(*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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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