www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 16: 523-530 (2016) DOI: 10.4194/1303-2712-v16_3_04

RESEARCH PAPER

The Synergistic Effect of Temperature and Hormonal Stimulation on Spawning Efficiency of Common Barbel, *Barbus barbus L.*

J. Nowosad[1], D. Kucharczyk[1], K. Targońska[1], E. Wyszomirska[2], R. Chwaluczyk[3], K Kupren[1,4]

[1] University of Warmia and Mazury, Faculty on Environmental Sciences, Department of Lake and River Fisheries, Poland.
[2] , National Medicines Institute, Department of Basic and Applied Pharmacy30/34 Chełmska St., 00-725 Warszawa, Poland.
[3] Fish Farm Czarci Jar, Poland.
[4] University of Warmia and Mazury, Department of Tourism, Recreation and Ecology, Poland.

* Corresponding Author: Tel.: +48 89 5234215; Fax: +48 895233969;
E-mail: nowosad.joanna@gmail.com

Received 29 January 2016
Accepted 16 April 2016

Abstract

Artificial reproduction of common barbel (Barbus barbus) under controlled conditions usually did not exceed 10% of ovulation success. The aim of the study was to optimise the process of artificial reproduction of the barbel (cultured generation F_4) with thermal and hormonal stimulation The first experiment examined the effect of stimulation with thermal conditions on the ovulation and the embryos survival rates. After the optimum conditions of thermal stimulation in barbel reproduction were determined, another experiment was conducted which examined how the effectiveness of reproduction is affected by four selected hormonal preparations (CPH, hCG, Ovaprim and Ovopel). Thermal stimulation for 58 days was found to be the most effective (748 degree-days). The second experiment examined the synergistic effects of different hormonal preparations under the optimal thermal conditions as, determined in the first experiment. Among the hormones evaluated as part of this study, CPH and Ovaprim were yielded the best results as compared to control. The use of the preparations resulted in the percentage of ovulations of 90-100% and the embryo survival rate at the hatching stage was about 90%. The study concludes that thermal stimuli and hormonal applications have a synergistic effect on artificial reproduction in *B, barbus*.

Keywords: *Barbus barbus*, embryos survival, hormonal stimulation, spawning, temperature.

Introduction

Barbel, *Barbus barbus* L., is a fish species which is highly sensitive to adverse environmental changes (Kujawa and Glińska-Lewczuk, 2011). It is one of the most valuable cyprinid rheophilic species in Europe, both in terms of angling value and as part of the environment biocenosis. It usually chooses well-oxygenated and clean waters. It usually reproduces from May to June in water at a temperature from 15-18°C. The relative fertility of barbel ranges from 36 to 85 thousand eggs per kg of female body weight (Baran, 2000). The population of barbel has been seen to decrease, both in Poland and in other European countries. The species is actively protected in order to prevent its total extinction. In consequence, the demand for stocking material to stock open waters is growing (Cowx, 1994; Wojda, 2004; Bolland *et al.*, 2008). The production of barbel stocking material can generate considerable financial gains owing to its high price, caused by lower fertility and effectiveness of artificial reproduction compared to other rheophilic fish species (Kucharczyk *et al.*, 2008; Kupren *et al.*, 2008; Hakuć-Błażowska *et al.*, 2009;Targońska*et al.*, 2011a; Nowosad *et al.*, 2014b).The changes in the natural environment, including the aquatic environment, that have been taking place recently have depleted biocenoses and decreased global biodiversity (Ross *et al.*, 2008).

Fish reproduction under controlled conditions requires the right environmental conditions, such as temperature, photoperiod and salinity (Nowosad *et al.*, 2014a, b) as well as - frequently - stimulation with hormonal agents (Yaron, 1995). Only a combination of these methods makes it possible to achieve final gamete maturation and high quality of gametes and larvae in captivity (Brooks *et al.*, 1997). The thermal conditions play a crucial role during the initial phase of stimulation of fish maturing under controlled conditions. Improperly chosen thermal conditions as well as their fluctuations either delay spawning or cause gametes to mature with defects (Wang *et al.*, 2010; Targońska *et al.*, 2010, 2012; Nowosad *et al.*, 2014a). Final gamete maturation, especially in cyprinids, is induced by hormonal stimulation (Yaron, 1995; Policar *et al.*, 2007; Kujawa *et al.*, 2011; 2015). There is no uniform reproduction protocol for this group of fish. Since there are different hormonal

agents applied at different doses in different species, a separate reproduction protocol should be developed for each species, taking into account the optimum environmental conditions and hormonal stimulation applied. The production of stocking material of different species of the genus *Barbus* is difficult under controlled conditions and it meets with a number of obstacles in European and Asian countries (Kahkesh *et al.*,2010; Targońska *et al.*, 2011b). The bottleneck of this process is the induction of ovulation, whose rate under controlled conditions in barbel is usually about 10% or lower. One of the methods of optimisation involves the use of partly domesticated fish in reproduction, with the average ovulation rate in such fish being 50% (Targońska *et al.*, 2011b). However, this method also requires further development and optimisiation ovulation rate as well as embryo survival. No effective method of reproduction stimulation of barbel with environmental conditions combined with hormonal treatment has been developed so far which offers a repeatable outcome and high ovulation rate.

The aim of the study was to optimise the process of artificial reproduction of the barbel with synergistic effect of thermal and hormonal stimulation.

