CRYOPRESERVATION OF SPERM FROM TWO ENDANGERED SALMONID SPECIES FROM THE RIVER SOČA IN SLOVENIA

Ákos Horváth[1*], Dušan Jesenšek[2], Balázs Csorbai[1], Zoltán Bokor[1], Béla Urbányi[1], Aleš Snoj[3]

1 Department of Aquaculture, Institute of Environmental and Landscape Management, Faculty of Agriculture and Environmental Science, Szent István University, Páter Károly u. 1. H-2103 Gödöllő, Hungary
2 Angling Club of Tolmin, Trg 1. maja 7, SI-5220 Tolmin, Slovenia
3 University of Ljubljana, Biotechnical Faculty, Department of Animal Science, Groblje 3, SI-1230 Domžale, Slovenia
* Corresponding author: Phone: +36 28522000 ext. 2311, Fax: +36 410804, e-mail: Horvath.Akos@mkk.szie.hu

INTRODUCTION

The Soča river system in Western Slovenia belongs to the drainage of the Adriatic sea and is inhabited by several endemic salmonid species including a phylogenetically distinct lineage of the grayling (*Thymallus thymallus*) and the Natura-2000 listed marble trout (*Salmo marmoratus*). Both species are seriously endangered due to the introduction of a non-native grayling lineage as well as brown trout (*Salmo trutta m. fario*) from the Danubian drainage and subsequent hybridization (Povž 1995). At present only hybrid grayling stocks with different proportions of “Adriatic” genes exist in the river, while pure marble trout populations remained in the upper sections of several streams that were inaccessible to brown trout (Fumagalli et al. 2002). However, these populations are also endangered due to natural hazards such as earthquakes, landslides, floods and avalanches which are frequent in this area.

The Angling Club of Tolmin that manages the Soča river basin has, based on genetic analysis, conducted extensive selection of individuals with the highest share of Adriatic genes and carries out systematic spawning and stocking of these fish into the river (Sušnik et al. 2004; Jesenšek & Šumer 2004). The objective of this work was to create a cryopreserved germplasm repository of the two Adriatic species and to carry out test fertilizations using the cryopreserved sperm.

MATERIALS AND METHODS

Grayling sperm (3 samples – two individuals and a pooled one) was collected from the broodstock at the Tolminka fish farm of the Angling Club of Tolmin as well as from wild individuals caught directly from the river. Marble trout sperm (3 samples) was collected from individuals of a pure population in different sections of the Trebuščica river. Eggs for fertilization tests were collected from female individuals of the broodstock of both species at the fish farm. Sperm of both species was diluted with an extender composed of 200 mM Glucose, 40 mM KCl, 30 mM Tris (pH 8.0). Methanol at 10% concentration was used as the cryoprotectant. Diluted sperm was loaded into 0.5-ml straws and frozen in the vapor of liquid nitrogen at 3 cm above the level of the liquid for 3 minutes. Straws were thawed following storage at a 40°C water bath for 13 seconds.

Two experiments were conducted in this study. In the first, the effect of the dilution ratio (1:1, 1:4 and 1:9) of sperm with extender was tested on the percentage of eyed eggs (fertilization) and hatched larvae in both species. In the second, the effect of sperm-egg ratio was investigated on the percentage of eyed eggs and hatched larvae in the grayling.

RESULTS

The highest post-thaw motility (50 ± 0 %) of grayling sperm measured in the first experiment, corresponded to the pooled sperm sample with 1:1 dilution ratio. In general, significant main effects of dilution ratios ($P < 0.0001$) and sperm samples ($P = 0.0090$) were observed on post-thaw motility of cryopreserved grayling sperm. In case of the marble trout the highest post-thaw motility (23 ± 6%) was observed in sperm sample 1 at a diltuion ratio of 1:9. Significant main effects of the dilution ratio (P = 0.0110) as well as the individual sample (P = 0.0003) on the post-thaw motility of marble trout sperm were found.

