after chronic exposure to moderate and high levels of NH3. An individual assessment was implemented wherein shrimp hemolymph metabolite profiles were assessed pre- and post-exposure to identify individual responses to 0 ppm (control), 10 ppm and 20 ppm NH3 across a 72 h period. Key metabolites that have previously been linked to condition and stress responses in penaeid shrimp were quantified, including total protein, glucose, triglycerides, and hemocyanin. Additionally, shrimp were held in individual respirometers throughout the trial and the oxygen consumption of shrimp was measured with fiber optic probes as a proxy of activity, or metabolism. Oxygen consumption in response to NH3 exposure showed wide variance in controls, while shrimp exposed to 10 ppm and 20 ppm NH3 decreased oxygen consumption in the 24 h period post-exposure. After 48h shrimp exposed to 10 ppm showed evidence of recovery through increased oxygen consumption, while 20 ppm remained low. Hemolymph triglycerides at 96 h post-exposure are significantly decreased in shrimp exposed to 20 ppm NH3 compared to 10 ppm and control shrimp, indicating an increase in energy expenditure. Other biochemical parameters measured including total protein, hemocyanin and glucose decreased pre-to-post exposure due to feed restriction, and were not significantly different between treatments. To compliment physiological data, the transcriptomic profile of gill tissue from shrimp post-exposure were obtained by Illumina RNASeq. Differentially expressed genes were identified in response to NH3 exposure, as well as in shrimp that showed physiological evidence of recovery. The pathways enriched and genes co-expressed were identified and demonstrate how shrimp respond to ammonia stress, and potentially how some animals are able to tolerate NH3.

keywords: Transcriptomics, physiology, stress, aquaculture, shrimp

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P-068.

CQSIRT1 from red claw crayfish *Cherax quadricarinatus* promotes white spot syndrome virus infection VIA positively regulating PI3K-AKT-mTOR pathway

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Abstract
Sirtuins (SIRT) are a family of evolutionarily conserved nicotinamide adenine dinucleotide (NAD+)-dependent deacylases that participate in antiviral immunity. In this report, the *SIRT1* gene (named as *CqSIRT1*) was identified with an open reading frame of 2256 bp that encoding 751 amino acids from red claw crayfish *Cherax quadricarinatus.* Tissue distribution analysis showed that *CqSIRT1* was ubiquitously expressed in all tissue tested with high expression in haematopoietic tissue (Hpt), haemocyte and gill, while low expression in hepatopancreas, musle and eyestalk. Unexpectedly, dysfunction of *CqSIRT1* by gene knockdown in red claw crayfish Hpt cell cultures resulted in markedly decrease expression of an early gene *ie1* and an envelope protein gene *vp28* of white spot syndrome virus (WSSV) at late stages post WSSV infection, indicating that CqSIRT1 was hijacked by WSSV to promote its replication. Importantly, the expression of *PI3K*, *AKT* and *mTOR,* which were involved in energy metabolism and autophagy, was significantly reduced after gene silencing of *CqSIRT1* during WSSV infection. Overall, these data suggest that CqSIRT1 could promote WSSV replication through positively modulating PI3K-AKT-mTOR signaling pathway in red claw crayfish *C. quadricarinatus*, which benefits further understanding of the molecular mechanism underlying the pathogenesis of WSSV in crustacean.

keywords: Sirtuins 1(SIRT1); PI3K-AKT-mTOR; White spot syndrome virus (WSSV); Haematopoietic tissue (Hpt); *Cherax quadricarinatus*.

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P-069.

The role of CD8α in the immune response of sea bass against nodavirus

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Abstract
The adaptive immune response is mediated by different mechanisms including humoral factors, cellular elements (T and B lymphocytes) and other specific proteins as major histocompatibility complex which act in coordination with diversified antigen receptors. Regarding cytotoxic T cells (CTLs) of vertebrates, they are direct effector lymphocytes in the fight against virus-infected cells by killing their cellular targets. The cluster of differentiation 8α (CD8α) is the distinctive marker of the lymphocyte subset of cytotoxic CTLs. However, teleost CTLs function is largely unknown mainly because of the lack of population-specific antibodies. European sea bass (*Dicentrarchus labrax*) is a very susceptible species to nodavirus (NNV) in which causes devastating mortality rates and, up to date, no solutions are available to prevent them. Previous studies pointed to the relevance of cell-mediated cytotoxicity in the response of European sea bass juveniles against NNV at transcriptional level. Thus, we have investigated the distribution, production and potential role on European sea bass CD8α+ cells by flow cytometry using a polyclonal antibody against European sea bass CD8α in naïve and NNV-infected cells. This work represents the first attempt to characterize CD8α+ cells in European sea bass.

Work partly funded by projects from MINECO and FEDER (AGL2013-43588-P and AGL2016-74866-C3-1-R), *Instituto Español de Oceanografía* (NODAMED), *Fundación Séneca* (*Grupo de Excelencia de la Región de Murcia* 19883/GERM/15) and National Commission for Scientific & Technological Research Chile (FONDECYT Nº 1140797).
keywords: *Dicentrarchus labrax*, nodavirus, CD8α, polyclonal antibodies.

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P-070.

