

**keywords:** Turbot, myeloperoxidase, neutrophils, immune system

# Corresponding author.

E-mail address: [jesus.lamas@usc.es](mailto:jesus.lamas@usc.es) (J. Lamas).

#### P-062.

##### Characterization and function of a group I type I interferon in the cartilage and hard scale fish Chinese sturgeon (*Acipenser sinensis*)

Youshen Li, Panpan Han, Guangming Tian, Lifeng Zhang, Huizhi Guo, Qiaoqing Xu<sup>#</sup>.

School of Animal Science, Yangtze University, Jingzhou, China

#### 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.

# Correspondence. School of Animal Science, Yangtze University, Jingzhou, China,

E-mail address: [xuqiaoqing@163.com](mailto:xuqiaoqing@163.com) (Q. Xu).

#### P-063.

##### Thermal experience during embryogenesis impacts the microRNA transcriptome in the spleen of adult zebrafish (*Danio rerio*)

Q. Zhang<sup>#</sup>, I. Babiak, J. Fernandes.

Faculty of Biosciences and Aquaculture, Nord University, 8049 Bodø, Norway

#### 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

# Corresponding author.

E-mail address: [qirui.zhang@nord.no](mailto:qirui.zhang@nord.no) (Q. Zhang).

#### 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*

R. González-Stegmaier<sup>1,2,#</sup>, I. Aguirre<sup>3</sup>, F. Villarreal-Espíndola<sup>4,5</sup>, R. Enríquez<sup>3</sup>, J. Figueroa<sup>1,2</sup>.

<sup>1</sup> Laboratorio de Biología Molecular de peces. Instituto de Bioquímica y Microbiología. Universidad Austral de Chile, Valdivia, Chile

<sup>2</sup> Centro FONDAP: Interdisciplinary Center for Aquaculture Research (INCAR), Chile

<sup>3</sup> Laboratorio de Biotecnología y Patología Acuática. Instituto de Patología Animal, Universidad Austral de Chile, Valdivia, Chile

<sup>4</sup> Department of Pathology, School of Medicine, Yale University, New Haven, USA

<sup>5</sup> Laboratorio Medicina Traslacional. Instituto Clínico Oncológico. Fundación Arturo López Pérez, Santiago, Chile

#### Abstract

Flagellin is the major component of the flagellum in Gram negative and positive bacteria, it binds and 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

# Corresponding author.

E-mail address: [roxana.gonzalez@uach.cl](mailto:roxana.gonzalez@uach.cl) (R. González-Stegmaier).

#### 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

Ruth Montero<sup>1, #</sup>, Lara Munkler<sup>1, 2</sup>, Sven Ostermann<sup>1</sup>, Bernd Köllner<sup>1</sup>.

<sup>1</sup> Friedrich-Loeffler-Institute, Institute of Immunology, Greifswald-Insel Riems, Germany

<sup>2</sup> Hochschule Esslingen, Department of Applied Natural Sciences, Esslingen am Neckar, Germany

#### Abstract

Infectious diseases cause serious economic losses due to the high-density fish stocks. Diverse vaccines were developed to prevent this; however, they are not effective enough and the injection 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 vaccine 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.

# Corresponding author.

E-mail address: [ruth.montero.m@gmail.com](mailto:ruth.montero.m@gmail.com) (R. Montero).

#### P-066.

##### Immune and pathogen interactions during experimental co-infection with *Piscirickettsia salmonis* and Piscine Orthoreovirus in *Salmo salar*

I. Aguirre<sup>1, #</sup>, A. Mancilla<sup>1</sup>, M. Navarrete<sup>1</sup>, E. Paredes<sup>1</sup>, R. González-Stegmaier<sup>1, 2</sup>, R. Enríquez<sup>1, #</sup>.

<sup>1</sup> Laboratorio de Biotecnología y Patología Acuática. Instituto de Patología Animal. Facultad de Ciencias Veterinarias. Universidad Austral de Chile, Valdivia, Chile

<sup>2</sup> Centro FONDAF: Interdisciplinary Center for Aquaculture Research (INCAR), Chile

#### 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-1 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 up-regulated 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-1 $\beta$  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

# Corresponding author.

E-mail addresses: [isabel.aguirre@uach.cl](mailto:isabel.aguirre@uach.cl) (I. Aguirre), [renrique@uach.cl](mailto:renrique@uach.cl) (R. Enríquez).

#### P-067.

##### Stress regulation and tolerance in shrimp: The transcriptomic and physiological response to chronic ammonia exposure in the black tiger shrimp, *Penaeus monodon*

S.E. Berry<sup>1, 2, 3, #</sup>, R. Huerlimann<sup>1, 2</sup>, S. McWilliam<sup>3</sup>, A.R. Foote<sup>1, 2, 3</sup>, D.R. Jerry<sup>1, 2</sup>, N.M. Wade<sup>1, 3</sup>.

<sup>1</sup> Australian Research Council Industrial Transformation Research Hub for Advanced Prawn Breeding, James Cook University, Townsville, QLD, 4811, Australia

<sup>2</sup> James Cook University, College of Science and Engineering, James Cook Drive, Townsville, QLD, 4811, Australia

<sup>3</sup> CSIRO Agriculture and Food, Aquaculture Program, 306 Carmody Road, St Lucia, QLD, 4067, Australia

#### Abstract

Elevated ammonia (NH<sub>3</sub>) 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 NH<sub>3</sub> levels to the well-being of farmed penaeids, little is known about physiological and transcriptomic responses to chronic NH<sub>3</sub> exposure. NH<sub>3</sub> is a toxic byproduct of the break-down of uneaten feed, faeces and metabolic processes, and high NH<sub>3</sub> 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*,