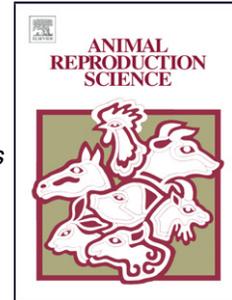


Journal Pre-proof

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PII: S0378-4320(19)30902-9

DOI: <https://doi.org/10.1016/j.anireprosci.2019.106222>

Reference: ANIREP 106222

To appear in: *Animal Reproduction Science*

Received Date: 23 September 2019

Revised Date: 13 October 2019

Accepted Date: 26 October 2019

Please cite this article as: Kucharczyk D, Kucharczyk DJ, Nowosad J, Omirzhanova N, Optimization of artificial insemination outcomes of African catfish (*Clarias gariepinus*) with differing hatchery conditions, *Animal Reproduction Science* (2019), doi: <https://doi.org/10.1016/j.anireprosci.2019.106222>

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**Optimization of artificial insemination outcomes of African catfish (*Clarias gariepinus*)
with differing hatchery conditions**

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ABSTRACT

The African catfish is one of the most promising warm-freshwater species for aquaculture development in the upcoming years, although there are two primary limitations in its production: less-than-desirable survival rates and low larvae quality. In this study, a novel method of egg fertilization for this species was evaluated which has already been successfully utilized in the production of other finfish. The results indicate that dividing the semen designated for fertilization of eggs into smaller aliquots and adding it to the eggs at 30s intervals (i.e., 0, 30 and 60s), after activation of the eggs with water, increased the hatching rate percentage (97.1%) compared to control groups (87.9%) in which the entire portion of semen was added to eggs at the time of water addition for egg activation. Results with repeated evaluations in the field confirmed that eggs with greater biological quality were fertilized at a similar rate using the modified and control methods for fertilization, although when the eggs were of a lesser biological quality, the fertilization rate was greater using the modified methods than the control methods.

Keywords: *In vitro* fertilization; New methods, Larvae, Freshwater

1. Introduction

With the growth of the human population, there is a constantly increasing demand for food, including protein. One of the possible sources of easily digestible protein are fish and other seafood. Because water resources for procuring seafood are currently exploited to the maximum, the supply of more fish to the market requires intensive culture for the production of seafood (Teletchea and Fontaine, 2014). For this reason, total aquaculture production has developed very rapidly: from 1 million metric tons in 1950 to 80 million metric tons in 2016 (FAO, 2018). Because the global production of some species has reached its maximum, there

has been increasing interest in different finfish species for aquaculture (Kristan et al., 2018; Kucharczyk et al., 2018; Teletchea, 2019). Of particular interest are species of fish, both freshwater and saltwater, which can be bred in confined conditions (i.e., the African catfish; *Clarias gariepinus*). The global production of this species increased from about 5,000 metric tons in 1992 to 248,000 metric tons in 2015 (FAO, 2019). Considering the relatively lesser production costs when this species is used for aquaculture than with many other fish species, as well as lesser environmental control requirements for breeding and the excellent quality of the meat as a protein source, African catfish production is a viable aquaculture endeavor. Further intensive production growth should be expected, particularly considering African catfish are a warm water species, which can be successfully cultured on a mass scale on different continents (e.g., Africa, Asia and Europe; Olaniyi and Omitogun, 2013; Bhattacharya et al., 2018; Müller et al., 2018) and there is also the possibility of larvae production throughout the year.

In aquaculture, reproductive efficiency is one of the limiting factors of fish meat production. Obtaining larvae of acceptable quality when attempting to produce viable quality gametes for aquaculture purposes is a particularly difficult task (Cejko et al., 2013; Szabo et al., 2015; Kristan et al., 2018). For this reason, there has been considerable emphasis on developing reproductive protocols for using biotechnologies in reproduction (Kucharczyk et al., 1997; 2019), gamete collection, collecting high quality gametes, short-term gamete storage (Kucharczyk et al. 2018) and fertilization (Cejko et al. 2016; Kristan et al., 2018; Müller et al., 2018). This is especially important, if non-domesticated- or cultured- (semi-domesticated or domesticated) spawning specimens are used for meat production (Kujawa et al., 2011; Targońska et al., 2011; Nowosad et al. 2017). In many cases, especially in species that have a relatively greater fecundity, there is often a limited number of spawning specimens used. In such situations, precisely-developed protocols for artificial reproduction, that when used, result in desirable egg fertilization percentages are necessary (Szabo et al., 2010; Kucharczyk et al.,

2016; Müller et al. 2019). The success of finfish artificial reproduction depends on many factors (i.e., genetic origin of spawning specimens, temperature and fluctuations in temperature, salinity and breeder feeding regimens; Nowosad et al., 2014, 2018) and all of the factors that affect the survivability of larvae need to be precisely controlled (Kupren et al., 2011).

