cost	**STSM** ***Short-term Scientific Mission***	AQUAGAMETE
"Application of cryopreservation to aquaculture and the conservation of salmonid species" Tolmin, Slovenia		

Aims:

i. Increase the collaboration between the groups from de ULE and the Department of Aquaculture of Szent István University.

ii. Collaborate in the cryopreservation programs developed in the Angling Club of Tolmin with the grayling (Thymallus thymallus), working with a different salmonid species to those commonly used for the applicant, at field conditions.

INTRODUCTION

The Angling Club of Tolmin is committed since some years ago in developing conservation programs for the Adriatic grayling strains, indigenous to the north Adriatic basin. This grayling lineage is located in the Soča river system, where it is endangered due to the introgression with non-indigenous lineages and species. The Angling Club of Tolmin is carrying out selection of individuals based on genetic analysis of molecular markers: mtDNA and microsatellite loci, and gene banking methodologies are useful tools to achieve this goal.

DEVELOPED ACTIVITIES.

Ripe males and females were captured by electrofishing in different tributaries of the Soča river: Glijun, Ucja and Tolminca. Males were anesthetised at the location of capture and sperm was collected by stripping into 15-ml tubes after drying the urogenital pore. Moreover, fin clips were sampled for genetic analysis. Eggs from captured ovulated females were also collected to be used in artificial fertilization experiences.

Sperm motility was analysed using a CASA system, and samples showing more than 70% progressive spermatozoa were frozen in 0.5ml straws using a dilution 1:1 in the extender (10% methanol as cryoprotectant in the final volume). Straws were frozen on a horizontal rack 3 cm above liquid nitrogen for 3 minutes and stored in a LN_2 container to be used in future fertilization, according to the results of genetic analysis. After thawing, motilities recorded ranged from 20,00% to 55,69%, indicating a good quality post-thaw.

In order to check the effects of the stz/egg ratio in artificial fertilization success, fresh sperm from 5 males from the brood stock of the Angling Club of Tolmin were separately used to fertilize batches of 800 (10g) eggs with 4 different stz/egg ratios,

ranging from 50.000 to 1.000. Sperm concentration was calculated using a Neubawer chamber. After spreading the appropriate volume of sperm over eggs, motility was activated with DIA solution. Results will be provided by the Slovenian team.

Apart from the use of cryopreserved sperm, the advantages of germ cells (mainly spermatogonia) for cryobanking genetic resources in the specific context of grayling, was discussed.

CONCLUSION

The activities developed for the Angling Club of Tolmin provide a very good example of the application of sperm cryopreservation technologies in wildlife preservation. Our first aim (collaboration between the groups from de ULE and the Szent István University) was accomplished; the collaboration in developing the experimental procedure was very helpful for the exchange of knowledge and the discussion about standardization of procedures. The second objective (collaborate in the cryopreservation of grayling genetics) was also achieved. The STSM resulted in a very good platform to establish new possible collaborations between COST partners (ULE – Angling Club of Tolmin) for the preservation of diploid genome of grayling and/or marble trout using germ cells, which should be explored in deep.

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