3rd INTERNATIONAL CONFERENCE ON FISH & SHELLFISH IMMUNOLOGY

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# 3rd INTERNATIONAL CONFERENCE ON FISH & SHELLFISH IMMUNOLOGY

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# P-001

## MOLECULAR CHARACTERIZATION OF A NEW FISH SPECIFIC CHEMOKINE CXCL_F6 IN LARGE YELLOW CROAKER (*Larimichthys crocea*) AND ITS ROLE IN INFLAMMATORY RESPONSE

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**ABSTRACT**
Chemokines are a superfamily of structurally related chemotactic cytokines exerting significant roles in regulating cell migration and activation. Currently, five subgroups of fish specific CXC chemokines, named CXCL_F1-CXCL_F5, have been identified in teleost fish. However, understanding of the functions of these fish specific CXC chemokines is still limited. Here, a new member of fish specific CXC chemokines, LcCXCL_F6, was cloned from large yellow croaker *Larimichthys crocea*. Its open reading frame (ORF) is 369 nucleotides long, encoding a peptide of 122 amino acids (aa). The deduced *Lc*CXCL_F6 protein contains a 19-aa signal peptide and a 103-aa mature polypeptide, which has four conserved cysteine residues (C28, C30, C56, and C72), as found in other known CXC chemokines. Phylogenetic analysis showed *Lc*CXCL_F6 formed a separate clade with sequences from other fish species, tentatively named CXCL_F6, distinct from the clades formed by fish CXCL_F1-5 and mammalian CXC chemokines. The *Lc*CXCL_F6 transcripts were constitutively expressed in all examined tissues and significantly up-regulated in the spleen and head kidney tissues by poly (I:C) and *Vibrio alginolyticus*. Its transcripts were also detected in primary head kidney leukocytes (HKLs), peripheral blood leucocytes (PBLs), and large yellow croaker head kidney (LYCK) cell line, and significantly up-regulated by poly(I:C), lipopolysaccharide (LPS), and peptidoglycan (PGN) in HKLs. Recombinant *Lc*CXCL_F6 protein (r*Lc*CXCL_F6) could not only chemotactically attract monocytes/macrophages and lymphocytes from PBLs, but also enhance NO release and expression of proinflammatory cytokines (TNF-α, IL-1β, and CXCL8) in monocytes/macrophages. These results indicate that *Lc*CXCL_F6 plays a role in mediating the inflammatory response.

**KEYWORDS**
CXC chemokine, CXCL_F6, Chemotaxis, Large yellow croaker (*Larimichthys crocea*), inflammatory response

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# P-002

## MOLECULAR MARKERS ASSOCIATED WITH ANTIGEN PRESENTATION PROCESS IN ATLANTIC SALMON DURING OUTBREAKS OF *Piscirickettsia salmonis* AT SEA FARMING CENTERS

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**ABSTRACT**

Chile is one of the main producers of Atlantic salmon (*Salmo salar*) in the world, with a currently marine culture biomass close to 350,000 tons. This biomass suffers mortalities close to 3,500 tons per month, being the infectious diseases the second primary cause of this problem (19.6%). The major agent of this mortality is the bacteria *Piscirickettsia salmonis* (67.9% of cases). *P. salmonis* is an intracellular facultative pathogen that generates systemic infections evading the immune response of the host by infection of macrophages and avoiding the respiratory burst. To control this pathogen, Chilean aquaculture has used antibiotics, immunostimulants and vaccines. However, no strategy has given optimal results. In this context, more data of immunological parameters in field conditions are necessary for a more accurate characterization of the real state of the fish when is facing a *P. salmonis* infection. In this work, we have focused on quantifying molecular markers associated with the process of antigen presentation, which is crucial to achieve coordination between innate and adaptive immune response. For this, we evaluated the gene expression of interferon gamma (ifng), transforming growth factor beta (tgfb) and interleukins (il-10, il-12 and il-15); markers of cell lineage (cd83 and cd80/86); major histocompatibility complex I and II (mhci and mhcii); T cell receptor alpha (tcra); immunoglobulin M (igm); and annexin1 (anxa1) by qPCR from spleen of *S. salar* at two sea farm centers (Puelche and Punta Islotes). Puelche reported two outbreaks of *P. salmonis*, while Punta Islotes didn't report any fish infected with the pathogen during the sampling time. Gene expression results showed that fish from Puelche increased the gene expression of ifng, tgfb, cd83, cd80/86, mhcii, il-10, il-12, igm and anxa1 at different sampling points. On the other hand, fish from Punta Islotes showed an increase of the gene expression of il-10 and cd80/86, mhcii and il-12. Finally, the correlation of data showed a proportional detection between markers of the same sea farm center and inversely proportional between centers with *P. salmonis* (Puelche) and without *P. salmonis* (Punta Islotes).

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**KEYWORDS**
*Salmo salar*, Chile, gene expression, spleen, Piscirickettsiosis
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# P-003

## EVALUATION OF ALPHA-LIPOIC ACID ANTI-INFLAMMATORY PROPERTIES USING ZEBRAFISH AS IN VIVO MODEL

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**ABSTRACT**

Chronic diseases remain the primary root cause of death and disability worldwide. It is now well established that several agents (aging, oxidative stress, iron overload, etc,) induce inflammation and dysregulate inflammatory pathways, which lead to the development of chronic diseases. Acute inflammation is a part of innate immunity initiated by the immune cells that persists only for a short time. However, if the inflammation continues, the second stage of inflammation called chronic inflammation commences which instigates various kinds of chronic diseases, including arthritis, cancer, cardiovascular diseases, diabetes, and neurological diseases via dysregulation of various signaling pathways. Therefore reducing inflammation by therapeutic strategies would decrease the risk of various chronic diseases. Alpha-lipoic acid (ALA) is a natural antioxidant compound which is naturally found in plant and animal sources but small quantity of ALA can be absorbed as free ALA. The pivotal action of ALA is the antioxidant activity due to its ability to scavenge and inactivate free radicals, protecting against oxidative damage in several diseases, including neurodegenerative disorders.

New evidence suggests that ALA might be a useful supplement for inflammation induced by oxidative stress in chronic diseases. This study investigated whether ALA has a protective role under oxidative stress induced inflammation in *Danio rerio*. Zebrafish has emerged as a powerful model system to examine mechanisms of human disease. The presence of both innate and adaptive utility in zebrafish allows its use as a tool to examine the role of immune cells in normal development and in the pathogenesis of disease states. A gene expression analysis of several proinflammatory marker genes (il4, il13, tnfa, ifng1, nos2b) was carried out in adult zebrafish gut after LPS (alone) and LPS plus ALA administration. Our preliminary data showed that ALA administration was capable to reduce ($p<0.001$) the inflammation induced by LPS treatment. Furthermore, we will also evaluate the effect of ALA on immunological aspects of chronic inflammation, using spint1a mutant zebrafish larval model which exhibit chronic skin inflammation characterized by epidermal hyperproliferation and neutrophil infiltration.

**KEYWORDS**

Chronic disease, inflammation, alpha Lipoic Acid, zebrafish, spint1 mutant

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# P-004

## IMMUNE RESPONSE ASSESSMENT OF ATLANTIC SALMON AGAINST *P. salmonis* IN SEA-CAGE FARMING CENTERS

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**ABSTRACT**

The monitoring of sea farming centers at the south of Chile was performed to obtain data on the expression of 39 immune-related genes related of Salmo salar in three fish organs: gill, spleen and head kidney. The data was obtained from farms with and without occurrences of outbreaks of *Piscirickettsia salm*onis, and a generalized mixed linear model (GMLM) was established, considering the environmental variables and fish necropsy. This model allowed (i) the establishment of a baseline of expression of immune response genes, and (ii) molecular gene markers of susceptibility to the pathogen, which is, if fish are P. salmonis positive. For the identification of the baseline expression of immune genes, the condition of normal (healthy) fish was defined and differences in the expression of these genes were established in the gill, spleen and head kidney. In addition, genes exhibiting temporal variation in their expression were identified and therefore, an annual historical reference of this value was considered to the baseline determination. Regarding the genes proposed as markers of P. salmonis infection, it was determined that the expression of the genes coding for TNF-α, cathelicidin, NLRX1 and IL-1β, in gills; cathelicidin and hepcidin in anterior kidney; and hepcidin and IL-10 in spleen are indicators of infected fish, and thus, susceptible to P. salmonis. This result also highlights the data obtained at the gill level, an easy-sampling organ in the field with validated molecular indicators. While these studies were based on the genes expression levels, we also obtained result on the availability at the protein level of several of these molecules, using specific antibodies obtained in this project. The GMLM will also allow to propose molecules whose increase in expression over time can be predictive indicators of P. salmonis infection. One of the molecular markers with the best application perspective and whose availability was also evaluated at the protein level is cathelicidin expressed in gills. The use of the proposed molecular tools in sea farming centers to evaluate the expression of gene markers will be useful for the identification of critical windows for therapeutic treatment. Consequently, if the expression of gene markers is detected between reference values, it will indicate infection by *P. salmonis* with an associated probability, and industry could applied productive strategies such as the use of medicated diets.

This work was funded by the Program for Sanitary Management in Aquaculture of the Ministry of Economy, Development and Tourism of Chile (FIE-2015-V014 201708070149).

**KEYWORDS**
Immune response, P. salmonis, cathelicidin, gill immunity, culture center
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# P-005

## TRANSCRIPTOME PROFILING OF ATLANTIC SALMON KIDNEY CELLS (ASK) AFTER STIMULATION WITH POLY (I:C) AND INFECTION WITH INFECTIOUS SALMON ANEMIA VIRUS (ISAV)

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**ABSTRACT**
Viral diseases are of great concern in fish farming. Close to 20% of salmon put out to sea are lost during production and a large part of this is due to infections. Oil-adjuvanted multivalent vaccines against bacteria/viruses are available and confer good protection against bacterial disease but efficiency against viral disease under field conditions has been questioned. Our goal is to test if known agonists for human Toll-like receptors (TLRs) can be used as adjuvants in vaccines formulations, against fish viruses. Poly (I:C) is a synthetic analog of double-stranded RNA (dsRNA) that mimics a viral infection and could be used for immunostimulation in therapeutics and vaccines. But before developing a vaccine it is necessary to understand more about how the immune system responds to these ligands.
Using ASK cells as an in vitro model, we have compared the transcriptome response of cells infected with ISAV and cells stimulated by poly (I:C) in different time points (12 and 48h). RNA sequencing analysis revealed a total of 3111 differential expressed genes (DEGs) in the treated groups compared to control. From these DEGs, 2815 and 1309 genes were differentially expressed in the poly (I:C) and, ISAV groups respectively. Poly (I:C) treated cells showed stronger response both at 12h and 48 hours when compared with ISAV infected cells. Using the recently annotated salmon genome, pathway and gene ontology (GO) enrichment analyses were performed using Ingenuity and the R package "ClusterProfiler". Most of the shared DEGs were immune-related and were overrepresented in pathways and GO terms related with immune response and response against virus. Some genes were only differentially expressed in one of the groups (e.g., CD28 – poly (I:C) group and interleukin 1β – ISAV group) while others were related only with early or late response against virus or poly I:C, for example interferon (IFNa3) was only detected in early poly (I:C) group (12h) and late ISAV group (48h) .
Our results can help to comprehend the molecular mechanism of Atlantic salmon immune response against ISA virus infection. They can also help identify biomarkers for ISA virus early detection and to investigate the possible role of poly (I:C) as adjuvant for future vaccines in aquaculture.

**KEYWORDS**
Atlantic salmon, RNA sequencing, Poly (I:C), Toll-like receptor, ISA virus

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# P-006

## DIETARY ARGININE AND CITRULLINE SUPPLEMENTATION DURING A SHORT-TERM FEEDING PERIOD IMPROVES THE GILTHEAD SEABREAM (*Sparus aurata*) IMMUNE STATUS.

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**ABSTRACT**

Several amino acids (AA) are known to regulate key metabolic pathways that are crucial for immune response. In particular, arginine (ARG) appears to have important roles regarding immune modulation since it is required for macrophage responses and lymphocyte development. Moreover, citrulline (CIT) is a precursor of arginine, and it was reported as an alternative to ARG for improving macrophage function in mammals. The present study aimed to explore the effects of dietary ARG or CIT supplementation on the gilthead seabream immune status. Triplicate groups of fish (23.1 ± 0.4 g) were either fed a control diet (CTRL) with a balanced AA profile, or the CTRL diet supplemented with graded levels of ARG or CIT (0.5% and 1% of feed); ARG1, CIT1, ARG2 and CIT2, respectively.

After 2 and 4 weeks of feeding, fish were euthanized and blood was collected for blood smears, plasma for humoral immune parameters and shotgun proteomics, and head kidney for the measurement of health-related transcripts. A total of 94 proteins were identified in the plasma of all treatments. Among them, components of the complement system, apolipoproteins, as well as some glycoproteins were found to be highly abundant. After performing a PLS of the proteins of interest, differences between the two sampling points regardless dietary treatment were observed. In this regard, component 1 (61%) justified the effect of sampling time, whereas component 2 (18%) represents the individual variability within diet. It is particularly interesting that fish fed ARG2 and CIT2 at 4 weeks were more distant tan fish fed all dietary treatments at 2 weeks and fish fed the CTRL diet at 4 weeks , suggesting that the modulatory effects of AA supplementation at the proteome level were more effective after 4 weeks of feeding. The bactericidal activity increased in fish fed the highest supplementation level of both AAs after 4 weeks. A tendency of increased monocytes was observed for the relative proportion of peripheral blood leucocytes in fish fed diets with the highest supplementation level of both AAs after 2 weeks of feeding period, compared to their counterparts fed the lower supplementation level. Peripheral monocyte numbers also correlated positively with nitric oxide, which showed an increasing trend in a dose-dependent manner. The colony stimulating factor 1 receptor tended to be up-regulated at the final sampling point regardless of dietary treatments. These results suggest that dietary supplementation with ARG or its precursor (CIT) have an immunostimulatory effect after 4 weeks of feeding. More

healthrelated biomarkers are being processed which will enlighten the effects of these functional diets.

**KEYWORDS:**
Amino acids, immunology, aquaculture, functional feeds, gilthead seabream

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# P-007

## IMMUNE RESPONSE OF GILTHEAD SEABREAM (*Sparus aurata*) AFTER EXPERIMENTAL INFECTION WITH LYMPHOCYSTIS DISEASE VIRUS (LCDV-SA)

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**ABSTRACT**
Lymphocystis disease (LCD) is caused by the lymphocystis disease virus (LCDV), a double-stranded DNA virus belonging to the genus *Lymphocystivirus* (family *Iridoviridae*), affecting more than 150 fish species from both marine and freshwater environments. A few studies have been focused on the immune defensive mechanisms of fish against LCDV, but only one was conducted during a natural LCD outbreak in gilthead seabream, which is one of the most important cultured fish species in the Mediterranean and the European Atlantic coasts. The aim of this study was the analysis of 23 genes related to the immune response in gilthead seabream specimens after experimental infection with LCDV-Sa using real-time PCR (qRT-PCR) in samples of head kidney and intestine at 1, 3, and 8 dpi. To study the progression of LCDV-Sa infection in gilthead seabreams, the number of viral DNA copies and the expression of *mcp* were determined in samples of caudal fin, head kidney and intestine. LCDV-Sa was detected by qPCR in all the samples from inoculated fish analysed, whereas no amplification was obtained in samples from the control group. Regarding the gene expression following LCDV-Sa infection, a total of 22 of the 23 genes studied were differentially expressed in head kidney or intestine samples at some time points analysed. The *pkr* was the only gene showing no differential expression compared to control samples through the entire experiment. Different gene expression profiles were obtained between the organs studied, detecting 18 differentially expressed genes (DEGs) in head kidney samples, four of them exclusively up- or down-regulated (*nccrp1*, *il10*, *mhcII*, and *tnfα* genes), and 5 genes with a significant change in the expression tendency from 1 to 8 dpi (*irf3*, *isg15*, *il10*, *ck10*, and *c3*). In the intestine, 18 DEGs were also detected (14 shared with head kidney), being *mx1*, *casp1*, *ck3* and *tlr9* genes exclusively detected in these samples, and *mx1*, *mx3, irf9* and *ighm* differentially regulated over time. The results obtained allow us to understand which genes are essential for host-pathogen interactions and could be used as molecular markers for vaccine efficacy evaluation.
This study was funded by the project P12-RNM-2261, (proyecto de Excelencia de la Junta de Andalucía).

**KEYWORDS**
*Sparus aurata*, LCDV-Sa, experimental infection, immune response, differentially expressed genes

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# P-008

## ANALYSIS OF GENE EXPRESSION IN NODAVIRUS-INOCULATED SENEGALESE SOLE USING A NEW OPENARRAY® PLATFORM

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**ABSTRACT**

Nervous necrosis virus (NNV) is the causative agent of the viral encephalopathy and retinopathy, a disease that affects cultured Senegalese sole (*Solea senegalensis*). A NNV reassortant (Ss160.03), combining genomic segments from red-spotted grouper nervous necrosis virus (RGNNV) and striped jack nervous necrosis virus (SJNNV) genotypes, has been previously isolated from Senegalese sole, being highly virulent to this fish species. The RNA-Seq technology has been used in a previous study to comparatively analyse Senegalese sole transcriptomes in two organs (head kidney and eye/brain) after infection with two NNV virus with different levels of virulence to that fish species, a highly virulent reassortant isolate (wSs160.03) and a less virulent mutant reassortant obtained by reverse genetics (rSs160.03247+270). To validate previous RNA-Seq results, a 112-assay OpenArray® platform (Thermofisher) has been designed. This platform included 89 genes chosen according to transcriptomic changes observed by RNA-Seq (covering PRRs, type I IFN response, signal transduction, inflammation, virus responsive genes, and apoptosis), 17 genes selected based on their previously described relation with the immune response against fish viral infections, and 6 control genes (including 3 endogenous genes and 3 viral genes). A total of 63.25% differentially expressed genes (DEGs) detected by RNA-Seq were validated by the OpenArray designed, showing similar expression levels and a 100% expression tendency accuracy. Furthermore, this tool brings new information about the infection process that was not shown by the RNA-Seq analysis, such as the expression profiles of *mda5*, *ifng*, *c9*, *c3*, *mx*, *ifit-1*, *myd88*, *tbkbp1*, and *ube1* genes in different samples at 48 h post-infection (pi). Moreover, a consistent decrease in the number of DEGs was observed at 72 hpi, confirming that 48 h is an adequate time point to study innate immune response of sole against NNV infection. In conclusion, this molecular platform has been confirmed as a good tool for further studies on the sole immune response against NNV mutant infections, which will contribute to the knowledge of the mechanisms of the pathogen-host interaction.

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**KEYWORDS**

*Solea senegalensis*, Reassortant Nervous Necrosis Virus, OpenArray®, differentially expressed genes (DEGs), Immune response.

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# P-009

## SALMO SALAR GLUCOCORTICOID RECEPTORS ANALYSIS OF ALTERNATIVE SPLICING VARIANTS UNDER STRESS CONDITIONS

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**ABSTRACT**

Cortisol is the main glucocorticoid in teleost, where exerts multiple functions mediated through the glucocorticoid receptors (GR). Currently, it is known that many fish species have two GR genes, gr-1 and gr-2. Additionally, some teleost has also two different splice variants for GR1; gr-1a and gr-1b. In this study, we report for first time the identification of 2 gene copies for GR1 and GR2, located on chromosomes 4q-13q (gr1) and 5p-9q (gr2) of Salmo salar genome. Furthermore, our results describe gr1 splice variants in each chromosome, sharing typical teleost GR elements, such as the 9 amino acids insertion in DNA binding domain (DBD) and variations in length of the ligand binding domain (LBD). For GR2 gene copy on chromosome 5, three splice variants were predicted and differentiated by 5 amino acids insertion and length in the DBD. Also, we identified an uncommon truncated gr-2 gene copy on chromosome 9, lacking the DBD and LBD domains and expressing its mRNA in salmon. Finally, through of specific primers design for each predicted splice variants, we validate and determine the expression of its transcripts in S. salar subjected to stress by stoking density. The results showed differences in the expression of all identified mRNAs, revealing that gr1 and gr2 splice variants were upregulated in head kidney and gills of post-stressed fish. In conclusion, our findings suggest that from specific salmonid genomic duplication (125 MYA), two gene copies of each GR receptor were generated in S. salar and the splice variants identified, could contribute to the variability of the complex modulation of the receptors expression during stressful events, leading to different physiological responses in fish. Fondap 15110027.

