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keywords: Nervous necrosis virus (NNV), immune system, *Dicentrarchus labrax*, PCR

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P-019.

CpG oligodeoxynucleotides modulate innate and adaptive functions of IgM+ B cells in rainbow trout

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Abstract

Oligodeoxynucleotides (ODN) containing unmethylated CpG motifs have been widely postulated as vaccine adjuvants both in mammals and teleost fish. However, to date, the effects that CpGs provoke on cells of the adaptive immune system remain mostly unexplored in fish. Given that rainbow trout (*Oncorhynchus mykiss*) IgM+ B cells from spleen and blood transcribe high levels of toll like receptor 9 (TLR9), the receptor responsible for CpG detection, in the current work, we have investigated the effects of CpGs on both spleen and blood IgM+ B cells from this species. CpGs were shown to exert strong proliferative effects on both spleen and blood IgM+ B cells, also increasing their survival. The fact that CpGs increase the size of IgM+ B cells, reduce the expression of surface IgM and IgD and upregulate the number of IgM-secreting cells strongly suggest that IgM+ B cells differentiate to plasmablasts / plasma cells in response to CpG stimulation. Additionally, CpGs were shown to modulate the antigen presenting capacities of trout IgM+ B cells through an increased surface MHC II expression and transcriptional up-regulation of co-stimulatory molecules, although in this case, significant differences were observed between the effects exerted on spleen and blood cells. Similarly, differences were observed between spleen and blood IgM+ B cells when CpG stimulation was combined with B cell receptor (BCR) crosslinking. Finally, CpGs were also shown to affect innate functions retained by teleost IgM+ B cells such as their phagocytic capacity. These results demonstrate that CpGs regulate many adaptive and innate functions of teleost B cells, supporting their inclusion as adjuvants in novel vaccine formulations.

keywords: Rainbow trout, CpG, IgM, B cells, BCR.

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P-020.

Insights into the functions of piscidins in the European sea bass (*Dicentrarchus labrax*)

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ABSTRACT

Antimicrobial peptides (AMPs) are one of the host's first line of defenses against a wide range of infectious agents. Apart from the antimicrobial activity, AMPs are known to influence other biological processes, such as immunomodulation and iron metabolism. Fish present a specific group of AMPs, the piscidins. These peptides have been characterized in several fish species, acting on multiple pathogens, being also altered when fish are subjected to an infection. Furthermore, several studies have shown the potential of using synthetic peptides to promote fish survival upon infection. However, in the European sea bass (*Dicentrarchus labrax*), a commercially important fish produced in aquaculture, only hepcidin has been extensively characterized. Thus, a comprehensive study on the functions of other AMPs, particularly piscidins, is missing. Here, we identified and characterized the different piscidins of sea bass. We evaluated the antimicrobial activity of piscidins against several bacteria known to cause massive mortalities in cultured marine fish. Furthermore, the expression of the different genes belonging to the piscidin family was assessed under distinct experimental conditions, particularly infection and iron modulation, at pre-determined time points. Our results show a diverse piscidin antimicrobial activity *in vitro* against the different bacteria, indicating that these AMPs have a direct role against these pathogens, depending on the pathogen and piscidin peptide. Our data also shows a piscidin response after infection, suggesting that piscidins are involved in the response against infection. Furthermore, preliminary data shows that piscidins also respond to iron modulation, indicating that these AMPs may have other yet undisclosed functions besides antimicrobial activity, such as a role in iron metabolism. Our findings imply that piscidins might be a complementary or alternative way that fish possess to deal with this essential element, apart from the major iron regulator hepcidin. It is known that iron is also essential for bacterial progression during infection. Thus, iron is in a continuous regulation to be available for body processes, being also modulated to ensure that is inaccessible to pathogenic microorganisms. Further work is necessary to fully understand the role and mechanisms of action of piscidins under the context of immune response and iron metabolism regulation, and to possibly uncover a novel function for these particular peptides in fish.

keywords: Antimicrobial peptides; Piscidins; Sea bass (*Dicentrarchus labrax*); Infection; Iron metabolism

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P-021.

Effects of 17 α -ethynylestradiol (EE2) on the immune system of juvenile European sea bass with a special focus on B and T cells

