

spleen. In addition, vaccinated fish showed PCNA +/IgM + and PCNA+/IgT+ B cells in the mass of vaccine and cells (CVM) associated with the spleen, which may indicate an importance of the CVM during vaccination, which goes beyond a mere place of phagocytosis and exchange of material. This work was funded by EU H2020 program through ParaFishControl Project (634429), by the Ministerio de Economía y Competitividad (Spain) and FEDER (European Union) (AGL2017-83577-R) and by grant ED431C2017/31 from the Xunta de Galicia. I.E. was contracted under APOSTD/2016/037 grant by the “Generalitat Valenciana”, and F.F. was contracted by the Xunta de Galicia.

**keywords:** Turbot, Spleen, Vaccination, B lymphocytes, Immunoglobulins

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### P-032.

#### Field validation of immunotoxic responses in the carpet shell clam (*Ruditapes decussatus*) from contaminated sites in the South Lagoon of Tunis (Tunisia)

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#### Abstract

The aim of this study was to validate immunological alterations as ecotoxicological biomarkers to detect and monitor the biological effects of anthropogenic pollution in the South Lagoon of Tunis (Tunisia). Carpet shell clams (*Ruditapes decussatus*) were collected during summer and winter from four locations: three of them within the polluted lagoon of Tunis (S1, S2 and S3) and from a clean site on the Mediterranean coast (Louza, Tunisia). To study the immunity of clams, the phenoloxidase, lysozyme, alkaline phosphatase, esterase, peroxidase, protease, anti-protease and bactericidal activities were measured in the haemolymph. Phenoloxidase activity was significantly lower in clams sampled from the three contaminated areas of the lagoon (S1, S2 and S3) than in those from the control point in both summer and winter. Lysozyme, esterase, protease and anti-protease activities were higher in the clams from site S3 than in the clams collected from the control site during winter. No significant variations were detected in the alkaline phosphatase, peroxidase and bactericidal activities of the clams collected from the four experimental sites. A significant seasonal effect was observed in clam immune status in winter. The results clearly showed that the affected biomarkers (mainly phenoloxidase) could be useful tools for biomonitoring clams in the study area.

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**keywords:** Biomarkers; Biomonitoring; Seasonality; Innate immunity; carpet shell clam (*Ruditapes decussatus*).

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### P-033.

#### Hepcidin, an antimicrobial and iron-regulated peptide that provides an ability to prevent bacterial diseases in grass carp (*Ctenopharyngodon idella*).

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#### Abstract

Hepcidin is an antimicrobial peptide and a regulator of iron homeostasis which has three isoforms: -20, -22 and -25. While hepcidin-25 has been studied extensively, the physiological significance of other isoforms remains poorly understood. Herein, we focused on the analysis of the differences in antibacterial and iron regulatory functions of hepcidin-20 and hepcidin-25, looking for a derivative of hepcidin as a preventive drug for bacterial diseases. In this study, we examined the antimicrobial potentials of the two hepcidins in the form of synthesized peptides, hepcidin-25 and hepcidin-20. We found that hepcidin-25 and hepcidin-20 exhibited apparent bactericidal activities against both Gram-positive and Gram-negative bacteria in a dose-dependent manner. *In vitro*, the hepcidin-20 had better antibacterial activity than the hepcidin-25. However, the antimicrobial activity on the cellular level has the opposite effect. We suspected that the iron-regulating function of hepcidin limits the available iron content of extracellular bacteria to enhance its bactericidal activity. Further tests indicated that only hepcidin-25 can block iron release from liver cell line L8824 via internalization and degradation of cellular iron exporter ferroportin, and restrain the use of iron in extracellular bacteria. This result also confirms our hypothesis. *In vivo*, recombinant *Ctenopharyngodon idella* hepcidin improved the survival rate of *C. idella* challenged with *Flavobacterium columnare*. In addition, the fish fed diet containing recombinant *C. idella* hepcidin had a higher survival rate than other pretreatment groups. The study showed that recombinant *C. idella* hepcidin regulated iron metabolism, causing iron redistribution, decreasing serum iron levels and increasing iron accumulation in the hepatopancreas. Immune-related genes were also evaluated, showing higher expression in the groups pretreated with recombinant *C. idella* hepcidin at an early stage of infection. In general, *C. idella* hepcidin not only has a direct killing effect on bacteria, but also reduces the available iron content of bacteria to inhibit bacterial growth. Our findings revealed a new role for hepcidin in fighting against bacterial infections and indicate a potential in controlling the bacterial infection in aquaculture.

**keywords:** *Ctenopharyngodon idella*; hepcidin; antibacterial;

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### P-034.