**KEYWORDS**

Glucocorticoids Receptor, splicing variant, Salmo salar, cortisol, fish stress

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# P-010

## MYD88 DEPENDENCE ON THE ACTIVATION OF INDUCED INNATE EFFECTOR MECHANISMS BY TLR5M AND TLR5S IN SALMONIDS

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**ABSTRACT**
The innate immune response (IIR) in teleosts is essential in the defense against pathogens because of adaptive immune response limitations. Also, the innate immune effector mechanisms are activated by the recognition of conserved structures among pathogens, through Pattern Recognition Receptors, such as Toll-like Receptors (TLRs). Specifically, the membrane-anchored and soluble Toll-like Receptor 5 (TLR5M and TLR5S, respectively) from teleosts recognize bacterial flagellin as do orthologs in mammals. However, it has not been demonstrated whether the induced signaling pathway by these receptors depends on the Myeloid Differentiation Protein 88 (MyD88) to generate a pro-inflammatory response, in addition to activating of IIR effector mechanisms in salmonids. Therefore, in this work we study the MyD88 dependence on the induction of TLR5M/TLR5S signaling pathway mediated by flagellin as ligand, at both predictive and experimental level. CellDesigner program was used for the construction and mathematical characterization of the TLRs model, and simulations were carried out to predict the its dynamics. On the other hand, at the experimental level, we studied the key components response of the TLR5M/TLR5S signaling pathway against to flagellin stimulation, as well as the functional participation of MyD88. Additionally, the activation of some RII effector mechanisms was evaluated against the induction of the signaling pathway under study and related dependence of MyD88. For these experimental assays, treatment kinetics were performed by immuno-stimulants and pre-treatments with a MyD88 inhibitor in *S. salar* Head Kidney Leukocytes (HKLs) primary culture, as cell model; and the key components expression of the signaling pathway was analyzed by RT-PCR. Moreover, the stimulation of some IIR effector mechanisms was evaluated (like Reactive Oxygen Species -ROS- production) in RT-S11 cells stimulated with flagellin and pre-treated with a MyD88 inhibitor.
Our results for the simulations predicted that the MyD88 inhibition produced a delayed response downstream of the signaling pathway against stimulation with flagellin. Hence, the stimulation assay in HKLs corroborated the previous predictions, and also showed a relative expression kinetic of genes concordant with a positive feedback mechanism between TLR5M/MyD88/TLR5S. Finally, the assays of RII effector mechanisms demonstrated that flagellin stimulates ROS production (extracellular and intracellular) and that these processes also depend on MyD88. In

conclusion, we demonstrate that the activation of flagellin-mediated TLR5 (M and S) signaling, as well as the activation of final effector functions, depend on MyD88.

**KEYWORDS**
Toll-like Receptor, salmonids, MyD88, flagellin, system biology.
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# P-011

## DIFFERENTIAL IMMUNE RESPONSES OF EUROPEAN SEA BASS (*Dicentrarchus labrax*) UPON NODAVIRUS INFECTION BY BATH OR INTRAMUSCULAR INJECTION.

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## ABSTRACT

Viral nervous necrosis virus (VNNV) produces the viral encephalopathy and retinopathy (VER) disease causing great mortalities in aquaculture fish. As is known, the innate system of teleost fish plays an important role in the defence against viral infection. Therefore, the aim of this study was to evaluate several immune-related enzymes (i.e. lysozyme, peroxidase and proteases) in serum, as well as the expression of immune-related genes (i.e. lysozyme, IgM, MHC I, TCR β, CD8α, IL-1β, IFNα, IL-10, IL-8, MAVS and Mx) in European sea bass (Dicentrarchus labrax) infected with VNNV by two methods with demonstrating effectiveness. In order to do this, European sea bass juveniles were infected with VNNV by either bath (1 hour, 106 TCID50 mL-1) or intramuscular injection ($10^6$ TCID50 mL-1). After 7, 15 and 30 days post-infection (dpi), fish serum was collected to measure innate immune parameters whilst the brain (tissue target of VNNV), spleen and intestine (as lethal samples), caudal fin, gills and skin (as low-invasive samples) were collected into RNA-later for the analysis of relative gene expression using Real-Time PCR (qPCR). The results demonstrated that serum peroxidase activity decreased in both infected groups at 15 dpi respect to the values found in control groups whilst this activity remained unchanged. In the case of protease activity, the results showed an increase in the serum of fish infected by bath method at the end of the trial (30 dpi) compare to unchallenged fish. The rest of immune-related enzymes measured not showed significant variations. Regarding to gene expression, the results revealed a modulation dependent on the type of infection and the organ studied (invasive or minimally invasive sample), which could be of great interest for the detection and treatment of this viral disease in aquaculture industry.

## ACKNOWLEDGEMENTS

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**KEYWORDS**
Viral Encephalopathy and Retinopathy (VER), nodavirus (VNNV), innate immune system, RT-qPCR, European sea bass (*Dicentrarchus labrax*).

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# P-012

## IDENTIFICATION OF PERITROPHINS AS POTENTIAL VACCINE CANDIDATES AGAINST SEA LICE: A REVERSE VACCINOLOGY APPROACH.

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**ABSTRACT**

Peritrophins are structural proteins of the peritrophic membrane (PM). This protein has been described in arthropods and have been described in some species of crustaceans. Peritrophins contribute to the maintenance of structural characteristics, including strength, elasticity and, permeability of the PM, and are capable to affect homeostatic regulation at intestinal level. In some copepod species of commercial interest such as *Caligus rogercresseyi* and *Lepeophtheirus salmonis* have not been characterized. These species are ectoparasite pathogens known as sea lice. The development of vaccine-based control strategies for sea lice control in salmonid aquaculture has been desired. The aim of this study was to use a reverse vaccinology approach to identify the *C. rogercresseyi* and *L. salmonis* peritrophins isoforms and identified by *in silico* analysis linear epitopes. We analyzed databases obtained by Illumina sequencing of the different stages of sea lice development. The contigs were compared against crustacean peritrophic membrane proteins database using BLAST and UniProt. Expression levels of different isoforms were evaluated by RNA-Seq and validated by RT-qPCR. *In silico* prediction tests of linear T and B epitopes were performed by the online software BepiPred and TEPITOPE, respectively. As results, in both sea lice species was identified different peritrophin isoforms, with differences in the number of chitin-binding domains. Furthermore, these proteins were differently expressed among sea lice developmental stages. In addition, linear epitopes of B lymphocytes were identified. These results demonstrate the antigenic potential of the peritrophins of both species. Further subsequent research will demonstrate the immunogenic action of perithrophins through *in vivo* tests against sea lice in farmed salmons.

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**KEYWORDS**

Peritrophins; sea lice; reverse vaccinology; *Caligus rogercresseyi*; *Lepeophtheirus salmonis.*

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# P-013

## ARE RELATED GABA AND ACETYLCHOLINE WITH THE SYNTHESIS OF IL-1β, TNF-α AND TGF-β IN THE IMMUNE SYSTEM ORGANS OF THE NILE TILAPIA?

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**ABSTRACT**

Background: External and internal changes are factors that can disrupt the balance between neuroendocrine and immune systems; however, currently studies that evaluate the modulation of the immune system by neuroendocrine factors in fish are scarce, and for some aspects are null. Objective: To evaluate the effects of sub-basal increase of GABA and acetylcholine (ACh) in pro and anti-inflammatory cytokines (IL-1β, TNF-α, and TGF-β) levels in the immune system organs and serum of Nile tilapia. Experimental procedures: Basal value of GABA was quantified in the serum by liquid chromatography using 2-hydroxynaphthaldehyde as derivatizing reagent. ACh was estimated by Hestrin method with modifications. Fish were intravenously dosed into the caudal vein at day 0, 3 and 6 with three doses below tenth of the basal levels of ACh and one hundred of GABA. Based on genome assemblies of *O. Niloticus* (BioProject: PRJNA354796), recombinant IL-1β, TNF-α and TGF-β were obtained by Gateway® method, and polyclonal antibodies against the recombinants were induced in mice. On the eighth day, specimens were sedated by freezing on ice for sampling the blood and after, the fish were euthanized by rapid freezing (-80°C). Levels of IL-1β, TNF-α and TGF-β were evaluated by indirect ELISAs. Results: By effect of sub-basal increases of neurotransmitters, a dose-dependent response of pro and anti-inflammatory cytokines synthesis was detected. In pronefros and thymus of ACh-treated fish, an increase was found ($p \leq 0.001$), as in the spleen ($p \leq 0.05$). Similarly in fish treated with GABA; however, a noticeable enhancement of IL-1β and TNF-α synthesis was noted in pronefros and spleen ($p \leq 0.001$). In contrast, the great induction of TGF-β was estimated in thymus. Nevertheless, high basal values of these cytokines as well as a clear dose-dependent response were quantified in serum. Discussion: Contrasting with this study, an inhibitory effect of GABAergic agents in macrophages and APC from a mouse multiple sclerosis model evaluated as IL-1β and IL-6 levels was detected. In macrophages, ACh suppresses the release of TNF-α, IL-1β and IL-18. Contrasting, in splenectomized mice dosed with an α7nAChR agonist (nicotine), an increase of serum TNF-α and IL-1β was observed. Despite there are not preceding reports about the role of dosed ACh or GABA on TGF-β level in serum or immune system organs, TGF-β and ACh present in the tumoral microenvironment of colon adenocarcinoma switch-on in agreement with tumor development, as facilitating IL-1β production. Conclusion: Results of this study suggest that GABA and ACh function as paracrine or autocrine factors in the immune system organs of the Nile tilapia.

**KEYWORDS**
Autocrine and paracrine factors; IL-1β; TNF-α; TGF-β.
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# P-014

## ALPINONE EXHIBIT IMMUNOSTIMULANT EFFECTS IN ATLANTIC SALMON KIDNEY

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**ABSTRACT**
Viral diseases are very harmful in aquaculture since they cause high mortalities in fish that have great economic losses. At present, vaccines to prevent viral diseases in fish are limited and their efficiency is not satisfactory. Therefore, it is of great importance for aquaculture to find new antiviral agents that can modulate the innate antiviral immune response in salmonids and this way can be used as a measure of prophylactic control against viral diseases. In this work, we evaluated the immunostimulant effect of the Alpinone in primary culture of Atlantic salmon kidney cells throught of analysis of the levels of transcripts of key genes involved in the activation of the innate antiviral immune system. **Materials and Methods**: Cells from head kidney of Atlantic salmon were treated with 5 µg /mL, 10 µg / mL and 15 µg / mL of the Alpinone flavonoid. We used as negative control untreated cells and cells treated with DMSO corresponding to the solvent of the compound, and as positive control we used 10 µg / mL of Poly I: C. After 8, 24 and 48 hours of treatment with the Alpinone flavonoid, the total RNA extraction of the cultures was carried out, the cDNA synthesis of the samples and we analyzed the levels of transcripts of genes involved in the early antiviral response such as MX, IFNα, IRF3, MDA-5 and RIG-I by real-time PCR. **Results**: Alpinone flavonoid increases the transcript levels of the transcription factor IRF-3, the cytokine IFN-α, the antiviral protein Mx and RIG-1 receptors, while the TLR3 and TLR9 receptors they do not present changes in the levels of transcripts with respect to the control. **Conclusion**: Alpinone flavonoid increases the levels of transcripts of genes involved in the innate antiviral immune response, proposing this flavonoid as a potential candidate to be used as an antiviral agent for the treatment of diseases in salmonid fish. Acknowledgements: Fondecyt 11170984 & Fondecyt 1180265

**KEYWORDS**
Flavonoid, Alpinone, Imnunostimulant, Antiviral, Salmonid.
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# P-015

## EVIDENCES OF *Tenacibaculum maritimum* EVADING STRATEGIES AGAINST SENEGALESE SOLE (*Solea senegalensis*) PRIMARY HEAD-KIDNEY LEUCOCYTES

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**ABSTRACT**
*Tenacibaculum maritimum* evading strategies are currently unknown and many aspects regarding the host-pathogen interaction are still not fully elucidated. Hence, the present study aimed to assess Senegalese sole cellular immune responses following stimulation with either live or UV killed *T. maritimum* through both functional (e.g. superoxide anion and nitric oxide production, leucocytes killing capacity) and gene expression approaches. Senegalese sole head-kidney leucocytes were isolated and exposed to several live or inactivated *T. maritimum* strains during 4 h, 12 h, 24 h and 48 h. In the present study, primary head-kidney leucocytes exposed to different live bacterial strains did not show significant changes in superoxide anion nor nitric oxide production, whereas UV killed *T. maritimum* strains seemed to stimulate leucocytes' nitric oxide release. Interestingly, leucocytes stimulated with both live and UV killed bacterial strains reacted with relatively low superoxide anion production. Regarding gene expression data, stimulation with live strains induced very low increase in interleukin-1β (*il1β*), hepcidin antimicrobial peptide (*hamp*), cyclooxygenase 2 (*cox2*) and g-type lysozyme (*glys*) transcripts at 4 h compared to non-stimulated cells, which decreased similarly until 48h. Although interleukin-10 (*il10*) expression levels presented a similar pattern, an upregulation was observed at 48 h post stimulation. In contrast, the expression levels of *il1β*, *cox2*, *hamp* and *il10* from host primary cell culture stimulated with inactivated bacterial strains increased more than those from leucocytes exposed to live bacteria and up to 100-fold. The downregulation of inflammatory, iron regulating genes and toll-like receptor 2, as well as the extensive destruction of phagocytes, in cells exposed to live bacteria could be considered as part of *T. maritimum* evading strategies.

**KEYWORDS**
Bacterial pathogens, innate immune response, respiratory burst, host defences.

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# P-016

## FILIFOLINONE, A NATURAL COMPOUND WITH ADJUVANT EFFECT FOR OPTIMIZATION OF A COMMERCIAL VACCINE FOR IPNV.

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**ABSTRACT**
Filifolinone is a natural compound isolated from resin of *Heliotropium huascoense*, that increases the expression level of pro-inflammatory and anti-inflammatory cytokines in kidney cells of salmon. Given that cytokines are generated in response to the presence of pathogens, we have studied their potential adjuvant effect for the optimization of commercial vaccines against IPNV. In Chile, infectious pancreatic necrosis (IPN) is an endemic, prevalent, highly contagious and economically important disease, since it is widely distributed in Chilean salmon farms, causing heavy economic losses, in freshwater crops such as sea crops. The negative economic impact is directly related to the increase in losses due to mortalities, elimination of carrier breeders, progeny from carrier breeders, decrease in growth rates and increase in control measures. Among the control measures implemented, is the application of biosecurity measures, egg disinfection, individual incubation systems and application of vaccines. Currently, there is a wide variety of vaccines, however none of them has been efficient enough to control or eradicate the disease. Therefore, we evaluated the adjuvant effect of Filifolinone searching new strategies for control. In addition to mortality, its effectiveness was determined in terms of the presence of erratic swimming, anorexia and exophthalmia. Besides, we evaluated the presence of total IgM antibodies and the viral load in the anterior kidney in terms of VP2 gene expression.

**KEYWORDS**
Adjuvant activity, Filifolinone, IPNV,vaccine, antiviral activity.
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# P-017

## CELL SURFACE MARKERS FOR THE IDENTIFICATION OF SUBPOPULATIONS OF SPLENOCYTES FROM SALMONIDS

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**ABSTRACT**

The phylogenetic development of the immune system shows that the adaptive response appears in fish. However the structure of this response and the antigen presentation process that coordinates the transit between the innate and adaptive immunity has been poorly characterized in teleost, due to the low number of phenotypic tools available for aquatic organisms. The spleen is a secondary lymphoid organ in fish and the present study aimed to identify subpopulations of splenocytes in salmonids that express molecules associated with the antigen presentation (CD83, CD86 and MHC II). For this, splenocytes were co-cultivated, activated or not by interferon gamma, with lymphocytes. The results indicated the existence of cells expressing surface molecules capable of presenting antigens to T lymphocytes. These cells, derived from splenocytes, increase the expression of surface molecules induced by interferon gamma and decrease their phagocytic capacity over time. Activation of these cell types *in vitro* shows that there is a tendency to activate populations of T cells (previously isolated with anti-CD4.1 and anti-IgM antibodies) to T regulatory lymphocytes. Further research is necessary to validate if the events here described also occur *in vi*vo, to understand if the link between innate and adaptive immunity in fish has an inhibitory phase component, which could explain the absence of memory or the low protection capacity of vaccines used in aquaculture. This work was support by the Program for Sanitary Management in Aquaculture of the Ministry of Economy, Development and Tourism of Chile (FIE-2015-V014 201708070149) and by the Norwegian Research Council (281052). BML is a fellow of Advanced Human Capital Formation of CONICYT, Chile (21151176).

**KEYWORDS**

*Salmo salar*, *Oncorhynchus mykiss*, primary culture, flow cytometry, qPCR

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# P-018

## BETANODAVIRUS INFECTION MODULATES EUROPEAN SEA BASS IMMUNE SYSTEM AT SHORT-TERM

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**ABSTRACT**
Viral diseases are responsible for high mortality rates of several marine species and the associated economic losses on actual aquaculture. Special attention has been paid to *Nervous Necrosis Virus* (NNV), which affects many fish species and causes viral encephalopathy and retinopathy disease. Particularly, the European sea bass (*Dicentrarchus labrax*) is a very susceptible fish species to NNV and suffer mortalities up to 100% at larvae and juveniles stages. In this work, we aimed to evaluate the transcription of genes related to the cell-mediated cytotoxic activity (CMC), which is the main cellular immune response triggered by this virus, in different tissues of European sea bass. For this purpose, the virus was intra-muscularly injected to each individual and the effects upon NNV infection were evaluated in head-kidney and brain after 1, 7 and 15 days post-infection. Several immune-related genes were studied by real time PCR including those that code for interleukin (IL)−27 subunit beta-like (EBI3), the lymphocyte antigen 6-like secreted (SLURP1L), IL-12 beta subunit (IL12BA), the cytotoxic and regulatory T cell molecule (CRTAM) and the co-stimulatory receptor for the activation of naive T cells (CD28). In general, the expression of these genes were up-regulated after infection with NNV. Results of this study evidence that NNV modulate fish immune system at short-term (1 day post-infection). These findings suggest that CMC has an important role on immune response against virus-infected cells.
This work was funded by projects from MINECO and FEDER (AGL2016-74866-C3-1-R), the *Spanish Institute of Oceanography* (NODAMED) and *Fundación Séneca* (*Grupo de Excelencia de la Región de Murcia* 19883/GERM/15).

