Application of Genome Engineering and Hormonal Sex Reversal in the Reproduction of European Grayling (*Thymallus thymallus*)

mgr inż. Rafał Rożyński

Abstract

Environmental pollution, intense anthropogenic pressure, and the loss of genetic integrity resulting from the mixing of gene pools (genetic contamination) among different populations have led to a significant decline in the abundance of European grayling (*Thymallus thymallus*) across many regions of its natural distribution. Effective management of this species should be based on stocking programs using fish originating exclusively from local broodstock populations. However, when this is not feasible, the use of sterile triploid stocking material may represent a viable alternative, as such fish are unable to interbreed with native populations while still maintaining the high attractiveness of recreational fisheries. Unfortunately, the technology for producing triploid grayling remains at an early stage of development. Therefore, the main objective of this doctoral dissertation was to determine the effects of possessing three chromosome sets (3n) on gonadal development, survival, growth performance, and angling attractiveness of European grayling, as well as to explore the possibility of developing a method for producing all-female triploid individuals of this species.

The obtained results demonstrated that triploid fish exhibited survival rates comparable to those of diploids. Growth performance was significantly higher in triploids only during the period when diploid individuals reached sexual maturity. Under communal rearing conditions, triploid fish showed slightly higher growth rates and better condition than diploids; however, these differences were not statistically significant. Recreational fishing results indicated that triploid individuals maintained high angling attractiveness and were at least comparable to, and in some cases slightly more attractive than, diploid grayling. Gonadal analyses revealed that the ovaries of triploid females were severely reduced, consisting mainly of connective tissue strands and lacking gametes. In contrast, the testes of many triploid males displayed a typical morphological appearance; however, spermiation was not observed. Given these findings, an important component of the study was the ambitious attempt to produce all-female triploid grayling. The application of gynogenesis using rainbow trout (*Oncorhynchus mykiss*) sperm enabled the production of exclusively all-female offspring. Unfortunately, the initial attempts at hormonal masculinization using androgens (OHA and MT) did not result in the production of fully functional neomales, and intersex characteristics were observed in some individuals.

In conclusion, the production of triploid European grayling represents a promising biotechnological tool for conserving the genetic resources of this species while maintaining its high utility value in recreational fisheries. This dissertation provides a foundation for further research aimed at developing technologies for the production of triploid, all-female stocking material of European grayling.

Keywords: European grayling; genetic integrity conservation; triploidization; gynogenesis; hormonal masculinization.