



**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.

Applicant: Martim Magro
STSM title: The use of molecular markers in the analysis of egg quality
Dates: 24<sup>th</sup> February 2015 to 31<sup>st</sup> March 2015
Local: Instituto de Acuicultura de Torre de la Sal, CSIC-IATS, Spain
Host: Dra. Inmaculada Varó, CSIC-IATS, Spain (inma@iats.csic.es)

## Description of the work

During this short stay and under the supervision of Dra. Inmaculada Varó, I analyzed two biomarkers: RNA/DNA related with growth and Hsp70 as stress indicator from *Octopus vulgaris* eggs incubated at two different temperatures. In the first place, I learned how to prepare samples for determination of DNA, RNA and total proteins as well as for Hsp70. RNA and DNA were quantified following the procedure described in Varó et al. (2007), using RiboGreen ™ RNA Quantitation Kit and PicoGreen ™ DNA Quantitation Kit respectively (Molecular Probes). HSP70 were separated by 1D-SDS-PAGE using a Mini-Protean Tetra cell system (Bio-Rad), and transferred onto PVDF membranes in a Trans-Blot<sup>R</sup> Turbo Blotting system (Bio-Rad). Blots were visualized on a VERSADOC Imaging system (Bio-Rad) using ELC-PRIME reagent (Amersham), and quantified by densitometry.

The results obtained confirm the hypothesis that at lower temperatures the embryonic development is longer and the embryos are bigger. Although, differences between temperatures are not significantly. It is highlight the variability in the egg size coming from the same brood.

The knowledge and results acquired in CSIC-IATS will contribute for my master thesis. The skills I gained with this STSM are in accordance with the objective of the WG1: development of new protocols for field, lab and industry applications. The Sixth Call for STSM (Short-Term Scientific Mission)







Figure 1 – Main Laboratory; Figure 2 – Preparing samples; Figure 3 – Using a black multiwell plate for DNA analyses; Figure 4 – Reading DNA flourescence using a spectophotometer; Figure 5 – Electrophoresis (Western blot); Figure 6 – Membrane transference (Western blot); Figures 7 and 8 – Doctor Inmaculada Varó and Martim Magro.