Study of the immunomodulatory and antiviral activity of bacterial lipopolysaccharides in seabass (*Dicentrarchus labrax*, Linnaeus 1758)

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Abstract
Diseases are the main cause of economic losses in the aquaculture sector that's why researching and improving this immunomodulation technique is fundamental. In this research work, the immunomodulatory effect of the lipopolysaccharides (LPS) of *Vibrio alginolyticus* was evaluated in a species of commercial interest, the seabass, through the cytokines, one kind of humoral components. Three groups of fish were evaluated, one unstimulated control, another positive stimulated with Poly I: C and another stimulated with LPS. The results showed statistically significant differences between the stimulants, where it was observed that the expression of the inflammatory (IL-1β, IL-6 and TNFα) and anti-inflammatory (IL-10) cytokines was directly related. The antiviral response (Mx) was different between the treatments and it was seen that with LPS the stimulating effect was more prolonged in time, although weaker.
keywords: Immunomodulation, cytokines, seabass, immunostimulants and lipopolysaccharides

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P-071.

Circadian rhythmic expression of TNF-α gene regulated by clock gene in the Japanese medaka (*Oryzias latipes*)

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Abstract
To date, little information is available on the effects of circadian oscillation on immune regulation in lower vertebrates, such as teleost fish. In the present study, regulation of circadian rhythmic expression of inflammatory cytokine: TNF-α gene by clock genes (Bmal1 and Clock1) was investigated using Japanese medaka (*Oryzias latipes*). Firstly, structural analysis of clock gene was performed, which revealed that medaka Bmal1 and Clock1 conserve functionally important domains, such as basic helix-loop-helix (bHLH) and period-aryl hydrocarbon receptor nuclear translocator-single-minded (PAS), seen in their counterparts in other vertebrates. Expression of medaka Bmal1, Clock1 and Per1 genes was confirmed in central and peripheral tissues. Moreover, the expression of these clock genes and TNF-α genes in medaka acclimated to a 12:12 light (L) - dark (D) cycle showed circadian rhythm. In addition, higher expression of TNF-α gene was detected in medaka embryo cells (Ol-Hdr R-e3) overexpressing Bmal1 and Clock1 genes. It was suggested that this increase was mediated by transcriptional regulation by clock proteins, which target E-box sequence in the cis-element of TNF-α gene as was detected by luciferase reporter gene assay. Moreover, in vitro head kidney stimulation with LPS at different zeitgeber time (ZT) under LD12:12 condition affected the degree of TNF-α gene expression, which shows high and low responsiveness to LPS stimulation at ZT18 and ZT10, respectively. These results suggested that medaka TNF-α exhibited circadian rhythmic expression regulated by clock proteins and its responsiveness against immune-stimulation depends on time zone.
keywords: Medaka, TNF-α, Circadian rhythm, Clock gene, Transcriptional regulation

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P-072.

Molecular characterizations and ligand-induced responds of type I interferon receptor (IFNR1) in orange-spotted grouper (*Epinephelus coioides*)

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Abstract
Grouper fishes are known as high economical species in aquaculture industry. However, outbreak of diseases caused chronic death rate of grouper juveniles. To increase survival rate of grouper, mechanism of first line of defense against virus diseases in teleost fish is studied. Previous result indicated up-regulation of gene expression level of grouper IFN and downstream antiviral Mx protein gene in response to NNV virus infection. However, signalling cascade of IFN system is unclear. To investigate relationship between type I 2C-IFN to its receptor (IFNR), full length orange-spotted grouper IFNR1 was firstly cloned and identified. Phylogenetic analysis indicated osgIFNR1 shared high homology similarities with other teleosts. Relative expression of osgIFNR1 was determined using real-time qPCR in regards to interferon treatment using immune organ primary cell culture. Gene expression of osgIFNR1 and downstream osgMx1 which act as marker was seen up-regulated approximately 0.5 and 10-fold respectively in 4 hours post-stimulation indicating involvement of transcriptional level responses. We also observed transcript level of osgIFNR1 in response to immune-stimulant (LPS and Poly I:C) with increased fold of around 2 and 6 times respectively and nodavirus infected larvae of around 0.5-fold downregulation. We further used GF-1 cell line to observe subcellular localization and interaction between ligand and receptor. The present study revealed effect of IFNR1 through up-regulation of gene expression level during 4 hours of interferón (IFN) treatment. This study contributed different insights in analysis of IFNR role in type I interferón system in orange-spotted grouper.
keywords: Orange-spotted grouper, interferon receptor, nervous necrosis virus, immune system, molecular cloning

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P-073.

Effects of recombinant IL-4/13A on the phagocytic capacity of salmonid leukocytes

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Abstract
The professional phagocytes described on teleost fish are macrophages, neutrophils and dendritic cells, and B lymphocytes. In mammals, several cytokines have effects on the phagocytic capacity of these cells, for example, IL-4 induces increase of phagocytosis by macrophages, as well as increase of reactive oxygen species production. In fish, it has been reported that IL-4/13 increases phagocytic capacity in Japanese pufferfish and Grass carp, however the function of this cytokine in salmonids has not been studied. In this work we evaluated the effect of recombinant IL-4/13A on the phagocytic capacity and ROS production of SHK-1 and