Material and Methods

Spawners and Manipulations of the Fish

The cultured barbel (generation F_4) originated from the Fish Farm in Czarci Jar near Olsztynek (north-eastern Poland). The cultured stock was obtained from fish living in the Vistula River basin (F_0 were caught at the outlet of the Świder River into the Vistula (52°05′59″N 21°12′53″E). Barbel generation F_4 was reared in $1m^3$ special breeder's tanks with the possibility of full controlling of environmental condition (Kujawa *et al.*, 1999) for 3 years. The photoperiod for the cultured fish was set with a control clock connected with photo-cell to the natural conditions. Fish were fed with trout pellets and frozen blood worm larvae at a weight proportion of 1:1 (maximally 1.5% biomass of the fish).

Two separate experiments were carried out. In the first experiment, different thermal regimes were tested to find the best time to apply hormonal stimulation to induce artificial reproduction. Fish from all groups, for first 40 days were kept at the same photo-thermal conditions (temperature 10–14°C) (Figure 1). Later in time, in treated groups water temperature was increased to 19°C (groups A, B, C and D), except groups K (control one). After two days of keeping fish in 19°C, the females obtain hormonal treatment. In the second experiment, the different spawning agents were tested in the best working (optimal) thermal regime obtained in Experiment I (groups D). The experiments were conducted during a single reproductive season for additional groups of the same stock. The photoperiod was constant during study: 14Light (L) :10Darkness (D). In all groups, number of females was 10.

During the spring period, the water temperature started to increase. The temperature was increased at

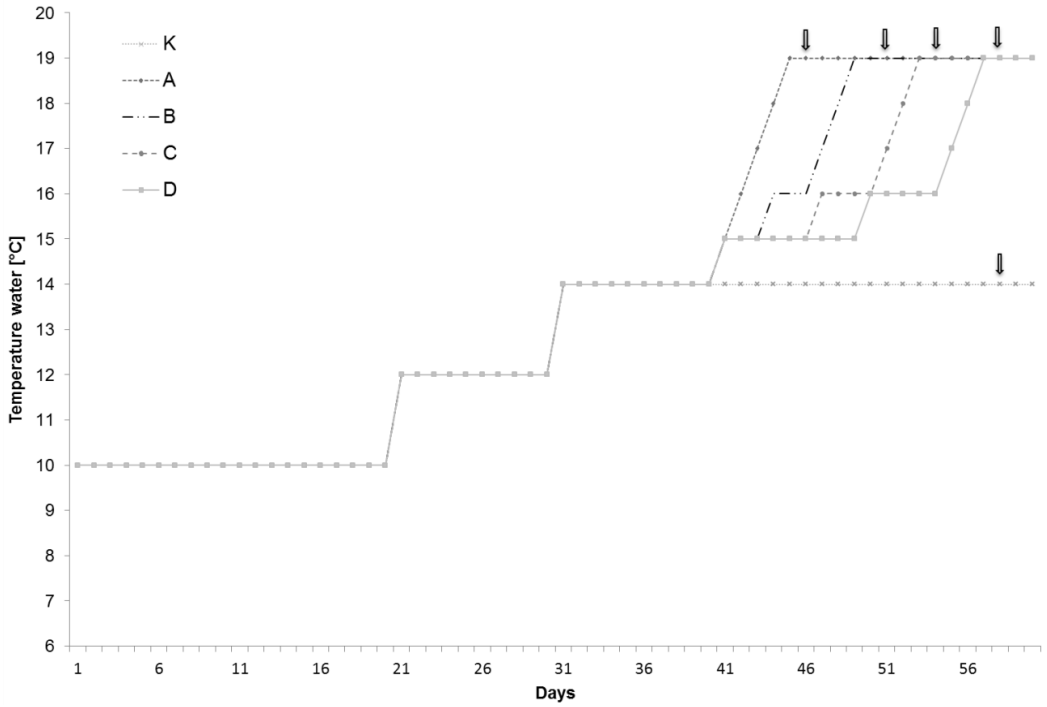

Figure 1. Thermal profiles applied for stimulation of fish maturation by means of temperature change in five different temperature regimens. The arrows indicate the time of performing hormonal injections in treated groups (A, B, C and D) and control one (group K). The number of females in each groups was 10.

the maximum level 0.2°C per hour. The thermal regimes are presented in Figure 1. The arrow in this figure indicated the time of applied hormonal injection. For the first 40 days, all groups were kept at the same conditions. Hormonal injections were administered in each group at different times (Figure 1). All manipulations with the fish were conducted after anaesthetizing them in a solution of 2-phenoxyethanol (0.5 cm^3 dm^{-3}) (Sigma-Aldrich, Germany) (Nowosad *et al.*, 2014a). Before the first injection, the fish (mean weight mean± SD: males 142 ± 18 g, females 168±29 g), were divided into 5 groups with different thermal regimes. The oocyte maturation stages were not developed because this is not possible in multi-batch spawners (Targońska *et al.*, 2012b). Stimulation was conducted using the Ovaprim commercial preparation. It contains 20 μg of salmon GnRH analogue [D-Arg^6, Pro^9-Net]-sGnRHa and 10 mg domperidone in 1 mL of propylene glycol (Peter *et al.*, 1993). All fish (females and males) were stimulated with a dose of 0.5 mL kg^{-1}. The injections were administered intraperitoneally, under the base of the ventral fin. Fifteen hours after the injection, females were assessed in order to detect signs of ovulation. It was checked by manual pressure of females belly. This was repeated every 3 h for the subsequent 15 h to determine the latency time. Gametes were obtained by applying gentle pressure on the abdominal covers of the. of the spawners, and the numbers of females and males rendering the gametes were recorded. The eggs from each female were collected separately into a different plastic bowl and weighed. The quality of the eggs was also determined macroscopically by the colour and transparent shape. To determine the influence of the applied thermal regime on the biological quality of gametes in each group, three samples of eggs were collected from each female (100–200 eggs each), and were fertilized within the same experimental group with pooled, freshly-obtained semen collected from 5 randomly-chosen males. These samples were incubated separately in water at 19.5°C (±0.5°C). The survival rates of the embryos were determined at the hatching time. After the completion of the experiments, the fish were maintained for 14 days to determine the spawner's survival.