The production of catfish stocks for aquaculture depends strictly on the quality of gametes and, consequently, on larvae quality. For this reason, different techniques of artificial reproduction, including assessments of different spawning agents (Brzuska, 2004; Brzuska et al., 2004; Mansour et al., 2004; Karami et al., 2011; Sharaf, 2012; Bhattacharya et al., 2018; Müller et al., 2018) and conditions for rearing larvae (Olaniyi and Omitogun, 2013) have been evaluated. The further development of methods for obtaining viable gametes and the short- and long-term storage conditions might also be useful for the production of “special lines” or different kinds of catfish hybrids for aquaculture purposes (Mansour et al., 2004; Szabo et al., 2010; Okomoda et al., 2017; Müller et al., 2018; 2019). In many finfish species, gametes are collected separately without contact with water or urine (Kupren et al., 2011; Cejko et al., 2016). This method is usually called the “dry” method of fertilization, which involves the placement of gametes together in a vessel with an addition of water or activation solution. In some freshwater finfish species, there is the possibility of fertilizing oocytes within a few minutes (e.g., about 3 minutes) after egg activation (e.g., Eurasian perch - *Perca fluviatilis*, common ide - *Leuciscus idus*, asp - *Leuciscus aspius* and burbot - *Lota lota*; (Żarski et al., 2012; Kucharczyk et al., 2016). In some other species, progressive motility of spermatozoa when placed in the water is not induced until about 60 minutes (range 20 to 60 s) after the placement of sperm in water or an activation induction solution. This is the situation with the common carp (*Cyprinus carpio*), crucian carp (*Carassius carassius*) and African catfish (Biegniewska et al., 2010; Cejko et al., 2013; 2016; Bozkurt and Yavas, 2017). The development of protocols to increase the fertilization rate, enhance development of embryos and improve the production of viable

larvae are extremely important for large-scale mass fish production (Kucharczyk et al., 2018). There has been recent progress in developing aquaculture conditions for African catfish, especially in the field of artificial reproduction (Brzuska, 2004; Brzuska et al., 2004; Sharaf, 2012; Müller et al., 2018), gamete management and cryopreservation (Viveiros et al., 2000; Mansour et al., 2004; Samarin et al., 2018; Müller et al., 2019) and biotechnological progress (Karami et al., 2011; Okomoda et al., 2017; Müller et al., 2018), although there have not been any studies conducted that relate to the optimization of the *in vitro* fertilization process in this species. For this reason, the aim of this study was to optimize the fertilization of African catfish eggs in laboratory conditions and to assess the effectiveness of this method for the production of African catfish in field conditions.

2. Material and methods

2.1. Broodstock and artificial reproduction

The research was conducted in accordance with the permission of the local ethics committee for animal experiments (27/2010N for years 2010–2015).

The African catfish broodstock was cultured in the Department of Lake and River Fisheries, University of Warmia and Mazury in Olsztyn, Poland (northern Poland). The spawning specimens originated from two African catfish farms: Przęsin Fish Farm (north-western Poland) and Jurki Fish Farm (north-eastern Poland). The fish were maintained in 1 m³ tanks which were a component of an RAS system (Kujawa et al., 1999). The water temperature was constant at 24.5 °C. Fish were fed dry feed with a protein content of 40% to 45% daily in amounts equivalent to 1% to 2% of the total body biomass. The fish were not fed for three days before imposing the reproduction induction treatment regimen. On the day before gamete collection, the water temperature was increased to 25 °C and 12 hours before the planned time of spawning induction the breeding specimens of both sexes were treated with Ovaprim

(Syndel, Canada) in a single dose of 0.5 mL/kg (Karami et al., 2011; Sharaf, 2012). The spawning specimens were captured for obtaining gametes 12 hours after the Ovaprim injection. All manipulations with fish occurred after anesthesia induction (0.15 g/L for females) or euthanasia (males for testis collection: 0.25 g/L) with MS-222 (Finquel, USA).

2.2. Collection of gametes

Eggs were collected separately from each female and placed in plastic bowls. For milt collection, the males were euthanized and the testis were collected as whole organs after dissection. There was an incision of the testis and semen was squeezed from the testes into small plastic containers (Brzuska et al., 2004; Sharaf, 2012). The spermatozoa motility were assessed using the method described by Viveiros et al. (2000). Only sperm with a motility of 90% or greater was used for fertilization of the eggs. The gametes were stored at room temperature ($\approx 22\text{ }^{\circ}\text{C}$) before activation for a maximum of 1 hour (Viveiros et al., 2000; Samarin et al., 2018).

