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: Paulo Gavaia
STSM Reference: ECOST-STSM-FA1205-060415-055499-55499
Title: Improvements on the techniques of larval quality evaluation in grayling (*Thymallus thymallus*) and huchen (*Hucho hucho*) produced from cryopreserved sperm
Dates: from 06-04-2015 to 12-04-2015
Location: Angling association of Tolmin, Slovenia
Host: Dr Dušan Jesenšek

Purpose of the STSM

The objectives of this STSM were to analyse previous results of larval *Thymallus thymallus* quality evaluation, to implement techniques for skeletal analysis and new production protocols for quality improvement of hatchery produced grayling.

Description of the work

In the course of STSM ECOST-STSM-FA1205-060415-055499-55499 I have actively collaborated in the conservation efforts with the Angling club of Tolmin under coordination of Dr Dušan Jesenšek. The lineage of Adriatic grayling is genetically distinct of other populations of European grayling and its conservation is threatened by the introduction of *T. thymallus* from other rivers that introduced an exogenous genetic pool. In order to reverse the hybridization the Angling club of Tolmin have been collecting and cryopreserving sperm from wild males in the local rivers, that is used for in vitro fertilization after genetic analysis. Hatchery production of larvae and fry resulting from this process has shown some skeletal quality problems that render the fish unfit for reintroduction into the wild. To overcome this problem, a collaboration was established for application of evaluation methods for determining the incidence of skeletal deformities in those fish, for establishing quality criteria. We have established an experimental protocol for larval production with nutritional supplementation in vitamins that will ensure a better skeletogenesis and improve quality of hatchery produced fry to have a more "wild-like" phenotype and maximize survival of introduced *grayling*.
The objective of this STSM fits into the working group 2 objectives of the Aquagamete action.

Future collaboration with host institution

A deeper collaboration will be established with the Angling club of Tolmin to try and overcome the quality problems in the hatchery production of *T. thymallus* fry conditions. The cryopreserved sperm samples were used for *in vitro* fertilization and samples of hatched larvae will be sent to Faro for further analysis.

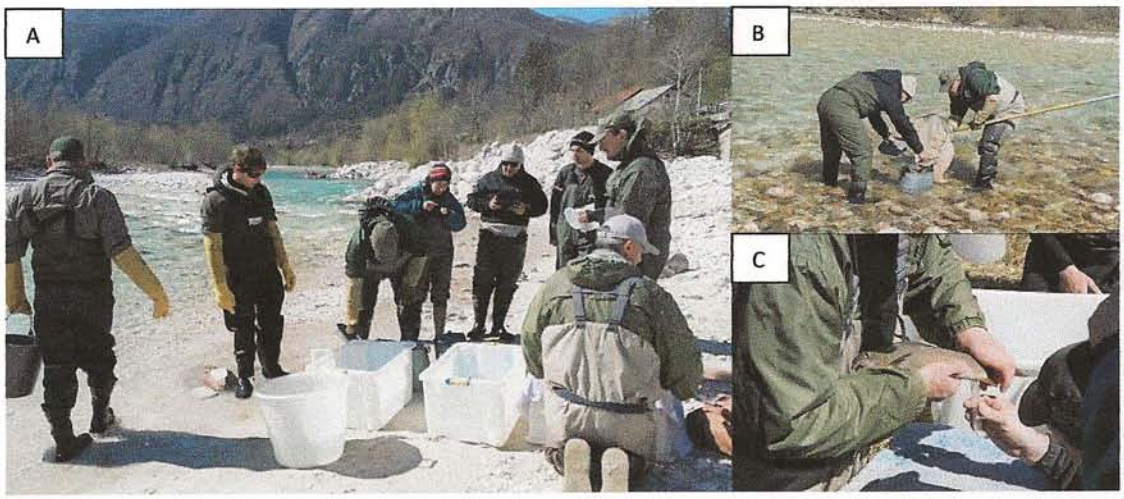

Figure 1- The team involved in field sampling work: A and B- Sampling wild grayling by electrofishing. C- Collecting sperm samples *in situ*.

Figure 2- Hatchery experiments: A- Incubating egg samples after *in vitro* fertilization. B- Larval production tanks.

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

Dr. Dušan Jesenšek (Angling association of Tolmin) certifies that Dr. Paulo Gavaia from the BIOSKEL group (CCMAR) has completed a Short-Term Scientific Mission awarded by the COST Action FA1205 between April 6-12, 2015.

Paulo Gavaia

Dušan Jesenšek
(Angling association of Tolmin -Host institution)

Paulo Gavaia
(STSM applicant)

RIBIŠKA DRUŽINA TOLMIN R/S