

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:** Zoran Marinović

**STSM Reference:** ECOST-STSM-FA1205-010615-063104

**Dates:** 01.06.2015. – 31.07.2015.

**Location:** Department of Aquaculture, Szent István University, Gödöllő, Hungary

**Host:** Dr Ákos Horváth

### **Purpose of the STSM**

This STSM was carried out at the Department of Aquaculture, Szent István University, Gödöllő, Hungary under the supervision of Dr Ákos Horváth, with the main objective of developing techniques for cryopreservation of the whole testicular tissue, as well as isolated testicular cells.

### **Description of the work**

The main focus of the present STSM was to develop optimal cryopreservation techniques for whole testicular tissue and isolated cells in different Central European fish species. Experiments were conducted on tench *Tinca tinca*, goldfish *Carassius auratus*, common carp *Cyprinus carpio* and brown trout *Salmo trutta*.

For cryopreservation of whole tissue, excised testes were cut into smaller pieces, equilibrated in cryoprotective media and then frozen in 1.8-ml cryovials. For the isolated cells, testes were firstly digested using 0.3 – 0.5% trypsin. After digestion, the suspension was filtered, centrifugated, and pelleted cells were resuspended in extender solution (PBS + 50 mM glucose + 0.5% BSA in most cases). Additionally, in common carp, Percoll gradient centrifugation was employed in order to separate germ cells from spermatozoa. Viability of cells following digestion was > 99%. Single cell suspension was then equilibrated in cryoprotective media, and frozen in 1.8-ml cryovials. As cryoprotectants we used methanol, DMSO, ethylene glycol and glycerol. We also tested different concentrations of these cryoprotectants (1, 2 and 3 M). Cryopreservation was conducted in a controlled-rate freezer with cooling rates of 1 °C/min. Thawing was conducted in a 38 °C water bath for 40 s. During these trials I learned basics of cryopreservation of testicular tissue and isolated cells, operating with a controlled-rate freezer and Percoll gradient centrifugation.

The main objective of this STSM fits into the working group 2 objectives of the Aquagamete action. Obtained results will be presented at the 5th International Workshop on the Biology of Fish Gametes and published later in a peer-reviewed journal.

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

Dr. Ákos Horváth (SZIE, Hungary) certifies that Zoran Marinović (UNS, Serbia) has completed a Short-Term Scientific Mission (STSM) awarded by the COST Action FA1205 AQUAGAMETE during the period 01.06.2015. – 31.07.2015.



AQUAGAMETE

Dr Ákos Horváth  
(SZIE-Host institution)



AQUAGAMETE

Zoran Marinović  
(STSM applicant)