2.3. Experimental design

The eggs from one female were used in conducting these experiments. Three separate experiments were conducted. In the first experiment, the eggs were mixed with sperm at different times subsequent to egg activation by placement of eggs in water: (0 s; control group – “C”) and after a delay of 30, 60 and 90 s (Groups A-30, A-60 and A-90). About 160 eggs were fertilized with 50 μL of pooled sperm added at one time to the vessel containing the eggs. In the second experiment, eggs were mixed with sperm at the time water was added for egg activation (0 s; control groups – “C”) and at: 0 and 30 s (B-0-30), 0 and 60 s (B-0-60) and 0 and 90 s (B-0-90) after activation of eggs by placement in water. In the latter three groups, the sperm were divided into two equal aliquots of 25 μL each, whereas in Group C the sperm were all

added at one time. In the third experiment, the sperm were divided into three (17 μL each) or four (12.5 μL each) aliquots and the eggs were placed with sperm at 0, 30 and 60 s (D-0-30-60), at 0, 30 and 90 s (D-0-30-90) and at 0, 30, 60 and 90 s (D-0-30-60-90) after activation of eggs by placement in water. The results were compared to the control group in which eggs were placed with sperm at the time of water addition for egg activation (control group – “C”).

The water used for oocyte activation (10 mL) had the following characteristics: conductivity (25 °C): 516; pH: 7.43; total water hardness: 255 mg/L CaCO_3 ; Na^+ : 10.8 ppm; K^+ : 4.9 ppm; Fe: 12 $\mu\text{g/L}$; Mn: 26 $\mu\text{g/L}$. The same water was used for egg activation in other freshwater finfish species (e.g., ide, asp, burbot; Kucharczyk et al. 2016, 2018). After adding the water for egg activation, the water was stirred for 120 s. The water was then changed and the eggs were transferred to 100 mL cylindrical plastic containers with a lid and mesh sides (200 μm), which ensured the free flow of water through the containers. The marked containers were then placed in the tanks. All treatments were conducted in triplicate.

2.4. Incubation and hatching

Eggs were incubated in marked containers which were placed in shallow pools that were part of an RAS (Sikora et al., 2018). The water temperature during incubation was 25 °C. After hatching, the larvae and dead eggs were transferred from each incubator to large Petri dishes for counting. Hatched larvae were divided into normal and abnormal development specimens. The hatched larvae without visible deformities, as compared with the total number of eggs used for fertilization, were used to determine the percentage hatching rate (Nowosad et al., 2018; Samarin et al., 2018).

2.5. Field application of the method

The field experiment was conducted at two African catfish farms using the methods previously described in this manuscript. For each experiment, there were about 100,000 eggs used for each replicate. Twelve groups were established: a control group with about 100,000 eggs being mixed with 5 mL of spermatozoa and 500 mL of tap (hatchery) water being added at “zero” time; and groups fertilized using the modified methods with about 100,000 eggs mixed with 5 mL spermatozoa divided into three equal aliquots and 500 mL of tap (hatchery) water added at 0, 30 and 60 s after egg activation by placement in water. The eggs were mixed with water until there was egg “stickiness” detected. The eggs were then placed in a tannin solution (0.5 ppt) two times for a total time of about 60 s.

The other aspects of the procedure were the same as previously described in this manuscript, except the egg incubation was conducted in 7 L Weiss jars as a part of a closed system (re-circulating aquaculture system), in which water is re-conditioned and re-used. Shortly before hatching, three samples of about 300 eggs per each replication were transferred to Petri dishes. The hatched larvae without any visible deformities, as compared with the number of eggs placed in vessels for fertilization, were used to determine the percentage hatching rate (Nowosad et al., 2018; Samarin et al., 2018).

2.6. Statistical analysis.

All of the data are presented in means \pm SD and were expressed in percentages. Arc-sine transformation of the data was conducted before the statistical analyses were conducted. The data regarding the survival rate of embryos (hatching rate) were analyzed using a one-way analysis of variance (ANOVA) and subjected to the Tukey’s *post hoc* test at a significance level of 5% ($P = 0.05$) for laboratory experiments. For field experiments, the survival rate of embryos (hatching rate) was analyzed using a one-way analysis of variance (ANOVA) and subjected to

a t-test at a significance level of 5% ($P = 0.05$). The statistical analyses were performed using STATISTICA 9.1 (StatSoft, Inc., USA).

3. Results

The biological quality of eggs in the laboratory study was considered to be very good and 87.9% of hatched larvae were without developmental anomalies in the control group. In the field test, the egg quality varied from 11.2% to 92.3% with an average of 71.5%. The hatching rate of African catfish embryos was less for longer periods from egg activation to the addition of the spermatozoa to the vessel containing the oocytes (Fig. 1). The greatest hatching rate was noted when the oocytes and spermatozoa were added at the same time in the water (time of activation – 0 s). A delay of 15 s and 30 s in adding spermatozoa to oocytes activated by water resulted in hatching rates of 71.2% and 45.7%, respectively. There was a hatching rate of 7.9% in the group in which spermatozoa were added 90 s after adding water for activation.

In the second experiment, the greatest hatching rates were in groups in which sperm was divided into two groups and were added 0 s and 30 s or 0 s and 60 s after adding water for oocyte activation (Fig. 2). In all three groups in which there were additions of sperm two times, the hatching rate was greater than in the control group (in which the sperm was added only once).