In the second experiment, the effectiveness of different hormonal agents were tested on ovulation rate, latency time and gamete quality. Stimulation was conducted using four commercial preparations: Ovopel (Unic-trade, Hungary) at a dose of 2.0 pellet kg-1, Ovaprim (Syndel, Canada) at a dose of 0.5 mL kg^{-1}, CPH – common carp pituitary homogenate (Argent, USA) at a dose of 6 mg kg^{-1} and hCG – human chorionic gonadotropin (Argent, USA) at a dose of 600 IU kg^{-1}. One pellet (commercial form) of Ovopel typically contained 18–20 μg mammalian GnRH analogue [D-Ala6, Pro9- Net]-mGnRHa and 8–10 mg of metoclopramide (Horváth *et al.*, 1997). In the control group, injections of physiological saline solution (0.9% NaCl) were applied. The injections were administered intraperitoneally, under the ventral fin.

The manipulation with spawners, collection of gametes, fertilization and other handling and manipulation was the same as described in experiment I: ovulation rates, latency time, spawners mortality and embryos survival.

Statistical Analysis

The statistical differences between the groups were evaluated by an analysis of variance (ANOVA–Statistica 2015) and, after obtaining significant values, Tukey's test ($P<0.05$) was applied as a *post hoc* test. All the values expressed as percentages were arcsine transformed prior to statistical analysis

Results

Experiment 1

This study found that the highest percentage of ovulating females was about 90%, and consequently, the highest percentage of females producing good quality eggs (80%) was achieved in group D, in which 58-day thermal stimulation (in temperature below 15°C) was applied (748 degree-days). Females in the control group (K) did not ovulate. In group A, in which the water temperature was raised from 14ºC to 19ºC over 5 days (45 days of stimulation, 564 degree-days) (Figure 1), the percentage of ovulation was 30%, with only 10% of females producing viable eggs (Figure 2, Figure 3A). The percentage of hatched embryos in this group (group A) was 56.7±1.0 (mean ± SE) and it was significantly different than in the other groups ($P<0.05$). The embryo hatching rate in group D was 87.7±1.2% (mean±SE) and it was significantly different ($P<0.05$) in groups A and B (Figure 3B). The best reproductive outcome (% of ovulation, embryo survival rate at the hatching level), was achieved in groups where the number of degree-days was 687–748, in group C and D, respectively. Thermal stimulation for 58 days (748 degree-days; group D) proved to be the best variant of thermal stimulation.

Experiment 2

The highest percentage of ovulating females of 90-100% was achieved in groups where CPH, Ovopel and Ovaprim were used for fish stimulation. The latency time in the fish was between 16.5±0.8 and 18.5±0.9 hours. When Ovaprim and CPH were used to stimulate females, high quality oocytes were obtained from 90% of females and the embryo survival rate was 90.3±3.1 and 84.6±7.9%, respectively (Table 1). In the control group, in which ovulation was induced only by thermal stimulation and the injection of 0.9% physiological saline, 30% of

Figure 2. Eggs of barbel, high (on left) and low (on right) quality.

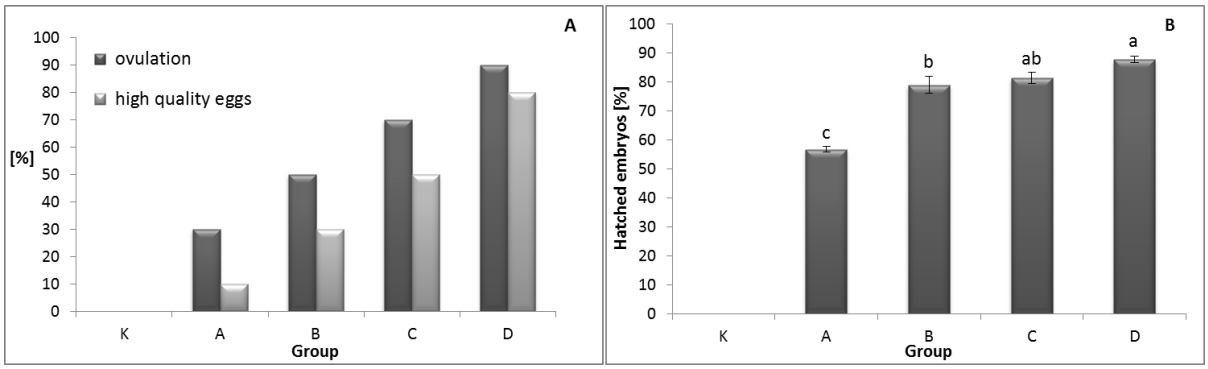

Figure 3. The outcome of barbel reproduction depending on the temperature regimen: female ovulation rate, quality of eggs (A) and percentage of hatched embryos (B). Data presented as a mean ± SE. The data denoted with different letters were statistically different (P<0.05).

