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Short communications

PRELIMINARY OBSERVATIONS ON INDUCING HOMOZYGOUS GYNOGENESIS IN NORTHERN PIKE (*ESOX LUCIUS*) USING PRESSURE SHOCK

Marek J. Łuczyński, Andrzej Szczerbowski*, Dariusz Kucharczyk**, Igor Babiak****

*The Stanisław Sakowicz Inland Fisheries Institute in Olsztyn, Poland
**Departament of Fisheries, University of Warmia and Mazury in Olsztyn, Poland
***Department of Animal Biochemistry, University of Warmia and Mazury in Olsztyn, Poland; present address: Bodoe Regional University, Departament of Fisheries and Natural Sciences, 8049 Bodoe, Norway

ABSTRACT. We induced homozygous gynogenesis in Northern pike (*Esox lucius* L.). The eggs obtained from two females and inseminated with UV inactivated milt were subjected to a pressure shock. The shock (600 atm, 3 min duration) was applied 60, 75, 90, 105, 120 or 135 min after activation of gametes. Application of the pressure shock resulted in interruption of the first mitotic division and gynogenetic larvae were obtained. The best results (5.20 ± 0.7% of survival, expressed as average ± SD) were observed when the eggs from the first female (A) were subjected to a pressure shock 75 min after insemination. Shock applied 90 min after activation resulted in 3.51 ± 2.4% of gynogenetic individuals. Pressure treatments applied 105, 120 or 135 min were ineffective. The results demonstrate that pressure shock can be applied to induce homozygous development in Northern pike.

Keywords: NORTHERN PIKE (*ESOX LUCIUS*), SEX CONTROL, REPRODUCTION, HOMOZYGOUS GYNOGENESIS, PRESSURE SHOCK

Interruption of first mitotic division of zygote enables obtaining tetraploid, mitotic gynogenetic or androgenetic specimens (Ihssen et al. 1990). In the case of mitotic gynogenesis or androgenesis, resulting embryos are presumably completely homozygous and carries chromosomes from only one maternal or paternal source, respectively. Such manipulations have many potential applications as establishment of inbred lines or recovery of valuable or endangered fish stocks using cryopreserved milt (Arai et al. 1992, Myers et al. 1995, Babiak et al. 2002). The blockage of first mitosis is most often achieved by the means of pressure or thermal shocks (Ihssen et al 1990). Induced homozygous gynogenesis showes considerably lower efficiency than het-

CORRESPONDING AUTHOR: Dr Marek J. Łuczyński, Instytut Rybactwa Śródlądowego, Zakład Akwakultury, ul. Oczapowskiego 10, 10-719 Olsztyn, tel./fax: +48 89 5241014,+48 89 5240505; e-mail: marekjl@infish.com.pl

erozygous (meiotic) gynogenesis, what is attributed to homozygosity (Pandian and Koteeswaran 1998). Heterologous gynogenesis was reported several times in esocids (Lin and Dabrowski 1996, Luczynski et al. 1997, Dabrowski et al. 2000); the data on homozygous gynogenesis are limited to muskellunge (*Esox masquinongy* Mitchill) (Lin and Dabrowski 1998). The aim of this study was to optimize the time of initiation of the pressure shock to produce homozygous gynogenetic northern pike (*Esox lucius* L.) larvae.