In the third experiment, where sperm were divided into three or four aliquots, the hatching rate in the treated groups was markedly greater than in the control group (Fig. 3). The greatest hatching rate was in the group in which sperm were added to the water containing the oocytes four times (0, 30, 60 and 90 s) after the addition of water for oocyte activation.

In the field test, the differences were compared between the hatching rates between using the fertilization method that is currently used commercially to the hatching rates for the modified method by dividing the semen into three samples and adding it to a vessel containing

eggs at 0 s, 30 s and 60 s after adding water for egg activation (Fig. 4). In eight of 12 samples, the hatching rate was greater using the modified method. In both cases, there were no differences in the hatching percentage between the control and modified groups with hatching percentages in the control group being greater than 92%.

4. Discussion

In the present study, a method was developed, for the first time, for optimizing fertilization in African catfish that had a positive effect on the survival of embryos and hatching percentage. The African catfish is a species in which males are sacrificed during artificial reproduction (Szabo et al., 2010; Müller et al., 2018; 2019; Samarin et al., 2018) because sperm cannot be obtained from the males of this species by stripping in field conditions (Van der Waal and Polling, 1985; Viveiros et al., 2002; 2003). This, however, results in losses of males from the spawning stock populations. For this reason, as well as the relatively greater fecundity of the females of this species compared with many other fish species, the number of spawning specimens used for a single spawning in a fish farm is usually very limited (e.g., one female and one male). The quality of the gametes and the fertilization rate are as important as in other species in which a limited number of spawning specimens are used for artificial reproduction (Kucharczyk et al., 2016; Müller et al., 2018; Samarin et al., 2018).

The biological quality of eggs is determined by the percentage of developing embryos or the percentage of hatched larvae with typical morphology, and is often used in both research and aquaculture production to estimate reproductive efficiency when there is use of artificial reproduction practices (Kucharczyk et al., 1997; 2019; Kujawa et al., 2011; Blecha et al., 2018; Kristan et al., 2018). For some species (e.g., zander or perch), methods have been developed to verify the egg quality before the eggs are fertilized (Żarski et al., 2011; 2012), but for many other species, including African catfish, there are no such methods available. The quality of

African catfish eggs, therefore, can only be ascertained after fertilization. In this species, many factors affect the biological quality of eggs, including environmental conditions or hormonal agents administered to induce the final stages of gamete maturation and ovulation (Brzuska, 2004; Brzuska et al., 2004). For this reason, Ovaprim is generally used to induce ovulation in African catfish because the eggs that develop with its use are generally of excellent biological quality (Karami et al., 2011; Sharaf et al., 2012). The findings in previous studies in this regard were similar to those in the present studies (in ten of 14 treatment groups the spawning percentage of hatched larvae was greater than 70%).

Fish sperm usually have a rather short period of motility after activation before a loss of viability. After contact with water or an activating fluid, the sperm generally lose the capacity for motility and for fertilization of eggs within seconds (Cejko et al., 2013; 2016). Biegniewska et al. (2010) reported that sperm of African catfish lose the capacity for motility in a very short period of time after activation. The period for which the capacity for motility is retained, however, is in contrast to length of the time during which fertilization can continue to occur after the time of egg activation. In some species, it takes as long as 180 s from the time of egg activation to fertilization, during which time there is no decrease in the percentage of fertilized eggs (Żarski et al., 2012; Kucharczyk et al. 2016). The situation in African catfish is somewhat different. If fertilization in this species has not occurred within 90 s after egg activation, the percentage of hatched larvae is about ten times less than if sperm and eggs are placed in the same vessel at the time of sperm and egg activation (Fig. 1). This indicates that the process of closing the micropyle in oocytes occurs more rapidly than for several other species (e.g., ide, asp, burbot; Kucharczyk et al., 2016).

Extending the time of contact between sperm and eggs by dividing semen into aliquots and adding aliquots of sperm at certain times after egg activation in some fish species has a positive effect on fertilization efficiency (Kucharczyk et al., 2016). There were similar results

in the current study, where the division of semen into several aliquots and adding the sperm to the container with the eggs at varying times after water was added for egg activation. Thus, the hatching percentage with the modified methods was greater than with the traditional method used for fertilization of African catfish eggs (Figs. 2 and 3). Using the same amount of sperm (with the last of the three or four additions occurring at 90 s after water was added for egg activation) increased the hatching rate by 9.2% compared with the control group and there was an excellent hatching rate (97.1%).

In African catfish, as well as other finfish, the percentage of fertilized eggs or hatched larvae varies and depends on many factors. These include the origin of the fish, type of stimulation with environmental conditions and hormonal agents, having a proper gonadal harvest time and storage conditions, feeding management of the spawning specimens and many other factors (Nowosad et al., 2014; 2016; 2017; Kristan et al., 2018). For example, in studies conducted with African catfish (Brzuska, 2004; Brzuska et al., 2004), the percentage of developing embryos was less than 60%. In a study conducted by Müller et al. (2019), there was an average survival rate of 61% (range 43.7%-72.4%), however, in a study of Samarin et al. (2018) it was 81% to 88%. This variability was confirmed in the control groups of field experiments in the current study: 11.2% to 92.3% with an average of 71.5% (Fig. 4). In experimental groups where there was fertilization of eggs using the modified method, the values were greater (+7.2%) for hatching percentage (average 78.7%). Furthermore, when there was a lesser biological quality of the eggs produced, there were greater relative differences between the groups in which the modified and control methods were used for fertilization in the field portion of the research of the present study.