Table 1. Barbel reproduction in an optimal thermal regime (group D in 1st experiment) with stimulation of different hormonal agents (2nd experiment). Data presented as a mean ± SD. The data in rows denoted with different letters were statistically different (P<0.05).

Group	I (control)	II	III	IV	V
Spawning agent	0.9% NaCl	hCG	Ovopel	Ovaprim	CPH
Hormonal dose		1000	2 pellet kg^{-1}	0.5 ml L^{-1}	6 mg kg^{-1}
Ovulating females [%]	30	70	90	90	100
Latency time [h]	22.5 ± 1.1^{c}	22.0 ± 1.4^{c}	18.0 ± 0.8^{b}	18.5 ± 0.9^{b}	16.5 ± 0.8^{a}
Females producing eggs of high quality [%]	20	70	80	90	90
Hatched embryos [%]	75.6 ± 3.2^{b}	92.6 ± 1.8^{a}	76.8 ± 2.8^{b}	90.3 ± 3.1^{a}	84.6 ± 7.9^{ab}

females ovulated, of which 20% produced good quality oocytes and the embryo survival rate was 75.6±3.2%. Although the embryo survival rate in the control group and in the group where Ovopel was used for stimulation (76.8±2.8%) were not statistically different (P>0.05), the values differed significantly (P<0.05) from the survival rate for embryos obtained from the females in which hCG (92.6±1.8%) and Ovaprim (90.3±3.1%) were used for hormonal stimulation (Table 1).

Discussion

The increasing production of cyprinid fishes stocking material necessitates the development and perfection of reproduction techniques. Reproduction of fish under controlled conditions is very difficult and it frequently requires developing a protocol which takes into account stimulation with environmental conditions (i.e. temperature, photoperiod, salinity) and hormonal agents. It must be noted that the techniques

of manipulations in reproduction, as well as the possibility of using the right hormonal agent, depend on the species, fish origin (wild or domesticated fish) and on the spawning time (within or outside the reproductive season).

The efficiency of artificial spawning is governed by the synergistic effect of a wide range of environmental parameters, what was found in the present study when the temperature regimes strongly influenced on spawning efficiency. Longer in time thermal stimulation of barbel females, caused in higher ovulation rate, higher production of eggs with high quality and high embryo survival. Two long keeping the spawners in too low temperature (control groups) did not caused ovulation. From environmental factors, the most important of fish reproduction are environmental conditions and their changes (Brooks *et al.*, 1997; Bromage *et al.*, 2001; Angius and Canavate, 2005; Wang *et al.*, 2010; Nowosad *et al.*, 2014a,b), feeding brooders before the reproduction period (Król *et al.*, 2014; Kucharczyk *et al.*, 2014), applying the right hormonal stimulation in order to induce final gamete maturation (Podhorec and Kouril, 2009), origin of fish (Krejszeff *et al.*, 2009) or a combination of these factors (Nowosad *et al.*, 2014a, 2015). Depending on the species, the most important conditions include temperature and its regime or the photoperiod and its regime. On the other hand, the thermal regime is more important in thermophilic fish, such as the barbel. This study has shown that the effectiveness of reproduction, understood to denote the number of ovulating females, quality of eggs and embryo survival rate, is affected by the temperature change rate during the pre-spawn period. The more prolonged the temperature growth period was, the better the reproduction results that were achieved. Fish reproduction during the reproduction period does not usually pose any difficulties in other cyprinids. There is only a short period of gradual temperature increase required as well as administering the right hormonal agents at the right doses (Kucharczyk *et al.*, 2005; Kujawa *et al.*, 2011; Nowosad *et al.*, 2014a). In some cases, for example, in the reproduction of domesticated shoals of some cyprinids species, it is enough to control the thermal conditions to achieve effective reproduction under controlled conditions (Krejszeff *et al.*, 2009). However, if thermal anomalies occur just before spawning, a disturbance in the ovulation process may occur as well as a considerable deterioration of spawn quality, which manifests itself by low embryo survival rate and a high percentage of larvae deformation. A temperature decrease in such species as asp (*Aspius aspius* L.) or ide (*Leuciscus idus* L.) considerably delays ovulation (latency time) (Targońska *et al.*, 2010, 2012a). However, when the temperature increases beyond the set thermal optimum (maximum), one should expect a considerable decrease in the percentage of ovulation and the biological quality of oocytes. However, thermal fluctuations in some stenothermic species, such as common dace (*Leuciscus leuciscus* L.), even if they lie within the optimum reproduction temperatures for the species under controlled conditions (10 – 14°C), may decrease the ovulation rate, relative fertility and embryo survival rate (Nowosad *et al.*, 2014a).

However, if reproduction is conducted outside the season, stimulation with environmental conditions, mainly with temperature, plays a very important role (Angius and Canavate, 2005, Kucharczyk *et al.*, 2008, Targońska *et al.*, 2014a). This experiment employed the optimum thermal stimulation (group D) during the out-of-season barbel reproduction, owing to which it was possible to achieve a very high percentage of ovulation in females, a high percentage of good quality spawn and a high percentage of larvae hatching. With the right thermal regime, it is possible to achieve an outcome comparable to in-season reproduction in freshwater fish (Targońska-Dietrich *et al.*, 2004; *et al.*, 2008; Targońska *et al.*, 2014a). However, thermal stimulation must be selected individually for the species and it sometimes lasts for several months. In out-of-season reproduction of perch (*Perca fluviatilis* L.), an excessively short period of thermal stimulation produces a considerable percentage of non-ovulating females and a very low biological quality of spawn (Targońska *et al.*, 2014b). Extending the period of thermal stimulation by 30 days (from 90 to 120) had a considerable effect on reproduction effectiveness. Similar results were obtained for barbel. Extending the period of thermal stimulation considerably extended the reproduction effectiveness. Even a few days of difference in the stimulation period had a significant effect on the results.