#### A novel *CQTRIM32* from red claw crayfish *Cherax quadricarinatus* inhibits white spot syndrome virus infection

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#### Abstract

Tripartite motif-containing (TRIM) proteins are highly conserved molecules that participate in a variety of biological processes such as regulation of development, apoptosis, and innate immunity in vertebrates. In this report, we identified a *TRIM32* homolog (named *CqTRIM32*) in red claw

crayfish *Cherax quadricarinatus*. *CqTRIM32* was widely distributed in the tissues selected, with the highest expression in muscle, relatively abundant in haematopoietic tissue (Hpt) and the lowest presence in eyestalk. Multiple amino acid alignment showed that *CqTRIM32* contained a conserved RING-finger domain but without B-BOX domain and coiled-coil region, which was different from the traditional TRIMs family. Interestingly, the expression of *CqTRIM32* was significantly up-regulated at both 24 h and 48 h after white spot syndrome virus (WSSV) challenge *in vivo* in crayfish Hpt tissue. Meanwhile, the expression of *CqTRIM32* was significantly up-regulated at both 12 h and 24 h after WSSV challenge *in vitro* in Hpt cells. The quantity of WSSV was increased in red claw crayfish Hpt cell cultures after gene knockdown of *CqTRIM32* post WSSV infection, in which the transcription of both an immediate early gene *ie1* and a late envelope protein gene *vp28* of WSSV were clearly up-regulated. Taken together, our data provide the first evidence that *CqTRIM32* exerts the antiviral activity in a crustacean.

**keywords:** Tripartite motif-containing (TRIM); Antiviral; White spot syndrome virus (WSSV); Haematopoietic tissue (Hpt); *Cherax quadricarinatus*.

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### P-035.

#### Lipid deposits and foamy macrophage-like cells in focal red and melanised muscle changes in Atlantic salmon (*Salmo salar*)

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#### Abstract

Focal melanised changes or “black spots” in farmed Atlantic salmon (*Salmo salar*) fillet is a common quality problem seen at slaughter. The changes develop during the seawater phase, starting as acute focal hemorrhages or “red changes” which progress into chronic inflammatory changes with melanisation. Regeneration in most changes remains ongoing without proper healing; a process that has been associated with the chronic persistence and replication of *Piscine orthoreovirus* (PRV).

Another chronically persistent feature in this condition is the histopathological presence of what appears as fat (seen as empty vacuoles) in both focal red and melanised changes. Previous studies have described vacuoles of various sizes assumed to be fat-containing, but as most studies have been carried out on formalin fixed and paraffin-embedded tissues, the content in such vacuoles has diminished during processing and histological investigations of lipids have hitherto been inconclusive.

Here, we use glutaraldehyde-fixed and frozen material, thus preserving the fat. Sections from both acute red and chronic melanised changes were stained with two different special stains (Sudan Black and Oil Red O) for detection of lipids. We show that most vacuoles indeed contain fat and that these are highly prevalent in the acute manifestations in areas of necrosis, haemorrhage and inflammation. We also show fat-containing vacuoles in chronic changes with melanisation, though with a different appearance; often in association with melano-macrophages. In addition, cells though to be foamy macrophages are identified and investigated by transmission electron microscopy. Based on our results, we discuss the potential role of fat in the development of focal melanised changes.

**keywords:** Inflammation; lipids; Macrophage; Melano-macrophage; Myositis

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### P-036.

#### Immune response in turbot exposed to the ciliate parasite *Philasterides dicentrarchi*

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#### Abstract

*Philasterides dicentrarchi* is a marine scuticociliate that causes scuticociliatosis in farmed fish worldwide and is currently considered one of the most important pathogens of cultured flatfish. Although there is abundant information about the infections caused by *P. dicentrarchi* in fish and about how the ciliates and fish immune cells interact *in vitro*, little is known about the interaction between this ciliate and the fish immune system *in vivo*. In the present study, turbot (*Scophthalmus maximus*) were exposed twice to the parasite (on days 1 and 21). Immersion infection was performed by adding ciliates to tanks of seawater (18 °C) to yield a final concentration of 4.5 x 10<sup>4</sup> ciliates/mL. Fish were exposed to the ciliates by immersion in the seawater for 20 min and were then transferred to tanks of clean seawater for 60 days. Control fish were immersed in seawater with no ciliates, and were subjected to the same conditions as the experimental fish. Four fish died of scuticociliatosis during the experiment. Fish (eight per group) were sampled on days 3, 7, 21 after the first exposure to *P. dicentrarchi* and on days 3, 7 and 40 after the second exposure. The presence of ciliates on the skin and gills was evaluated by qPCR. The IgM, IgT and IgD levels were measured in serum on days 3, 7 and 40 and in mucus on day 40 after the second exposure. Changes in gene expression of immunoglobulins, MHCII and other immune-related genes were determined by qPCR, in gills, skin, and spleen at all sampling times. There were no significant differences in serum IgM, IgD and IgT levels between experimental and control groups at any of the sampling times; however, there was a significant increase in mucus IgT levels 40 days after administration of the second exposure. The results of the qPCR analysis showed few changes of the immunoglobulin expression in the analyzed organs and a mild inflammatory response with the current infective dose.

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**keywords:** Turbot, *Philasterides dicentrarchi*, immunoglobulins, immune response, infection

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### P-037.

#### Development of a reverse genetics system for snakehead vesiculovirus

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