5. Conclusions

Modifying the fertilization method in African catfish increases the percentage of larvae hatching. This method involves dividing the semen used for insemination into smaller aliquots and adding it to eggs within 90 s after adding water for egg activation. Field assessments confirmed the effectiveness of this method. Furthermore, when eggs were of a lesser biological quality, the relative effectiveness of the modified method for larvae production as compared with the conventional method was greater than when the eggs were of greater biological quality.

Conflicts of interest

The authors have no conflict of interest to declare.

Acknowledgements

The authors thank the African catfish fish-farmers: Rafał Sztobnicki and Rafał Stanisławski for help during field experiments. This study was financed by UWM Olsztyn, project No. 18.610.005-110. The project financially supported by Minister of Science and Higher Education in the range of the program entitled "Regional Initiative of Excellence" for the years 2019-2022, Project No. 010/RID/2018/19, amount of funding 12.000.000 PLN.

References

- Bhattacharya, D., Sarkar, S., Juin, S.K., Nath, P., 2018. Induction of fertilizable eggs by conspecific vitellogenin implantation in captive female walking catfish, *Clarias batrachus* (Linn.). *Aquacult. Res.* 49, 3167–3175.
- Biegnewska, A., Zietara, M.S., Rurangwa, E., Ollevier, F., J. Swierczynski, J., Skorkowski, E.F., 2010. Some differences between carp (*Cyprinus carpio*) and African catfish (*Clarias gariepinus*) spermatozoa motility. *J. Appl. Ichthyol.* 26, 674–677.
- Blecha, M., Dzyuba, B., Boryshpolets, S., Horokhovatskyi, Y., Dadras, H., Malinovskyi, O., Sampelsa, S., Policar, T., 2018. Spermatozoa quality and sperm lipid composition in intensively cultured and wild burbot (*Lota lota*). *Anim. Reproduct. Sci.* 198, 129–136.
- Bozkurt, Y., Yavas, I., 2017. Effect of extender compositions, glycerol levels, and thawing rates on motility and fertility of cryopreserved wild African catfish (*Clarias gariepinus*) sperm. *Israel. J. Aquacult. – Bamidgah* 69, 1357.
- Brzuska, E., 2004. Artificial propagation of African catfish (*Clarias gariepinus*): the application of a single dose of pellets containing D-Ala6, Pro9NEt-mGnRH and dopamine inhibitor metoclopramide. *Czech J. Anim. Sci.*, 49(7), 289–296.
- Brzuska, E., Kouril, J., Adamek, J., Stupka Z., Bekh, V., 2004. The application of [D-Tle6, ProNHet9]mGnRH (Lecirelin) with the dopaminergic inhibitor metoclopramide to stimulate ovulation in African catfish (*Clarias gariepinus*). *Czech J. Anim. Sci.*, 49(7), 297–306.
- Cejko B.I., Żarski D., Krejszeff S., Kucharczyk D., Kowalski R.K., 2013. Effect of hormonal stimulation on milt volume, number of sperm and sperm motility in the crucian carp, *Carassius carassius* (L.) *Isr. J. Aquacult. – Bamidgah* 65.2013.912, 7 pages.

- Cejko B.I., Słowińska M., Judycka S., Kowalski R.K., 2016. Substrate specificity of proteolytic activity in the testes fluid and seminal plasma of the common carp, *Cyprinus carpio* L. J. Fish Biol. 88, 1904–1917.
- FAO. 2018. The State of World Fisheries and Aquaculture 2018 - Meeting the sustainable development goals. Rome. Licence: CC BY-NC-SA 3.0 IGO.
- FAO. 2019. Fisheries & Aquaculture - Cultured Aquatic Species Information Programme - *Clarias gariepinus* (Burchell, 1822). 13pp. 21.06.2019: http://www.fao.org/fishery/culturedspecies/Clarias_gariepinus/en
- Karami, A., Christianus, A., Zokaefar, H., Saad, K.Z., Imraan, F.T.J., Shakibazadeh, S., Negarestan, H., Courtenay, S.C., 2011. Ovaprim treatment promotes oocyte development and milt fertilization rate in diploid and triploid African catfish (*Clarias gariepinus*). Aquacult. Int. 19, 1025–1034.
- Kristan, J., Źarski, D., Blecha, M., Policar, T., Malinovskyi, O., Samarin, A.M., Palinska-Zarska, K., Nowosad, J., Krejszeff, S., Kucharczyk, D., 2018. Fertilizing ability of gametes at different post-activation times and the sperm–oocyte ratio in the artificial reproduction of pikeperch *Sander lucioperca*. Aquacult. Res. 49(4), 1383–1388.
- Kucharczyk, D., Kujawa, R., Mamcarz, A., Wyszomirska, E., 1997. Induced spawning in rudd (*Scardinius erythrophthalmus* L.). Pol. Arch. Hydrobiol. 44(1–2), 209–213.
- Kucharczyk, D., Nowosad, J., Łuczyński, M.J., Targońska, K. 2016. New technique for fertilizing eggs of burbot, asp and ide under hatchery conditions. Anim. Reprod. Sci. 172, 143–147.
- Kucharczyk, D., Nowosad, J., Kujawa, R., Dietrich, G., Biegaj, M., Sikora, M., Luczynski, M.J. 2018. Comparison of spontaneous and hormone-induced reproduction of burbot (*Lota lota* L.) under hatchery conditions. Aquaculture 485, 25–29.

