

Third Call for STSM-Applications

COST Action FA1205: AQUAGAMETE - Assessing and improving the quality of aquatic animal gametes to enhance aquatic resources. The need to harmonize and standardize evolving methodologies, and improve transfer from academia to industry

Name: Sonia Martínez-Páramo

STSM Reference: ECOST-STSM-FA1205-110514-043031

Title: Separation and identification of gilthead seabream and European seabass sperm proteins by western blot analyses.

Dates: from 23-05-2014 to 31-05-2014

Location: University of Salento, Lecce, Italy

Host: Dr Sebastiano Vilella. University of Salento. (sebastiano.vilella@unile.it)

STSM report:

The STSM was carried out at the Department of Biological and Environmental Sciences and Technology, at the University of Salento (Lecce, Italy), to work with the Dr Sebastiano Vilella's research team. The objective of this STSM was to optimize the western blot protocol for the identification of the antioxidant enzymes glutathione peroxidase (GPx), glutathione reductase (GSR) and superoxide dismutase (SOD) on sperm from European seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*). During this STSM several western blot trials were performed with seabass and seabream sperm protein extracts to determine the optimal conditions to identify the target proteins using the anti-GPx, anti-GSR and anti-SOD antibodies. The results obtained allowed optimizing the western blot protocol to identify specifically GPx, GSR and SOD in seabass sperm (Figure 1). However, in the seabream samples it was observed a high unspecific response of the antibodies. During the STSM I learned the basis of the western blot needed to continue working on this technique. Further trials to optimize the protocol in seabream sperm will be performed with the support of the Professor Vilella's team as consultants. This technique will be used to quantify GPx, GSR and SOD in fresh and cryopreserved sperm from European sea bass and gilthead seabream fed antioxidant supplemented diets. It was demonstrated that antioxidant supplemented diets improved sperm quality before and after cryopreservation, increasing total antioxidants in blood plasma, improving sperm viability and diminishing lipid peroxidation in these species (Martínez-Páramo et al., 2013). Now, we will analyse the effect of these diets on the antioxidant enzymes by western blot using the technique learned during this STSM. The results obtained, together with the previous results obtained from this experiment will provide a complete description of the effects of the antioxidant supplemented diets on the sperm quality of two commercially valuable species, gilthead seabream and European sea bass. These results will allow articulate knowledge within the main work groups of the AQUAGAMETE Cost Action, providing new data about oxidative stress and the use of antioxidants (WG3) to improve sperm quality (WG1) and reduce cryodamage (WG2).

The main results will be published in international peer review journals related to the aquaculture industry, physiology of reproduction and cryobiology (Theriogenology, Cryobiology, Aquaculture, and Biology of Reproduction) contributing to the objectives of the AQUAGAMETE COST Action (FA1205).

References:

Martínez-Páramo, S., Dinis, M.T., Soares, F., Pacchiarini, T., Sarasquete, C., Cabrita, E., 2013. A nutritional approach to enhance the antioxidant system of fish teleosts In: Martínez-Páramo, S., Oliveira, C.C.V., Dinis, M.T. (Eds.), 4th International Workshop on the Biology of Fish Gametes. Centre of Marine Sciences (CCMAR), Albufeira, Portugal, pp. 120-121.

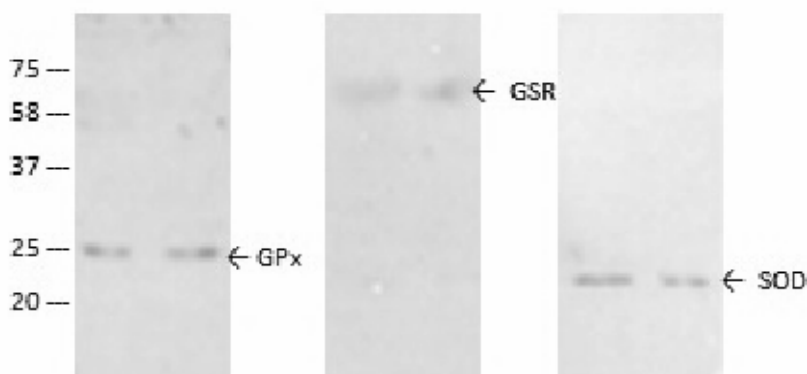


Figure 1- Western blotting with anti-GPx, anti-GSR and anti-SOD antibodies of European seabass sperm proteins separated by separated by SDS-PAGE on 15% polyacrylamide gels and then transferred on nitrocellulose.

Confirmation by the host institution of the successful completion of the STSM

Dr. Sebastiano Vilella (Department of Biological and Environmental Sciences and Technology, University of Salento, Lecce, Italy) certifies that Dr. Sonia Martínez-Páramo (Centre of Marine Sciences, Faro, Portugal) has completed a Short-Term Scientific Mission awarded by the COST Action FA1205 between May 23-31, 2014.

Professor Sebastiano Vilella
(Host)



Sonia Martínez-Páramo, PhD.
(STSM applicant)

