

**O-054.****Stomach metabolic alterations in response to AHPND infection in shrimp**

Teng-Chun Tung<sup>1, #</sup>, Ramya Kumar<sup>1</sup>, Tze Hann Ng<sup>1</sup>, Che-Chih Chang<sup>1</sup>, Yi-Ming Chen<sup>1</sup>, Shih-Shun Lin<sup>2</sup>, Wen-Chi Chang<sup>3</sup>, Han-Ching Wang<sup>1</sup>.

<sup>1</sup> Department of Biotechnology and Bioindustry Sciences, National Cheng Kung University, Tainan, Taiwan

<sup>2</sup> Institute of Biotechnology, National Taiwan University, Taipei, Taiwan

<sup>3</sup> Institute of Tropical Plants Sciences, National Cheng Kung University, Tainan, Taiwan

**Abstract**

Acute hepatopancreatic necrosis disease (AHPND), previously termed early mortality syndrome, is an emerging shrimp disease with very serious impacts on Asian shrimp aquaculture. This disease is caused by pathogenic bacteria *Vibrio parahaemolyticus* (Vp) containing a unique virulence plasmid (pVA), with genes encoding a binary toxin PirABVp. Molecular pathogenesis of this emerging disease and host responses against AHPND remain unclear. To further explore AHPND pathogenesis, a systems biology approach was used to identify the significant pathway[s] in transcriptome and metabolome of AHPND-infected shrimp stomach. Using UHPLC-QTOF-MS-based metabolomics profiling, 503 and 634 differentially expressed metabolites were selected from positive and negative ion modes. These metabolites were combined with our in-house transcriptome database to obtain global host responses in stomachs of AHPND-infected shrimp. With this strategy, it was determined that several lipid metabolism related pathways in shrimp stomachs were dysregulated during AHPND infection. A gene-to-gene correlation network was created to identify candidate genes, with gene expression subsequently confirmed with real-time PCR. Cytosolic phospholipase A2 (cPLA2) and JHE-like carboxylesterase (JHE-LCE) were significantly expressed in stomachs of AHPND-infected shrimp. These findings are new knowledge regarding AHPND pathogenesis and will contribute to development of an evidence-based biosecurity approach for shrimp aquaculture industry.

**Keywords:** AHPND, metabolomics, lipid metabolism, Cytosolic phospholipase A2, JHE-like carboxylesterase

# Corresponding author.

E-mail address: [dannyw840805@gmail.com](mailto:dannyw840805@gmail.com) (T.-C. Tung).

**O-055.****Characterization and development of focal red and melanised changes in the Atlantic salmon**

H. Bjørgen<sup>1</sup>, R. Haldorsen<sup>2</sup>, Ø. Oaland<sup>2</sup>, I. Hordvik<sup>3</sup>, E. Rimstad<sup>4</sup>, E.O. Koppang<sup>1, #</sup>.

<sup>1</sup> Section of Anatomy and Pathology, Veterinary Faculty, The Norwegian University of Life Sciences, Oslo, Norway

<sup>2</sup> Mowi ASA, Sandviksboder 77AB, Bergen, Norway

<sup>3</sup> Institute of Biology, University of Bergen, Bergen, Norway

<sup>4</sup> Section of Virology, Veterinary Faculty, The Norwegian University of Life Sciences, Oslo, Norway

**Abstract**

The occurrence of melanised changes in the muscle fillets in the Norwegian salmon industry is approximately 20% and leads to quality reduction and severe economic losses. The condition is common for Atlantic salmon farming in general, but prevalence are not available outside Norway. Here, we investigated the occurrence of focal red and melanised changes in a regular field production net-pen population, throughout the saltwater production period. We found that the prevalence of focal acute red changes was stable throughout the production period and that these changes develop into focal melanised changes over time. The presence of bacteria and virus in focal red changes was addressed but could not explain the acute manifestations, however, accumulations of lipids was a common

finding. Lipid depositions were located within degenerative muscle cells. Infection with *Piscine orthoreovirus* 1 (PRV-1), which is ubiquitous in sea farmed Atlantic salmon, appeared in the population at Week 23 post sea transfer. Chronic melanised changes appeared after the identification of PRV-1, and cells with replicating virus was always present in the chronic melanised changes that were characterized as granulomatous inflammation. We further addressed the inflammatory responses by targeting different cell markers.

**Keywords:** Granuloma; inflammation; lipids: melano-macrophage; PRV

# Corresponding author.

E-mail address: [erling.o.koppang@nmbu.no](mailto:erling.o.koppang@nmbu.no) (E.O. Koppang).

**O-056.****Endocrine regulation of the thymic function in European sea bass: Interactions between environmental factors and the reproductive system development**

M. Paiola<sup>1, #</sup>, C. Moreira<sup>1</sup>, A. Duflot<sup>1</sup>, J. Hétru<sup>2</sup>, G. Scapigliati<sup>3</sup>, T. Knigge<sup>1</sup>, T. Monsinjon<sup>1</sup>.