- Kucharczyk, D., Nowosad, J., Kucharczyk, D.J., Kupren, K., Targońska, K., Wyszomirska, E., Kujawa, R., 2019. Out-of-season artificial reproduction of common dace (*Leuciscus leuciscus* L.) under controlled conditions. *Anim. Reprod. Sci.* 202, 21–25.
- 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. *Aquacult. Int.* 19 (3), 513–521.
- Kupren, K., Mamcarz, A., Kucharczyk, D., 2011. Effect of variable and constant thermal conditions on embryonic and early larval development of fish from the genus *Leuciscus* (Cyprinidae, Teleostei). *Czech J. Anim. Sci.* 56(2), 70-80.
- Mansour, N., Lahnsteiner, F., Patzner, R.A., 2004. Seminal vesicle secretion of African catfish, its composition, its behaviour in water and saline solutions and its influence on gamete fertilizability. *J. Exp. Zool.* 301A, 745–755.
- Müller, T., Kucska, B., Horvath, L., Ittzes, A., Urbanyi, B., Blake, C., Guti, C., Csorbai, B., Kovacs, B., Szabo, T., 2018. Successful, induced propagation of African catfish (*Clarias gariepinus*) by ovarian lavage with sperm and hormone mixture. *Aquaculture* 485(2), 197–200.
- Müller, T., Szabo, T., Kollar, T., Csorbai, B., Marinovic Z., Horvath, L., Kucska B., Bodnar, A., Urbanyi, B., Horvath, A., 2019. Artificial insemination of African catfish (*Clarias gariepinus*) using cryopreserved sperm. *Theriogenology* 123, 145–150.
- Nowosad J., Targońska K., Chwaluczyk R., Kaszubowski R., Kucharczyk D., 2014. Effect of temperature on the effectiveness of artificial reproduction of dace [Cyprinidae (*Leuciscus leuciscus* (L.))] under laboratory and field conditions. *J. Ther. Biol.* 45: 62-68.
- Nowosad J., Kucharczyk D., Targońska T., Wyszomirska E., Chwaluczyk R., Kupren K., 2016. The Synergistic Effect of Temperature and Hormonal Stimulation on Spawning Efficiency of Common Barbel, *Barbus barbus* L. *Turk. J. Fish. Aquat. Sci.* 16, 523-530.

- Nowosad, J., Kucharczyk, D., Targońska, K. 2017. Enrichment of Zebrafish *Danio rerio* (Hamilton, 1822) Diet with Polyunsaturated Fatty Acids Improves Fecundity and Larvae Quality. *Zebrafish* 14(4), 364–370.
- Nowosad, J., Sikora, M., Kucharczyk, D. 2018. Survival rates and the occurrence of larval malformations, including Siamese twins, following fertilization of post-ovulatory aged oocytes in ide *Leuciscus idus*. *Dis. Aquat. Org.* 127(3), 237–242.
- Okomoda, V.T., Koh, I.C.C., Shahreza, M.S., 2017. First report on the successful hybridization of Pangasianodon hypophthalmus (Sauvage, 1878) and *Clarias gariepinus* (Burchell, 1822). *Zygote* 25, 443–452.
- Olaniyi, W.A., Omitogun, O.G., 2013. Stages in the early and larval development of the African catfish *Clarias gariepinus* (Teleostei, Clariidae). *Zygote* 22, 314–330.
- Samarin, A.M., Sampels, S., Policar, T., Rodina, M., Hematyar, N., Samarin, A.M., 2018. mRNA abundance changes during in vitro oocyte ageing in African catfish *Clarias gariepinus* (Burchell, 1822). *Aquacult. Res.* 49, 1037–1045.
- Sharaf, S.M., 2012. Effect of GnRHa, pimozide and Ovaprim on ovulation and plasma sex steroid hormones in African catfish *Clarias gariepinus*. *Theriogenology* 77, 1709–1716.
- Sikora, M., Nowosad, J., Biegaj, M., Kucharczyk, D., Dębowski, M., 2018. The possibility of application of agglomerate elastomers (EPP) as media for biological bed in aquaculture. *Aquacult. Res.* 49(9), 2988–2994.
- Szabo, T., Erdei, P., Urbányi, B., 2010. Delayed in vitro fertilization of African catfish (*Clarias gariepinus*) and common carp (*Cyprinus carpio*) eggs in ovarian fluid of African catfish and rainbow trout (*Onchorhynchus mykiss*). *J. Appl. Ichthyol.* 26, 823-825.
- Szabo, T., Radics, F., Borsos, Á., Urbányi, B., 2015. Comparison of the results from induced breeding of European catfish (*Silurus glanis* L.) broodstock reared in an intensive system or in pond conditions. *Turk. J. Fish. Aquat. Sci.*, 15, 385-390.

