.31%) contigs were subsequently annotated. In addition, three de novo specific assemblies were performed from clean reads obtained from each tissue cDNA libraries for their comparison. Gene ontology (GO) and KEGG analyses revealed that annotated sequences from hemocytes, gill and digestive gland could be classified into both general and specific subcategory terms and known biological pathways, respectively, according to the tissue nature. Finally, several immune related candidate genes were identified, and the differential expression of a mannose receptor, C-type lectin, C1q, MPEG-1, IL-17 and a scavenger receptor CD163 genes was established as tissue-specific, suggesting they could display specific roles in the host defense. The data presented in this study provides the first insight into the tissue specific transcriptome profiles of *A. purpuratus*, which should be considered for further research on the interplay between the hemocytes and mucosal immune responses. WORK FUNDED BY FONDECYT 11150009.

keywords: RNA-seq; tissue-specific immune genes; scallop; mucosal interfaces; antimicrobial effectors

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P-059.

Cellular and humoral immune responses of meagre (*Argyrosomus regius*) juveniles to bacterial infection with *Photobacterium damsela* piscicida

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