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

Corresponding author.
E-mail address: mat.paiola@gmail.com (M. Paiola).

O-057.

Nodavirus modulates immune-relevant proteins in European sea bass

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

[1] 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
[2] Oceanogeaphic Center of Murcia, Spanish Institute of Oceanography (IEO), Carretera de la Azohía s/n. 30860, Puerto de Mazarrón, Murcia, Spain
[3] Grupo de Marcadores Inmunológicos, Laboratorio de Genética e Inmunología Molecular, Instituto de Biología, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile
[4] 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 Nº 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: yuvalero@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[#].

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 (Z. Xu).

O-059.

17α-ethinylestradiol or tamoxifen alters the humoral innate immune function in male gilthead seabream

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

[1] Oceanogeaphic Center of Murcia, Spanish Institute of Oceanography (IEO), Carretera de la Azohía s/n. 30860, Puerto de Mazarrón, Murcia, Spain
[2] 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. 17α-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

responses are altered by both compounds in a manner than depends on the age and the reproductive stage of fish. The innate immune function in fish is the first line of defense against pathogens and it is of great importance in poikilothermic animals. In this work we have studied the effect on different humoral innate immune responses of gilthead seabream upon dietary exposure to EE2 or Tmx at different ages and reproductive stages. Our results show that both compounds modulate the humoral innate immune response and that the exposure animals needed different times to recover control values upon the cease of the treatment depending on fish age, the reproductive stage and the length of the treatments.

Work partly funded by projects from MINECO and FEDER (AGL2014-53167-C3-2-R), *Instituto Español de Oceanografía* (NODAMED) and *Fundación Séneca* (04538/GERM/06). Y. Valero thanks *Spanish Institute of Oceanography* for her predoctoral fellowship.

Keywords: 17α-ehinylestradiol, tamoxifen, innate immune system, *Sparus aurata*, males

Corresponding author.
E-mail address: yuvalero@um.es (Y. Valero).

O-060.

Trypanosome-host interaction revealed through the zebrafish looking glass

S.H. Jacobs [1,#], E. Dóró [1], F. Hammond [1], S. Brugman [1], M. Nguyen-Chi [3], G.F. Wiegertjes [1,2], M. Forlenza [1].

[1] Cell Biology and Immunology Group, Wageningen University, P.O. Box 338, 6700, AH, Wageningen, the Netherlands
[2] Aquaculture and Fisheries Group, Wageningen University, P.O. Box 338, 6700, AH, Wageningen, the Netherlands
[3] Laboratoire de Dynamique des Interactions Membranaires Normales et Pathologiques, Centre National de la Recherche Scientifique Unité Mixte de Recherche 5235, Université Montpellier 2, 34095, Montpellier, France

Abstract

Trypanosoma carassii is an extracellular blood parasite of cyprinid fish phylogenetically closely related to *Trypanosoma brucei*, the causative agent of the sleeping sickness disease in humans and livestock. Motility is crucial for trypanosome pathogenicity, but real-time visualization of parasite movement *in vivo*, in the natural host environment, has not been reported thus far. In this study, we report the establishment of *T. carassii* infection in zebrafish (*Danio rerio*), which allowed us, for the first time in a vertebrate host, to characterize in details the movement of trypanosomes *in vivo*. By combining the transparency of zebrafish larvae with the availability of several transgenic lines marking macrophages, neutrophils, cytokine-expressing leukocytes and endothelial cells, we were able to study in real-time: 1) parasite movement *in vivo*; 2) the kinetics of innate immune responses; and 3) parasite interaction with host (immune) cells. Our results indicate that during *T. carassii* infection of young zebrafish a differential macrophages and neutrophils response is observed. Macrophages responded more prominently than neutrophils by proliferating, and were massively recruited to blood vessels. Macrophages also exhibited heterogeneous morphologies and a strong pro-inflammatory profile. In fact, they were strongly positive for Tnfα and Il-1β and had a morphology characteristic of foamy macrophages. Large foamy macrophages accumulated in the portal vein of highly infected individuals, and were strongly positive for lipid staining, which revealed the abundance of lipid bodies in their cytoplasm. Finally, with respect to parasite movement and interaction with the host, using high-speed videography, we were able to capture novel mechanisms of parasite-host cell interaction, and to follow the onset of anemia, vasodilation and extravasation typical of trypanosome infections. Altogether, this is the first report of an *in vivo* trypanosome infection model in a natural vertebrate host describing both, the pathogen behavior and the host response. Considering that trypanosomes can infect all vertebrates, including humans, livestock and fish, our infection model is a relevant complementary tool to gain more insights in the underlying mechanisms of trypanosome infections.