**KEYWORDS**
Nervous necrosis virus (NNV), immune system, *Dicentrarchus labrax*, PCR

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# P-019

## CPG OLIGODEOXYNUCLEOTIDES MODULATE INNATE AND ADAPTIVE FUNCTIONS OF IGM+ B CELLS IN RAINBOW TROUT

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**ABSTRACT**
Oligodeoxynucleotides (ODN) containing unmethylated CpG motifs have been widely postulated as vaccine adjuvants both in mammals and teleost fish. However, to date, the effects that CpGs provoke on cells of the adaptive immune system remain mostly unexplored in fish. Given that rainbow trout (Oncorhynchus mykiss) IgM+ B cells from spleen and blood transcribe high levels of toll like receptor 9 (TLR9), the receptor responsible for CpG detection, in the current work, we have investigated the effects of CpGs on both spleen and blood IgM+ B cells from this species. CpGs were shown to exert strong proliferative effects on both spleen and blood IgM+ B cells, also increasing their survival. The fact that CpGs increase the size of IgM+ B cells, reduce the expression of surface IgM and IgD and upregulate the number of IgM-secreting cells strongly suggest that IgM+ B cells differentiate to plasmablasts / plasma cells in response to CpG stimulation. Additionally, CpGs were shown to modulate the antigen presenting capacities of trout IgM+ B cells through an increased surface MHC II expression and transcriptional up-regulation of co-stimulatory molecules, although in this case, significant differences were observed between the effects exerted on spleen and blood cells. Similarly, differences were observed between spleen and blood IgM+ B cells when CpG stimulation was combined with B cell receptor (BCR) crosslinking. Finally, CpGs were also shown to affect innate functions retained by teleost IgM+ B cells such as their phagocytic capacity. These results demonstrate that CpGs regulate many adaptive and innate functions of teleost B cells, supporting their inclusion as adjuvants in novel vaccine formulations.

**KEYWORDS**
Rainbow trout, CpG, IgM, B cells, BCR.

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# P-020

## INSIGHTS INTO THE FUNCTIONS OF PISCIDINS IN THE EUROPEAN SEA BASS (*Dicentrarchus labrax*)

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**ABTRACT**
Antimicrobial peptides (AMPs) are one of the host's first line of defenses against a wide range of infectious agents. Apart from the antimicrobial activity, AMPs are known to influence other biological processes, such as immunomodulation and iron metabolism. Fish present a specific group of AMPs, the piscidins. These peptides have been characterized in several fish species, acting on multiple pathogens, being also altered when fish are subjected to an infection. Furthermore, several studies have shown the potential of using synthetic peptides to promote fish survival upon infection. However, in the European sea bass (*Dicentrarchus labrax*), a commercially important fish produced in aquaculture, only hepcidin has been extensively characterized. Thus, a comprehensive study on the functions of other AMPs, particularly piscidins, is missing. Here, we identified and characterized the different piscidins of sea bass. We evaluated the antimicrobial activity of piscidins against several bacteria known to cause massive mortalities in cultured marine fish. Furthermore, the expression of the different genes belonging to the piscidin family was assessed under distinct experimental conditions, particularly infection and iron modulation, at pre-determined time points. Our results show a diverse piscidin antimicrobial activity *in vitro* against the different bacteria, indicating that these AMPs have a direct role against these pathogens, depending on the pathogen and piscidin peptide. Our data also shows a piscidin response after infection, suggesting that piscidins are involved in the response against infection. Furthermore, preliminary data shows that piscidins also respond to iron modulation, indicating that these AMPs may have other yet undisclosed functions asides antimicrobial activity, such as a role in iron metabolism. Our findings imply that piscidins might be a complementary or alternative way that fish possess to deal with this essential element, apart from the major iron regulator hepcidin. It is known that iron is also essential for bacterial progression during infection. Thus, iron is in a continuous regulation to be available for body processes, being also modulated to ensure that is inaccessible to pathogenic microorganisms. Further work is necessary to fully understand the role and mechanisms of action of piscidins under the context of immune response and iron metabolism regulation, and to possibly uncover a novel function for these particular peptides in fish.

**KEYWORDS**
Antimicrobial peptides; Piscidins; Sea bass (*Dicentrarchus labrax*); Infection; Iron metabolism

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# P-021

## EFFECTS OF 17α-ETHYNYLESTRADIOL (EE2) ON THE IMMUNE SYSTEM OF JUVENILE EUROPEAN SEA BASS WITH A SPECIAL FOCUS ON B AND T CELLS

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**ABSTRACT**

Synthetic compounds are known for their persistence and bioaccumulation in the environment. 17α-ethynylestradiol (EE2), a synthetic derivative of the natural hormone oestradiol, is present in human contraceptive pills, but also in livestock and aquaculture activity. Therefore, municipal wastewaters are one of the most important sources of this compound in the aquatic environment. Because EE2 induces oestrogenic effects even at trace level concentration, it has potent endocrine disrupting properties. In wildlife, and especially in jawed vertebrates, EE2 is classically recognized for its negative effects on the reproductive functions. As endogenous oestrogens, however, EE2 can also modulate the immune system. Consequently, EE2 may affect the individual fitness by altering the capacity to cope with pathogenic agents. Adverse effects of EE2 on immune system function and ontogenesis remain to be fully elucidated, both for mammals and teleost fish. Juvenile European sea bass (90 days post-hatch) were exposed to waterborne EE2 (5ng/L) for one month at 17ºC in order to investigate the modes of actions of EE2 on the developing immune system. Exposure concentrations were verified by LC-MS/MS and the oestrogenic activity assessed by yeast estrogen screen assay. Following exposure, several lymphoid organs including the thymus, the head-kidney and the gills were sampled for analysis by qPCR, immunohistochemistry and flow cytometry on isolated leucocytes. The leucocytes were analyzed for their phagocytic capacity as well as their proportion of DLIg3+ and DLT15+ lymphocytes. DLIg3 and DLT15 are monoclonal antibodies, which specifically recognize sea bass IgM and a pan-T cell marker, respectively. First results validate the exposure, which did not significantly impact the biometric measurements of the fish (growth, spleno- and hepatosomatic indices). Considering the proportion of DLIg3+ and DLT15+ lymphocytes and the phagocytic capacity, the treatment increased significantly the proportion of DLIg3+ cells in the head-kidney only but had no effect on the other measured immune parameters. Ongoing work aims at evaluating the effect of EE2 on T cell and B cell differentiation in their respective primary organs (thymus and head-kidney) as well as the secondary immune organs in order to understand the capacity of EE2 on the establishment of the immunocompetence in sea bass.

**KEYWORDS**
Immune system; European sea bass; endocrine disruptor; T Cell; B Cell

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# P-022

## ANTI-INFLAMMATORY MEDIATORS AND APPETITE REGULATORY NEUROPEPTIDES ARE AFFECTED BY CHRONIC STRESS IN *Salmo salar*

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**ABSTRACT**

In fish farming, there are different long-term stress conditions, some of which are so severe that fish can no longer reestablish homeostasis. Factors that cause prolonged stress can drastically alter the defense against pathogens by upregulation of anti-inflammatory response. In addition, chronic stress modifies the fish energy balance diminishing the appetite. In vertebrates, the stimuli that generate stress are initially perceived by the central nervous system sensors of hypothalamus, stimulating the release of cortisol into the bloodstream. Interestingly, the feeding control center of fish, like in mammals, is also found in the hypothalamus. Thus, extensive cultivation under inappropriate conditions chronically can affect the inflammatory response and the energy uptake from the food. In this study, the expression of the glucocorticoid-regulated protein Annexin A1 (AnxA1), an important endogenous anti-inflammatory mediator was analyzed in smolt salmon kept at different stocking densities for 40 days. The highest stocking density (HSD) (40 and 60 Kg/m3) simulated a chronic stress by crowding. In addition, the mRNA expression of NPY, substance P, VIP and CGRP appetite regulatory neuropeptides, and the anorexigenic hormone leptin were also analyzed. The results of ELISA assay showed that AnxA1 expression was significantly increased in the gill and muscle of specimens kept at highest stocking density. While gene expression analysis by Real-time PCR showed upregulation of VIP mRNA in the fish gut of HSD group. Moreover, the mRNA expression of SP neuropeptide increased three fold in liver of specimens held at 60 Kg/m3. For the other hand, dowregulation of NPY mRNA in fish brain of HSD group was observed. Finally, leptin mRNA expression was maintained at high levels in the liver of specimens held at the highest stocking density. These alterations reflect the effect of high stocking density on inflammatory and appetite molecular signals. Therefore understanding how these signals are affected during the productive processes of fish farming is required. A reduced appetite involves a lower uptake of energy from food, which affects the functioning of different physiological processes such as the immune system.

This work was support by the Program for Sanitary Management in Aquaculture of the Ministry of Economy, Development and Tourism of Chile (FIE-2015-V014 201708070149).

**KEYWORDS**
Anti-inflammatory, Annexin A1, appetite regulatory molecules, stock density, stress
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# P-023

## INTERLEUKIN (IL-17) AND RECEPTORS IN EUROPEAN SEA BASS AND GILTHEAD SEABREAM. REGULATION BY NNV INFECTION

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**ABSTRACT**
Interleukin-17 (IL-17) is a cytokine family composed of six ligands (A-F) being IL-17A and IL-17F the best characterized. These are produced by Th17 cells and induce the expression of many inflammatory mediators. In addition, several IL-17 receptors have been also identified with different cell distribution. The main objective of this work was to identify IL-17 forms, as well as some IL-17 receptors, in two fish species: the European sea bass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*). In addition, we have evaluated the transcription of IL-17 forms and their receptors in seabream and sea bass after the Nervous necrosis virus (NNV) infection, which is a disease that produces viral encephalopathy and retinopathy in fish, by real time PCR. This study revealed the presence of IL-17 and their receptors in both fish species. Moreover, a regulation of fish immune system was observed in fish exposed to NNV infection. To our knowledge, this is the first study addressing the IL-17 expression on the European sea bass and the gilthead seabream infected by NNV.
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**KEYWORDS**
Interleukin IL-17, Il-17 receptors, sea bass, gilthead seabream, Nodavirus

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# P-024

## HYDROGEN PEROXIDE TREATMENT MODULATES THE IMMUNE AND DETOXIFICATION RESPONSES IN THE SEA LOUSE *Caligus rogercresseyi*

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**ABSTRACT**
The sea louse *Caligus rogercresseyi* is the main ectoparasite affecting Chilean salmon industry. Hydrogen peroxide (H2O2) has been applied as a chemical treatment to control sea lice infestations. The mode of action is based on forming bubbles in the copepod hemolymph inducing a mechanical paralysis detaching the parasite from the host. However, there are critical mechanisms underlying the defense responses of the ectoparasite to this chemical, but are poorly understood. This study is aimed to describe the molecular responses of *C. rogercresseyi* to H2O2 by gene expression analyses of selected candidate genes on parasites exposed to the chemical. Bioassays were conducted using 6 concentrations of H2O2 (180, 360, 540, 744, 900, 1080 ppm) plus one control group. Median-effective concentration (EC50) and median-effective time (ET50) values were obtained. This evaluation consisted in prolonged exposure of sea lice to H2O2 and counting affected animals during different intervals of time. Affected parasites at each examination time were collected for gene expression analyses. RT-qPCR was conducted to evaluate the expression of several immune-related genes, and others associated with the antioxidant system. Increased expression levels of genes related to defense response were obtained, such as genes of toll-like receptors and immune deficiency pathways. Genes of the antioxidant system associated as catalase and superoxide dismutase were also modulated. Here, novel immune and detoxification responses during exposure to hydrogen peroxide are evidenced. This study contributes to a better understanding of the innate immune response in sea louse and also provide new insights into the mechanism of action of hydrogen peroxide as a chemical treatment.

**KEYWORDS**
Hydrogen peroxide, *Caligus rogercresseyi*, immune response, gene expression, bioassays.
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# P-025

## SELECTION OF PROTEIN CANDIDATES FOR VACCINE DEVELOPMENT AGAINST *Piscirickettsia salmonis* USING A REVERSE VACCINOLOGY APPROACH

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## ABSTRACT
*Piscirickettsia salmonis* is an intracellular γ-proteobacteria, belonging to the order Thiotricales and the etiological agent of Piscirickettsiosis, which causes massive economic losses in the Chilean salmon industry and generates an extremely high consumption of antibiotics during the production cycle. Despite experimental evidence for effective subunit vaccine formulations, currently available commercial vaccines for *P. salmonis* are mostly whole cell vaccines and vaccine combinations, which have been proven to be ineffective in generating long-term protection, thus the importance of developing new therapeutic tools.
Reverse vaccinology is the name given to a novel approach to vaccine/adjuvant design, which takes full advantage of the genome and protein information available for pathogens. The main goal in this method is to identify antigenic regions or epitopes in proteins that can stimulate different components of the immune system, using several *in silico* tools and immunological databases. This approach has been applied to several bacteria, such as *A. baumanii*, *Campylobacter*, *M. tuberculosis, S. pneumoniae, V. anguillarum*, among others. To our knowledge, no study has used this methodology to determine potential candidates for *P. salmonis* vaccine formulations. In this study, two datasets were used, the complete set of non-clustered protein sequences of the LF-89$^T$ strain and a clustered *P. salmonis 'pangenome'* set of protein sequences, and their outputs were combined. As a result of our substractive workflow, 12 potential proteins were identified, and annotated as hypothetical porins (mainly from the LbtU-like family), outer membrane proteins (OmpW), proteins belonging to secretion systems (TolC), LPS-related proteins and other lipoproteins. In addition, several T-cell and B-cell epitopes were determined for these proteins. Future work should focus on the validation of the epitopes obtained using structural *in vitro* tools for them to be applied in a hypothetical vaccine design for *P. salmonis*.

## KEYWORDS
Reverse vaccinology, *Piscirickettsia salmonis*, Structural vaccinology, B-cell epitopes, T-cell epitopes

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# P-026

## RECOMBINANT IMMUNOTHERAPY AGAINST *Ichthyophthirius multifiliis* IN *Oncorhynchus mykiss*

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**ABSTRACT**
*Ichthyophthirius multifiliis* (Ich) is a fish protozoan parasite and the causative agent of white spot disease. During the Ich life cycle, fish epidermis and gill epithelia are disrupted, which could increase the susceptibility to a secondary infection, and even could cause mortalities when the fish are under a severe infection. Therefore, it is important to develop an effective measure to control this parasitic infection in farmed fish. Currently, the Ich control relies on treating the water containing infective Ich with chemical compounds that have a negative impact on both human health and the environment. Vaccines have raised as an alternative strategy to control Ich infection in farmed fish. Early observations have shown that rainbow trout were able to acquire protection after either a non-lethal infection or an intraperitoneal injection of live parasites. Antibodies seemed to play an essential role in the defense mechanism since fish were protected after being passively immunized with immobilizing monoclonal antibodies against Ich. Although several vaccines against this parasite have been evaluated, currently there is no commercial vaccine available. This work aims to develop an immunotherapy based on a recombinant Ich-immobilizing single chain variable fragment (Ich-scFv), which is a fusion of the variable regions of the heavy and light chains of an Ich immobilizing monoclonal antibody, connected by a short linker peptide. The steps to reach this objective include: selecting a hybridoma clone that produces immobilizing monoclonal antibody against Ich, sequencing the variable regions of the IgG gene of the hybridoma clone, designing *in silico* an Ich-svFv, and evaluating *in vivo* the effectiveness of different delivery systems of the Ich-svFv. In this conference, preliminary results will be presented.
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**KEYWORDS**
*Ichthyophthirius multifiliis*, Ich, rainbow trout, single chain antibody, scFv.

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# P-027

## EFFECT OF SALINITY ON IMMUNOLOGICAL RESPONSES IN STRIPED CATFISH (*Pangasianodon hypophthalamus*) IN LARVAE AND JUVENILE STAGES

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## ABSTRACT

Effect of salinity in aquatic animals has been investigated in many species with special attention to osmoregulation capacities but how it affects the immunomodulatory responses in fish remains largely unknown. In this study, striped catfish larvae (10-day post-hatching) and juveniles (20-25 g) were chronically exposed to different salinities (namely 0, 5, 10, 15 and 20 ppt during 10 and 20 days respectively). Then the larvae were heat shocked at 390C while the juveniles were submitted to a challenge test with a virulent bacteria *Edwardsiella ictaluri.* The results showed that lysozyme activity in larval fish increased proportionally to the increase of salinity, with significant differences in groups submitted to 15 and 20 ppt (28.3 and 30.6 U/mg protein respectively). Additionally, lethal time LT50 after heat shock were 5 times higher in fish exposed to 5 and 10 ppt treatments in comparison with 0 and 15 ppt treatments ($p<0.05$). In juveniles, hyperosmotic exposure led to a significant decrease of hematocrit during both salinity exposure period and bacterial challenge, with the highest hematocrit values found in 0 ppt treatment and the lowest in 20 ppt treatment ($p<0.05$). On the other hand, blood osmolality and ion concentrations significantly increased with salinity exposure, confirming the low capacity of striped catfish to osmoregulate. The lysozyme activity reached the highest values in fish reared at 10 ppt after bacterial challenge ($p<0.05$), without significant differences with fish at 15 ppt. Histopathological observations indicated some alterations of gills (*e.g.* reduction of interlamellar cell mass, increase of epithelial cell thickness) and head kidney (*e.g.* edema between melanomacrophages, reduction of hematopoietic tissue) after salinity exposure. Complement activity did not show any significant difference between salinity treatment, neither in larvae nor in juveniles. These results support the hypothesis that hyperosmotic stress may affect the striped catfish larvae and juveniles, with a higher sensitivity observed at the larval stage.

## KEYWORDS

Hyperosmotic exposure, striped catfish, immunity, histopathology, salinity.

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# P-028

## RECOMBINANT VP1 AND VP2 OF INFECTIOUS PANCREATIC NECROSIS VIRUS TRIGGER LYMPHOID CELL CHANGES AND INDUCED CYTOKINE TRANSCRIPTIONAL EXPRESSION IN RAINBOW TROUT HEAD KIDNEY

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## ABSTRACT

Infectious pancreatic necrosis (IPN) is a disease that affects salmonid fish producing mortality and economic losses in the salmon industry. The agent responsible for this disease is the IPN virus (IPNV), which has a double-stranded RNA genome of two segments. The segment A encodes proteins VP2, VP3, VP4 and VP5 and the segment B encodes the VP1 protein, which is an RNA- dependent RNA polymerase. In this work, we assessed the effects of VP1 and VP2 recombinant proteins on the lymphoid cell populations and cytokine expression in the head kidney of the rainbow trout (Oncorhynchus mykiss). Fish were i.p immunized with VP1 or VP2 and the IgM+, CD3+ and CD4+ lymphoid cells of the anterior kidney were analyzed by flow cytometry. The results showed that the percentage of IgM+ B cells did not change in the kidney of immunized fish whilst significant variations of CD3+ and CD4+ lymphoid cells were observed after VP1 and VP2 immunization, respectively. In addition, transcripts of cytokines related to the lymphoid immune response, i.e., IFNg, IL-4/13A, IL-4/13B1, IL-4/13B2, IL-2, IL-22, IL-10 and TGFb were quantified by qRT-PCR. The results showed significant increase of the cytokine transcripts and distinct expression profile in VP1 and VP2 immunized fish. In summary, recombinant VP1 and VP2 trigger distinct immune responses in the head kidney of rainbow trout, which can be essential to develop fish immunity against IPNV.

## KEYWORDS

Rainbow trout, Immune response, IPNV, lymphoid cells, cytokines.

