

analysis of *Epinephelus coioides* in metamorphosis stages revealed that dissimilarity in distribution of the individuals in 12dph, 18dph and 50dph. Analysis of immune gene (TLR-3, IL-1 $\beta$ , IFN- $\alpha$ , IFN- $\gamma$ , NF- $\kappa$ B and Mx gene) showed the fish were in pathogen-free environment. The Proteobacteria in intestine microbiome is most abundant microbes at each development stages. In the other hand, NNV-, GIV-, and Vibrio- infected fish exhibit different phylum composition of microbes. The relationship of pathogen and microbiome in grouper will be investigated in the future, which might be the solution for the grouper disease control.

**Keywords:** Grouper, intestinal microbiota, development stages, disease, 16S metagenomics

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### O-136.

#### Identification of novel biomarkers associated with infection and chronic stress in Russian sturgeon

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#### Abstract

*Acipenser* spp (sturgeon) are non-teleost fish species of ecological and economic value due to their exquisite meat and caviar. During 20th century, overfishing and pollution have led to a drastic decline of its natural reserves and nowadays many species are listed as critically endangered. Sturgeon aquaculture is thus an important tool for conservation and restoration of sturgeon populations, representing also an activity with valuable socio-economic impact. *A. gueldenstaedtii* (Russian sturgeon), one of the most cultured sturgeon species worldwide, is successfully farmed in Uruguay. However, for achieving a sustainable development, sturgeon aquaculture has to deal with the adaptation of sturgeon to a warmer climate, since increased mortality rates and economic losses are observed during Uruguayan summer. Our previous work demonstrated that warm temperatures ( $\geq 24^\circ\text{C}$ ) alter fish innate defences by a chronic stress-dependent mechanism. Unfortunately, molecular tools for monitoring chronic stress and innate defences are not commercially available for sturgeons. Therefore, identification of biomarkers associated with inflammation and/or chronic heat stress in sturgeon is crucial for improving sturgeon aquaculture. With this aim, we follow two different experimental approaches: i) analysis of sturgeon acute phase proteins as putative biomarkers of inflammation and ii) analysis by RNA sequencing of differentially expressed genes in sturgeons subjected to chronic heat stress and challenged with pathogenic bacteria. Firstly, we identified several putative acute phase proteins in Russian sturgeon: hepcidin, haptoglobin, hemopexin, transferrin, serum amyloid A protein (SAA) and serum amyloid P protein (SAP). Hepatic expression analysis of these proteins in bacteria-challenged sturgeons showed that SAA could be a good inflammation biomarker candidate. We also obtained specific antibodies against SAA and SAP and analysed their serum protein levels by ELISA or Western blot. We found that SAA, but no SAP levels were significantly increased in serum of bacteria-challenged sturgeons, suggesting that SAA is a positive acute phase protein in Russian sturgeon. However, neither SAA nor SAP showed to be good biomarkers of chronic heat stress. Analysis of differentially

expressed genes in liver and spleen of bacteria-infected sturgeons subjected to chronic heat stress are currently being undertaken by Illumina sequencing in order to identify novel inflammation and chronic stress biomarkers. This identification will open new possibilities for monitoring sturgeon health in farms, contributing to less economic impact from disease outbreaks and favouring aquaculture development.

**Keywords:** *Acipenser gueldenstaedtii*, infection and chronic-stress biomarkers

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### O-137.

#### A lectin with LDL receptor domain suppress the replication of WSSV

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#### Abstract

In invertebrates, C-type lectins (CTLs) play important roles in innate immunity against pathogens. Previous studies have focused mainly on the functions of CRD of CTLs in immune recognition, activation, and regulation. Conversely, limited research has been conducted on the relationship of CTLs and low-density lipoprotein (LDL). In this work, a lectin with LDL receptor domain was identified in *Procambarus clarkii* and designated as CTL-R. CTL-R was widely distributed in all tested tissues, and it was induced expression after white spot syndrome virus (WSSV) challenge. The recombinant CTL-R enhanced the anti-virus action of crayfish, whereas the anti-virus action was inhibited after CTL-R knockdown. The expression level of AMPs were suppress after CTL-R knockdown in crayfish. Furthermore, CTL-R protein induce the activity of dorsal. NF- $\kappa$ B is associated with anti-virus by regulating the expression of AMPs. Therefore CTL-R plays a key role in immune response of WSSV by positive regulation the activity of dorsal.

**Keywords:** Lectin; White spot syndrome virus; NF- $\kappa$ B; *Procambarus clarkii*; Innate immunity

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### O-138.

#### A comparative study on intestinal immune cells in two elasmobranchs species

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#### Abstract

The immune system of fish is of great relevance as it provides information on the evolution of immunity in vertebrates. All sharks, skates and rays are cartilaginous fish and together form a group called the elasmobranchs.