- Targońska, K., Kucharczyk, D., Żarski, D., Cejko, B., Krejszeff, S., Kupren, K., Król, R., Dryl, K., Kowalski, R., Glogowski, J., 2011. Artificial reproduction of wild and cultured barbel (*Barbus barbus*, Cyprinidae) under controlled conditions. *Acta Vet. Hung.* 59 (3), 363–372.
- Teletchea, F., Fontaine, P. 2014. Levels of domestication in fish: Implications for the sustainable future of aquaculture. *Fish Fisher.* 15, 181-195.
- Teletchea, F., 2019. Fish domestication in aquaculture: reassessment and emerging questions. *Cybium* 43(1), 7-15.
- Van der Waal, B.C.W., Polling, L. 1985. Stripping male *Clarias gariepinus* of semen. *Aquaculture* 48, 137-142.
- Viveiros, A.T.M., So, N., Komen, J. 2000. Sperm cryopreservation of African catfish *Clarias gariepinus*: cryoprotectants, freezing rates and sperm: egg dilution ratio. *Theriogenology* 54, 1395–1408.
- Viveiros, A.T.M., Y., ter Veld M., Schulz, R., Komen, H. 2002. Hand-stripping of semen and semen quality after maturational hormone treatments, in African catfish *Clarias gariepinus*. *Aquaculture* 213(1-4), 373-386.
- Viveiros, A.T.M., Jatzkowski, A., Komen, J. 2003. Effects of oxytocin on semen release response in African catfish (*Clarias gariepinus*). *Theriogenology* 59, 1905-1917.
- Żarski, D., Palińska, K., Targońska, K., Bokor, Z., Kotrik, L., Krejszeff, S., Kupren, K., Horváth, A., Urbányi, B., Kucharczyk, D., 2011. Oocyte quality indicators in Eurasian perch, *Perca fluviatilis* L., during reproduction under controlled conditions. *Aquaculture* 313(1-4), 84-91.
- Żarski, D., Krejszeff, S., Horváth, Á., Bokor, Z., Palińska, K., Szentes, K., Łuczyńska, J., Targońska, K., Kupren, K., Urbányi, B., Kucharczyk, D., 2012. Dynamics of composition

and morphology in oocytes of Eurasian perch, *Perca fluviatilis* L., during induced spawning. Aquaculture 364-365, 103-110.

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Figure captions:

Fig. 1. Association between the hatching rate of African catfish (*Clarias gariepinus*) larvae and different experimental fertilization procedures; Groups description: C – control groups (eggs mixed with sperm at the time of adding water for egg activation) and after a delay of 30 s, 60 s and 90s as groups A-30, A-60 and A-90; Data among the groups with different letters were different ($P < 0.05$)

Fig. 1.

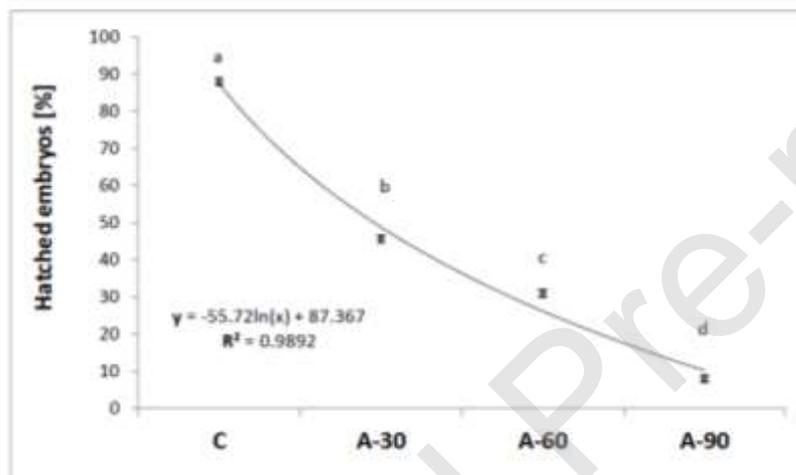


Fig. 2. Association between hatching rate of African catfish (*Clarias gariepinus*) larvae and different experimental fertilization procedures; Groups description: C – control groups (eggs mixed with sperm at the time of adding water for egg activation) and eggs mixed with sperm at 0 s and 30 s (as B-0-30), 0 s and 60 s (as B-0-60) and 0 s and 90 s (as B-0-90) relative to the time of adding water for egg activation; Data among the groups with different letters were different ($P < 0.05$)

Fig. 2.