The findings of this study indicate the great importance of temperature in the maturing of fish and the outcome of reproduction, both under controlled conditions and in the natural environment. In the present study, fish kept in too low temperature (14 °C, groups K, 1st experiment) did not matured what was in opposite in results obtained from groups where temperature was raised to 19 °C. This results also indicate that weather anomalies, including thermal fluctuations during the pre-spawn period or an overly rapid increase in water temperature in natural water courses, can have a considerable effect on the outcome of reproduction and natural recruitment (Hilder and Pankhurst 2003; Moran *et al.*, 2009; Rijnsdorp *et al.*, 2009; Nowosad *et al.*, 2014a). Reproduction of cyprinids under controlled conditions is usually impossible without hormonal stimulation during the final gamete maturation. This applies both to fish with one-off spawning during the season, such as common dace (*Leuciscus leuciscus* (*L.*)), carp (*Cyprinus carpio* L.), ide, asp and batch-spawning fish, such as common tench (*Tinca tinca* (L.)), crucian carp (*Carassius carassius* (L.)) or rudd (*Scardinius erythrophthalmus* L.) (Kucharczyk 1997a,b,c; Brzuska 2005, 2006; Kujawa *et al.*, 2011; Podhorec

and Kouril, 2009; Targońska *et al.*, 2012b; Nowosad *et al.*, 2014a). Only in domesticated fish can spawn be obtained without hormonal stimulation, but this is not always the case (Krejszeff *et al.*, 2009, 2010; Targońska *et al.*, 2011a). Hormonal stimulation is applied differently in two groups of cyprinid fish (Podhorec and Kouril, 2009). In fish with one-off spawning, carp pituitary homogenate (CPH) is usually used in in-season reproduction, as well as preparations containing GnRH analogue with dopamine inhibitor (DA) or combinations of CPH with hCG (Kucharczyk *et al.*, 2005; Brzuska, 2005, 2006; Kristan *et al.*, 2013; Nowosad *et al.*, 2014b). In batch-spawning fish, like in percids (such as perch *Perca fluviatilis* L.), there can be other agents used successfully except for the preparations mentioned above, such as hCG, FSH+LH, or analogues of GnRH (Kucharczyk et al, 1998; Kujawa *et al.*, 2011; Targońska and Kucharczyk, 2011; Targońska *et al.*, 2012a). Therefore, even those preparations which do not work, or which work only to a limited extent with cyprinid fish with one spawn in the season, can be used to induce final gamete maturation in fish which spawn in portions. Since barbel is a multi-batch spawner, it should be expected that different hormonal agents will work in this species, too. These are the findings of this study in out-of-season reproduction of barbel. Ovulation was observed even in the control group in 30% of females. This was proof of an excellent reproductive protocol and the appropriate stimulation with environmental conditions (mainly the temperature of water) as well of the effect of domestication on the outcome of the species reproduction (Krejszeff et 2009; Teletchea and Fontaine, 2014). Stimulation with environmental conditions (temperature, photoperiod) in cyprinids, even those bred by humans for centuries, does not induce maturation, especially in females (Brzuska, 2005, 2006; Kucharczyk *et al.*, 2008; Targońska and Kucharczyk, 2011). Environmental stimulations in fish living in the wild sometimes does not cause a visible change in the position of nuclei in the oocytes and, even if it does, the change is only slight (Kucharczyk *et al.*, 1997a,b, 2005).

In present study, the synergistic effect of thermal stimulation and administering of hormonal agents to barbel brooders in order to induce final gamete maturation increased the ovulation rate considerably compared to the control group. The lowest ovulation rate among the study groups was observed after administering hCG. These findings are consistent with those observed in in-season barbel reproduction (Targońska *et al.*, 2011a) as well as in that of other batch-spawning cyprinids (Kucharczyk *et al.*, 1997c; Targońska and Kucharczyk, 2011; Targońska *et al.*, 2012b; Kujawa *et al.*, 2013). As in other species, embryo survival rate after this measure was applied was the highest, although it was not statistically different than observed after Ovaprim was administered. The best outcome of reproduction in benni (*Barbus sharpeyi* [Günther, 1874]), such as the percentage of ovulations and embryo hatching of 87.5% and 78.427±1.7%, was achieved by Kahkesh *et al.* (2010) by using a combination of synthetic analogues of lutenizing hormone-releasing hormone (LHRHa) and CPH. Those authors achieved ovulation in merely 37.5% of females after the administration of Ovaprim, which contains the salmon analogue of GnRH and domperidon as DA (Peter *et al.*, 1993) and usually works excellently in cyprinids. This was also confirmed in studies conducted on carp, asp and other fish species (e.g. Kucharczyk *et al.*, 2008; Targońska *et al.*, 2010). The other hormonal agents applied in out-of-season reproduction of barbel, i.e. CPH and Ovopel (which contains a mammalian analogue of GnRH and metoclopramide as DA – Horvath *et al.*, 1997) had a positive impact on final gamete maturation, the ovulation rate and the embryo survival rate until the hatching stage.