Elasmobranchs have a low incidence of disease and their immune cells have been identified as possible sources of novel tumour cell inhibitors. The basic anatomical structure of the elasmobranch gut is similar to that of other vertebrates, with a striking exception of the presence of a spiral intestine that provides an enlarged surface area for digestion and absorption of food by means of spiral folds. During recent years, considerable number of studies have described the immune system of teleosts, whereas little effort has been directed towards studying immunity in elasmobranchs. The lack of knowledge on immune cells in the intestine of elasmobranchs prompted us to carry out present study. During 2018, 13 blackmouth catshark *Galeus melastomus* and 15 specimens of the thornback ray *Raja clavata* were provided by a trawl fleet from the Gulf of Asinara (Sardinia, western Mediterranean Sea). Histochemical, immunohistochemical and ultrastructural observations were conducted on a subsample of spiral intestine of these fish species. Regarding *R. clavata*, three types of granular cells were identified; type I in epithelium, types II and III in lamina propria-submucosa, with each of them containing cytoplasmic granules with distinct ultrastructural characteristics. Immunostaining of intestinal sections showed the reactivity of granular cells: type I cells were positive for lysozyme, mast cell tryptase and tumor necrosis factor- $\alpha$  (TNF- $\alpha$  based on antibody staining; type III cells were immune-reactive to anti-interleukin 6 (IL-6) antibody, whilst type II cells were negative to all the antibodies used. Additionally, in *G. melastomus*, our studies identified unique, large granular cell type in intestinal epithelium. Electron microscopy showed that the epithelial granular cell type made intimate contact, by means of junctional complexes, with adjacent epithelial and mucous cells. Histochemical staining showed the cytoplasmic granules to be strongly eosinophilic and stained positively to periodic acid-schiff and Alcian blue. Immunostaining of intestinal sections revealed immunoreactivity of the granular cell to TNF- $\alpha$  antibody. However, no reactivity to inducible-nitric oxide synthesis, IL-6, interleukin IL-1 $\beta$ , lysozyme, serotonin 5-HT antibodies was detected. Comparison of each granular cell type in both elasmobranch species with immune cells of teleosts or mammals and a hypothesis on their nature and function would be described.

**Keywords:** Elasmobranchs; spiral intestine; lysozyme; cytokines; transmission electron microscopy

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## O-139.

### *Liza ramada* innate immunity against intestinal microparasite, *Myxobolus mugchelo* (Myxozoa)

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#### Abstract

Thinlip mullet *Liza ramada* is the most abundant of mullet species that live in the Comacchio lagoons (Northern Adriatic Sea, Italy). Histological and ultrastructural sections of the intestine of *L. ramada* in 30 specimens evaluated, showed that over 80% of specimens were infected with intestinal parasite of mullets, *Myxobolus mugchelo* (Myxozoa). The 'Myxozoa Grassé, 1970', is a phylum composed of over 2400 species. With reference to parasites of mullets, records on myxozoans species encountered in intestine and intestinal mesentery of worldwide mullets revealed that among 12 species of myxosporeans that infect *Liza ramada*, five belong to genus *Myxobolus*. In histological sections of the infected intestine, plasmodia of *M. Mugchelo* appeared rounded to ovoid and spindle shaped. *M. mugchelo* conspicuous plasmodia were encysted often in muscle and submucosa layers. In the muscle layer, there was no intense host inflammatory response. Plasmodia containing mature spores were situated closer to mucosal folds and were surrounded by numerous immune cells, mainly mast cells (MCs). Mature spores were generally oval in shape, they were noticed in paracellular space among the enterocytes or within them. Near the infected epithelial cells, several MCs, rodlet cells and few neutrophils were noticed. Indeed, degranulation of the MCs was very frequent in this region. In intestinal epithelium, some large cells with intracellular spores of *M. mugchelo*, resembling macrophages were documented. Some of these macrophages appeared foamy in aspect and possess elongate striated granules and frequently with enclosed necrotic debris. In some parasitized intestines, sections of epithelium were either completely substituted by spores of *M. mugchelo* or the spores were noticed inside the damaged enterocytes with spores exuded into the intestinal lumen due to destruction of these cells. These findings enabled us to postulate upon the life cycle of *M. mugchelo* and the migration of the mature spores through the intestinal wall to reach the lumen. Immunohistochemical analysis on sections of intestinal tissue of uninfected and infected *L. ramada* revealed positivity of epithelial macrophages to anti-histamine, -leuencephalin or -serotonin antibodies. The macrophages often showed at the plasma membrane and were seen close to the mucous cells and epithelial cells with intracellular spores of *M. mugchelo*. In the areas of epithelium infected with spores, epithelial cells positive to proliferating cell nuclear antigen (PCNA) were also observed. The current study is the first record on occurrence of intraepithelial macrophages that engulfed myxozoan spores.

**Keywords:** Mullet, *Liza ramada*; intestine, microparasite, mast cells, macrophages

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