<sup>1</sup> UMR-I 02 INERIS-URCA-ULH SEBIO, Environmental stresses and biomonitoring of aquatic ecosystems, FR CNRS 3730 Scale, Université Le Havre Normandie, F-76063, Le Havre Cedex, France

<sup>2</sup> Adaptive Physiology, Institute of Life, Earth and Environment, University of Namur, Namur, Belgium

<sup>3</sup> Department for Innovation in Biological, Agro-food and Forest Systems, Tuscia University, 01100 Viterbo, Italy

**Abstract**

Jawed vertebrates have a powerful immune system that must be tightly regulated to maintain homeostasis and to ensure trade-offs with other energy-demanding functions, such as reproduction. With respect to the latter, immune system regulation is become apparent by the immunomodulatory function of gonad-derived hormones. In mammals, the thymus, involved in T cell-maturation, is a highly plastic organ, the function of which is drastically modulated by high levels of estrogen and testosterone. In teleost fish, recent findings indicate that the estrogenic regulation may be evolutionary conserved. A knowledge gap exists, however, with regard to the immunomodulatory role of testosterone in teleosts. Moreover, in addition to physiological changes related to reproductive investment, poikilotherm animals living in seasonally changing environments must adapt their immune system to widely varying temperatures. Consequently, to discriminate environmental and endogenous factors that regulate thymus function in European sea bass, *Dicentrarchus labrax*, we analyzed the thymuses of juvenile fish with monthly sampling from 95 to 430 days post-hatching covering the sexual differentiation-period and the first seasonal gonad investment, which were kept separately in either constant conditions, or in conditions mimicking the natural variation of photoperiod and temperature. The thymuses were sampled for qRT-PCR-analysis, thymocytescount, viability, in vitro steroid-responsiveness as well as immunofluorescence measurement of DLT15+ and DLlg3+ cells by flow cytometry. DLlg3 and DLT15 are antisera for sea bass IgM and a Pan-T cell marker. We observed that temperature regulates thymus size without affecting the proportion of DLT15+ and DLlg3+ thymocytes or thymocyte viability. A decrease of the DLT15+ cell-proportion and thymocyte-viability was observed concomitantly with an increase of the gonadosomatic index, but without a significant decrease in the intraperitoneal adiposomatic index. Overall, the results suggest that temperature regulates thymocyte number and is likely to modulate the amount of T cell-egress. However, with reproductive investment and the associated increase of plasmatic steroid hormone levels, thymic T cell proportions as well as viability of immature T cell are modulated. As the adiposomatic index was not altered, this suggests that steroid hormones modulate thymic T cell-maturation and -fate, i.e., apoptosis or differentiation, which are likely to alter the TCR-repertoire of exported mature T cells. Ongoing measurements aim to (1) quantify changes in plasma steroid levels and to (2) determine the effect on thymic T maturation.

**Keywords:** Thymus, T cell, energy trade-off, eco-immunology, reproduction

# Corresponding author.

E-mail address: [mat.paiola@gmail.com](mailto:mat.paiola@gmail.com) (M. Paiola).

#### O-057.

##### **Nodavirus modulates immune-relevant proteins in European sea bass**

Y. Valero<sup>1, #</sup>, M. Arizcun<sup>2</sup>, Jimena Cortés<sup>3</sup>, Fanny Guzmán<sup>4</sup>, Luis Mercado<sup>3</sup>, M. Ángeles Esteban<sup>1</sup>, E. Chaves-Pozo<sup>2</sup>, A. Cuesta<sup>1</sup>.

<sup>1</sup> Fish Innate Immune System Group, Department of Cell Biology and Histology, Faculty of Biology, Regional Campus of International Excellence "Campus Mare Nostrum", University of Murcia, 30100, Murcia, Spain

<sup>2</sup> Oceanographic Center of Murcia, Spanish Institute of Oceanography (IEO), Carretera de la Azohía s/n. 30860, Puerto de Mazarrón, Murcia, Spain

<sup>3</sup> Grupo de Marcadores Inmunológicos, Laboratorio de Genética e Inmunología Molecular, Instituto de Biología, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile

<sup>4</sup> Núcleo Biotecnológico de Curauma (NBC), Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile

#### Abstract

European sea bass (*Dicentrarchus labrax*) is the most important fish species in Spanish aquaculture in terms of biomass (Tm) production. One of the biggest problems facing its farming is its great susceptibility to nodavirus infection, which causes mortality rates up to 100% in larvae and juveniles. The knowledge of immune responses triggered upon nodavirus infection in European sea bass specimens and their regulatory mechanisms is mandatory to maintain the production of this specie. Nodavirus up-regulates the transcription of genes coding for antimicrobial peptides, cellular markers of T and B lymphocytes and pro-inflammatory cytokines whether inhibits those genes related to the interferon type I pathway in the brain. At this point, an insufficient antiviral response at transcriptional level is shown leading to develop the viral disease. In this work, and due to the lack of tools to characterize the leucocytes using specific cell populations markers, we have produced polyclonal antibodies specific to European sea bass antimicrobial peptides (NK-lysin and dicentracin), interferon gamma and perforin to quantify them through larval development and study their regulation in control and nodavirus-infected juveniles. Our results show basal levels of these proteins during the entire larval development from eggs up to 69 days post-fertilization, increasing at two different time points in the case of several proteins. After nodavirus infection, the quantification of most proteins decreased instead of increasing as expected upon activation of an immune response. These data suggest a post-translational modulation of these proteins by the virus since the enhancement of antimicrobial activities was previously demonstrated. Taking into account the lack of preventive or palliative solutions to nodavirus infections, further investigations should be developed to understand how nodavirus evades the immune response to spread.

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 N° 1140797). Y. V. thanks to *Becas Iberoamérica. Santander Investigación 2016-2017* for her postdoctoral fellowship.

**Keywords:** Nodavirus, protein, polyclonal antibodies, immune response, *Dicentrarchus labrax*

# Corresponding author.

E-mail address: [yvalero@um.es](mailto:yvalero@um.es) (Y. Valero).

#### O-058.

##### **Mucosal immunoglobulin IgT plays a key role in the oral immunity of teleosts**

Y.Y. Yu, W.G. Kong, H.Y. Xu, Z.Y. Huang, X.T. Zhang, L.G. Ding, Z. Xu<sup>#</sup>.

Department of Aquatic Animal Medicine, College of Fisheries, Huazhong Agricultural University, Wuhan, Hubei, 430070, China

#### Abstract

The oral gustatory organ of vertebrates is crucial to obtain energy for survival and reproduction, and it is simultaneously exposed to pathogenic organisms. Thus, oral-associated lymphoid tissue (OALT) is considered the first line of immune and by far, it has only been described in terrestrial animals. Since teleost fish represent the most ancient bony vertebrates containing the oral mucosa, we hypothesized that the relationship between the oral gustatory surface and mucosal immunity represents an ancient association. Supporting this hypothesis, we show for the first time that OALT is present in teleost fish and is similar to other mucosa-associated lymphoid tissues (MALTs). Moreover, we discover that the majority of bacterial microbiota in the oral mucosa is coated with IgT and, to a much lesser degree with IgM and IgD. In addition, following parasitic infection, significant specific-IgT immune responses were observed in the oral mucus, while IgM responses were almost exclusively detected in the serum. In contrast, parasite-specific IgD was absent both in oral mucus and serum. Importantly, we detect significant IgT+ B cell proliferative responses in the oral mucosa but not in head kidney and spleen of fish that survived parasitic infection, providing the first demonstration that IgT is the main immunoglobulin player in oral mucosal immunity and that IgT responses are probably induced locally in the oral mucosa. More critically, we reveal that the teleost oral mucosa is a novel and effective site of immunization for the control of aquatic parasitic infection. Overall, our findings further broaden the understanding of oral immunity in not only terrestrial animals but also in early vertebrates.

**Keywords:** Oral immunity, IgT, B cells, Mucosal immune, Rainbow trout (*Oncorhynchus mykiss*)

# Corresponding author.

E-mail address: [zhenxu@mail.hzau.edu.cn](mailto:zhenxu@mail.hzau.edu.cn) (Z. Xu).

#### O-059.

##### **17 $\alpha$ -ethinylestradiol or tamoxifen alters the humoral innate immune function in male gilthead seabream**

Y. Valero<sup>1, #</sup>, A.E. López-Cánovas<sup>2</sup>, M.C. Rodenas<sup>2</sup>, I. Cabas<sup>2</sup>, P. García-Hernández<sup>2</sup>, M. Arizcun<sup>1</sup>, A. García-Ayala<sup>2</sup>, E. Chaves-Pozo<sup>1</sup>.

<sup>1</sup> Oceanographic Center of Murcia, Spanish Institute of Oceanography (IEO), Carretera de la Azohía s/n. 30860, Puerto de Mazarrón, Murcia, Spain

<sup>2</sup> Department of Cell Biology and Histology, Faculty of Biology, University of Murcia, 30100 Murcia, Spain

#### Abstract

The presence of pharmacological compounds in the marine water have increased the concern about their unpredicted effects in aquatic organisms. 17 $\alpha$ -ethinylestradiol (EE2), a potent estrogenic compound, is widely used in oral contraceptive pills treatments and hormonal therapies. Tamoxifen (Tmx), an antagonist or agonist of the estrogen receptor alpha depending on the cell types, is commonly used in breast cancer therapies. Both drugs are present in aquatic environments. The gilthead seabream (*Sparus aurata*) is one of most important species in Mediterranean aquaculture and the effects of these compounds in its physiology are of especial relevance. It is demonstrated that cellular and adaptive humoral immune