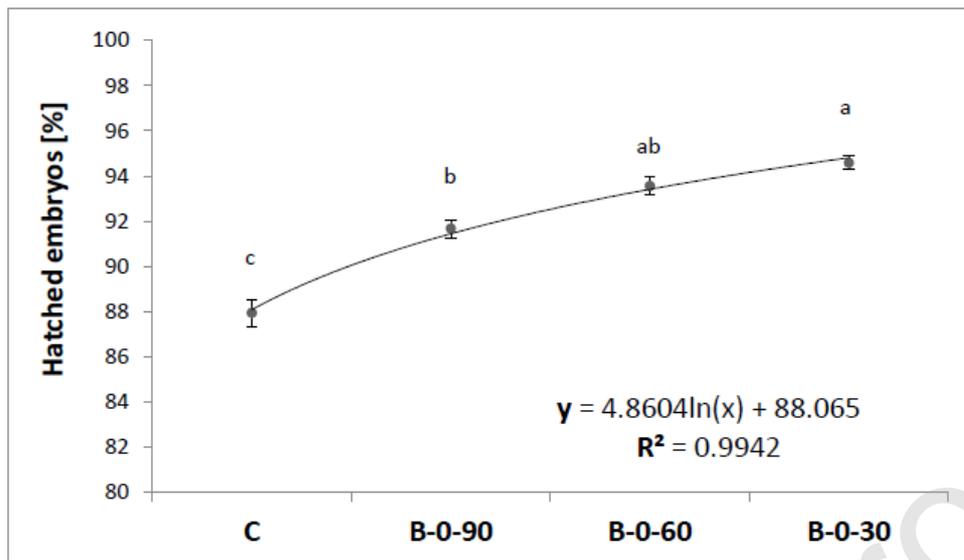


Fig. 3. Association between hatching rate of African catfish (*Clarias gariepinus*) larvae and different experimental fertilization procedures; Groups description: C – control groups (eggs mixed with sperm at the time of adding water for egg activation) and eggs were mixed with sperm at 0 s, 30 s and 60 s (D-0-30-60), at 0 s, 30 s and 90 s (D-0-30-90) and at 0 s, 30 s, 60 s and 90 s (D-0-30-60-90) relative to the time of adding water for egg activation; Data among the groups with different letters were different ($P < 0.05$)

Fig. 3.

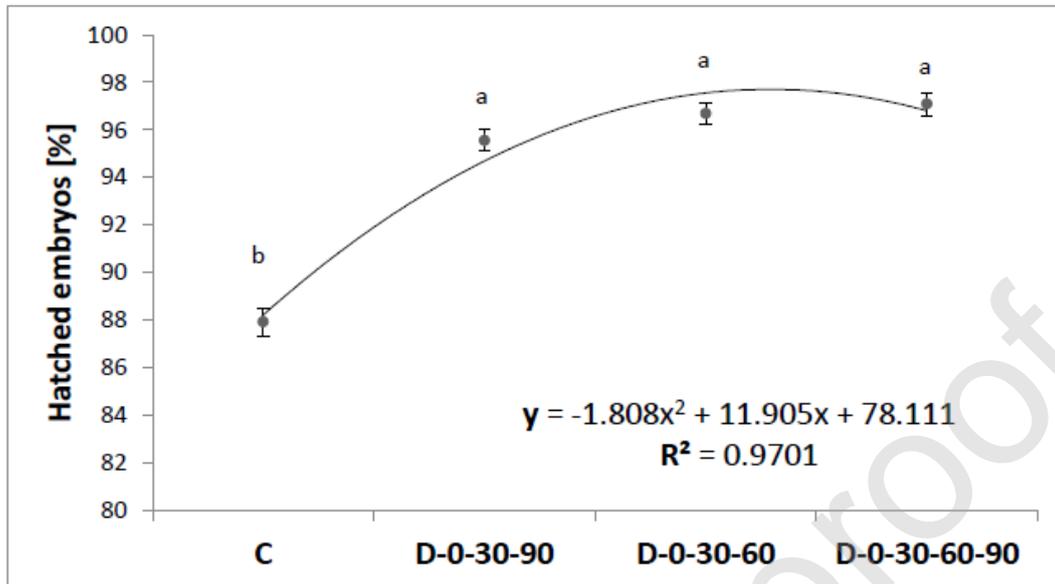


Fig. 4. Hatching rate of African catfish (*Clarias gariepinus*) larvae from the field test; Groups description: control groups (eggs mixed with sperm at the time of adding water for egg activation) and treated groups in which eggs were mixed with sperm at 0 s, 30 s and 60 s after adding water for egg activation; Data among the groups with different letters were different ($P < 0.05$)

Fig. 4.