In conclusion, a combination of thermal stimulation and the application of hormonal stimulation results in a high ovulation rate and a high embryo hatching rate in common barbel. The ovulation rates and embryos survival was very high in cultured form (generation F_4) and cultured fish might be used for reproduction and stocking material production of this species. This is also conclude, that the synergistic effect of temperature regime and hormonal treatment influenced the final gametes maturation in barbel which caused by high ovulation rate and high embryos survival.

Acknowledgements

This study was partially supported by the projects: “Innovations in finfish aquaculture with special reference to reproduction” (InnovaFish), Operational Programme for Sustainable Development of the Fisheries Sector and Coastal Fishing Areas 2007-2013 (OR14-61724-OR1400003/09/10/11).

Animal Care and Use Guidelines

The experiments were performed with compliance of Local Ethical Committee in Olsztyn, Poland (No. 30/2011).

References

Anguis, V., Canavate, J.P. 2005. Spawning of captive Senegal sole (*Soleasene galensis*) under a naturally fluctuating temperature regime. Aquaculture, 243: 133–145. doi:10.1016/j.aquaculture.2004.09.026.

Baran, A. 2000. Barbel *Barbus barbus* (Linnaeus, 1758). W: M. Brylińska (Ed.), Polish freshwater fish, 186–191 Wyd. PWN, Olsztyn.

Bolland, J.D., Cowx, I.G., Lucas, M.C. 2008. Movements and habitat use of wild and stocked juvenile chub, *Leuciscus cephalus* (L.), on a small lowland river. Fisheries Management and Ecology, 15: 401–407. doi: 10.1111/j.1365-2400.2008.00631.x

Bromage, N., Porter, M., Randall, C., 2001. The environmental regulation of maturation in farmed finfish with special reference to the role of photoperiod and melatonin. Aquaculture, 197: 63– 98. doi: 10.1016/S0044-8486(01)00583-X

Brooks, S., Tyler, C.R., Sumptem, J.P. 1997. Egg quality in fish: what makes a good egg? Reviews in Fish Biology and Fisheries, 7: 387–416.

Brzuska, E., 2005. Artificial spawning of carp (*Cyprinus carpio* L.), differences between females of Polish strain 6 and Hungarian strain W treated with carp pituitary homogenate, Ovopel or Dagin. Aquaculture Research, 36: 1015–1025.

Brzuska, E. 2006. Artificial spawning of female Lithuanian strain B carp (*Cyprinus carpio* L.) after treatment with carp pituitary homogenate, Ovopel or [D–Tle^{6,} ProNHEt^{9}] LHRH–a (Lecirelin). Aquaculture Research, 37: 264–271.

Cowx, I.G. 1994. Stocking strategies. Fisheries Management and Ecology, 1: 15–30.

Hakuć–Błażowska, A., Kupren, K., Turkowski, K., Targońska, K., Jamróz, M., Krejszeff, S., Kwiatkowski, M., Żarski, D., Kucharczyk, D. 2009. Comparison of economic effectiveness of applying different hormonal preparations for reophile cyprinid fish reproduction stimulation based on the example of asp *Aspius aspius* (L.) and ide *Leuciscus idus* (L.). Polish Journal Natural Sciences, 24: 224–234. doi: 10.2478/v10020-009-0021-y

Hilder, M.L., and Pankhurst, W. 2003. Evidence that temperature change cues reproductive development in the spiny damselfish, *Acanthochromis polycanthus*. Environmental Biology of Fishes, 66: 187–196.

Horvath, L., Szabo, T., Burke, J. 1997. Hatchery testing of GnRH analogue-containing pellets on ovulation in four cyprinid species. Polskie Archiwum Hydrobiologii, 44: 221–226.

Kahkesh, F.B., Feshalami, M.Y., Amiri, F., Nickpey, M. 2010. Effect of Ovaprim, Ovatide, HCG, LHRH-A2, LHRHA2+CPE and carp pituitary in benni (*Barbus sharpeyi*) artificial breeding. Global Veterinaria, 5(4): 209– 214.

Krejszeff, S., Targońska, K., Żarski, D., Kucharczyk, D. 2009. Domestication affects spawning of the ide (*Leuciscus idus*) – preliminary study. Aquaculture, 295: 145–147. doi:10.1016/j.aquaculture.2009.06.032

Krejszeff, S., Targońska, K., Żarski, D., Kucharczyk, D. 2010. Comparison of artificial reproduction of two different spawn-forms of the chub. Aquaculture Research, 10: 67–74. doi:10.1016/S1642-431X(12)60038-2

Kristan, J., Alavi, S.M.H., Stejskal, V., Policar, T. 2013. Hormonal induction of ovulation in pikeperch (*Sander lucioperca* L.) using human chorionic gonadotropin (hCG) and mammalian GnRH analogue. Aquaculture International, 21: 811–818. doi: 10.1007/s10499-012-9572-y

Król, R., Targońska, K., Nowosad, J., Kujawa, R., Kupren, K., Mamcarz, A., Łuczyńska, J., Kaszubowski, R., Czarkowski, T.K., Furgała-Selezniow, G., Kucharczyk, D. 2014. Effect of environmental conditions and feeding on the effectiveness of ide *Leuciscus idus* (L.) reproduction. Wulfenia Journal, 21: 125 – 135.

