Centro de
Ciências do Mar

STSM Scientific Report

The purpose of the STSM was to continue some joint experiments developed in May-June 2014 regarding the detection of the testis expression of PLCZ1 gene in the testis by means of in situ hybridization techniques.

Task 1. Testis sampling and PLCZ1 gene cloning

Before the travel to CCMAR, 2 male eels were sacrificed, and testis samples were fixed in 4% PFA and preserved at 4 ºC. Once in the CCMAR facilities the samples were included in paraffin with the cautions for this type of studies. From the European eel *plcz1* sequences (Genbank JX868847.1 (*Anguilla anguilla* phospholipase C zeta 1; plcz1, by Nourizadeh-Lillabadi, R., Asturiano, J.F., Pérez, L., Weltzien, F-A.), specific primers were designed to obtain a 500 bp gene fragment to be cloned in a plasmid.

Task 2. Slide preparation and in situ hybridization

Paraffin sections of 6 µm were prepared with the cautions for this type of studies. To produce and label the target RNA we used the "DIG RNA Labeling Kit (SP6/T7)" (Roche), following the instructions of the fabricant. Restriction enzymes were used in order to cut the target fragment from the cloned DNA. The protocol for in situ hybridization was described by Pachiarini et al. (2013).

Moreover, one experiment testing different cryoprotectants for zebrafish (*Danio rerio*) sperm was carried out. Post-thawing viability was determined using live/dead fluorescent staining as well as CASA evaluation.

First experiments are related with *WG3. Basic and applied research on gametes biochemistry and physiology, including omics,* while second type of experiment is related with *WG2. Gametes storage and preservation.*

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

Professor Elsa Cabrita, as responsible researcher at the Aquaculture Research Group (Aquagroup), at the Centre of Marine Sciences (CCMAR) certifies that Dr. Juan F. Asturiano visited us as a guest researcher from 21th September to 4th October, thanks to a Short Term Scientific Mission funded by AQUAGAMETE COST Action FA1205.

Professor Elsa Cabrita

Dr. Luz Pérez

Faro, 4th October, 2014

Universidade do Algarve - Campus de Gambelas - 8005-139 - FARO - Portugal
+ 351 289 800 051 + 351 289 800 900 ext. 7374 ccmar@ualg.pt http://ccmar.ualg.pt