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# P-029

## IMMUNE RESPONSES OF EUROPEAN SEABASS (*Dicentrarchus labrax)* JUVENILES TO CHRONIC INFLAMMATION

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**ABSTRACT**
The immune system is responsible for maintaining homeostasis by the initiation and control of the cellular and humoral inflammatory mechanisms in response to inherent or external factors. Innate immune system repertoire, as the first response system, is used as indicator of fish immune status. Most studies performed have been focused on the acute responses of the innate immune mechanisms of fish after inflammation and therefore few data exist about their long-term response. Therefore, this study intends to gather deeper insights on the European seabass (*Dicentrarchus labrax*) immune responses to chronic inflammation in a way to assess new biomarkers for this health condition. A total of 48 European seabass juveniles (initial body weight: ± 300 g) were randomly distributed by 2 tanks (24 individuals/tank) and injected with 100 ml of Hank's Balanced Salt Solution (Control-CTRL) or 100 ml of FIA in the peritoneal cavity, after a 24 h fasting period. Fish were fed two times a day (1.5% total biomass) during 21 days. Six European seabass were sampled from each tank at 7, 14 and 21 days post-injection. Fish hematology (total red and white blood cells and hematocrit), total peritoneal leucocytes counting, analysis of plasma humoral parameters (lysozyme, proteases activities, immunoglobulin M and proteins) and blood respiratory burst were performed. Regardless time, fish from FIA presented an increased proteases activity compared to CTRL. At 7 days following intraperitoneal infection, individuals from FIA increased the lysozyme activity compared to CTRL. Regarding to respiratory burst activity, values from European seabass injected with FIA increased at 21 days post-injection compared to individuals sampled at 7 and 14 days. Moreover, a higher activity was also observed in individuals from FIA treatment at 21 days compared to those from CTRL. Total peritoneal cells also increased in European seabass injected with FIA from 7 to 21 days, compared to CTRL individuals. Results from the present study suggest that European seabass immune response to FIA is still increasing after 21 days with higher levels of cells migration to the
peritoneal cavity and increased blood respiratory bust activity at that time. Other molecular and metabolic markers are being assessed to get deeper insights on the response of European seabass to chronic inflammation.

**KEYWORDS**
Chronic inflammation, immune status, neutrophils, leucocytes, peritoneal cells.

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# P-030

## MOLECULAR CHARACTERIZATION OF A PATTERN RECOGNITION PROTEIN LGBP HIGHLY EXPRESSED IN THE EARLY STAGES OF MUD CRAB *Scylla paramamosain*

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## ABSTRACT

The early developmental stages of the mud crab *Scylla paramamosain* suffer from high mortality caused by pathogen infections; however, few immune associated factors are known. Lipopolysaccharide and β-1,3-glucan-binding protein (LGBP) functions as a typical pathogen recognition receptor and plays an important role in the innate immune system of invertebrates. In this study we characterized a LGBP gene (SpLGBP) which was highly expressed in the late embryonic, zoea I larval stage and hepatopancreas of S. paramamosain.. It encodes 364 amino acids, composed of several conserved domains like the bacterial glucanase motif. The recombinant SpLGBP protein (rSpLGBP) was obtained through the *E.coli* expression system, in which two 6.His-tags were added to both C and N terminals during vector construction for the improvement of purification efficiency. In vivo the study showed that the SpLGBP mRNA was significantly up-regulated under *Vibrio parahaemolyticus* and a lipopolysaccharide (LPS) challenge in the hemocytes and hepatopancreas. The ELISA binding assay in vitro indicated that the rSpLGBP was capable of binding to LPSs and peptidoglycan (PGN). The rSpLGBP could agglutinate both G+ and G- bacteria in the presence of Ca2+. Our results suggest that SpLGBP may play an immunological role against pathogenic infection in the early developmental stages of *S. paramamosain*.

## KEYWORDS

Ca2+ dependent, lipopolysaccharide and β-1,3-glucan-binding protein (LGBP), LPS, recognition, *Vibro parahaemolyticus*

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# P-031

## THE SPLEEN IN THE HUMORAL IMMUNE RESPONSE OF TURBOT (*Scophthalmus maximus*) TO VACCINATION WITH THE CILIATE PARASITE *Philasterides dicentrarchi*

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**ABSTRACT**

Fish spleen is an organ rich in lymphocytes, particularly, in B lymphocytes, playing a relevant role in the adaptive immune response after vaccination. After fish vaccination by intraperitoneal injection, there is a strong migration of antigen containing cells to the spleen, where antigen presentation occurs. In the present study, we have analysed the B cell populations (IgM, IgT or IgD positive cells) and the gene expression (IgM, IgT, IgD, MHCII, and several immune related genes) in the spleen of turbot immunized with a vaccine containing an oleous adjuvant and a particulated *Philasterides dicentrarchi* antigen. Fish were immunized at days 0 and 30, and samples were obtained at dasy 3, 7, 33, 37 and 60. The vaccine provoked a significant increase in specific and total serum IgM at 37 dpi and at 60 dpi, but the specific IgT levels did not vary significantly in vaccinated fish. No significant regulation of sIgT, mIgT, sIgM, mIgM and IgD was found before 37 dpi, whereas at 37 and 60 dpi, overexpression of sIgT and mIgT was detected in fish injected with adjuvant alone or with the vaccine. The identification of B lymphocytes by immunofluorescence allowed their classification in four groups: IgM+IgD-IgT- (the majority of IgM+ cells), IgM+IgD+IgT-, IgM-IgD+IgT- (very few cells; which probably correspond with lymphocytes with low levels of IgM), and IgM-IgD-IgT+ cells. The IgM+ and IgT+ cells were scattered throughout the parenchyma, and grouped around large vessels and surrounding melanomacrophage centres (MMC). IgM+ cells, IgT+ cells or a mixture of the two populations were associated frequently to MMC. Cell proliferation was estimated by using a combination of antibodies anti-IgT, anti-IgM and anti-PCNA. Proliferation of both IgM+ and IgT+ B cells was found in several areas of the spleen. In addition, vaccinated fish showed PCNA +/IgM + and PCNA+/ IgT+ B cells in the mass of vaccine and cells (CVM) associated with the spleen, which may indicate an importance of the CVM during vaccination, which goes beyond a mere place of phagocytosis and exchange of material. This work was funded by EU H2020 program through ParaFishControl Project (634429), by the Ministerio de Economía y Competitividad (Spain) and FEDER (European Union) (AGL2017-83577-R) and by grant ED431C2017/31 from the Xunta de Galicia. I.E. was contracted under APOSTD/2016/037 grant by the "Generalitat Valenciana", and F.F. was contracted by the Xunta de Galicia.

**KEYWORDS**
Turbot, Spleen, Vaccination, B lymphocytes, Immunoglobulins

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# P-032

## FIELD VALIDATION OF IMMUNOTOXIC RESPONSES IN THE CARPET SHELL CLAM (*Ruditapes decussatus*) FROM CONTAMINATED SITES IN THE SOUTH LAGOON OF TUNIS (TUNISIA)

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**ABSTRACT**

The aim of this study was to validate immunological alterations as ecotoxicological biomarkers to detect and monitor the biological effects of anthropogenic pollution in the South Lagoon of Tunis (Tunisia). Carpet shell clams (*Ruditapes decussatus*) were collected during summer and Winter from four locations: three of them within the polluted lagoon of Tunis (S1, S2 and S3) and from a clean site on the Mediterranean coast (Louza, Tunisia). To study the immunity of clams, the phenoloxidase, lysozyme, alkaline phosphatase, esterase, peroxidase, protease, antiprotease and bactericidal activities were measured in the haemolymph. Phenoloxidase activity was significantly lower in clams sampled from the three contaminated areas of the lagoon (S1, S2 and S3) than in those from the control point in both summer and winter. Lysozyme, esterase, protease and antiprotease activities were higher in the clams from site S3 than in the clams collected from the control site during winter. No significant variations were detected in the alkaline phosphatase, peroxidase and bactericidal activities of the clams collected from the four experimental sites. A significant seasonal effect was observed in clam immune status in winter. The results clearly showed that the affected biomarkers (mainly phenoloxidase) could be useful tools for biomonitoring clams in the study area.

This study was supported by the Ministry of Scientific Research and Technology, the University of Monastir (Tunisia), the Spanish Ministry of Economy and Competitiveness co-funded with Fondos Europeos de Desarrollo Regional/European Regional Development Funds (Grant no. AGL2014- 51839-C5-1-R) and Fundación Séneca de la Región de Murcia (Grupo de Excelencia. Grant no. 19883/GERM/15).

**KEYWORDS**

Biomarkers; Biomonitoring; Seasonality; Innate immunity; carpet shell clam (*Ruditapes decussatus*).

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# P-033

## HEPCIDIN, AN ANTIMICROBIAL AND IRON-REGULATED PEPTIDE THAT PROVIDES AN ABILITY TO PREVENT BACTERIAL DISEASES IN GRASS CARP (*Ctenopharyngodon idella*).

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**ABSTRACT**
Hepcidin is an antimicrobial peptide and a regulator of iron homeostasis which has three isoforms: -20, -22 and -25. While hepcidin-25 has been studied extensively, the physiological significance of other isoforms remains poorly understood. Herein, we focused on the analysis of the differences in antibacterial and iron regulatory functions of hepcidin-20 and hepcidin-25, looking for a derivative of hepcidin as a preventive drug for bacterial diseases. In this study, we examined the antimicrobial potentials of the two hepcidins in the form of synthesized peptides, hepcidin-25 and hepcidin-20. We found that hepcidin-25 and hepcidin-20 exhibited apparent bactericidal activities against both Gram-positive and Gram-negative bacteria in a dose-dependent manner. *In vitro*, the hepcidin-20 had better antibacterial activity than the hepidin-25. However, the antimicrobial activity on the cellular level has the opposite effect. We suspected that the iron-regulating function of hepcidin limits the available iron content of extracellular bacteria to enhance its bactericidal activity. Further tests indicated that only hepcidin-25 can block iron release from liver cell line L8824 via internalization and degradation of cellular iron exporter ferroportin, and restrain the use of iron in extracellular bacteria. This result also confirms our hypothesis. *In vivo*, recombinant *Ctenopharyngodon idella* hepcidin improved the survival rate of *C. idella* challenged with *Flavobacterium columnare*. In addition, the fish fed diet containing recombinant *C. idella* hepcidin had a higher survival rate than other pretreatment groups. The study showed that recombinant *C. idella* hepcidin regulated iron metabolism, causing iron redistribution, decreasing serum iron levels and increasing iron accumulation in the hepatopancreas. Immune-related genes were also evaluated, showing higher expression in the groups pretreated with recombinant *C. idella* hepcidin at an early stage of infection. In general, *C. idella* hepcidin not only has a direct killing effect on bacteria, but also reduces the available iron content of bacteria to inhibit bacterial growth. Our findings revealed a new role for hepcidin in fighting against bacterial infections and indicate a potential in controlling the bacterial infection in aquaculture.

**KEYWORDS**
*Ctenopharyngodon idella*; hepcidin; antibacterial;

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# P-034

## A NOVEL *CQTRIM32* FROM RED CLAW CRAYFISH *Cherax quadricarinatus* INHIBITS WHITE SPOT SYNDROME VIRUS INFECTION

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**ABSTRACT**
Tripartite motif-containing (TRIM) proteins are highly conserved molecules that participate in a variety of biological processes such as regulation of development, apoptosis, and innate immunity in vertebrates. In this report, we identified a *TRIM32* homolog (named *CqTRIM32*) in red claw crayfish *Cherax quadricarinatus*. *CqTRIM32* was widely distributed in the tissues selected, with the highest expression in muscle, relatively abundant in haematopoietic tissue (Hpt) and the lowest presence in eyestalk. Multiple amino acid alignment showed that *CqTRIM32* contained a conserved RING-finger domain but without B-BOX domain and coiled-coil region, which was different from the traditional TRIMs family. Interestingly, the expression of *CqTRIM32* was significantly up-regulated at both 24 h and 48 h after white spot syndrome virus (WSSV) challenge in *vivo* in crayfish Hpt tissue. Meanwhile, the expression of *CqTRIM32* was significantly up-regulated at both 12 h and 24 h after WSSV challenge in *vitro* in Hpt cells. The quantity of WSSV was increased in red claw crayfish Hpt cell cultures after gene knockdown of *CqTRIM32* post WSSV infection, in which the transcription of both an immediate early gene *ie1* and a late envelope protein gene *vp28* of WSSV were clearly up-regulated. Taken together, our data provide the first evidence that *CqTRIM32* exerts the antiviral activity in a crustacean.

**KEYWORDS**
Tripartite motif-containing (TRIM); Antiviral; White spot syndrome virus (WSSV); Haematopoietic tissue (Hpt); *Cherax quadricarinatus*.

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# P-035

## LIPID DEPOSITS AND FOAMY MACROPHAGE-LIKE CELLS IN FOCAL RED AND MELANISED MUSCLE CHANGES IN ATLANTIC SALMON (*Salmo salar*)

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**ABSTRACT**
Focal melanised changes or "black spots" in farmed Atlantic salmon (*Salmo salar*) fillet is a common quality problem seen at slaughter. The changes develop during the seawater phase, starting as acute focal hemorrhages or "red changes" which progress into chronic inflammatory changes with melanisation. Regeneration in most changes remains ongoing without proper healing; a process that has been associated with the chronic persistence and replication of *Piscine orthoreovirus* (PRV).
Another chronically persistent feature in this condition is the histopathological presence of what appears as fat (seen as empty vacuoles) in both focal red and melanised changes. Previous studies have described vacuoles of various sizes assumed to be fat-containing, but as most studies have been carried out on formalin fixed and paraffin-embedded tissues, the content in such vacuoles has diminished during processing and histological investigations of lipids have hitherto been inconclusive.
Here, we use glutaraldehyde-fixed and frozen material, thus preserving the fat. Sections from both acute red and chronic melanised changes were stained with two different special stains (Sudan Black and Oil Red O) for detection of lipids. We show that most vacuoles indeed contain fat and that these are highly prevalent in the acute manifestations in areas of necrosis, haemorrhage and inflammation. We also show fat-containing vacuoles in chronic changes with melanisation, though with a different appearance; often in association with melano-macrophages. In addition, cells though to be foamy macrophages are identified and investigated by transmission electron microscopy. Based on our results, we discuss the potential role of fat in the development of focal melanised changes.

**KEYWORDS**
Inflammation; lipids; Macrophage; Melano-macrophage; Myositis

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# P-036

## IMMUNE RESPONSE IN TURBOT EXPOSED TO THE CILIATE PARASITE *Philasterides dicentrarchi.*

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**ABSTRACT**

*Philasterides dicentrarchi* is a marine scuticociliate that causes scuticociliatosis in farmed fish worldwide and is currently considered one of the most important pathogens of cultured flatfish. Although there is abundant information about the infections caused by *P. dicentrarchi* in fish and about how the ciliates and fish immune cells interact in vitro, little is known about the interaction between this ciliate and the fish immune system in vivo. In the present study, turbot (*Scophthalmus maximus*) were exposed twice to the parasite (on days 1 and 21). Immersion infection was performed by adding ciliates to tanks of seawater (18 ºC) to yield a final concentration of 4.5 x 104 ciliates/mL. Fish were exposed to the ciliates by immersion in the seawater for 20 min and were then transferred to tanks of clean seawater for 60 days. Control fish were immersed in seawater with no ciliates, and were subjected to the same conditions as the experimental fish. Four fish died of scuticociliatosis during the experiment. Fish (eight per group) were sampled on days 3, 7, 21 after the first exposure to *P. dicentrarchi* and on days 3, 7 and 40 after the second exposure. The presence of ciliates on the skin and gills was evaluated by qPCR. The IgM, IgT and IgD levels were measured in serum on days 3, 7 and 40 and in mucus on day 40 after the second exposure. Changes in gene expression of immunoglobulins, MHCII and other immune-related genes were determined by qPCR, in gills, skin, and spleen at all sampling times. There were no significant differences in serum IgM, IgD and IgT levels between experimental and control groups at any of the sampling times; however, there was a significant increase in mucus IgT levels 40 days after administration of the second exposure. The results of the qPCR analysis showed few changes of the immunoglobulin expression in the analyzed organs and a mild inflammatory response with the current infective dose.

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**KEYWORDS**
Turbot, *Philasterides dicentrarchi*, immunoglobulins, immune response, infection

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# P-037

## DEVELOPMENT OF A REVERSE GENETICS SYSTEM FOR SNAKEHEAD VESICULOVIRUS

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## ABSTRACT
Snakehead vesiculovirus (SHVV) is a new rhabdovirus isolated from diseased hybrid snakehead fish (*Channa maculate* ♀ x *Channa argus* ♂) and has caused serious economic losses in snakehead fish culture in China. To better understand the pathogenicity of SHVV, we developed a reverse genetics system for SHVV by using human and fish cells. In detail, human 293T cells were cotransfected with four plasmids encoding the full-length SHVV antigenomic RNA or the supporting proteins including nucleoprotein (N), phosphoprotein (P), and large polymerase (L), followed by the cultivation in Channel catfish ovary (CCO) cells. We also rescued a recombinant SHVV expressing enhanced green fluorescent protein (EGFP), which was inserted into the 3' non-coding region (NCR) of the glycoprotein (G) gene of SHVV. Our study provides a potential tool for unveiling the pathogenicity of SHVV and a template for the rescue of other fish viruses by using both human 293T and fish cells.

## KEYWORDS
Snakehead vesiculovirus, reverse genetics, EGFP, vaccine

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# P-038

## EVIDENCE OF TRAINED IMMUNITY IN TELEOST FISH: CONSERVED FEATURES IN CARP MACROPHAGES

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**ABSTRACT**

Trained immunity is a form of innate immune memory best described in mice and humans. Trained immunity is defined as a heightened response to a secondary infection that can be exerted toward both homologous and heterologous microorganisms. Typical criteria of trained immunity include: 1) induction upon primary infections or immunizations and subsequent protection against a secondary infection, in a T- and B-lymphocyte independent manner, 2) a response that is less specific than an adaptive immune response but that still confers increased resistance upon reinfection of the host and, 3) the involvement of innate cell types such as NK cells and macrophages involved in improved pathogen recognition and an increased inflammatory response. Clear evidence of the evolutionary conservation of trained immunity in teleost fish is lacking. Given the evolutionary position of teleosts as early vertebrates with a fully developed immune system, we hypothesize that teleost myeloid cells show features of trained immunity common to those observed in mammalian macrophages. These would at least include the ability of fish macrophages to mount heightened responses to a secondary stimulus in a non-specific manner. We established an *in vitro* model to study trained immunity in fish by adapting a well-described culture system of head kidney-derived macrophages of common carp. A soluble NOD-specific ligand and a soluble β-glucan were used to train carp macrophages, after which cells were rested for six days prior to exposure to a secondary stimulus. Unstimulated trained macrophages displayed evidence of metabolic reprogramming, as well as heightened phagocytosis and increased expression of the inflammatory cytokines *IL6* and *TNFα*. Stimulated, trained macrophages showed heightened production of reactive oxygen and nitrogen species as compared to the corresponding stimulated but untrained cells. Measurement of the production of reactive oxygen species proved particularly informative to identify ligands able to train carp macrophages. We discuss the value of our findings for future studies on trained immunity in teleost fish.

**KEYWORDS**

Trained immunity, Cyprinidae, Monocytes/Macrophages, innate immunity,

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# P-039

## TLR5 ACTIVATION SITE IN *EDWARDSIELLA TARDA* FLAGELLIN IS IMPORTANT TO INDUCE EXPRESSION OF INTERLEUKIN-1B AND NF-κB GENES IN JAPANESE FLOUNDER, *Paralichthys olivaceus*

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**ABSTRACT**
Flagellin is the subunit protein that composes bacterial flagella and is recognized by toll-like receptor 5 (TLR5) as a ligand. Flagellin protein (*e.g.*, FliC and FlaA) contains the D1, D2, and D3 domains; the D1 domain is important for recognition by TLR5 for activation of the innate immune system. In teleosts, there are two types of TLR5, the membrane form (TLR5M) and soluble form (TLR5S), the latter of which is not present in mammals. In this study, the potential of flagellin from *Edwardsiella tarda* (EtFliC) to induce inflammation-related genes interleukin (IL)-1β and NF-κB-p65 through TLR5S in Japanese flounder (*Paralichthys olivaceus*) was elucidated. A transient overexpression system was developed in flounder natural embryonic (HINAE) cells using constructs encoding two flagellin genes derived from *E. tarda* (pEtFliC) and *Escherichia coli* (pEcoFliC) and the flounder TLR5S gene (pPoTLR5S). Expression of inflammation-related genes in EtFliC- and PoTLR5S-overexpressing HINAE cells was significantly lower than in EcoFliC- and PoTLR5S-overexpressing cells. To clarify the difference between EtFliC and EcoFliC potency, the amino acid sequence of EtFliC was compared with that of other bacterial flagellin. The 91st arginine residue, known as the mammalian TLR5 activation site, was conserved in the flagellin of *E. coli* and other bacteria but not in EtFliC. To reveal the importance of the 91st arginine residue in FliC, a pEtFliC construct in which the 91st asparagine was mutated to arginine (pEtFliC_N91R) was generated. Expression of the IL-1β and NF-κB-p65 genes in the HINAE cells co-transfected with pEtFliC_N91R and pPoTLR5S was significantly higher than that in cells co-transfected with pEtFliC and pPoTLR5S. The results suggested that the 91st arginine residue of bacterial flagellin is involved in inflammatory response through TLR5S in teleosts. Thus, EtFliC improved by site-directed mutagenesis could be an effective adjuvant against *E. tarda* infection in Japanese flounder.

