



STSM REPORT

COST-STSM-FA1205-24056

Description of the work carried out during the STSM

The optimal hormonal treatment was fixed in previous studies. We studied the evolution of the sperm quality through the spermiation period, trying to determine the weeks with the highest percentage of samples with high quality sperm, so a protocol could be design.

Sperm collection

The first sperm samples were collected 24 h after hormone injection, from the 10th week of treatment, by making an abdominal massage. In order to proceed to sperm collection, the fish were anesthetized with benzocaine (60 ppm), after cleaning the genital area with distillated water, to avoid the contamination of the samples (feces, urine, or sea water) the eel was dried and sperm was collected, in one plastic tube for each eel. Sperm samples will be collected from the 10th week on. The sperm samples will be maintained in plastic tubes at 4 °C during 1 to 2 hours before the analyses.

Quality analysis of fresh sperm samples

Sperm motility was determined by CASA (ISAS, Proiser, Spain; as described by Gallego *et al.* 2013). The analyses of the sperm parameters were started by activating the sample with 1μ L of sperm and 200 μ L of artificial sea water (Aqua Medic Meersalz, 37 g/L, with 2% BSA [wt/vol] with pH adjusted to 8.2 (Peñaranda *et al.* 2010). All motility analyses was performed in triplicate using the motility module of ISAS v1 and an ISAS 782M camera recorder capturing 60 frames per second (fps). Minimum 400 to 700 spermatozoa were captured in each field, by adjusting the brightness and contrast in the CASA settings. Range size particles was defined between 2-20 μ m in the CASA settings. The software provided analysis of curvilinear velocity (VCL), rectilinear velocity (VSL), angular velocity (VAP) and flagellar beat frequency (BCF). Spermatozoa was considered immotile if their VCL will be <10 μ m/s.

Description of the main results obtained

At this point of the experiment results were not achieved.



Future collaboration with host institution

Since the aim of the study is to standardize a cryopreserving protocol for European eel, by standardizing the best cryoprotectant, volumes, freezing media, grade for dilution, temperature of frost and thawing of the samples, it would be an asset to continue the research project with the group.

Since I also will applied to the 6th STSM call of AQUAGAMETE in order to continue in the research project.

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

Dr. Juan F. Asturiano, as responsible researcher at the Instituto de Ciencia y Tecnología Animal (UPV) certifies that Ana Leonardo visited us as a guest researcher from August 28th to October 31th, thanks to a Short Term Scientific Mission funded by AQUAGAMETE COST Action FA1205.

Valencia (Spain), 16th April, 2015

Dr. Juan F. Asturiano

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