IMPACT OF SEMEN CRYOPRESERVATION ON SKELETAL AND MUSCULAR DEVELOPMENT OF MARBLE TROUT (SALMO MARMORATUS) LARVAE FROM THREE RIVER BASINS

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The marble trout (*Salmo marmoratus*) is a freshwater species from the family of the salmonids. Due to several human activities and to the hybridization with brown trout, marble trout was included in the European Union Habitat Directive and in the 2013 IUCN Red List, classifying it as "Critically Endangered" (1). In this context, the existence of a sperm cryobank assumes a great importance on this species preservation and natural stocks enhancement. The aim of this study was to assess the viability of the marble trout larvae from three river basins, Adige, Piave and Brenta, obtained with fresh (CTR) and cryopreserved semen (CRYO, from the Spallanzani cryobank). It is important to refer that, within each river basin, the genetic line of this species is independent. Therefore, differences between rivers are not relevant for the purpose of this study.

Sperm samples were collected and cryopreserved according to the protocol developed ad hoc for marble trout. Fertilization took place and hatching occurred at 44 dpf (days post-fertilization). Larvae were evaluated in terms of weight and total length, muscle development (histometruc analyses for fibers density, FD and cross-sectional area, CSA) as well as the occurrence of skeletal abnormalities, using a double staining whole-mount technique with Alcian Blue and Alizarin for cartilage and bone tissues respectively. The fish handling procedures and sampling methods used in the trial followed the guidelines of the E.U directive 2010/63/EU on the protection of animals used for scientific purposes.

For each river basin, no significant differences were found between treatments regarding larvae total length. Moreover, no differences were observed in Adige in weight between treatments. Interestingly, in Piave, larvae from the CTR group were heavier than the CRYO ones; on the opposite, in the river Brenta, the heavier larvae were the ones obtained with CRYO. For each river basin no differences were found regarding FD and CSA, except for Brenta, where larvae from the CRYO group showed the highest CSA. These differences may be due to a different balance between hyperplasia/hypertrophy as well as to exogenous factors related to the different basins and fish genotypes. Regarding skeletal development, whole-mount double staining showed no abnormalities in larvae from all the river basins and from all treatments.
Overall, taking into account these results, it would seem safe to state that cryopreserved semen produces equally good quality larvae as the ones obtained with traditional, fresh semen, for all the three river basins. Acknowledging no negative effects on the use upon the descendants, semen cryopreservation from cryobank can be an additional strategy to protect endangered species.

[1] Rondinini et al, 2013. Lista Rossa IUCN dei Vertebrati Italiani. Comitato Italiano IUCN e Ministero dell'Ambiente e della Tutela del Territorio e del Mare, Roma.