**KEYWORDS**
*Edwardsiella tarda*; FliC; TLR5S; IL-1β; NF-κB-p65; Japanese flounder (*Paralichthys olivaceus*)

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# P-040

## EFFECT OF ALOE VERA NATURAL ADDITIVE ON ENTERITIS CAUSED BY DIETS THAT INCLUDE SOYBEAN MEAL IN ATLANTIC SALMON

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## ABSTRACT
Soybean meal is one of the most widely used alternatives to replace fishmeal. However, its ingestion triggers an intestinal inflammatory process that compromises fish health. Finding strategies that reduce its deleterious effects will be relevant. In this work we analyzed the effects of aloe vera (Aloe barbadensis miller, AV) as additives in a soybean meal-based diet on intestinal inflammation in Atlantic salmon. To determine the immunomodulatory effect of AV, we supplemented fishmeal (FM) and soybean meal (SBM) based diets with AV. 4 groups in duplicate of 40 Atlantic salmons each, with an average weight of 75 gr at the beginning of the study were fed for 28 days with the FM, SBM, FM + AV and SBM + AV diets. At the end of the feeding period, the length of fish fed with SBM was significantly lower than that of fish fed with the FM, FM + AV or SBM + AV diets. Weight gain was similar between fish fed with SBM, FM and FM + AV diets, whereas fish fed SBM + AV gained 11% more weight than the SBM group. Samples of the distal intestine of 12 fish per treatment were taken for histological analysis. A semi-quantitative scoring system was used to assess the degree of morphological changes induced by different diets. A higher score was evidenced for the SBM group compared with the FM group, suggesting SBM triggered an inflammatory process. On the other hand, the SBM + AV group had a significantly lower score compared to the SBM group, evidencing the intestinal protection granted by AV. To complement our result, we characterized the expression of cytokine markers *il-1β* _and *il-10* by qPCR, obtaining higher expression of inflammatory related genes in the SBM group when compared with SBM+AV or FM group. The present study suggests that aloe vera could be used as additive in farmed fish diets to facilitate the replacement of fishmeal by soybean meal without affecting intestinal health.

## KEYWORDS
Soybean meal, enteritis, natural additive, distal intestine, histology
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# P-041

## IDENTIFICATION AND BIOACTIVITY OF A GRANULOCYTE COLONY-STIMULATING FACTOR B HOMOLOGUE FROM LARGE YELLOW CROAKER (*Larimichthys crocea*)

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**ABSTRACT**
Granulocyte colony-stimulating factor (GCSF) is a pleiotropic cytokine that plays a key role in regulation of hematopoiesis, innate and adaptive immune responses in mammals. However, bioactivity of GCSF in teleost fish remains largely unknown. In this study, a GCSFb homologue from large yellow croaker (*Larimichthys crocea*) (*Lc*GCSFb) was cloned by RACE-PCR techniques. The open reading frame (ORF) of *Lc*GCSFb is 603 bp long and encoded a protein precursor of 200 amino acids (aa), with a 19-aa signal peptide and a 181-aa mature peptide. In healthy fish, the *Lc*GCSFb was constitutively expressed in all examined tissues, with the highest levels in mucous tissues, such as gills, intestine, and stomach. Its transcripts in head kidney, spleen and gills were significantly induced by *Vibrio alginolyticus* challenge. *Lc*GCSFb transcripts were also detected in primary head kidney leukocytes (PKL), primary head kidney macrophages (PKM), primary head kidney granulocytes (PKG) and head kidney cell line (LYCK), and markedly up-regulated by inactivated *Vibrio alginolyticus*. These data suggested that *Lc*GCSFb may play a role in immune response against bacterial infection. *In vivo* administration of recombinant *Lc*GCSFb protein (r*Lc*GCSFb) significantly up-regulated the expression levels of the inflammatory cytokines (IL-6 and TNFα), and transcription factor C/EBPβ, which is required for proliferation of neutrophils. Furthermore, r*Lc*GCSFb showed an ability to strengthen the phagocytosis of PKL *in vitro*. Taken together, *Lc*GCSFb may be involved in antibacterial immunity via promoting the inflammatory response and the phagocytic activity of leukocytes. To our knowledge, this is the first report on immunoregulatory roles of GCSF in teleost.

**KEYWORDS**
Granulocyte colony-stimulating factor (GCSF); Large yellow croaker (*Larimichthys crocea*); inflammatory response; bacterial infection; phagocytosis

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# P-042

## ROLE OF THE SPLEEN IN THE IMMUNE RESPONSE OF TURBOT (*Scophthalmus maximus*) TO VACCINATION WITH THE CILIATE PARASITE *Philasterides dicentrarchi*

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**ABSTRACT**

Fish spleen is rich in lymphocytes, particularly in B lymphocytes, and plays an important role in the adaptive immune response after vaccination. After vaccination of fish by intraperitoneal injection, there is a strong migration of antigen-containing cells to the spleen, where antigen presentation occurs. In the present study, we analysed the B cell populations (IgM, IgT or IgD positive cells) and gene expression (IgM, IgT, IgD, MHCII, and several immune-related genes) in the spleen of turbot immunized with a vaccine containing an oleous adjuvant and a particulate *Philasterides dicentrarchi* antigen. Fish were immunized on days 0 and 30, and samples were obtained on days 3, 7, 33, 37 and 60. The vaccine provoked a significant increase in specific and total serum IgM at 37 dpi and at 60 dpi, but the specific IgT levels did not vary significantly in vaccinated fish. No significant regulation of sIgT, mIgT, sIgM, mIgM and IgD occurred before 37 dpi, whereas at 37 and 60 dpi, overexpression of sIgT and mIgT was detected in fish injected with adjuvant alone or with the vaccine. Immunofluorescence analysis enabled classification of the B lymphocytes into four groups: IgM+IgD-IgT- (the majority of IgM+ cells), IgM+IgD+IgT-, IgM-IgD+IgT- (very few cells, probably corresponding to lymphocytes with low levels of IgM) and IgM-IgD-IgT+ cells. The IgM+ and IgT+ cells were scattered throughout the parenchyma, and grouped around large vessels and surrounding melanomacrophage centres (MMC). Cell proliferation was estimated using a combination of anti-IgT, anti-IgM and anti-PCNA antibodies. Proliferation of both IgM+ and IgT+ B cells was observed in several areas of the spleen. In addition, vaccinated fish showed a mass of vaccine and cells (CVM) associated to the spleen. The CVM contained scattered PCNA+/IgM + and PCNA+/ IgT+ B cells, possibly indicating its importance during vaccination, above and beyond its role as a site for phagocytosis and material exchange.

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**KEYWORDS**
Turbot, Spleen, vaccination, B lymphocytes, Immunoglobulins

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# P-043

## PROTECTIVE IMMUNE RESPONSES OF RECOMBINANT OUTER MEMBRANE PROTEINS OMPF AND OMPK OF *Aeromonas hydrophila* IN EUROPEAN EEL (*Anguilla anguilla*)

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**ABSTRACT**

Outer membrane proteins (Omps) of Gram-negative bacteria were proved to be efficient subunit vaccines against bacteriosis. In this study, OmpF and OmpK of *Aeromonas hydrophila* were expressed and evaluated their immune protective effects on European eel (*Anguilla anguilla*). The genomic DNA of *A. hydrophila* 322A was used as the template, and two kinds of prokaryotic expression plasmids pET-32a-OmpF and pET-32a-OmpK were constructed, respectively. Recombinant OmpF protein (r-OmpF) and r-OmpK were purified and proved to have antigenicity by Western-blot analysis. The r-OmpF and r-OmpK were used as immunogens to immunize European eel by the intraperitoneal injection. The mRNA expression of 6 immune-related genes (*IgM*, *IL-10*, *IRF3*, *IRF7*, *LysG4*, and *HexB*) in liver tissues of eels at 1 h, 3 h, 6 h, 12 h, 24 h, 72 h, and 10 d post-immunization was analyzed by real-time PCR. At 30 dpi, serum antibody response was measured by ELISA. Fish were attacked at 15 dpi by live 322A in order to assess the protective immunity of r-OmpF and r-OmpK. Both r-OmpF and r-OmpK could up-regulate the expression of all 6 genes in varying degrees. The serum antibody titer of r-OmpF- and r-OmpK-immunized groups was 1: 1600 and 1: 3200, respectively. In addition, r-OmpF could give 35.5% of relative immune protection rate to European eels, while r-OmpK gave 70.0%. By analyzing the protective immunity and the regulatory role in the immune-related gene expression of the two recombinant proteins provided, it could be found that r-OmpK was a potential vaccine candidate of *A. hydrophila*.

**KEYWORDS**

*Aeromonas hydrophila*; outer membrane protein; subunit vaccine; protective immunity; European eel (*Anguilla anguilla*)

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# P-044

## TRANSCRIPTOME ANALYSIS OF IMMUNE-RELATED GENE EXPRESSION IN HYBRID SNAKEHEAD (*Channa maculata* ♀ X *Channa argus* ♂) AFTER CHALLENGE WITH *Nocardia seriolae*

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**ABSTRACT**

Hybrid snakehead fish (*Channa maculata* ♀ x *Channa argus* ♂ ), a new species used in freshwater aquaculture in China, is the common host of an epizootic bacterial infection by *Nocardia seriolae*. However, the information on the functions and mechanisms of hybrid snakehead immune pathways with the *N. seriolae* infection is limited. Thus, the peripheral blood lymphocytes from hybrid snakehead were used for transcriptome analysis to understand the host immune response after challenge with N. seriolae. A total of 49,839,332 and 50,059,283 raw reads were obtained from the *N. seriolae*-challenged group (Ns group) and phosphate-buffered saline control group (Ctr group), respectively. The 75.50% and 74.25% reads from the Ns and Ctr groups were matched to reference genomic sequence after cleaning the raw reads, respectively. Additionally, there were 2892 significant differentially expressed genes (DEGs) among the 17,196 expressed genes between the Ns and Ctr groups, including 1387 upregulated and 1505 downregulated genes. All the DEGs were classified into three gene ontology categories, and 2502 DEGs had significant matches, which were allocated to 246 Kyoto Encyclopedia of Genes and Genomes pathways. Immune-related genes were detected from immune system pathways among the top 20 enriched pathways. Moreover, the regulation of several observed effective genes was confirmed by real-time quantitative Polymerase chain reaction. Altogether, this study offers deep-sequence data of hybrid snakehead peripheral blood lymphocyte via transcriptome analysis and lays the foundation for further study on the immunogenetics of hybrid snakehead. Moreover, it provides insights into the pathogenic mechanism of N. seriolae, facilitating the prevention and treatment of fish nocardiosis.

**KEYWORDS**

*Nocardia seriolae*, Hybrid snakehead, Transcriptome analysis, Immune-related genes, Fish nocardiosis

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# P-045

## A RECOMBINANT VACCINE TARGETING THE PARASITIC CILIATE *Ichthyophthirius multifiliis*

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**ABSTRACT**

New vaccine candidates were identified targeting the one celled parasite I. multifiliis, which negatively affects aquaculture freshwater fish productions all over the world. In silico selection with the use of artificial intelligence identified several potential vaccine candidates and three of these were recombinantly expressed using *E. coli* and insect cells. Following a vaccine trial one protein (a so-called neurohypophysial n-terminal domain protein, #10) was found to induce moderate protection against I. multifiliis in rainbow trout (*Oncorhynchus mykiss*). To develop a highly protective heterologous vaccine we aim to combine #10 with a protective epitope from the already known homologous protective antigen Iag52b, which is a GPI-anchored cysteine rich surface protein. To be able to produce #10 at low costs, recombinant expression has been conducted in an eukaryotic host. Purified Iag52b does not induce immunity in fish without the use of adjuvants, thus the most potentially protective epitope of Iag52 was selected in silico and coupled to a viruslike particle. This coupling enables the epitope to be presented in a virus-like conformation, which theoretically should be immunogenic to the fish. Results are discussed.

**KEYWORDS**

Recombinant vaccine, Ichthyophthirius multifiliis, protective epitope, vaccine candidates, virus-like particle

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# P-046

## TRANSCRIPTOMIC PROFILES OF POST-SMOLT ATLANTIC SALMON CHALLENGED WITH PISCIRICKETTSIA SALMONIS REVEAL A STRATEGY TO EVADE THE ADAPTIVE IMMUNE RESPONSE AND MODIFY CELL-AUTONOMOUS IMMUNITY

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**ABSTRACT**
Piscirickettsiosis is the main bacterial disease affecting the Chilean salmon farming industry and is responsible for high economic losses. The development of effective strategies to control piscirickettsiosis has been limited in part by insufficient knowledge of the host response. The aim of this study was to use RNA sequencing to describe the transcriptional profiles of the responses of post-smolt Atlantic salmon infected with LF-89-like or EM-90-like *Piscirickettsia salmonis*. Enrichment and pathway analyses of the differentially expressed genes revealed several central signatures following infection, including positive regulation of DC-SIGN and TLR5 signalling, which converged at the NF-kB level to modulate the pro- inflammatory cytokine response, particularly in the PS-EM-90-infected fish. *P. salmonis* induced an IFN-inducible response (e.g., IRF-1 and GBP-1) but inhibited the humoral and cell-mediated immune responses. *P. salmonis* induced significant cytoskeletal reorganization but decreased lysosomal protease activity and caused the degradation of proteins associated with cellular stress. Infection with these isolates also delayed protein transport, antigen processing, vesicle trafficking and autophagy. Both P. salmonis isolates promoted cell survival and proliferation and inhibited apoptosis. Both groups of Trojan fish used similar pathways to modulate the immune response at 5 dpi, but the transcriptomic profiles in the head kidneys of the cohabitant fish infected with PS-LF-89 and PS-MS-90 were relatively different at day 35 post-infection of the Trojan fish, probably due to the different degree of pathogenicity of each isolate. Our study showed the most important biological mechanisms used by P. salmonis, regardless of the isolate, to evade the immune response, maintain the viability of host cells and increase intracellular replication and persistence at the infection site. These results improve the understanding of the mechanisms by which interacts with its host and may serve as a basis for the develop- ment of effective strategies for the control of piscirickettsiosis.

**KEYWORDS**
RNA-seq, Piscirickettsiosis, *Piscirickettsia salmonis*, LF-89, EM-90.

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# P-047

## PIVOTAL ROLE OF IMMUNOGLOBULIN IGT IN RAINBOW TROUT SKIN AFTER BACTERIAL INFECTED WITH *Flavobacterium columnare*

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**ABSTRACT**
In contrast to mammalian skin, teleost skin has been considered as mucosal surface which serves as the first line of defense against invading pathogens. Moreover, teleost skin contains skin-associated lymphoid tissue (SALT) that elicits gut-like immune responses against parasitic infection. However, little is known so far about the B cells and immunoglobulins (Igs) responds to bacterial infection in the skin mucosal immune system of teleost. We hypothesized that, microbial exposure can elicit a dedicated mucosal Igs response and locally specific immune responses would be generated within its mucosa. To address our hypothesis, we construct an infected model with rainbow trout (*Oncorhynchus mykiss*), which was experimentally exposed to *Flavobacterium columnare*. H & E staining of trout skin shows the morphological changes and qRT-PCR indicates the increased mRNA expression levels of immune-related genes, which were further studied by RNA-Seq analysis, in trout skin after infected with *Flavobacterium columnare*. Moreover, strikingly increased IgT concentration and strong pathogen-specific IgT responses were detected in the cutaneous mucus, and the accumulation of IgT+ B cells were also noted in the skin epidermis of experimental group. Critically, IgT responses against the pathogen were mainly limited to the skin whereas IgM responses were almost exclusively detected in the serum. Moreover, local IgT+ B cells proliferation and pathogen-specific IgT generation were found in the trout skin, providing new evidence for the local mucosal immune responses in trout skin. Overall, our findings indicate that, following bacteria exposure, IgT and IgT+ B cells play the prevailing role in skin mucosal immunity. To our knowledge, our results provide the first example of locally induced immunoglobulin in the skin of rainbow trout after *Flavobacterium columnare* infection.

**KEYWORDS**
Skin, B cells, Immunoglobulins, *Flavobacterium columnare*, Rainbow trout (*Oncorhynchus mykiss*)

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# P-048

## EARLY IMMUNE RESPONSE IN ATLANTIC SALMON VACCINATED WITH INACTIVATED WHOLE-CELL BACTERIN OF *Piscirickettsia salmonis* AND PATHOGENIC ISOLATES.

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**ABSTRACT**
Piscirickettsiosis (SRS) is the main bacterial disease affecting the Chilean salmon farming industry. The aim of this study was to describe and comparatively quantify the immune response of Atlantic salmon intraperitoneally infected with LF-89 and EM-90 *Piscirickettsia salmonis* and vaccinated with inactivated whole-cell bacterin of P. salmonis. A positive correlation of the overexpression of IFNγ, IL-2, IL-10, IL-12ß, MHC-II and CD4 was seen in the PS-LF-89- and PS-EM-90-infected fish, but the proinflammatory response in the PS-EM- 90-infected fish was more exacerbated. The fish infected with PS-LF-89 showed an anti- inflammatory response, whereas this finding was not observed in the PS-EM-90-infected fish. Conversely, a positive correlation of the downregulation of IFNγ, IL-2, IL-12ß, MHC-I and CD8 was seen in the vaccinated fish. Fish infected with both *P. salmonis* isolates showed mhc1-mhc2, cd4-cd8b and igm overexpression, suggesting that P. salmonis promotes a CD4+ T- and CD8+ T cell response and a humoral immune response. The vaccinated-fish exhibited mhc1, mhc2 and cd4 overexpression but a significant downregulation of cd8b and igm, suggesting that the vaccine supported the CD4+ T-cell response but did not induce an immune response mediated by CD8+ T cells or a humoral response.

