cost	**STSM** ***Short-term Scientific Mission***	AQUAGAMETE

"Application of cryopreservation to aquaculture and the conservation of salmonid species"
Tolmin, Slovenia

Aims:

i. Introduce to techniques used to collect samples from wild fish.
ii. Carry out sperm cryopreservation of the grayling (*Thymallus thymallus*) with conservation purpose.
iii. Get to know the methodology to do fertilization at a fish farm.

INTRODUCTION

Adriatic grayling is indigenous to the north Adriatic basin. It is a morphological and genetically distinct lineage and it is located in the Soča river system, where it is endangered, due to the phenomenon of introgression with non-indigenous species. The Angling Club of Tolmin is carrying out selection of individuals based on genetic analysis of molecular markers: mtDNA and microsatellite loci.

OBJECTIVE 1. Sample collection.

To reach the first aim, 21 individuals were captured in different rivers: Učja, Glijun and Soča by electrofishing. Males were anesthetised at the location of capture and sperm was collected into 15-ml tubes after drying the urogenital pore. Moreover, fin clips and some scales were taken for genetic and morphometric analysis, respectively. Fin clips and scales were collected only from individuals that had sperm. Eggs were also collected from occasionally captured ovulated females.

OBJECTIVE 2. Sperm cryopreservation.

Grayling sperm was cryopreserved using a dilution 1:1 in the extender (10 % methanol as cryoprotectant in the final volume). Sperm was loaded into 0,5 ml straws and frozen placed on a horizontal rack 3 cm above liquid nitrogen for 3 minutes. Sperm samples were thawed in a water bath at 40 ºC during 13 seconds.

Sperm from wild and captive animals were frozen and sperm motility was analysed using CASA system before sperm cryopreservation.

OBJECTIVE 3. Fertilization.

Fertilization was performed using cryopreserved sperm and eggs, both from captive animals. 15 grams eggs were fertilised with 500 µl of thawed sperm. After spreading of sperm over eggs, motility was activated with DIA solution and gently mixed. Eggs were maintained in the dark and in a steady place.

Sperm from wild animals will be used in a future fertilization, according to results of genetic analysis.

CONCLUSION

Hatching rate of the fertilization was not measured yet due to the long incubation time. Percentages of eyed eggs and hatch will be counted later by the fish farm personnel and they will be conveyed to us for statistical analysis. However, with this stay it has been clear that the application of the short-term cryopreservation methodology with standard protocols is an important tool for animal conservation. Not only it can reduce the negative effects of species introgression but also it can improve the genetic variability in the captive individuals.

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