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

Corresponding author.
E-mail address: lvgj@sund.ku.dk (L.V.G. Jørgensen).

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

M. Rozas-Serri [1,2,#], A. Peña [1,#], L. Maldonado [1].

[1] Laboratorio Pathovet Ltda., Puerto Montt, Chile
[2] Newenko Group SpA., Puerto Montt, Chile

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.

Corresponding author
E-mail address: marco.rozas@pathovet.cl (M. Rozas-Serri).

P-047.

Pivotal role of immunoglobulin IgT in rainbow trout skin after bacterial infected with *Flavobacterium columnare*

X.T. Zhang, Z. Xu[#].

Department of Aquatic Animal Medicine, College of Fisheries, Huazhong Agricultural University, Wuhan, Hubei, 430070, China

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*)

Corresponding author.
E-mail address: zhenxu@mail.hzau.edu.cn (Z. Xu).

P-048.

Early immune response in Atlantic salmon vaccinated with inactivated whole-cell bacterin of *Piscirickettsia salmonis* and pathogenic isolates

M. Rozas-Serri [1,2,#], A. Peña [1], L. Maldonado [1].

[1] Laboratorio Pathovet Ltda., Puerto Montt, Chile
[2] Newenko Group SpA., Puerto Montt, Chile

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.

Corresponding author.
E-mail address: marco.rozas@pathovet.cl (M. Rozas-Serri).

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

M. Cortés[#,], V. Wong[#,*], C. Navarro, M. Imarai[#].*

Laboratorio de Inmunología, Centro de Biotecnología Acuícola, Universidad de Santiago de Chile, Santiago, Chile

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.

Acknowledgment. Proyecto Fondecyt Postdoctorado 3180560; Proyecto Fondecyt 1161015.

keywords: Atlantic salmon, macrophages, Piscirickettsia salmonis, apoptosis, phagocytosis.

Corresponding authors.
E-mail addresses: marcos.cortes@usach.cl (M. Cortés), monica.imarai@usach.cl (M. Imarai).
* These authors have contributed equally to this work.

P-050.

Transcriptional profile and serological studies of the European sea bass immune response against betanodavirus infections

P. Moreno [1], B. Lopez-Jimena [2], G. Scapigliati [3], F. Buonocore [3], E. Garcia-Rosado [1], J.J. Borrego [1], J. Bejar [1], M.C. Alonso [1,#].

[1] Universidad de Málaga, Departamento de Microbiología, Facultad de Ciencias, 29071 Málaga, Spain
[2] LifeArc Centre for Diagnostics Development. Nine, Edinburgh Bioquarter, 9 Little France Road, Edinburgh, EH16 4UX, Scotland, United Kingdom
[3] Department for Innovation in Biological, Agrofood and Forest Systems, University of Tuscia, Viterbo, Italy

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.

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).

Corresponding authors;
E-mail address: mdalonso@uma.es (M.C. Alonso).

P-051.

The nucleotide polymorphism of histone H2A and their functions of H2A variants in pathogen infection

X.M. Wu [1], L. Cao [1], J. Zou [2], P. Nie [1], M.X. Chang [1,#].

[1] State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, Hubei Province, 430072, China