

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:** Elsa Cabrita

**STSM Reference:** COST-STSM-ECOST-STSM-FA1205-060415-055504

**Title:** Cryopreservation of grayling sperm for conservation purposes

**Dates:** from 06-04-2015 to 12-04-2015

**Location:** Angling association of Tolmin, Slovenia

**Host:** Dr Dušan Jesenšek

### **Purpose of the STSM**

This STSM was carried out at the Angling association of Tolmin under the supervision of Dr Dušan Jesenšek, with the main objective to learn the techniques used in the cryopreservation of Adriatic grayling (*Thymallus thymallus*) sperm for the conservation of this species.

### **Description of the work**

During this STSM I join the conservation work carried out by the Angling club of Tolmin since 2009. The Adriatic grayling is a phylogenetically distinct lineage of the European grayling with both phenotypical and genetic differences from other grayling populations. Genetic integrity of this lineage has been compromised by the introduction of an allochthonous lineage of grayling from the Sava river. This has led to hybridization and introgression of the two lineages. Currently, only hybrids of varying degree of the original Adriatic genotype can be found in the local rivers. Sperm was collected from wild males at the spawning sites, after electrofishing, and cryopreserved. In addition to the conservation work, we carried out experiments on the fertilizing capacity of cryopreserved grayling sperm. Post-thaw storage time as well as sperm-egg ratio have previously been optimized for this species. However several protocols for sperm cryopreservation are found in the literature and need to be standardized. Therefore this year we tested the effect of several protocols for the cryopreservation of grayling sperm. The main objective of this STSM fit into the working group 2 objectives of the Aquagamete action.

### **Future collaboration with host institution**

Future collaborations will be established between the Dr Dušan Jesenšek and our group. We collected sperm samples for DNA analysis to test the effect of any damage produced by cryopreservation. This information will help in the management of stocks produced with cryopreserved sperm and will contribute to one of the goals of our action.

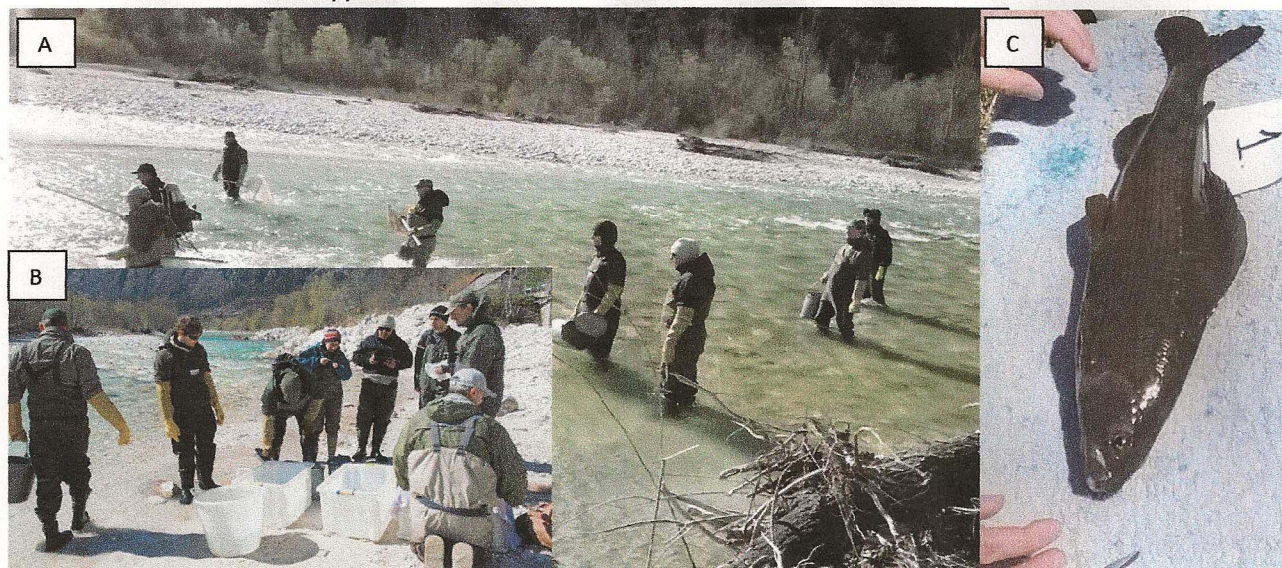


Figure 1- The team involved in the experiments: A-First day electrofishing to catch wild grayling (C). B-Preparation for *in situ* extraction of sperm.

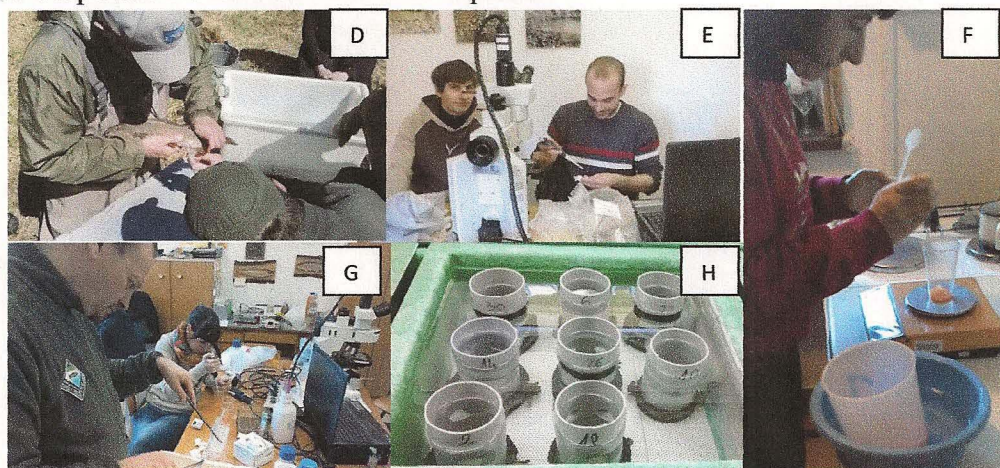




Figure 2- Sperm collection in the field (D). Motility analysis (E); Preparing oocytes for fertilization (F); Thawing sperm (G); Incubation of trials (H). All the team was involved in the work.

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

Dr. Dušan Jesenšek (Angling association of Tolmin) certifies that Dr. Elsa Cabrita (CCMAR\UA\lg) has completed a Short-Term Scientific Mission awarded by the COST Action FA1205 between April 6-12, 2015.

  
Dušan Jesenšek  
(Angling association of Tolmin -Host institution)

  
Elsa Cabrita  
(STSM applicant)