Northern pike gametes were collected by stripping spawners obtained from Szwaderki Hatchery (Olsztyn District, Poland). Eggs were kept in plastic container at 10.0°C for 2 hours before insemination. Milt was collected with syringes and kept on crushed ice at 0.0-4.0°C. Motility of sperm (in 120 mM NaCl activating solution) was examined using a microscope (500 ×), and only samples with more than 70% of motile spermatozoa were used (Babiak et al. 1995). Eggs were taken from two females (A and B) and milt from eight males. Samples of milt were pooled and eggs from each female were further divided into separate batches of approximately 900-1000 eggs. Control groups of eggs from females A and B (K-A and K-B) were fertilized with 0.05 ml of intact sperm. Milt was diluted in immobilizing solution (1:9) (Tris 2.42 g l^{-1}, glycine 3.75 g l^{-1}, NaCl 5.52 g l^{-1}, KCl 2.0 g l^{-1}) (Billard et al. 1976). Samples of milt were placed on Petri dishes (1 mm layer) and UV-irradiated (Philips 30 W UV tube, 253.7 nm, intensity of irradiation 6.4 W m^{-2}) for 8 min (Luczynski et al. 1997). Samples of irradiated milt were pooled. Control groups of eggs (I-A and I-B) (eggs not pressure shocked) and experimental groups of eggs (each in three repetitions) were inseminated, each with 0.5 ml of diluted and UV irradiated sperm. Gametes were activated with water at 14.0°C. Approximately 2.5 min after gamete activation each group of eggs was transferred into a small plastic sieve and immersed in 14.0°C water. Groups of eggs were subjected to a pressure shock of 600 atm for 3 min, beginning at 60, 75, 90, 105, 120 or 135 min after activation (pressure chamber HPC, Aquatic Eco-Systems). The efficiency of pressure treatment was evaluated based on the hatching percentage of normally developed gynogenetic diploid and abnormally developed haploid larvae. Data were transformed (arc sin $\sqrt{P}$) and one-way analysis of variance (ANOVA) was employed to test the differences between experimental groups (multiple range test Tukey's HSD intervals at $p \leq 0.05$).

The pressure shock at 600 atm lasting 3 min resulted in interruption of the first mitotic division and obtaining of gynogenetic larvae in almost all experimental

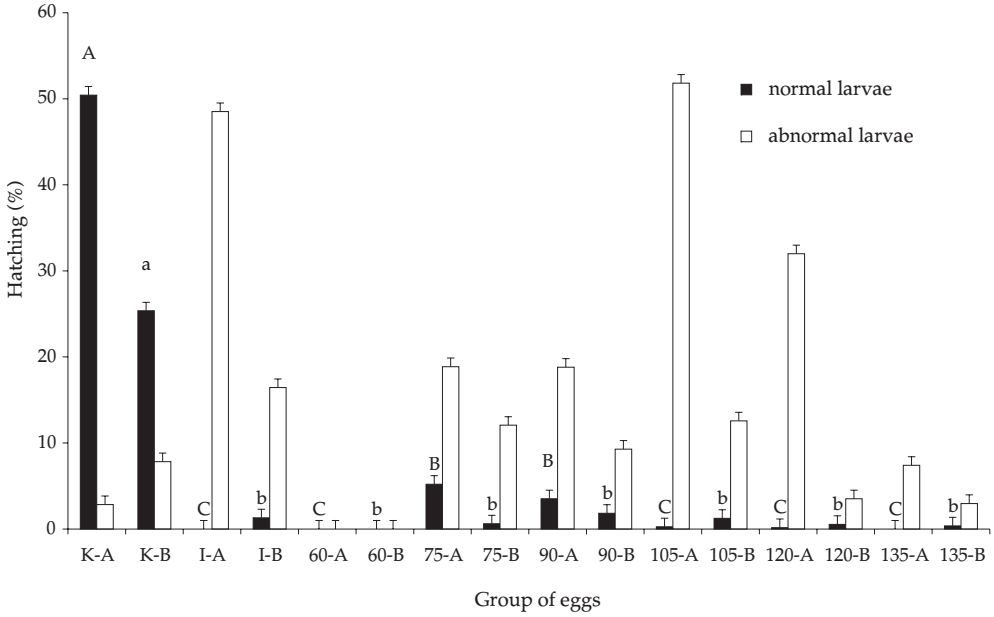

Fig. 1. The effect of a pressure shock on the hatching percentage of normal (diploid) and abnormal (haploid) larvae. The columns with the same letters are not significantly different at P = 0.05; statistics were carried out separately for female A and B – small letters mark columns showing percentage of diploid larvae obtained from female B, whereas capital letters mark columns refer to female A, respectively. Description of experimental groups of eggs i.e: K – control eggs fertilized with intact sperm; I – control eggs fertilized with diluted and irradiated sperm; "75-A": „75" – time of initiation of shock (min after egg activation); „A"– female A.

groups of northern pike eggs (Fig. 1). In the first female (A) pressure shock applied 75 min after gametes activation resulted in 5.20 ± 0.7% of gynogenetic larvae, whereas shock applied 90 min after activation resulted in 3.51 ± 2.4% of gynogenetic progenies. Pressure treatments applied 105, 120 or 135 min were ineffective. In the second female (B) the highest percentage of gynogenetic specimen (1.83%) was observed in group 90-B, however this value did not differ statistically from the percentage of gynogenetic larvae observed in control group I-B (1.30%).

