

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 (cocci predominant grouped in pairs) and size ranging among 0.5 to 1.5 μm. This pathogen is the etiological agent of Piscirickettsiosis, the main infectious disease causing around 70% of mortalities in Atlantic salmon in Chilean farming, according to 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.

Acknowledgment. Proyecto Fondecyt Postdoctorado 3180560; Proyecto Fondecyt 1161015.

**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-1, 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-1, 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 SJNNV-inoculated 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.

This study has been supported by the project AGL2017-84644-R (MINECO/AEI/FEDER, UE). P. Moreno was supported by a fellowship from Ministerio de Educación, Cultura y Deporte (FPU12/00265, Spanish Government).

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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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