Kucharczyk, D., Kujawa, R., Mamcarz, A., Wyszomirska, E. 1997a. Artificial spawning in bream (*Abramis brama* L.). Polskie Archiwum Hydrobiologii, 44: 203–207.

Kucharczyk, D., Kujawa, R., Łuczynski, M., Glogowski, J., Babiak, I., Wyszomirska, E., 1997b. Induced spawning in bream, *Abramis brama* (L.), using carp and bream pituitary extract and hCG. Aquaculture Research, 28: 139–144.

Kucharczyk, D., Kujawa, R., Mamcarz, A., Wyszomirska, E. 1997c. Induced spawning in rudd (*Scardinius erythrophthalmus* L.). Polskie Archiwum Hydrobiologii, 44: 207–211.

Kucharczyk, D, Kujawa, R., Mamcarz, A., Skrzypczak, A., Wyszomirska, E. 1998. Induced spawning in perch, *Perca fluviatilis* L., using FSH + LH with pimozide or metoclopramide. Aquaculture Research, 29: 131–136.

Kucharczyk, D., Kujawa, R., Mamcarz, A., Targońska-Dietrich, K., Wyszomirska, E., Glogowski, J., Babiak, I., Szabo, T. 2005. Induced spawning in bream (*Abramis brama* L.) using pellets containing LHRH. Czech Journal Animal Science, 50: 89–95.

Kucharczyk, D., Targońska, K., Hliwa, P., Gomułka, P., Kwiatkowski, M., Krejszeff, S., Perkowski, J. 2008. Reproductive parameters of common carp (*Cyprinus carpio* L.) spawners during natural season and out–of–season spawning. Reproductive Biology, 8: 285–289. doi:10.1016/S1642-431X(12)60018-7

Kucharczyk, D., Nowosad, J., Biłas, M., Stępień, P., Łuczyńska, J. 2014. The effect of feed enrichment on the fatty acid composition of body in model cyprinid rosy barb (*Puntius conchonius*). Pensee Journal, 76: 118 – 126.

Kujawa, R., Kucharczyk, D., Mamcarz, A. 1999. A model system for keeping spawners of wild and domestic fish before artificial spawning. Aquacultural Engineering, 20: 85–89.

Kujawa, R., and Glińska-Lewczuk, K. 2011. The impact of regulation and hydrotechnical works in river channels on the rheophilic Cyprinidae fish. Contemporary Problems of Management and Environmental Protection, 7: 233–248.

Kujawa, R., Kucharczyk, D., Mamcarz, A., Żarski, D., Targońska, K. 2011. Artificial spawning of common tench *Tinca tinca* (Linnaeus, 1758), obtained from wild and domestic stocks. Aquaculture International, 19: 513–521. doi: 10.1007/s10499-010-9366-z

Kujawa, R., Kucharczyk, D., Mamcarz, A., Furgała-Selezniow, G., Glińska-Lewczuk, K. 2013. Possibility of artificial reproduction of sichel *Pelecuscult ratus* (L.) under controlled conditions. Wulfenia Journal, 20: 82–91.

Kujawa, R., Furgała-Selezniow, G., Mamcarz, A., Lach, M., Kucharczyk, D. 2015. Influence of temperature on the growth and survivability of sichel larvae *Pelecus cultratus* reared under controlled conditions. Ichthyological Research, 62: 163 – 170. doi: 10.1007/s10228-014-0419-6

Kupren, K., Turkowski, K., Kucharczyk, D., Krejszeff, S., Żarski, D., Hakuć–Błażowska, A., Targońska, K., Kwiatkowski, M., Jamróz, M., Czarkowski, T. 2008. Economic aspects of rearing larval asp, *Aspius aspius* (L.), and ide, *Leuciscus idus* (L.), in closed recirculating systems. Archives of Polish Fisheries, 16: 413–420.

Moran, R., Harvey, I., Moss, B., Feuchtmayr, H., Hatton, K., Heyes, T., Atkinson, D. 2009. Influence of

simulated climate change and eutrophication on three-spined stickleback populations: a large scale mesocosm experiment. Freshwater Biology, 55: 315–325.

Nowosad, J., Targońska, K., Chwaluczyk, R., Kaszubowski, R., Kucharczyk, D. 2014a. Effect of temperature on the effectiveness of artificial reproduction of dace *[Cyprinidae (Leuciscus leuciscus (L.))]* under laboratory and field conditions. Journal of Thermal Biology, 45: 62 – 68. doi: 10.1016/j.jtherbio.2014.07.011.

Nowosad, J., Kucharczyk, D., Czarkowski, T.K., Kwasek, K. 2014b. Changes in body weight and eye size in female European eel kept in fresh and salt water. Italian Journal of Animal Science, 13: 382–386.

Nowosad, J., Kucharczyk, D., Łuczyńska, J., Targońska, K., Czarkowski, T.K., Biłas, M., Krejszeff, S., Horvath, L., Muller, T. 2015. Changes in European eel ovary development and body and ovary chemistry during stimulated maturation under controlled conditions: preliminary data. Aquaculure International, 23, 13 – 27. doi: 10.1007/s10499-014-9794-2

Peter, R.E., Lin, H.R., van der Kraak, G., Little, M. 1993. Releasing hormones, dopamine antagonists and induced spawning. In: J.P. Muir, P. Roberts, Recent Advances in Aquaculture vol. IV. Institute of Aquaculture, Blackwell Scientific Publications, Oxford: 25–30.