**KEYWORDS**
*Piscirickettsia salmonis*, immune response, vaccination.

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# P-049

## MACROPHAGE-LIKE CELLS FROM PRIMARY CELL CULTURE OF ATLANTIC SALMON ARE CAPABLE TO PHAGOCYTE *Piscirickettsia salmonis*: APOPTOSIS OF PHAGOCYTES ALSO OCCURS DURING INFECTION

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**ABSTRACT**
Innate immune mechanisms of response in fish are essential as a first defense mechanism to fight against pathogens. The phagocytosis is one key process able to eliminate the pathogens and stop the infection. Once the pathogen is internalized, the phagocyte should destroy the pathogen by fusion of the phagosome with lysosomes. *Piscirickettsia salmonis* is a Gram-negative intracellular facultative bacterium, pleomorphic (coccoid predominant grouped in pairs) and size ranging among 0.5 to 1.5 um. This pathogen is the etiological agent of Piscirickettsiosis, the main infectious disease causing around 70% of mortalities in Atlantic salmon in Chilean farming, according the last sanitary report from Sernapesca (2017). Until now, there are just a few articles that poorly described the infection induced by this bacterium. In this study, we proposed that macrophage-like cells from Head Kidney of Atlantic salmon are capable to phagocytose P. salmonis as a mechanism of defense. To test this, we labelled *P. salmonis* using FITC and after testing viability of the bacteria, we used them to inoculate primary cell cultures of Head Kidney obtained from Atlantic salmon. The cells were infected with bacteria at MOI 10. Using confocal microscopy, we observed that the adherent cells internalized the pathogen and later underwent apoptosis. A video of six hours infection was made, and the results showed that the bacteria and cells attachment occur as quickly as five minutes post inoculation. The internalization can be observed for thirty minutes to four hours post infection while apoptotic cells were observed for thirty minutes post infection until the end of the experiment. In addition, other cells could be seen moving and looking for infected cells to destroy them and eliminate the threat. This was observed from the beginning of the experiment until the end of infection. Thus, macrophage-like cells from Head Kidney of Atlantic salmon are capable to phagocyte *P. salmonis*. In addition, the infected macrophage-like cells experienced apoptosis probably to diminish pathogen viability and avoid the spread of the microorganism. How apoptosis is induced is not very clear yet, but further analysis must be performed to solve this important question.
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**KEYWORDS**
Atlantic salmon, macrophages, Piscirickettsia salmonis, apoptosis, phagocytosis.
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# P-050

## TRANSCRIPTIONAL PROFILE AND SEROLOGICAL STUDIES OF THE EUROPEAN SEA BASS IMMUNE RESPONSE AGAINST BETANODAVIRUS INFECTIONS

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**ABSTRACT**

European sea bass (*Dicentrarchus labrax*) culture is highly affected by outbreaks of viral nervous necrosis disease, provoked by the nervous necrosis virus (NNV). This virus displays a single-stranded, positive-sense RNA genome, which is composed of two segments, RNA1, encoding the viral polymerase; and RNA2, encoding the capsid protein. Only two genotypes of NNV have been detected in sea bass to date, although showing very different levels of virulence. Specifically, RGNNV is highly virulent to sea bass, causing high mortality, whereas SJNNV replicates in sea bass brain without causing clinical signs. In the present work, the comparative analysis of the European sea bass immune response against isolates belonging to both viral genotypes has been performed. The immune response has been evaluated in brain and head kidney of experimentally infected sea bass by relative real-time PCR of genes involved in the type I interferon (IFN I) system (ifn-I, mxA, isg15, isg12), and genes related to inflammatory (il-8, tnf-α, il-10, tgf-β) and adaptive responses (tr-γ, mhc-β). Ribosomal 18S RNA was used as reference endogenous gene. In addition, a serological study, consisting of the ELISA quantification of IgM in sera, was also performed. The transcription analyses of the innate defence-related genes point out the importance of this mechanism to control betanodavirus infections. The results obtained showed a strong induction of ifn-I, mxA, isg15 and isg12 in both organs analysed, especially in response to the virus highly virulent to sea bass (RGNNV). However, the response was quicker in head kidney of SJNNVinoculated sea bass, suggesting that this genotype induces a more rapid systemic response. Regarding the inflammatory response, RGNNV triggered a strong transcription of proinflammatory genes in brain, which provides evidences about the importance of the inflammatory process in betanodavirus infection. Thus, the massive inflammatory process may be responsible for the eventual damage in nervous tissues, which would lead to fish dead. Finally, the high values of tr-γ and mhc-β mRNA recorded in brain and the high IgM titer in sera, which was higher in SJNNV-inoculated fish, suggest that the adaptive response constitutes another important factor in the European sea bass immune response against betanodaviruses, both at systemic and at local level.

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# P-051

## THE NUCLEOTIDE POLYMORPHISM OF HISTONE H2A AND THEIR FUNCTIONS OF H2A VARIANTS IN PATHOGEN INFECTION

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**ABSTRACT**

Histones are well-known components of the nucleosome. Among core histones H2A, H2B, H3 and H4, the H2A family exhibits the greatest diversity including the largest number of various variants such as H2A.X, H2A.Z, MacroH2A and H2A.Bbd. In general, H2A variants differ mostly in their C-terminus, regarding both length and amino acid sequences. The importance of H2A variants in nucleosome stability and chromatin structure has been well established. Although a lot of progress was made regarding antibacterial peptides derived from the N-terminus of histone H2A in the past decade, the nucleotide polymorphism of H2A and their functions of H2A variants in pathogen infection are largely unknown. In the present study, we found that piscine H2A exhibited abundant nucleotide polymorphism. 15 H2A variants were cloned from zebrafish. Although only 1 to 2 amino acids differ, the similarities among these H2A variants are 90.1~99.5%. Strikingly, nucleotide polymorphism of H2A influenced the antibacterial and antiviral activities. Thus, our results provide insights into the functional differences of H2A variants in pathogen infection.

**KEYWORDS**

Histone H2a; nucleotide polymorphism; viral infection; bacterial infection

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# P-052

## THE EFFECTS OF IFNΓ, IL-1B, AND TNF-A ON IFNΓ-IFNΓ R1/R2 PATHWAY IN MACROPHAGES FROM RAINBOW TROUT (*Oncorhynchus mykiss*)

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**ABSTRACT**
In vertebrates, the cytokines: Interleukin-1β (IL-1β), Tumor Necrosis Factor-α (TNF-α) and Interferon-γ (IFNγ) are proinflammatory cytokines crucial for the inflammatory response. The cytokine IL-1β is important for inducing neutrophilia and imparting a signal required for optimal T and B cell function. The cytokine TNF-α is key for T and macrophage cell activation and has an important role in the induction of inflammatory mediators, such as nitric oxide and prostaglandins. While the IFNγ plays a central role controlling the host response to viral or bacterial infection, through the activation of the JAK/STAT pathway and the induction of iNOS in M1 macrophages. Studies described that IFNγ is capable of inhibits Il-1β and induce TNF-α in murine macrophages. Also, nitric oxide regulates IL-1β production in murine macrophages. In rainbow trout, the existence of IFNγ receptors (IFNγR1/R2) has been demonstrated, and Il-1β, TNF-α, IFNγ shows similar functions to their vertebrate's counterparts. However, there are no studies about the combinatory effect that different cytokines have on the IFNγ pathway and cytokine expression in rainbow trout. The aim of this work was to characterize at transcriptional level the IFNγ-IFNγ R1/R2 pathway, IL-1β and Interleukin-6 (IL-6) expression in trout macrophages. For this, a cell line of monocytes/macrophages from rainbow trout were stimulated with recombinant IFNγ/Il-1β and IFNγ/TNF-α during 4-6 and 12 hours. The results showed that IFNγR1 and TRIM8 were downregulated. While IFNγR2 has an oscillatory response and was not detected at 12 hours, also STAT1 was upregulated mainly at 12 hours for IFNγ/IL-1β treatment. The IFNγ/TNFα treatment showed an upregulation of STAT1 for all the hours, mainly at 4 hours, contrasting with the expression observed for the IFNγ/IL-1β. iNOS expression was downregulated in IFNγ/IL-1β at 12 hours. The evaluation of the cytokines showed downregulation of IL-1β at 6-12 hours and IL-6 at 4-6-12 hours for IFNγ/IL-1β experiment. However, in IFNγ/TNF-α just a downregulation of IL-6 was observed. An experiment with just IL-1β showed that IFNγR1 was upregulated at 12 hours. The results obtained suggest a different mechanism of regulation for IFNγ-IFNγ R1/R2 pathway and a difference in cytokine regulation, mainly with IL-6, a cytokine that was upregulated in mammals when IFNγ is used with IL-1β/TNF-α.

**KEYWORDS**
Cytokines - IFNγ - IL-1β - IFNγ-IFNγ R1/R2 pathway - Rainbow trout
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# P-053

## RAINBOW TROUT SHAPE-SHIFTED RED BLOOD CELLS

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## ABSTRACT

Rainbow trout Ficoll-purified red blood cells (RBCs) cultured *in vitro* undergo morphological changes, especially when exposed to stress, and derive into a cell stage that we have coined shape-shifted RBCs (shRBCs). We have characterized these shRBCs using transmission electron microscopy (TEM) micrographs, Wright-Giemsa staining, cell markers immunostaining, and transcriptomic and proteomic evaluation. shRBCs displayed reduced density of the cytoplasm, hemoglobin loss, decondensed chromatin in the nucleus. Also, shRBCs displayed striking expression of the B lymphocyte molecular marker IgM. shRBCs were transiently observed in heat-stressed rainbow trout bloodstream for three days. Functional network analysis of combined transcriptomic and proteomic studies resulted in the identification of proteins involved in pathways related to the regulation of cell morphogenesis involved in differentiation, cellular response to stress, and immune system process. In this regard, it has long been suggested that primitive nucleated erythroid cells in the bloodstream of mammals are more similar to nucleated red cells of fish, amphibians, and birds than the red cells of fetal and adult mammals. In addition, shRBCs increased interleukin 8 (IL8), interleukin 1 β; (IL1β), interferon ɣ (IFNɣ), and natural killer enhancing factor (NKEF) protein production in response to viral hemorrhagic septicemia virus (VHSV). Also, shRBCs conditioned medium triggered cytokine signaling in trout pronephros stroma (TPS-2) cell line. In conclusion, shRBCs may represent a novel cell stage that participates in roles related to immune response mediation, homeostasis, and the differentiation and development of blood cells.

## KEYWORDS

Rainbow trout; shape-shifted red blood cells; VHSV; transcriptome; proteome

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# 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 (*Onchorhynchus 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, inmunoprecipitate, 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 downregulation during reproduction. Recovery of them 3 months after the spawning moment, with exception of the phagocytic activity, supports the the trade-off theory. Moreover, 17β-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. Aquiculture 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 Aquiculture 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 where 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 asses 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 EXPRESSIONAL 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 chaperonines 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 hsps 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 damselae piscicida*

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**ABSTRACT**
One of the challenges of the fish farming industry is the occurrence of disease outbreaks that can lead to important monetary losses. In this context, the study of suitable biomarkers to assess the fish health status, such as haematological and immune responses during the first steps of infection could assist in the creation of measures of recognition and prevention of disease. The present study was conceived to evaluate meagre (*Argyrosomus regius*) innate immune response after infection with *Photobacterium damselae piscicida* (*Phdp*).
A time-course study was performed at CETEMARES (Instituto Politécnico de Leiria, Peniche, Portugal) facilities with 36 animals being sampled (79.3 ± 15.1 g). Among them, 12 fish were randomly selected and sampled before infection (time 0 h). Thereafter, the remaining animals were randomly selected and intraperitoneally injected (i.p.) with 100 µl PBS (control group) or 100 µl of bacteria (105 CFU/mL; infected group) and distributed as a randomized complete design in 6 recirculating systems (i.e. triplicates per experimental condition). Two animals per tank (n=6) were randomly selected and sampled at 6 and 24 h after i.p. injection. At each sampling point, fish were anaesthetized with 2-phenoxyethanol and blood samples were collected for haematological procedures such as total and differential counting of peripheral leukocytes and total circulating erythrocytes counts. The remaining blood was centrifuged and plasma was collected for innate humoral parameters determination (i.e. bactericidal, antiproteases and peroxidase activities). Results showed similarities among cellular and humoral parameters in challenged fish. Infected meagre presented an increased peripheral white blood cells concentration compared to control individuals. Peripheral lymphocyte numbers increased in infected meagre from 0 h to 24 h while circulating neutrophils decreased in challenged fish regardless time, most likely due to migration of these cells to the peritoneal cavity (inflammatory focus). Plasma bactericidal activity increased in infected specimens after 24 h. Samples of head-kidney tissue will be suited the assessment of mRNA immune-related gene expression in order to understand how *Phdp* infection influences meagre immune machinery.

**KEYWORDS**
Infection, leukocytes, immune response, bactericidal activity, neutrophils.

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# P-060

## IMPACT OF HIGH TEMPERATURE ON THE INNATE IMMUNE RESPONSE IN THE INTESTINE OF ORANGE-SPOTTED GROUPER

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**ABSTRACT**
Temperature is a well-known important factor to the immune response in poikilothermic animals, such as fish. Studies in teleost fishes have shown that production of many innate immunity and adaptive immunity factors are temperature-dependent. We report an investigation on the impact of increased water temperature on the innate immune response in different regions of the intestine (anterior gut, midgut, & posterior gut) of orange-spotted grouper (*Epinephelus coioides*). In the study, fish were subjected to two modes of temperature shifting: shock treatment (temperature increased from 28°C to 32°C sharply) and adaptive treatment (temperature increased from 28°C to 32°C at the rate of 1°C per day). Our study showed that the shock and adaptive treatments exerted differential impacts on the expression of immune-relevant genes in the three regions of intestine. In fish subjected to immune stimulations, including poly I:C, lipopolysaccharide (LPS) and CpG oligodeoxynucleotide (ODN), shock and adaptive treatments also exerted differential impacts on the induction of immune genes in the three regions of intestine. Interestingly, the negative impact by increased temperature on the induction of immune genes was most noted in the mid-section of the gut. The differential effects of increased temperature in different regions of intestine might reflect the diversity in cellular composition and even microbiota in the regions.

**KEYWORDS**
Temperature, immune stimulants, innate immunity, intestine, grouper

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# P-061

## THE TURBOT (*Scophthalmus maximus*) MYELOPEROXIDASE: CHARACTERIZATION AND FUNCTIONAL STUDIES

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**ABSTRACT**
Myeloperoxidase (MPO) is a major enzyme that is mainly present in fish neutrophils. This enzyme is well characterised in mammals but little is known about its structure and function in fish. In this study, we sequenced the turbot MPO and studied some of its functions in turbot. The 5690 bp turbot myeloperoxidase gene contains an ORF with 14 exons. In addition to the 13 introns of the ORF, there is one intron of 134 nucleotides located in the 5´UTR region. The untranslated 5' and 3´ regions have 111 bp and 970 bp respectively. The coding sequence contains 2301 nucleotides that encode a polypeptide of 767 aa with a predicted molecular mass of 86.21 kDa. BLASTp analysis revealed that turbot MPO displays high similarity to the MPO of other fish species (identity varied between 60 and 82%) and lower than those of mammals (identity 50%) and reptiles (identity 47%). Turbot MPO was found to have several conserved domains such as the signal peptide, propeptide (118 aa) and light (113 aa) and heavy chains (591 aa). Other important sites for regulation of MPO activity are also present in the turbot molecule, including distal haem cavities I and II and proximal haem cavities I and II. Several catalytic, haem linkage and cysteine residues, a Ca+2-binding motif and also eight potential N-linked glycosylation sites were identified. Western blot analysis and use of an anti-turbot MPO polyclonal antibody revealed that turbot MPO exists in its mature form as a homodimer of about 150 kDa in the anterior kidney, spleen, peritoneal fluid and serum, indicating that the protein loses the propeptide during maturation. The MPO transcripts were most strongly expressed in the anterior kidney, gill, white blood cells and spleen, and they were most weakly expressed in liver, muscle, heart and brain. Immunofluorescence was used to identify cells compatible with neutrophils containing MPO + granules in the anterior kidney, spleen, blood, gill and intestine. In an in vitro stimulation test, anterior kidney leukocytes (HKL) were isolated on a Percoll gradient and stimulated with purified MPO obtained by affinity chromatography. Incubation of HKL with turbot MPO generates positive regulation of the proinflammatory cytokines TNF-α and IL12-β, the cytokine IFNγ, the chemokine IL8 and the subunits of the CD11b / CD18 integrins, suggesting that, in addition to its microbicidal activity, the MPO may act as a mediator of the immune response in fish.
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**KEYWORDS**
Turbot, myeloperoxidase, neutrophils, immune system

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# P-062

## CHARACTERIZATION AND FUNCTION OF A GROUP I TYPE I INTERFERON IN THE CARTILAGE AND HARD SCALE FISH CHINESE STURGEON (*Acipenser sinensis*)

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**ABSTRACT**
The type I IFNs are a pleiotropic family of cytokines. Three kinds of IFNe were found in the Chinese sturgeon (*Acipenser sinensis*), named with IFNe1, IFNe2 and IFNe3. In the present study, we found that the Chinese sturgeon IFNe2 protein can stimulate the expression of antiviral genes (PKR, VIPERIN, Mx, and ADAR4) and interferon regulatory factors in the Chinese sturgeon fin (CSF) cell, and induce the phosphorylation of IRF3 and IRF7. In addition, IFNe2 can also induce change in self-expression and positively regulate the expression of IFNe3 during early induction. Similarly, IFNe2 can up-regulate the expression of interferon-stimulated genes in EPC cells. EPC cells showed significantly increase antiviral ability when cells were treated with conditioned medium containing Chinese sturgeon IFNe2 for 2 hours prior to SVCV infection. Among the antibacterial activities, we have not found that IFNe2 has a resistance to *Aeromonas hydrophila* isolated from Chinese sturgeon.

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# P-063

## THERMAL EXPERIENCE DURING EMBRYOGENESIS IMPACTS THE MICRORNA TRANSCRIPTOME IN THE SPLEEN OF ADULT ZEBRAFISH (*Danio rerio*)

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**ABSTRACT**
The thermal experience during early development is known to have a long-term effect on several adult phenotypes but its impact on the immune system is still poorly understood. MicroRNAs (miRNAs) are a class of small non-coding RNAs that fine-tune various biological processes, including the immune response. Moreover, miRNA expression can be affected by environmental temperature. In this study, we investigated the effect of embryonic incubation temperature (24, 28, or 32 °C for 3, 4 and 5 days, respectively) on the expression of miRNAs in the spleen of adult fish, and their potential involvement in the immune response to lipopolysaccharide (LPS). Small RNA-seq results revealed that the spleen transcriptome comprised of 150 miRNAs conserved in zebrafish, 130 mature miRNAs known in other species, and 53 novel miRNA candidates. A total of 30 miRNAs were differentially expressed in the spleen of fish from the high (32 °C) embryonic incubation temperature group compared to those from reference temperature group (28 °C). Enrichment analysis showed that the putative target genes of these miRNAs were involved in immune biological processes of "endocytosis", "vesicle-mediated transport", "negative regulation of leukocyte activation" and "induction of positive chemotaxis". No miRNAs were differentially expressed in the low temperature group compared to the reference temperature. LPS challenge induced three miRNAs in the spleen of fish kept at constant reference temperature. Immune processes such as "endocytosis", "vesicle-mediated transport", "cytokine production" and "NIK non-canonical NF-κB signaling" were enriched by their target mRNAs. In conclusion, high embryonic incubation temperature had a long-term effect on miRNA expression in the spleen of adult zebrafish, and the miRNAs differentially expressed with temperature may be involved in fine-tuning immune processes.

