European sea bass (*D. labrax*) gill response to *Amyloodinium ocellatum* infection: an immunohistochemical study

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The present study aims to characterize and describe some cellular components and mediators recruited or activated in the gills during the sea bass response to the infection by the parasitic dinoflagellate *A. ocellatum*. For this purpose, gill samples were collected from naturally infected farmed sea bass and experimentally infected sea bass in aquarium facilities (Univ. of Udine). Tissues were immediately fixed in Bouin’s solution, processed by routine histology methods, then 4 µm sections addressed to histological (H.E) and immunohistochemical evaluation. Immunohistochemical labelling was performed using an HRP-based anti-rabbit or anti-mouse kit (EnVisionTM FLEX, Dako) and DAB as chromogen. The procedure included a preliminary antigen retrieval treatment (High pH or Low pH solutions, Dako) and the use of a panel of mono- or polyclonal antibodies specific for the following antigens: Inducible Nitric Oxide Synthase (iNOS) (RB-1605, Thermo Scientific); Cytochrome P450 CYP1A (CO-226, Biosense Laboratories); GM-CSFRα (sc-690, Santa Cruz Biotech); sea bass IgM (rabbit polyclonal, Univ. of Trieste, Italy); CD35 (N-19, sc-7640, Santa Cruz Biotech.); CD16 (H-80, sc-20627, Santa Cruz Biotech.); TNF-alpha (ab6671, Abcam); TLR4 (76B357, Imgenex), TLR2 (ab1655, Abcam).

These markers were selected based on their relevance as indicators of tissue reactivity in terms of inflammatory/proliferative response and their previously assessed reactivity on sea bass tissues. The parasitic infections in sea bass were confirmed by the detection of trophonts attached to the secondary lamellae by the stomopode, induced the modification of gills architecture. Specific signs were the hyperplasia of the epithelial cells and the partial fusion of the secondary lamellae. Hyperplastic lamellae revealed the presence of cell populations positive to iNOS, CYP1A, GM-CSFRα, CD16, CD35, IgM, TNF-alpha, TLR4, and TLR2. The localization of the immunolabelling will be described in order to provide information on cell populations, cell receptors and chemical mediators potentially involved in the host response to *A. ocellatum*.