The time of application of the pressure shock affected the survival of embryos. Treatment applied 60 min after activation caused 100% mortality at early stages of development. Survival was also relatively low in groups of eggs pressure treated at 120 (female B) or 135 min (females A and B) after activation. In female A the best

results were obtained when the shock was applied at 75 min after egg insemination. In female B the strongest reaction on the pressure shock was observed when the shock was applied 15 min later. On the other hand, no obtained diploids and relatively high survival of haploids in experimental groups of eggs was observed after shock application at 105 min for female A, and similarly 15 min later, at 120 min for female B. Thus, the effective period of shock application can be evaluated as 75-90 min and 90-105 min, for female A and B, respectively. Lin and Dabrowski (1998) induced androgenesis and homozygous gynogenesis in muskellunge with the heat shocks administered at 1.0-1.5 τ_0 after fertilization. The best results were obtained when the shock was administered at 1.2-1.5 τ_0. The dimensionless factor $\hat{o}_o$ of muskellunge was determined to be 127 min, so in case of northern pike the effective shock disrupting first mitotic division should be applied earlier.

In female B there were few normally developed diploid larvae present in control group of eggs I-B, inseminated with UV-irradiated milt and not subjected to pressure shock. Such phenomenon was also recorded by other authors (Hollebecq et al. 1986, Goryczko et al. 1991, Lin and Dabrowski 1998). The percentage of diploid larvae in this group was low (1.3%) and may probably be caused by spontaneous gynogenesis, as it was observed after insemination of northern pike eggs with UV-irradiated Eurasian perch (*Perca fluviatilis* L.) sperm (Luczynski M.J., unpublished data). The same pool of UV-irradiated milt was used for activation of eggs of control group I-A, where no diploid larvae were observed. Normally developed larvae were observed in groups 75-B, 90-B, or 105-B, however, statistically significant differences between those groups and group I-B were not observed. The present results demonstrate possibility for using pressure shock to restore diploidy in homozygous gynogenetic northern pike. The future research will focus on optimization of other parameters and standarization of the procedure.

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STRESZCZENIE

WYWOŁYWANIE HOMOZYGOTYCZNEJ GYNOGENEZY U SZCZUPAKA (*ESOX LUCIUS*) PRZY UŻYCIU SZOKU CIŚNIENIOWEGO – OBSERWACJE WSTĘPNE

Zablokowanie pierwszego podziału mitotycznego rozwijających się jaj umożliwia otrzymywanie osobników tetraploidalnych, gynogenetycznych albo androgenetycznych. Ryby uzyskane w wyniku gynogenezy mitotycznej bądź androgenezy są homozygotyczne i posiadają chromosomy pochodzące z jednego źródła rodzicielskiego. Wymienione manipulacje genomowe mają wiele potencjalnych zastosowań, takich jak tworzenie stad ryb o wysokim stopniu inbredu bądź też odtwarzanie cennych albo zagrożonych stad przy użyciu kriokonserwowanego nasienia. Celem przeprowadzonych badań było wywołanie gynogenezy homozygotycznej u szczupaka (*Esox lucius* L.). Ikra szczupaka, zaplemniona za pomocą nasienia inaktywowanego genetycznie przy użyciu promieniowania UV, została poddana działaniu szoku (udaru) ciśnieniowego (600 atm, czas trwania 3 min), stosowanego po upływie 60, 75, 90, 105, 120 albo 135 min od momentu aktywacji gamet. Zastosowanie szoku ciśnieniowego spowodowało przerwanie pierwszego podziału mitotycznego i uzyskanie wylęgu gynogenetycznego w większości grup doświadczalnych (rys. 1). Najlepsze rezultaty (5,20 ± 0,7%) osiągnięto po zastosowaniu szoku po upływie 75 min od momentu zaplemnienia ikry, podczas gdy szok zastosowany po upływie 90 min pozwolił na uzyskanie 3,51 ± 2,4% wylęgu gynogenetycznego. Nieskuteczne okazało się stosowanie szoków po upływie 105, 120 albo 135 min od momentu zaplemnienia.