**KEYWORDS**
Temperature, miRNA, RNA-seq, immune system, zebrafish

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# P-064

## RECOMBINANT FLAGELLIN B AND ITS ND1 DOMAIN FROM *Vibrio anguillarum* PROMOTE IN VIVO OVEREXPRESSION OF IL-1β AND IL-8 CYTOKINES IN *Salmo salar*

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**ABSTRACT**

Flagellin is the major component of the flagellum in Gram negative and positive bacteria, it bindsand activates the Toll-like receptor 5 and promotes the expression of proinflammatory cytokines and chemokines in vertebrates. As reported, two recombinant molecules of *Vibrio anguillarum*, flagellin (rFLA) and the amino-terminus of the D1 domain (rND1) from the same molecule induce an in vitro upregulation of proinflammatory genes in gilthead seabream and rainbow trout. We have hypothesized that rFla and rND1 may function as universal immunomodulator molecules in teleost. In this work, we studied in vitro and in vivo the biological properties for each of those molecules in *Salmo salar* and measured proinflammatory cytokines by real time PCR. The results for in vitro assays using SHK-1 cells and isolated head kidney leucocytes (HKL) were comparable and overall showed that IL-8 transcript increased 6-10-fold using rFLA and 2-6-fold using rND1, IL-1β transcript increased 3-4-fold with rFLA and 1.1-1.8 using rND1. We compared the in vivo effectivity of rFLA and rND1 alone or in combination with a commercial vaccine (CV) against P. salmonis. IL-1β and IL-8 induction was measured in head kidney at 4, 24, and 72 hours after intraperitoneal (I.P) injection with 5 μg rFLA or 15 μg of rND1. Results showed that rFLA and rND1 induced a time-dependent acute pro-inflammatory response. IL-1β upregulation reached 25-fold above the PBS-control after 4 hours and it decreased progressively until 3 to 6-fold over the baseline. IL-8 showed an acute response, reaching a 13-fold change above basal levels using rFLA or rND1 at 4 hours post IP injection. After 24 hours IL-8 was almost undetectable. The combined challenge (CV plus one single recombinant) showed differential responses based on IL-8 and IL-1β overexpression. For both combinations, an acute IL-8 upregulation of 3-fold change in head kidney after 4 hours was observed. However, the rFLA effect on IL-8 had a shorter duration than rND1 which response was stable until 144 hours after challenge. IL-1β was shortly upregulated by 2-fold by rFLA but not by rND1, and this induction was sustained in time. Altogether, our results suggest that rFLA and rND1 can drive non-redundant cytokines upregulation and both recombinants are valid candidates to be used as an immuno-stimulant or adjuvant in farmed salmon.

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**KEYWORDS**
Flagellin, cytokines proinflammatory, immune response, adjuvant

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# P-065

## DEVELOPMENT OF A MODULAR ORAL VACCINE BASED IN OUTER MEMBRANE VESICLES FOR RAINBOW TROUT AND CHARACTERIZATION OF THE SYSTEMIC AND MUCOSAL B AND T CELL RESPONSE ASSEMBLED.

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**ABSTRACT**
Infectious diseases cause serious economic losses due to the high-density fish stocks. Diversevaccines were developed to prevent this; however, they are not effective enough and theinjection route is linked to side effects and stress. It is known Gram-negative bacteria produce Outer Membrane Vesicles (OMVs) and are used for human research purposes; therefore, fish bacteria OMVs could be also used as vaccines platforms. The present work is focused on the development of an oral vaccine based on recombinant *Aeromonas salmonicida* OMVs, expressing the G-protein of VHSV, and the evaluation of the B and T cell response at mucosal and systemic level in Rainbow trout. In order to validate the oral stimulation formula, first fish were stimulated with inactivated bacteria intra-peritoneally or orally using vaccine pellets. Distribution and proliferation of B and T-cell populations were analyzed in gut, peritoneum, spleen and head kidney using monoclonal antibodies by flow cytometry. Additionally, cell populations were sorted for characterization of membrane and secreted markers, expressed cytokines and transcription factors. The immune response is characterized by an early proliferation of intraperitoneal B and T-cells (24-48h post stimulation). Comparing the kinetics of the cell populations observed in the peritoneum and in the gut as well as the recruitment of cells from spleen or head kidney will be further analyze. The next upcoming trial will be done with the OMVs from *A. salmonicida* to further studies.

**KEYWORDS**
Rainbow trout, oral vaccine, OMVs, *Aeromonas salmonicida*, adaptive immunity.

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# P-066

## IMMUNE AND PATHOGEN INTERACTIONS DURING EXPERIMENTAL CO-INFECTION WITH *Piscirickettsia salmonis* AND PISCINE ORTHOREOVIRUS IN *Salmo salar*

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**ABSTRACT**

Piscine Orthoreovirus (PRV) infections are widespreadly distributed in Chilean salmon cultivation. And it is estimated that over 80% of freshwater Atlantic salmon is infected predominantly with PRV-1. In this scenario, mixed infections with other viruses or bacteria are likely to occur, and typical clinical signs could be misdiagnosed due to different responses triggered during a simultaneous infection with two or more pathogens. On the other side, *Piscirickettsia salmonis (P. salmonis)* is the most important bacterial pathogen for Chilean salmon cultivation. The objective of our pilot study was investigating viral and bacterial presence, some aspects of innate immune responses and histopathological features during an experimental challenge with P. salmonis in a population of Atlantic salmon smolt infected with PRV-1.

From a population of 240, PRV-1 positive smolt (100g), 84 shedder fish were intraperitoneally infected with *P. salmonis* and then allocated with 156 co-habitant smolts. Sampling was carried out at 14, 21, and 30 days post-challenge (dpc). Co-habitant fish were euthanized and denervated. Blood, head kidney and spleen samples were directed to molecular analysis and head kidney, spleen, liver, heart, and gills were obtained for histological examination.

Our results showed that viral loads diminished significantly from 14dpc to 21dpc and to 30dpc, but they did not disappear. Meanwhile, the percentage of *P.salmonis* positive fish increased from 21 to 30dpc. In accordance with the decreasing viral load, a significant drop of IFN-I transcripts was detected from 21-fold change at 14dpc to 11,7 at 21 and to 1,4-fold change at 30dpc. On the other hand, Mx transcripts did not show any considerable change during the experiment. Cytokine transcripts related to inflammatory bacterial infections such as IL-8 transcripts were upregulated 12,4-fold change at 14 and 7,9-fold change at 21dpc and decreased 2,7-fold change at 30dpc. However, IL-12 and IL-1b _transcripts showed no variation at any time point evaluated. Most of the fish showed no lesion, and just a few evidenced only mild to moderate lesions concordant with HSMI or SRS at different time points. These results suggest that PRV-1 infection could exert an apparent protective effect on the host against the bacterial infection diminishing the severity of SRS clinical and histopathological signs. Grants: FIE-Sernapesca 2015-V014, VIDCA UACH.

**KEYWORDS**
Co-infection, Piscine Orthoreovirus, Piscirickettsia salmonis, antiviral immune response, antibacterial immune response

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# P-067

## STRESS REGULATION AND TOLERANCE IN SHRIMP: THE TRANSCRIPTOMIC AND PHYSIOLOGICAL RESPONSE TO CHRONIC AMMONIA EXPOSURE IN THE BLACK TIGER SHRIMP, *Penaeus monodon*.

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## ABSTRACT

Elevated ammonia (NH3) is a significant challenge in penaeid shrimp aquaculture worldwide, and can compromise shrimp osmoregulation, growth, immune-competency, leading to substantial crop loss. Despite the impact of elevated NH3 levels to the well-being of farmed penaeids, little is known about physiological and transcriptomic responses to chronic NH3 exposure. NH3 is a toxic byproduct of the break-down of uneaten feed, faeces and metabolic processes, and high NH3 levels are often difficult to mitigate quickly in ponds. This study investigated the physiological and transcriptomic response of sub-adult black tiger shrimp, *Penaeus monodon*, after chronic exposure to moderate and high levels of NH3. An individual assessment was implemented wherein shrimp hemolymph metabolite profiles were assessed pre- and post-exposure to identify individual responses to 0 ppm (control), 10 ppm and 20 ppm NH3 across a 72 h period. Key metabolites that have previously been linked to condition and stress responses in penaeid shrimp were quantified, including total protein, glucose, triglycerides, and hemocyanin. Additionally, shrimp were held in individual respirometers throughout the trial and the oxygen consumption of shrimp was measured with fiber optic probes as a proxy of activity, or metabolism. Oxygen consumption in response to NH3 exposure showed wide variance in controls, while shrimp exposed to 10 ppm and 20 ppm NH3 decreased oxygen consumption in the 24 h period post-exposure. After 48h shrimp exposed to 10 ppm showed evidence of recovery through increased oxygen consumption, while 20 ppm remained low. Hemolymph triglycerides at 96 h post-exposure are significantly decreased in shrimp exposed to 20 ppm NH3 compared to 10 ppm and control shrimp, indicating an increase in energy expenditure. Other biochemical parameters measured including total protein, hemocyanin and glucose decreased pre-to-post exposure due to feed restriction, and were not significantly different between treatments. To compliment physiological data, the transcriptomic profile of gill tissue from shrimp post-exposure were obtained by Illumina RNASeq. Differentially expressed genes were identified in response to NH3 exposure, as well as in shrimp that showed physiological evidence of recovery. The pathways enriched and genes co-expressed were identified and demonstrate how shrimp respond to ammonia stress, and potentially how some animals are able to tolerate NH3.

**KEYWORDS**
Transcriptomics, physiology, stress, aquaculture, shrimp

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# P-068

## *CQSIRT1* FROM RED CLAW CRAYFISH *Cherax quadricarinatus* PROMOTES WHITE SPOT SYNDROME VIRUS INFECTION VIA POSITIVELY REGULATING PI3K-AKT-MTOR PATHWAY

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**ABSTRACT**
Sirtuins (SIRT) are a family of evolutionarily conserved nicotinamide adenine dinucleotide (NAD+)-dependent deacylases that participate in antiviral immunity. In this report, the *SIRT1* gene (named as *CqSIRT1*) was identified with an open reading frame of 2256 bp that encoding 751 amino acids from red claw crayfish *Cherax quadricarinatus*. Tissue distribution analysis showed that *CqSIRT1* was ubiquitously expressed in all tissue tested with high expression in haematopoietic tissue (Hpt), haemocyte and gill, while low expression in hepatopancreas, musle and eyestalk. Unexpectedly, dysfunction of *CqSIRT1* by gene knockdown in red claw crayfish Hpt cell cultures resulted in markedly decrease expression of an early gene *ie1* and an envelope protein gene *vp28* of white spot syndrome virus (WSSV) at late stages post WSSV infection, indicating that CqSIRT1 was hijacked by WSSV to promote its replication. Importantly, the expression of *PI3K*, *AKT* and *mTOR,* which were involved in energy metabolism and autophagy, was significantly reduced after gene silencing of *CqSIRT1* during WSSV infection. Overall, these data suggest that CqSIRT1 could promote WSSV replication through positively modulating PI3K-AKT-mTOR signaling pathway in red claw crayfish *C. quadricarinatus*, which benefits further understanding of the molecular mechanism underlying the pathogenesis of WSSV in crustacean.

**KEYWORDS**
Sirtuins 1(SIRT1); PI3K-AKT-mTOR; White spot syndrome virus (WSSV); Haematopoietic tissue (Hpt); *Cherax quadricarinatus*.

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# P-069

## THE ROLE OF CD8a IN THE IMMUNE RESPONSE OF SEA BASS AGAINST NODAVIRUS

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**ABSTRACT**

The adaptive immune response is mediated by different mechanisms including humoral factors, cellular elements (T and B lymphocytes) and other specific proteins as major histocompatibility complex which act in coordination with diversified antigen receptors. Regarding cytotoxic T cells (CTLs) of vertebrates, they are direct effector lymphocytes in the fight against virus-infected cells by killing their cellular targets. The cluster of differentiation 8α (CD8α) is the distinctive marker of the lymphocyte subset of cytotoxic CTLs. However, teleost CTLs function is largely unknown mainly because of the lack of population-specific antibodies. European sea bass (*Dicentrarchus labrax*) is a very susceptible species to nodavirus (NNV) in which causes devastating mortality rates and, up to date, no solutions are available to prevent them. Previous studies pointed to the relevance of cell-mediated cytotoxicity in the response of European sea bass juveniles against NNV at transcriptional level. Thus, we have investigated the distribution, production and potential role on European sea bass CD8α+ cells by flow cytometry using a polyclonal antibody against European sea bass CD8α in naïve and NNV-infected cells. This work represents the first attempt to characterize CD8α+ cells in European sea bass.

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**KEYWORDS**

*Dicentrarchus labrax*, nodavirus, CD8α, polyclonal antibodies.

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# P-070

## STUDY OF THE IMMUNOMODULATORY AND ANTIVIRAL ACTIVITY OF BACTERIAL LIPOPOLYSACCHARIDES IN SEABASS (*Dicentrarchus labrax*, Linnaeus 1758)

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**ABSTRACT**
Diseases are the main cause of economic losses in the aquaculture sector that's why researching and improving this immunomodulation technique is fundamental. In this research work, the immunomodulatory effect of the lipopolysaccharides (LPS) of *Vibrio alginolyticus* was evaluated in a species of commercial interest, the seabass, through the cytokines, one kind of humoral components. Three groups of fish were evaluated, one unstimulated control, another positive stimulated with Poly I: C and another stimulated with LPS. The results showed statistically significant differences between the stimulants, where it was observed that the expression of the inflammatory (IL-1β, IL-6 and TNFα) and anti-inflammatory (IL-10) cytokines was directly related. The antiviral response (Mx) was different between the treatments and it was seen that with LPS the stimulating effect was more prolonged in time, although weaker.

**KEYWORDS**
Immunomodulation, cytokines, seabass, immunostimulants and lipopolysaccharides
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# P-071

## CIRCADIAN RHYTHMIC EXPRESSION OF TNF-a GENE REGULATED BY CLOCK GENE IN THE JAPANESE MEDAKA (*Oryzias latipes*)

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## ABSTRACT
To date, little information is available on the effects of circadian oscillation on immune regulation in lower vertebrates, such as teleost fish. In the present study, regulation of circadian rhythmic expression of inflammatory cytokine: TNF-α gene by clock genes (Bmal1 and Clock1) was investigated using Japanese medaka (*Oryzias latipes*). Firstly, structural analysis of clock gene was performed, which revealed that medaka Bmal1 and Clock1 conserve functionally important domains, such as basic helix-loop-helix (bHLH) and period-aryl hydrocarbon receptor nuclear translocator-single-minded (PAS), seen in their counterparts in other vertebrates. Expression of medaka Bmal1, Clock1 and Per1 genes was confirmed in central and peripheral tissues. Moreover, the expression of these clock genes and TNF-α genes in medaka acclimated to a 12:12 light (L) - dark (D) cycle showed circadian rhythm. In addition, higher expression of TNF-α gene was detected in medaka embryo cells (Ol-Hdr R-e3) overexpressing Bmal1 and Clock1 genes. It was suggested that this increase was mediated by transcriptional regulation by clock proteins, which target E-box sequence in the cis-element of TNF-α gene as was detected by luciferase reporter gene assay. Moreover, in vitro head kidney stimulation with LPS at different zeitgeber time (ZT) under LD12:12 condition affected the degree of TNF-α gene expression, which shows high and low responsiveness to LPS stimulation at ZT18 and ZT10, respectively. These results suggested that medaka TNF-α exhibited circadian rhythmic expression regulated by clock proteins and its responsiveness against immune-stimulation depends on time zone.

## KEYWORDS
Medaka, TNF-a, Circadian rhythm, Clock gene, Transcriptional regulation

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# P-072

## MOLECULAR CHARACTERIZATIONS AND LIGAND-INDUCED RESPONDS OF TYPE I INTERFERON RECEPTOR (IFNR1) IN ORANGE-SPOTTED GROUPER (*Epinephelus coioides*)

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**ABSTRACT**
Grouper fishes are known as high economical species in aquaculture industry. However, outbreak of diseases caused chronic death rate of grouper juveniles. To increase survival rate of grouper, mechanism of first line of defense against virus diseases in teleost fish is studied. Previous result indicated up-regulation of gene expression level of grouper IFN and downstream antiviral Mx protein gene in response to NNV virus infection. However, signalling cascade of IFN system is unclear. To investigate relationship between type I 2C-IFN to its receptor (IFNR), full length orange-spotted grouper IFNR1 was firstly cloned and identified. Phylogenetic analysis indicated osgIFNR1 shared high homology similarities with other teleosts. Relative expression of osgIFNR1 was determined using real-time qPCR in regards to interferon treatment using immune organ primary cell culture. Gene expression of osgIFNR1 and downstream osgMx1 which act as marker was seen up-regulated approximately 0.5 and 10-fold respectively in 4 hours post-stimulation indicating involvement of transcriptional level responses. We also observed transcript level of osgIFNR1 in response to immune-stimulant (LPS and Poly I:C) with increased fold of around 2 and 6 times respectively and nodavirus infected larvae of around 0.5-fold downregulation. We further used GF-1 cell line to observe subcellular localization and interaction between ligand and receptor. The present study revealed effect of IFNR1 through up-regulation of gene expression level during 4 hours of interferón (IFN) treatment. This study contributed different insights in analysis of IFNR role in type I interferón system in orange-spotted grouper.

**KEYWORDS**
Orange-spotted grouper, interferon receptor, nervous necrosis virus, immune system, molecular cloning

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# P-073

## EFFECTS OF RECOMBINANT IL-4/13A ON THE PHAGOCYTIC CAPACITY OF SALMONID LEUKOCYTES

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Abstract: The professional phagocytes described on teleost fish are macrophages, neutrophils and dendritic cells, and B lymphocytes. In mammals, several cytokines have effects on the phagocytic capacity of these cells, for example, IL-4 induces increase of phagocytosis by macrophages, as well as increase of reactive oxygen species production. In fish, it has been reported that IL-4/13 increases phagocytic capacity in Japanese pufferfish and Grass carp, however the function of this cytokine in salmonids has not been studied. In this work we evaluated the effect of recombinant IL-4/13A on the phagocytic capacity and ROS production of SHK-1 and RTS-11 cell lines and salmon spleen B cells. The phagocytic capacity was evaluated with fluorescent latex beads and ROS production with the probe 2,7-dichlorohydrofluroescein diacetate by flow cytometry. The results showed that rIL-4/13A increases the percentage of phagocytosis in trout and salmon spleen B cells. Also this protein, increases the phagocytic capacity in RTS-11 and SHK-1 cell lines, and in addition the mean fluorescence intensity of all tested cells. Besides, rIL-4/13A increases ROS production in RTS-11 cells and trout and salmon spleen B cells. In conclusion, rIL-4/13A increases phagocytosis and the production of reactive oxygen species indicating that this cytokine plays a role stimulating phagocytes to effectively engulf and eliminate invading microorganisms in salmonids.

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# P-074

## ATLANTIC SALMON LYMPHOCYTES: INFECTION TARGET CELLS FOR *Piscirickettsia salmonis*?

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**ABSTRACT**
*Piscirickettsia salmonis* is a facultative intracellular gram-negative bacterium of variable size (0.5-1.5 μm of diameter) causing salmonid rickettsial septicaemia in salmonids. During 2017, *P. salmonis* was responsible of almost 70% of mortality of salmon due to infectious causes in Chilean marine aquaculture centers. The pathogen produces prominent lesions in the liver, kidney, spleen and intestine, and one of the main target cells are macrophages. To date, only few studies investigate the cell targets of infection and the strategies and kinetics of *P. salmonis* infection at the cellular level. Thus, this study aimed to characterize *P. salmonis* infection of Atlantic salmon leukocytes using confocal microscopy and flow cytometry. We hypothesized that not only macrophages, but also lymphocytes were able to internalize *P. salmonis*. Thus, leukocytes isolated from the spleen of Atlantic salmon were infected with *P. salmonis*-727 conjugated to FITC (MOI 10). First, a time-course study was performed to follow up salmon splenocytes infection with *P. salmonis* for 6 hours using confocal microscopy. Video recording showed that 5 min post-infection (p.i.), *P. salmonis* was attached to the cell membrane of salmon splenocytes, whilst the bacteria were on the cell surface and/or internalized 30 min p.i. Thirty minutes later almost all splenocytes had the bacteria internalized. During the 6 hours of infection, splenocytes of different size showed protrusions of cell membrane that seems to engulf the bacteria, which is a characteristic process during phagocytosis of a pathogen. Apoptotic bodies and dead cells were also observed during this period of time. We then examined if the lymphoid population internalize the bacterium. This was evaluated by flow cytometry, using salmon spleen lymphocytes, infected with *P. salmonis* 727-FITC (MOI 10) for 0.5, 4, 8 and 24 hours. The percentage of lymphocytes able to incorporate *P. salmonis*-FITC was evaluated. An increase in the number of live lymphocytes containing the bacteria was observed during the first 8 h, that was decreasing when approaching 24 hours after infection. Similarly, an increase in the percentage of dead lymphocytes with *P. salmonis* was observed, supporting results observed in confocal microscopy. In summary, we reported that spleen lymphocytes of Atlantic salmon can internalize *P. salmonis* that might be due to the phagocytic capabilities of fish lymphoid cells or because they are target cells for *P. salmonis* infection or both.
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**KEYWORDS**
Atlantic salmon, lymphocytes, *Piscirickettsia salmonis*, infectivity, phagocytosis.
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# P-075

## COMPARATIVE MODULATION OF LNCRNAS IN WILD-TYPE AND RAG1 HETEROZYGOUS MUTANT ZEBRAFISH EXPOSED TO AN IMMUNE CHALLENGE WITH SPRING VIREMIA OF CARP VIRUS (SVCV).