Podhorec, P., and Kouril, J. 2009. Induction of final oocyte maturation in *Cyprinidae* fish by hypothalamic factors: a review. Veterinarni Medicina, 54: 97 – 110.

Policar, T., Kozák, P., Hamáčková, J., Lepičová, A., Musil, J., Kouřil, J. 2007. Effects of short-time *Artemia* spp. feeding in larvae and different rearing environments in juveniles of common barbel (*Barbus barbus*)on their growth and survival under intensive controlled conditions. Aquatic Living Resources, 20: 175 –183. doi: http://dx.doi.org/10.1051/alr:2007029

Rijnsdorp, A.D., Peck, M.A., Engelhard, G.H., Möllmann, C., Pinnegar, J.K. 2009. Resolving the effect of climate change on fish populations. ICES Journal of Marine Science, 66(7):1570–1583.

Ross, L.G., Palacios, C.A.M., Morales, E.J. 2008. Developing native fish species for aquaculture: the interacting demands of biodiversity, sustainable aquaculture and livelihoods. Aquaculture Research, 39: 675 – 683. doi: 10.1111/j.1365-2109.2008.01920.x

Szczerbowski, A., Kucharczyk, D., Mamcarz, A., Łuczyński, M.J., Targońska, K., Kujawa, R. 2009. Artificial off-season spawning of Eurasian perch *Perca fluviatilis* L. Archives of Polish Fisheries, 17: 95–98.

Targońska-Dietrich, K., Zielazny, T., Kucharczyk, D., Mamcarz, A., Kujawa, R. 2004. Out-of-season spawning of cultured ide (*Leuciscus idus* L.) under controlled conditions. EJPAU 7(2) #02. http://www.ejpau.media.pl/volume7/issue2/fisheries/art-02.html ISSN 1505-0297

Targońska, K., Kucharczyk, D., Kujawa, R., Mamcarz, A., Żarski, D. 2010. Controlled reproduction of asp, *Aspius aspius* (L.) using luteinizing hormone releasing hormone (LHRH) analogues with dopamine inhibitors. Aquaculture, 306: 407–410. doi:10.1016/j.aquaculture.2010.05.027

Targońska, K., and Kucharczyk, D. 2011. The application of hCG, CPH and Ovopel in successful artificial reproduction of goldfish (*Carassius auratus auratus*) under controlled conditions. Reproduction in Domestic Animals, 46(4): 651–655. doi: 10.1111/j.1439-0531.2010.01723.x

Targońska, K., Kupren, K., Żarski, D., Król, R., Kucharczyk, D. 2011a. Influence of thermal conditions on successful ide (*Leuciscus idus* L.) artificial reproduction during spawning season. Italian Journal of Animal Science, 10:e50: 209–212. doi: 10.4081/ijas.2011.e50

Targońska, K., Kucharczyk, D., Żarski, D., Cejko, B., Krejszeff, S., Kupren, K., Król, R., Dryl, K., Kowalski, R., Glogowski, J. 2011b. Artificial reproduction of wild and cultured barbel (*Barbus barbus, Cyprinidae*) under controlled conditions. Acta Veterinaria Hungarica, 59: 363 – 372. doi: 10.1556/AVet.2011.021.

Targońska, T., Żarski, D., Krejszeff, S., Kucharczyk, D. 2012a. Influence of age of wild ide *Leuciscus idus* (L.) females on spawning effectiveness under controlled conditions. Italian Journal of Animal Science, 11: 342–346. doi: 10.4081/ijas.2012.e63

Targońska, K., Perkowski, T., Żarski, D., Krejszeff, S., Mamcarz, A., Kujawa, R., Kucharczyk, D. 2012b. Method of evaluation of wild common tench, *Tinca tinca* (L.), female suitability for artificial reproduction during the spawning season. Italian Journal of Animal Science, 11: 164–168. doi: http://dx.doi.org/10.4081/ijas.2012.e30

Targońska, K., Żarski, D., Kupren, K., Palińska-Zarska, K., Mamcarz, A., Kujawa, R., Skrzypczak, A., Furgała-Selezniow, G., Czarkowski, T.K., Hakuć-Błazowska, A., Kucharczyk, D. 2014a. Influence of temperature during four following spawning seasons on the spawning effectiveness of common bream, *Abramis brama* (L.) under natural and controlled conditions. Journal of Thermal Biology, 39: 17–23. doi:10.1016/j.jtherbio.2013.11.005

Targońska, K., Szczerbowski, A., Żarski, D., Łuczyński, M.J., Szkudlarek, M., Gomułka, P., Kucharczyk, D. 2014b. Comparison of different spawning agents in artificial out-of-season spawning of Eurasian perch, *Perca fluviatilis* L. Aquaculture Research, 45: 765–767. doi: 10.1111/are.12010

Teletchea, F., and Fontaine, P. 2014. Levels of domestication in fish: implications for the

sustainable future of aquaculture. Fish and Fisheries, 15: 181–195. doi: 10.1111/faf.12006

Wang, N., Teletchea, F., Kestemont, P., Milla, S., Fontaine, P. 2010. Photothermal control of the reproductive cycle intemperate fishes. Reviews in Aquaculture, 2: 209–222.

Wojda, R. 2004. Produkcja materiału zarybieniowego ryb reofilnych w Polsce w latach 1995–2002, możliwości i potrzeby jej dalszego zwiększania. Archives of Polish Fisheries, 12: 359–369.

Yaron, Z. 1995. Endocrine control of gametogenesis and spawning induction in the carp. Aquaculture, 129: 49–73.