Optimisation of biotechnology reproduction and selected aspects of early ontogenesis of common barbel (*Barbus barbus* L.) under controlled conditions

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Summary

The common barbel *Barbus barbus* (L.) is a rheophilic cyprinid fish, and is a valuable component of river ecosystems. However, for many years, a reduction in the number of fish of this species has been noted both in Poland and in other European countries. In order to prevent further decline in the common barbel population size, different activities are being taken to protect this species, both actively and passively. Therefore, in recent years, the common barbel has become the focus of research aimed at developing procedures for the artificial reproduction and rearing of stocking material under controlled conditions. The common barbel aquaculture is primarily associated with conservative aquaculture, which involves the production of stocking material under controlled conditions, and the stocking of open waters. The research carried out so far into the reproduction of this species has not enabled the establishment of a repeatable protocol for the breeding and production of the common barbel stocking material. This is due, *inter alia*, to a difficulty in inducing ovulation in wild and poorly domesticated common barbel females, the relatively low fecundity, or the absence of eggs fertilisation procedures.

Therefore, the main objective of this study was to optimise the common barbel artificial reproduction and larval rearing biotechnics under controlled conditions. The study focused on such aspects as the optimisation of reproduction, improving the fertilisation methods, and an analysis of the course of the early common barbel larval development using methods that enable an analysis of the morphological and anatomical change dynamics. The conducted

experiments used the reproduction common barbel spawners (F_4 and F_5 generations) originating from the Hatchery in Czarci Jar near Olsztynek (north-eastern Poland) or bred at the Department of Ichthyology and Aquaculture, Faculty of Animal Bioengineering, University of Warmia and Mazury in Olsztyn, Poland. The spawning stock (F_4 generation) was derived from fish originating from the Vistula River basin (the Świder River). The conducted study comprised three complementary stages. During the first stage, an attempt was made to optimise the common barbel's spawning under controlled conditions using different variants of thermal stimulation, followed by combining a selected (the most effective) thermal stimulation variant with the use of various hormonal preparations (Ovopel, Ovaprim, hCG, CPH). The aim of the study was to obtain a high percentage of ovulating females and a high embryo survival rate. The second stage of the study focused on optimising the fertilisation process, with particular attention paid to the ovarian fluid composition and its effect on sperm motility. The third stage of the study analysed the common barbel larvae development from hatching until the skin fold disappearance (0 – 22 days post hatch (DPH)). The focus was on such aspects as the behaviour, a change in allometric growth patterns and the morphogenesis of the common barbel larvae digestive system.

The results demonstrated that the simultaneous application of thermal and hormonal stimulation can result in a high common barbel *Barbus barbus* reproduction effectiveness under controlled conditions. Of all the hormonal preparations tested, CPH and Ovaprim were found to be the most effective. After the application of these preparations, the ovulation percentage was 90 –100%, and the 90% embryo survival rate at the hatching stage was achieved. It was observed that during the reproduction, along with the common barbel eggs, considerable amounts of ovarian fluid were obtained (14 – 68 % of the weight of the eggs), which had an adverse effect on sperm motility, thus inhibiting their activity. Moreover, a significant relationship was found between the pH of the ovarian fluid and the fertilisation efficiency.

The highest survival rate (exceeding 60%) was found in the pH range of 7.9 – 8.4. At higher or lower pH values, a significant reduction in the embryonic survival rate was noted. After being activated with water, the common barbel spermatozoa retained the ability to fertilise for a relatively short time. This duration, however, could be elongated by using Woynarovich's solution during the fertilisation process, which additionally contributed to an increase in the embryonic survival rate, and thus in the number of hatched larvae. It was observed that the common barbel larvae, despite their relatively large body size (as compared to other cyprinids), were developmentally less advanced at the time of hatching. An analysis of the course of the successive early ontogenetic stages for this species found that the most dynamic period of changes occurred during the first 11 days after hatching. This was reflected, *inter alia*, in the occurrence of a number of individual flexion points that indicated changes in the dynamics of the growth of the tested body parts or organs and, consequently, changes in the body proportions. Despite a poorly advanced developmental stage at the time of hatching, i.e. the absence of pigment in the eye, the absence of a distinct liver and pancreas and an obstructed gastrointestinal tract, the common barbel larvae passed through the larval period exceptionally quickly and entered the juvenile period (22 days) already possessing many characteristics observed in adult individuals.

Not only do the results presented in this paper provide scientific value by improving knowledge on both the spawning-affecting factors and the course of the early ontogenetic stages, but they also offer important practical guidance that can be used by breeders to achieve measurable results during the breeding and rearing of the common barbel under controlled conditions. They can significantly contribute to: an increase in the percentage of ovulating females, an increase in the percentage of fertilised eggs and the embryonic survival rate. The growth patterns and the course of larval development, with particular focus on the morphological and anatomical development as well as the larvae behaviour, as specified

in this study, provide a basis for the development of procedures for reducing the duration of the common barbel stock material rearing, including by selecting an appropriate larva and fry feeding protocol. Fish farmers using the valuable guidance provided in this study can achieve higher effectiveness of breeding and production of common barbel stocking material, which may indirectly contribute to an improvement in the common barbel population size in open waters through the stocking of waters with high quality stocking material. All of the above aspects provide an important contribution to the active protection of the common barbel.

Keywords: *fertilisation; eggs quality; larval development; ovary fluid; ovulation; morphogenesis; reproduction*