Keywords: Trypanosome, TNF-α, IL-1B, foamy macrophages, *in vivo*

Corresponding author.
E-mail address: sem.jacobs@wur.nl (S.H. Jacobs).

O-061.

Is pallial mucus involved in oyster defense against the parasite *Bonamia ostreae*?

S. Fernández-Boo [1,4,#], O. Gervais [1], M. Prado-Alvarez [3], B. Chollet [1], S. Claverol [2], C. Lecadet [1], C. Dubreuil [1], I. Arzul [1].

[1] *Institut Français de Recherche pour l'Exploitation de la Mer (IFREMER), Laboratoire de Génétique et Pathologie (LGP)*, Avenue Mus de Loup, 17390, La Tremblade, *France*
[2] *Université de Bordeaux, Centre Génomique Fonctionnelle de Bordeaux, Plateforme Protéome*, F-33000, *Bordeaux*, *France*
[3] *Aquatic Molecular Pathobiology Group. Marine Research Institute (IIM-CSIC)*, Vigo, Spain
[4] *Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR), University of Porto*, Avenida General Norton de Matos, S/N, 4450-208, *Matosinhos*, *Portugal*

Abstract

Bonamia ostreae is an intrahemocyte parasite responsible for severe mortalities in the flat oyster *Ostrea edulis* since 70's. The Pacific oyster *Crassostrea gigas* is considered resistant to the disease and seems to develop mechanisms to avoid the infection. Most of studies carried out on invertebrate immune system focus on the roles of hemolymph, although mucus could also act as a barrier against pathogens. In this study, the effect of mucus from both oyster species, *O. edulis* and *C. gigas*, on *B. ostreae* was investigated *in vitro* using flow cytometry. Results showed an increase in esterase activities and mortality rate of parasites exposed to mucus from both oyster species. Also, the mucus seems to have an effect on the internalization of the parasite inside the hemocytes of *O. edulis*, while parasites non-exposed to mucus were highly internalized, those with mucus exposition present a lower rate of internalization, suggesting some mechanisms to neutralize the parasite activities. In order to better understand the potential role of mucus in the defense of the oyster against parasite *B. ostreae*, liquid chromatography and tandem mass spectrometry were used to describe and compare protein composition between mucus from both oyster species. Whatever the oyster species is, pallial mucus displays a great variety of proteins. More than 1800 proteins were identified in *C. gigas* mucus while 767 proteins were identified in *O. edulis* mucus. This huge difference is derived from the availability of the protein database used, *C. gigas* proteome was used to identified the proteins and some mismatch in *O. edulis* could be derived from the lack of a proper genome reference. However, *C. gigas* mucus showed more proteins related to antiviral replication such as ferritin and Ras-related protein Rab7a, as well as, antioxidant proteins such as superoxide dismutase; which is 100 times more expressed in *C. gigas* than in *O edulis*. Conversely, more protease activity proteins such as Cethepsin β and Z and apoptotic related proteins like Ubiquitin conjugatin protein enzyme E2-N were identified in mucus from the flat oyster. Our results suggest an adaptation of oysters to develop a specific response against their specific pathogens; while *C. gigas* is more susceptible to herpes virus (OsHV-1) and bacterial infection (*Vibrio aesturianus*); *O. edulis* oyster is more susceptible to protozoan parasites (*B. ostreae* and *Marteilia refringens*). These results also provide new insights for further investigations in the immune response in oysters.

Keywords: *Bonamia ostreae*, oysters, flow cytometry, immune response, proteome

Corresponding author.
E-mail address: sboo@ciimar.up.pt (S. Fernández-Boo).