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**ABSTRACT**

Although the modulation of immune-related genes after viral infection have been largely described in vertebrates, the potential implication non-coding RNAs (ncRNAs), and especially the long non-coding RNAs (lncRNAs), in the immunity is still an incipient research field. The model species zebrafish could serve as a very useful organism to study the functionality of lncRNAs due to the numerous advantages of this teleost, including the existence of numerous mutant lines. In this work, we conducted whole-transcriptome analysis of kidney from wild-type (WT) and heterozygous *rag1* mutants (*rag1*+/-) after infection with the pathogen Spring Viremia of Carp virus (SVCV). The RAG1 of vertebrates is one of the endonucleases with a role in the assembly of immunoglobulins and T cell receptor (TCR) genes. The absence of functional RAG1 protein results in the impossibility to develop mature B and T lymphocytes. However, heterozygous *rag1* zebrafish, which are partially deficient in Rag1, could allow us to detect lncRNAs potentially involved in the adaptive immunity due to the compensatory processes induced after infection. We identified 12,165 putative lncRNA in zebrafish, most of them shared by both zebrafish lines. However, by comparing the lncRNA profile induced after SVCV infection in both WT and *rag1*+/-, we found that the majority of the lncRNAs significantly induced after viral challenge were exclusive of each line, reflecting a highly differential response to the virus. Analysis of the neighbor genes of lncRNAs exclusively modulated in WT revealed a high representation of metabolism-related terms, whereas those from *rag1*+/- showed enrichment in terms related to the adaptive immune response, among others. As was expected, commonly modulated lncRNAs were surrounded by genes involved in numerous antiviral processes. These results clearly indicate that, after SVCV infection, zebrafish are able to induce the expression of an array of lncRNAs with a function in different aspects of the immunity.

**KEYWORDS**

zebrafish, lncRNAs, RNA-Seq, SVCV, immunity, rag1

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# P-076

## TWO REGULATOR OF COMPLEMENT ACTIVATION GROUP 2 GENES IN RAINBOW TROUT: GENE DUPLICATION AND DIVERGENT EVOLUTION

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**ABSTRACT**

The complement system is a crucial part of the immune system of vertebrates, protecting hosts from invading pathogens. The optimal activation of the complement system is tightly regulated by regulators of complement activation (RCAs) for host cell protection. In vertebrates, the RCAs can be categorized into two groups, including group 1 and group2. Despite increasing researches on complement components of rainbow trout, the RCA group 2 genes are still unknown. In this study, two RCA group 2 genes were cloned and identified in rainbow trout, named TRC1 and TRC2. The TRC1 is comprised of a signal peptide and ten SCR domains, while TRC2 only comprised of six SCR domains. Protein sequence alignment, gene structure comparison, phylogenetic and syntenic analysis revealed that TRC1 and TRC2 are two homologous genes, which may duplicate and evolved from an ancestral gene by the salmonid-specific whole-genome duplication (WGD) event. Further analysis revealed that the SCR domains of fish group 2 RCAs can be clustered into four types (A, B, C and D), and the SCR orders of TRC1 and TRC2 are ADBAABADCA and ADBADC, respectively. Expression analysis showed that TRC1 and TRC2 are constitutively expressed in various tissues and leukocyte subpopulations, with the expression level of TRC1 higher than that of TRC2, especially in IgT+ and IgM+ B cells. Previous studies have shown that vertebrate RCA group 2 genes are closely located in one chromosome, the first time we report two homologous, but significantly different RCA group 2 genes located in two different chromosomes in rainbow trout, providing new insights into the duplication and evolution of RCA genes in vertebrates.

**KEYWORDS**

Complement, regulator, duplication, evolution, rainbow trout

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# P-077

## INFLUENCE OF TRADITIONAL CHINESE MEDICINE AND *BACILLUS* SPECIES (TCMBS) ON GROWTH, IMMUNE RESPONSE AND DISEASE RESISTANCE IN NILE TILAPIA, *Oreochromis niloticus*

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**ABSTRACT**

Multispecies herbs and probiotic bacteria offered to fish might enhance their immune response and increase disease resistance, but the dose effects of herbal-probiotic application remain unclear. Therefore, the effect of herbal-probiotic mixtures of traditional Chinese medicine (TCM) of composition *Astragalus membranaceus*, *Angelica sinensis*, *Crataegus hupehensis* and probiotic Bacillus species (BS) of composition *Bacillus subtilis* and *Bacillus lincheniformis* as natural immunostimulants in tilapia *Oreochromis niloticus* have been investigated. Fish were randomly divided into four groups: control diet (CT), TCMBS1 [TCM at 3 g/kg and BS at 7 g/kg], TCMBS2 [TCM at 5 g/kg and BS at 5 g/kg], TCMBS3 [TCM at 7 g/kg and BS at 3 g/kg]. Tilapia in the TCMBS3 group showed significant improvement in weight gain, specific growth rate, and lowered feed conversion ratio compared with other treated groups and the control. Concerning immune indexes, all treated groups significantly enhanced lysozyme, superoxide dismutase, catalase, protease and antiprotease activities, with highest values in catalase and antiprotease activities in TCMBS3 compared with control. TCMBS3 demonstrated higher expression of beta-defensin, lysozyme, heat shock protein 70, catalase and transforming growth factor-beta compared with other treated groups or the control group in both mid-intestines and head-kidney. After challenge with *Streptococcus agalactiae*, the best survival was found in TCMBS3 (97%), followed by TCMBS2 (73%), and TCMBS1 (69%) compared with the CT (35%). Collectively the present results suggest that TCMBS3 dose might potentiate a higher immune response and disease resistance in fish.

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# P-078

## SEROTRANSFERRIN AND C-TYPE LECTIN COULD MEDIATED NCC ACTIVITY BY INTERACTING WITH NCCRP-1 IN NILE TILAPIA (*Oreochromis niloticus*)

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**ABSTRACT**

Nonspecific cytotoxic cells (NCCs) are precursor cells of mammalian natural killer cells and play an important role in innate immunity of fish. NCCCRP-1, as an activated receptor of NCC, could combining many exogenous substances or endogenous proteins to mediate NCC activity. In this study, we obtained two proteins, On-C-type lectin and On-serotransferrin, that could interacting with NCCRP-1 through screening of Yeast Two-hybrid library. These two recombinant proteins can effectively induce some toxic effector molecules of NCC high expression. At the same time, On-C-type lectin and On-serotransferrin stimulated NCC could effectively activating FHM apoptotic signal, but blocking on-NCCRP-1 by anti-On-NCCRP-1 antibody could inhibit this apoptotic signal. These results indicate that on-C-type lectin and on-serotransferrin could mediate the killing activity of NCC through NCCRP-1. This study contributes in better understanding the mechanism of NCC activation in teleost.

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# P-079

## HEMATOLOGICAL ANALYSIS OF GRASS CARP, BLUNT SNOUT BREAM AND YELLOW CATFISH: MORPHOLOGY, ULTRASTRUCTURE, CYTOCHEMISTRY AND QUANTIFICATION OF PERIPHERAL BLOOD CELLS

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**ABSTRACT**
The grass carp (*Ctenopharyngodon idella*), blunt snout bream (*Megalobrama amblycephala*) and yellow catfish (*Pelteobagrus fulvidraco*) are economically important fishes in China. Fish hematological features, especially the type and number of peripheral blood cells, are crucial for the evaluation of fish health state and the diagnosis of fish diseases. Since the automatic blood cell count equipment for human is not suitable for fishes, the manual method is critical in the quantification of fish blood cells. To make sense of the comparison and interpretation of the blood cell count studies in different articles, the standardization of blood cell classification is necessary. In this study, erythrocytes (RBC), thrombocytes (TC) and leucocytes (i.e. white blood cells, WBC, including lymphocytes, neutrophils and monocytes) were well distinguished in blood smears with Giemsa staining and confirmed by transmission electron microscopy. RBC, TC and WBC were directly counted with an improved Neubauer counting chamber in a modified diluting solution. The differential leucocyte count (DLC) was carried out in blood smears. In view of the labeling characteristics of peroxidase (PO) positivity in neutrophils and non-specific esterase (α-ANAE) positivity in monocytes, PO positive cell percentage and α-ANAE positive cell percentage were also determined in cytochemical staining smears. No difference was found for the percentages of neutrophils and monocytes between Giemsa staining and cytochemistry staining. The standardized classification, normal count ranges and sizes of the peripheral blood cells by the present systemic studies will provide useful references for monitoring the health status of grass carp, blunt snout bream and yellow catfish.

**KEYWORDS**
Grass carp (*Ctenopharyngodon idella*); Blunt snout bream (*Megalobrama amblycephala*); Yellow catfish (*Pelteobagrus fulvidraco*); Hematological parameters; Morphology.

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# P-080

## DEVELOPMENT OF MONOCLONAL ANTIBODY AGAINST IGM OF LARGE YELLOW CROAKER (*Larimichthys crocea*) AND CHARACTERIZATION OF IGM+ B CELLS

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## ABSTRACT

In the present study, a monoclonal antibody (mAb) against large yellow croaker IgM was produced by immunizing mice with purified serum IgM. Western blotting showed that this mAb could specifically react with the heavy chain of large yellow croaker serum IgM. Indirect immunofluorescence assay (IFA) analysis suggested that the resulting mouse anti-IgM mAb could recognize membrane-bound IgM (mIgM) molecules of large yellow croaker. This mouse anti-IgM mAb also can be used for sorting of large yellow croaker IgM+ B cells through the magnetic-activated cell sorting (MACS) method, which was further confirmed by RT-PCR analysis of specific marker genes for B cells. Flow cytometry analysis showed that the percentages of IgM+ B cells in head kidney, spleen and peripheral blood lymphocytes were 29.00± 1.58%, 33.00 ± 1.64%, and 16.50 ± 2.39%, respectively. Additionally, the phagocytosis rates of IgM+ B cells for 0.5 mm beads in head kidney, spleen and peripheral blood were calculated to be 7.56 ± 0.58%, 4.053 ± 0.62% and 23.17 ± 2.26%, respectively, while only 2.36 ± 0.23%, 1.16 ±0.44% and 6.41 ± 0.45 of IgM+ B cells in these three tissues ingested 1 mm beads. Taken together, our data demonstrated that the mouse anti-IgM mAb produced in this study could be used as a tool to characterize IgM+ B cells of large yellow croaker, and rates of phagocytic IgM+ B cells varied in different tissues.

## KEYWORDS

Monoclonal antibody; Immunoglobulin M; large yellow croaker (*Larimichthys crocea*); phagocytosis; IgM+ B cells

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# P-081

## FISH-SPECIFIC FINTRIM FTR36 TRIGGERS IFN PATHWAY AND MEDIATES INHIBITION OF VIRAL REPLICATION

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**ABSTRACT**
The tripartite motif (TRIM) family involves many cellular processes, including fundamental functions in antiviral immunity. Antiviral activities of TRIMs are reported in a variety of patterns, and one of the most significant channels is related to the activation of the type-I interferon (IFN) pathway. In this study, we described a *fintrim (ftr*) gene named *ftr36*, which is mainly expressed in the gills, skin, and intestines. This study shows that *ftr36* encodes a protein affording a potent antiviral effect. *In vitro*, overexpression of FTR36 mediated an upregulated pattern of recognition receptor retinoic acid–inducible gene I (RIG-I), interferon regulatory factor 3/7(IRF3/7), IFN, and IFN-stimulated genes (ISGs) expression. Thereby, FTR36 expression could afford host defense against the spring viremia of carp virus (SVCV) and the giant salamander iridovirus (GSIV). With the deletion of the RING domain or B30.2 domain separately, the antiviral ability of FTR36 was abolished partially and almost lost its ability to activate the IFN-pathway. These findings indicate that both RING and B30.2 domains are indispensable for the antiviral activity of FTR36. Altogether, this study described a finTRIM FTR36, which can activate IFN-pathways and stimulate ISGs to provide host defense against viral infections.

**KEYWORDS**
FTR36; finTRIM; antiviral; interferon; mechanism

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# P-082

## INVOLVMENT OF MUCOUS CELLS IN THE PROTECTIVE RESPONSE OF FISH AGAINST ENTERIC PARASITES

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**ABSTRACT**
Fish immunology receives considerable attention due to the breeding of several species for human consumption. The pathogens of fish attach to mucosal membranes of skin, gills, and gut and can cause a significant loss of affected species, that result in economic constrains. In the intestinal mucosa, the presence of parasites causes a local inflammatory reaction with recruitment of several immune cell types at the sites of infection, such as mast cells, neutrophils, macrophages and rodlet cells. Moreover, parasites as intestinal helminths disrupt the mucosal layer at their attachment site and could facilitate entry of pathogenic bacteria or viruses. In the intestine, mucous cells produce and secrete mucins, different high molecular weight glycosylated protein, which first hydrate, lubricate and protect the intestinal epithelium from the mechanical injuries due to the transition of digesta. Mucous intestinal cells are considered elements of the innate immune system, as they secrete lectins, toxins, immunoglobulins, and anti-microbial peptides as well. Most studies show the hyperplasia and hypertrophy of mucous cells in the area of parasite attachment, and their high production and discharge of mucus at the epithelial surface and in the lumen. In the point of helminth attachment, intestinal mucus shows the prevalence of the high viscosity acid mucins, and worms can appear surrounded by an adherent mucus layer or blanket. Acid mucins can also envelope and eliminate other infected microorganisms and their possible role in the stimulation of the immune system has been previously reported. In the parasitized intestine, mucous epitelial cells often are close to endocrine cells of the diffuse endocrine system from which they possibly receive or send local signals. Additionally, mast cells that infiltrate the intestinal epithelium are observed near mucous cells. The current study reports quantitative data on the density of the different mucous cell types and the mucus composition in perch, Perca fluviatilis, and mullet, *Liza ramada*, infected with *Acantocephalus lucii* and *Neoechinorhynchus agilis* (Acanthocephala), respectively. Mucous cell types are discriminated by the histochemical reaction with Alcian Blue pH2.5 and Periodic Acid Schiff, and by lectin histochemistry. Eight lectins were used to characterize the differences in glycoconjugate composition of mucous cells in infected/uninfected fish. The results between these two fish-helminth systems are discussed and compared with the data we obtained previously in seven different fish-helminth systems.

**KEYWORDS**
Mucous cells; mucins; intestinal helminths; perch; mullet
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# P-083

## CELLULAR INFLAMMATORY RESPONSE ON MARBLED ROCKFISH SEBASTISCUS MARMORATUS EXPERIMENTALLY INFECTED WITH OCHROCONIS HUMICOLA

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**ABSTRACT**
The fungal infection with Ochroconis humicola was previously reported caused mortality in young stages of cultured marine fish in Japan, including devil stinger Inimicus japonicus and marbled rockfish *Sebastiscus marmoratus*. Occurrence of this infection is depending on the body size of the fish, adult fish appeared to be more resistant to this infection. This study compared the histopathological features of inflammatory response among the experimentally infected marbled rockfish of different body sizes. Fish were divided into small (25-35mm), medium (50-60mm) and large (70-80mm), then intraperitoneally injected with the conidia of *O. humicola* NJM1503 at concentration 1.105 per fish The fish in each group were kept in aquaria containing 40L artificial seawater and the temperatura was maintained at 22$^{O}$C. The dead fish were collected accordingly and survived fish was sacrificed by using over dose FA100 after 30 days post infection (d.p.i). Spleen, kidney and liver of the fish were fixed and routinely embedded in paraffin and sectioned at 5mm. The serial sections were stained with hematoxylin and eosin (H&E), periodic acid-Schiff (PAS) reaction and Schmorl method. First mortality of infected small and médium fish was recorded 7 d.p.i and 15 d.p.i respectively. Mortality was not observed in large fish. Histopathologically, severe mycotic necrosis with large number of hyphae was observed in the infected small fish. Infected medium fish showed granulomatous inflammation in infected organs. This features was also observed in infected small fish dead from 20 d.p.i onwards. All large fish survived and showed more evident granuloma, including appearance of epithelioid cells and the hyphae was found inside the granuloma. The hyphae in large fish was encapsulated inside the granuloma and prevent further penetration. Therefore, it is suggested that the granulomas formation in infected large fish may suppress the fungal growth and showing resistant to this infection.

**KEYWORDS**
Ochroconis, granuloma, inflammation, epithelioid cells, fungal infection

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# P-084

## THE INDUCIBLE MICRORNA-21 NEGATIVELY MODULATES THE INFLAMMATORY RESPONSE IN TELEOST FISH VIA TARGETING IRAK4

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**ABSTRACT**

Eradication of bacterial infection requires timely and appropriate immune and inflammatory responses, but excessive induction of inflammatory cytokines can cause acute or chronic inflammatory disorders. Thus, various layers of negative mechanisms and regulators are needed to control the homeostasis of the immune system. miRNAs are a family of small noncoding RNAs that emerged as significant and versatile regulators involved in immune response. Recently, the molecular mechanisms of miRNA in host-pathogen interaction networks have been extensively studied in mammals, whereas the underlying regulatory mechanisms in fish are still poorly understood. In this study, we identify miR-21 as a negative regulator involved in regulating teleost inflammatory response. We found that lipopolysaccharide and *Vibrio anguillarum* significantly upregulated the expression of fish miR-21. Upregulated miR-21 suppresses LPS-induced inflammatory cytokine expression by targeting IL-1 receptor-associated kinase 4 (IRAK4), thereby avoiding excessive inflammatory responses. Furthermore, we demonstrated that miR-21 regulates inflammatory responses through NF-kB signaling pathways. The collective findings indicate that miR-21 plays a regulatory role in host-pathogen interactions through IRAK4-mediated NF-kB singling pathway.

**KEYWORDS**

miR-21; inflammation; IRAK4; NF-kB signaling

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# P-085

## GENERATION OF TOOLS FOR CHARACTERISATION OF ADAPTIVE IMMUNE RESPONSE IN CARP

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**ABSTRACT**

Recently our group reported the development of an intramuscular DNA vaccine against Spring Viremia of Carp Virus (SVCV). This vaccine conferred up to 100% protection to carp even at a single dose of 0.1 µg DNA/g of fish. A further characterization of the adaptive immune response revealed the presence of serum neutralizing antibodies and of memory T cells in vaccine-protected animals. Nevertheless, with the current tools, it was not possible to determine which specific type of immunoglobulin (IgM, IgD, IgT1, IgT2 or all) contributed to the serum neutralizing titres, nor which specific subtype of T cells (CD4+, CD8α+ or both) contributed to the memory response.

In this study, we report the preliminary characterization of tools against B and t cells of carp. For example, we have developed polyclonal antibodies recognizing IgT1+ positive B cells and are further characterizing a monoclonal antibody recognizing putative mucosal T cells. These tools, combined with those already available in our group against IgM+ B cells, CD4-1+ and CD8a+ T cells, provide a panel of markers that will allow the characterization of B and T cell adaptive immune response in carp in general and to vaccination in particular.

**KEYWORDS:**

IgT, IgM, mucosal T cells, B cells, common carp

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