Abstract
One of the challenges of the fish farming industry is the occurrence of disease outbreaks that can lead to important monetary losses. In this context, the study of suitable biomarkers to assess the fish health status, such as haematological and immune responses during the first steps of infection could assist in the creation of measures of recognition and prevention of disease. The present study was conceived to evaluate meagre (*Argyrosomus regius*) innate immune response after infection with *Photobacterium damselae piscicida* (*Phdp*).
A time-course study was performed at CETEMARES (Instituto Politécnico de Leiria, Peniche, Portugal) facilities with 36 animals being sampled (79.3 ± 15.1 g). Among them, 12 fish were randomly selected and sampled before infection (time 0 h). Thereafter, the remaining animals were randomly selected and intraperitoneally injected (i.p.) with 100 μl PBS (control group) or 100 μl of bacteria (105 CFU/mL; infected group) and distributed as a randomized complete design in 6 recirculating systems (i.e. triplicates per experimental condition). Two animals per tank (n=6) were randomly selected and sampled at 6 and 24 h after i.p. injection. At each sampling point, fish were anaesthetized with 2-phenoxyethanol and blood samples were collected for haematological procedures such as total and differential counting of peripheral leukocytes and total circulating erythrocytes counts. The remaining blood was centrifuged and plasma was collected for innate humoral parameters determination (i.e. bactericidal, antiproteases and peroxidase activities). Results showed similarities among cellular and humoral parameters in challenged fish. Infected meagre presented an increased peripheral white blood cells concentration compared to control individuals. Peripheral lymphocyte numbers increased in infected meagre from 0 h to 24 h while circulating neutrophils decreased in challenged fish regardless time, most likely due to migration of these cells to the peritoneal cavity (inflammatory focus). Plasma bactericidal activity increased in infected specimens after 24 h. Samples of head-kidney tissue will be suited the assessment of mRNA immune-related gene expression in order to understand how *Phdp* infection influences meagre immune machinery.
keywords: Infection, leukocytes, immune response, bactericidal activity, neutrophils.

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P-060.

Impact of high temperature on the innate immune response in the intestine of orange-spotted grouper

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Abstract
Temperature is a well-known important factor to the immune response in poikilothermic animals, such as fish. Studies in teleost fishes have shown that production of many innate immunity and adaptive immunity factors are temperature-dependent. We report an investigation on the impact of increased water temperature on the innate immune response in different regions of the intestine (anterior gut, midgut, & posterior gut) of orange-spotted grouper (*Epinephelus coioides*). In the study, fish were subjected to two modes of temperature shifting: shock treatment (temperature increased from 28°C to 32°C sharply) and adaptive treatment (temperature increased from 28°C to 32°C at the rate of 1°C per day). Our study showed that the shock and adaptive treatments exerted differential impacts on the expression of immune-relevant genes in the three regions of intestine. In fish subjected to immune stimulations, including poly I:C, lipopolysaccharide (LPS) and CpG oligodeoxynucleotide (ODN), shock and adaptive treatments also exerted differential impacts on the induction of immune genes in the three regions of intestine. Interestingly, the negative impact by increased temperature on the induction of immune genes was most noted in the mid-section of the gut. The differential effects of increased temperature in different regions of intestine might reflect the diversity in cellular composition and even microbiota in the regions.
keywords: Temperature, immune stimulants, innate immunity, intestine, grouper

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P-061.

The turbot (*Scophthalmus maximus*) myeloperoxidase: Characterization and functional studies

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Abstract
Myeloperoxidase (MPO) is a major enzyme that is mainly present in fish neutrophils. This enzyme is well characterised in mammals but little is known about its structure and function in fish. In this study, we sequenced the turbot MPO and studied some of its functions in turbot. The 5690 bp turbot myeloperoxidase gene contains an ORF with 14 exons. In addition to the 13 introns of the ORF, there is one intron of 134 nucleotides located in the 5UTR region. The untranslated 5' and 3′regions have 111 bp and 970 bp respectively. The coding sequence contains 2301 nucleotides that encode a polypeptide of 767 aa with a predicted molecular mass of 86.21 kDa. BLASTp analysis revealed that turbot MPO displays high similarity to the MPO of other fish species (identity varied between 60 and 82%) and lower than those of mammals (identity 50%) and reptiles (identity 47%). Turbot MPO was found to have several conserved domains such as the signal peptide, propeptide (118 aa) and light (113 aa) and heavy chains (591 aa). Other important sites for regulation of MPO activity are also present in the turbot molecule, including distal haem cavities I and II and proximal haem cavities I and II. Several catalytic, haem linkage and cysteine residues, a Ca+2-binding motif and also eight potential N-linked glycosylation sites were identified. Western blot analysis and use of an anti-turbot MPO polyclonal antibody revealed that turbot MPO exists in its mature form as a homodimer of about 150 kDa in the anterior kidney, spleen, peritoneal fluid and serum, indicating that the protein loses the propeptide during maturation. The MPO transcripts were most strongly expressed in the anterior kidney, gill, white blood cells and spleen, and they were most weakly expressed in liver, muscle, heart and brain. Immunofluorescence was used to identify cells compatible with neutrophils containing MPO + granules in the anterior kidney, spleen, blood, gill and intestine. In an in vitro stimulation test, anterior kidney leukocytes (HKL) were isolated on a Percoll gradient and stimulated with purified MPO obtained by affinity chromatography. Incubation of HKL with turbot MPO generates positive regulation of the proinflammatory cytokines TNF-α and IL12-β, the cytokine IFNγ, the chemokine IL8 and the subunits of the CD11b / CD18 integrins, suggesting that, in addition to its microbicidal activity, the MPO may act as a mediator of the immune response in fish.
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keywords: Turbot, myeloperoxidase, neutrophils, immune system

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P-062.

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

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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.

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P-063.

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

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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

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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*

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