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 μl of Hank's Balanced Salt Solution (Control-CTRL) or 100 μl 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