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Abstract

Synthetic compounds are known for their persistence and bioaccumulation in the environment. 17 α -ethynylestradiol (EE2), a synthetic derivative of the natural hormone oestradiol, is present in human contraceptive pills, but also in livestock and aquaculture activity. Therefore, municipal wastewaters are one of the most important sources of this compound in the aquatic environment. Because EE2 induces oestrogenic effects even at trace level concentration, it has potent endocrine disrupting

properties. In wildlife, and especially in jawed vertebrates, EE2 is classically recognized for its negative effects on the reproductive functions. As endogenous oestrogens, however, EE2 can also modulate the immune system. Consequently, EE2 may affect the individual fitness by altering the capacity to cope with pathogenic agents. Adverse effects of EE2 on immune system function and ontogenesis remain to be fully elucidated, both for mammals and teleost fish. Juvenile European sea bass (90 days post-hatch) were exposed to waterborne EE2 (5ng/L) for one month at 17°C in order to investigate the modes of actions of EE2 on the developing immune system. Exposure concentrations were verified by LC-MS/MS and the oestrogenic activity assessed by yeast estrogen screen assay. Following exposure, several lymphoid organs including the thymus, the head-kidney and the gills were sampled for analysis by qPCR, immunohistochemistry and flow cytometry on isolated leucocytes. The leucocytes were analyzed for their phagocytic capacity as well as their proportion of DLlg3+ and DLT15+ lymphocytes. DLlg3 and DLT15 are monoclonal antibodies, which specifically recognize sea bass IgM and a pan-T cell marker, respectively. First results validate the exposure, which did not significantly impact the biometric measurements of the fish (growth, spleno- and hepatosomatic indices). Considering the proportion of DLlg3+ and DLT15+ lymphocytes and the phagocytic capacity, the treatment increased significantly the proportion of DLlg3+ cells in the head-kidney only but had no effect on the other measured immune parameters. Ongoing work aims at evaluating the effect of EE2 on T cell and B cell differentiation in their respective primary organs (thymus and head-kidney) as well as the secondary immune organs in order to understand the capacity of EE2 on the establishment of the immunocompetence in sea bass.

keywords: Immune system; European sea bass; endocrine disruptor; T Cell; B Cell

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P-022.

Anti-inflammatory mediators and appetite regulatory neuropeptides are affected by chronic stress in *Salmo salar*

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Abstract

In fish farming, there are different long-term stress conditions, some of which are so severe that fish can no longer reestablish homeostasis. Factors that cause prolonged stress can drastically alter the defense against pathogens by upregulation of anti-inflammatory response. In addition, chronic stress modifies the fish energy balance diminishing the appetite. In vertebrates, the stimuli that generate stress are initially perceived by the central nervous system sensors of hypothalamus, stimulating the release of cortisol into the bloodstream. Interestingly, the feeding control center of fish, like in mammals, is also found in the hypothalamus. Thus, extensive cultivation under inappropriate conditions chronically can affect the inflammatory response and the energy uptake from the food. In this study,

the expression of the glucocorticoid-regulated protein Annexin A1 (AnxA1), an important endogenous anti-inflammatory mediator was analyzed in smolt salmon kept at different stocking densities for 40 days. The highest stocking density (HSD) (40 and 60 Kg/m³) simulated a chronic stress by crowding. In addition, the mRNA expression of NPY, substance P, VIP and CGRP appetite regulatory neuropeptides, and the anorexigenic hormone leptin were also analyzed. The results of ELISA assay showed that AnxA1 expression was significantly increased in the gill and muscle of specimens kept at highest stocking density. Moreover, gene expression analysis by Real-time PCR showed upregulation of VIP mRNA in gut of HSD group. Furthermore, the mRNA expression of SP neuropeptide increased three fold in liver of specimens held at 60 Kg/m³. For the other hand, downregulation of NPY mRNA in fish brain of HSD group was observed. Finally, leptin mRNA expression was maintained at high levels in the liver of specimens held at 60 Kg/m³. These alterations reflect the effect of high stocking density on inflammatory and appetite molecular signals. Therefore understanding how these signals are affected during the productive processes of fish farming is required. A reduced appetite involves a lower uptake of energy from food, which affects the functioning of different physiological processes such as the immune system.

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keywords: Anti-inflammatory, Annexin A1, appetite regulatory molecules, stock density, stress

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P-023.

Interleukin (IL-17) and receptors in European sea bass and gilthead seabream. Regulation by NNV infection

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Abstract

Interleukin-17 (IL-17) is a cytokine family composed of six ligands (A-F) being IL-17A and IL-17F the best characterized. These are produced by Th17 cells and induce the expression of many inflammatory mediators. In addition, several IL-17 receptors have been also identified with different cell distribution. The main objective of this work was to identify IL-17 forms, as well as some IL-17 receptors, in two fish species: the European sea bass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*). In addition, we have evaluated the transcription of IL-17 forms and their receptors in seabream and sea bass after the Nervous necrosis virus (NNV) infection, which is a disease that produces viral encephalopathy and retinopathy in fish, by real time PCR. This study revealed the presence of IL-17 and their receptors in both fish species. Moreover, a regulation of fish immune system was observed in fish exposed to NNV infection. To our knowledge, this is the first study addressing the IL-17 expression on the European sea bass and the gilthead seabream infected by NNV. This work was funded by projects from MINECO and FEDER (AGL2016-74866-C3-1-R) and Fundación Séneca (Grupo de Excelencia de la Región de Murcia 19883/GERM/15).

keywords: Interleukin IL-17, IL-17 receptors, sea bass, gilthead seabream, Nodavirus

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