In case of fertilization percentages in the grayling at eyed stage, significant main effects of dilution ratios (*P = 0.0002*) and sperm samples (*P = 0.0285*) were found, however, only dilution ratios had an effect (*P = 0.0008*) on the hatch rates. The highest fertilization (74 ± 4 %) and hatch (63±6%) percentages were observed in sample number 10 using the 1:1 dilution ratio. In case of the marble trout, the highest percentage of eyed eggs (84 ± 4%) and hatched larvae (70 ± 3%) was observed with sperm sample 3 and dilution ratio of 1:1. A significant main effect of individual samples was observed on the percentage of eyed eggs and hatched larvae (both: *P<0.0001*), yet, no main effect of the dilution ratio was found.

In the second experiment, sperm-to-egg ratio significantly affected both fertilization (P = 0.0330) and hatch (P = 0.0455) percentages. The use of 5.687 ± 0.181 × 10^4 spermatozoa per egg gave the highest percentage of eyed eggs (78 ± 7 %) and best hatch (73 ± 7 %) results, although, no significant difference was observed between this ratio and half of it. Control fertilization and hatch results were not counted in this experiment due to human error.

DISCUSSION AND CONCLUSIONS

Although freezing of salmonid sperm is by far the most studied are of fish sperm cryopreservation, most studies concentrate on the male germplasm of the rainbow trout (*Oncorhynchus mykiss*). To the best of our knowledge this is the first attempt of cryopreservation of marble trout sperm and there are only two other studies on the cryopreservation of grayling sperm (Lahnsteiner et al. 1992, 1996).

As individual sperm samples had a significant main effect on the fertilizing capacity of both species (although to a higher degree in the marble trout), it is clear that collection of good quality sperm samples for cryopreservation is necessary. Urine-free collection of marble trout sperm was not possible on the site of collection and this could have had an effect on sperm quality. However, other quick and relatively simple methods of sperm quality determination could also be important which would allow an on-site evaluation of the suitability of sperm fro cryopreservation or prediction of its post-thaw fertilizing capacity.

ACKNOWLEDGEMENTS

This work was supported by the Hungaria-Slovenian bilateral S&T project SI-03/2007 and the Regional University Center of Excellence in Environmental Industry of Szent István University.

REFERENCES

Fumagalli L.; Snoj A.; Jesenšek D.; Balloux F.; Jug T.; Duron O.; Brossier F.; Crivelli A.J.; Berrebi P., 2002: Extreme genetic differentiation among the remnant populations of marble trout (*Salmo marmoratus*) in Slovenia. Mol. Ecol. **11**, 2711-2716.

Jesenšek D.; Šumer S., 2004: Adriatic grayling (*Thymallus thymallus*, Linnaeus, 1758) in the Soča river basin, Slovenia: action plan. Ribiška družina Tomin; Ente Tutela Pesca del Friuli Venezia Giulia, Udine.

Lahnsteiner F.; Weismann T.; Patzner R., 1992: Fine structural changes in spermatozoa of the grayling, *Thymallus thymallus* (Pisces: Teleostei) during routine cryopreservation. Aquaculture **103**, 73-84.

Lahnsteiner F.; Weismann T.; Patzner R., 1996: Cryopreservation of semen of the grayling (*Thymallus thymallus*) and the Danube salmon (*Hucho hucho*). Aquaculture **144**, 265-274.

Povž M., 1995: Status of freshwater fishes in the Adriatic catchment of Slovenia. Biol. Conserv. **72**, 171-177.

Sušnik S.; Berrebi P.; Dovč P.; Hansen M.M.; Snoj A., 2004: Genetic introgression between wild and stocked salmonids and the prospects for using molecular markers in population rehabilitation: the case of the Adriatic grayling (*Thymallus thymallus* L. 1785). Heredity **93**, 273-282.