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

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O-060.

Trypanosome-host interaction revealed through the zebrafish looking glass

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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*

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O-061.

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

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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

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