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RESEARCH ARTICLE

The induction of meiotic gynogenesis in Northern pike (*Esox lucius*) using the heterologous European perch (*Perca fluviatilis*) sperm

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Abstract. Northern pike (*Esox lucius* L.) is one of the fish species whose production in freshwater aquaculture may increase in the next few years. One method of producing this species is to create monosex stocks of pike, as females grow faster, mature later and gain larger body sizes. They can be obtained in the process of gynogenesis. The aim of this research was to determine and optimize the conditions of UV irradiating European perch (*Perca fluviatilis* L.) spermatozoa to inactivate them genetically (first experiment). The aim of this study was also to confirm whether perch spermatozoa can be used to induce northern pike gynogenesis using thermal shock (second experiment). During first experiment the highest rate of haploid larvae (29.9 ± 0.85%) was noted in the group inseminated with perch sperm irradiated for 6 min (1548 J m^{-2}). No viable embryos were observed in groups of eggs inseminated with sperm irradiated for more than 10 minutes (2580 J m^{-2}). The heat shock applied 12 or 14 min after gamete activation, for 3 or 5 min at 34.0°C, resulted in obtaining of gynogenetic specimen due to retention of the second polar body in all experimental groups. The most efficient was heat shock applied 14 min after gamete activation and lasting 3 min, and resulted in 18.5 ± 1.3% of gynogenetic larvae for female B. Heat shock applied 12 min after gamete activation, lasting 3 min was also effective in the case of female A, resulting in obtaining of 16.5 ± 2.1% gynogenetic specimen.

Keywords. *Esox lucius*; genomic manipulations; gynogenesis; *Perca fluviatilis*; heterologous sperm

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Introduction

Northern pike (*Esox lucius* L.) is one of the most common predators in fresh and brackish waters in the northern hemisphere (Vehanen et al. 2006, Birnie-Gauvin et al. 2018), and it is one of the most important elements of these aquatic ecosystems (Forsman et al. 2015). Northern pike is also of great importance to the inland commercial and recreational fisheries and is valued by consumers for its healthy, delicious meat. In 2016–2019, global capture production of northern pike ranged from 31,000 to 38,000 metric tons annually, while global

aquaculture production in 2018–2019 was 20 times less at just over 1,500 metric tons (FAO 2022). This means that the niche in northern pike production is very large, and it is expected that it will be filled in the coming years. A significant part of the northern pike aquaculture production is used for stocking surface waters. Pike stocking material is also used to biomanipulate and regulate populations of fishes that are less economically valuable (Martyniak et al. 1996). When northern pike feed on small cyprinids, pressure on zooplankton filtering forms is reduced, and this reduces phytoplankton biomass, which can lead to improved water quality (Mehner et al. 2004). Northern pike is a sexually dimorphic species with larger females that grow faster than do males of the same age (Łuczyński et al. 2007). In order to meet market requirements, new solutions are still being sought that will improve the effects of stocking material production under controlled conditions and bring measurable economic benefits (Hakuć-Błażowska et al. 2009, Kucharczyk et al. 2016, 2019b, Szabo et al. 2019, Cejko et al. 2020). It is justified to use direct gynogenesis, technique with northern pike to create female-only herds (Łuczyński et al. 2007) or use sex-reversed fish (Łuczyński et al. 2003) for further production, e.g., in ponds. This can be achieved through gynogenesis (Łuczyński et al. 2007, Nowosad et al. 2015), which is a process that produces specimens in which all the nuclear genetic material comes from the mother. Gynogenotes are obtained by fertilizing fish eggs with inactivated sperm. Inactivation, or the complete destruction of the sperm genome, can be performed with ionizing radiation, e.g., UV (Zou et al. 2011, Kucharczyk et al. 2014, Nowosad et al. 2015). Semen from the same species (Nowosad et al. 2015) or from a different one (Kucharczyk et al. 2008) can be used to fertilize or activate oocytes. For the formation of a diploid zygote (2n), the genetic material of the embryo must be replicated (Łuczyński et al. 1997, Ocalewicz et al. 2010). For this, the haploid zygote (1n) is subjected to an environmental (pressure, thermal, or chemical) shock (Komen et al. 1988) to retain the second polar body or disrupt the first mitotic division of the embryo (Refstie 1983, Ihssen et al. 1990). In the future, gynogenesis and the opposite process of androgenesis (Ocalewicz et al. 2010, Nowosad et al. 2015) could be methods used to restore or revive endangered populations of many fish species and other vertebrates. Inducing gynogenesis using semen from a different species provides many benefits, while when using sperm of the same species it is theoretically possible that some spermatozoa are not irradiated and inactivated. On the other hand, spontaneous diploidization can also happen. In both cases, diploid specimens can occur in haploid control groups of eggs. Heterologous sperm is used to improve the technique of inducing gynogenesis, when no viable hybrids are observed and all offspring obtained are exclusively of gynogenetic, or maternal, origin, with no possible paternal genetic contribution (Chourrout 1982, Dabrowski et al. 2000, Morgan et al. 2006, Chen et al. 2017, Polonis et al. 2018, Fu et al. 2022). Despite many studies on the application of gynogenesis in fish, the survival of the offspring obtained remains relatively low. The hatching rate of northern pike gynogenotes is about 24% (Łuczyński et al. 2007). Optimizing the procedures and techniques used to obtain gynogenotes can significantly improve the outcomes of gynogenesis. It was decided to verify whether it is possible to use European perch (*Perca fluviatilis* L.) sperm to induce gynogenesis in northern pike (*Esox lucius* L.). European perch spermiates well in captivity and produces relatively large amounts of sperm compared to northern pike. European perch sperm can be obtained easily during the natural spawning period of northern pike, which is also important. Our earlier observations (unpublished data, Łuczyński et al. 2000) revealed that it is possible to use genetically inactivated European perch semen to trigger the embryonic development of northern pike eggs. The first aim of this research was to determine and optimize the conditions of UV irradiating *P. fluviatilis* spermatozoa, destroy their DNA and to inactivate them genetically. The second aim of this study was to confirm whether perch spermatozoa can be used to induce *E. lucius* gynogenesis using thermal shock.

Materials and methods

Ethical content

All procedures conducted on the fish were in accordance with the regulations set forth in the Act on the Protection of Animals Used for Scientific or Educational Purposes (Polish Journal of Laws of 2015, item 266, of 15 January 2015 and European Union 2010/63/UE). The managers of fishing districts obtained the consent of the respective marshal's offices to catch northern pike spawners during the closed season (1 March–30 April) for use in artificial reproduction to produce stocking material. No endangered or protected species were involved in the study. The authors of the study (M.J.Ł., J.N., and D.K.) hold a certificate of professional competence for designing experiments and experimental projects.

Fish origin and gamete collection

Two separate experiments were conducted. Northern pike gametes were obtained from the Pasym Fish Hatchery (Olsztyn District, north-eastern Poland). Spawners were caught in Lake Kalwa Wielka (Pasym) using trap- and gill-nets and were transported live (in water) to the hatchery. After the fish were anesthetized gametes were obtained by manually massaging of bellies of the spawners (Łuczyński et al. 2022). The eggs were kept in plastic containers until insemination (less than 2 hours), at 10-12°C. Sperm was collected with plastic syringes (1 ml) and kept on crushed ice at a temperatures of 1.0 – 4.0°C (Łuczyński et al. 2007). Sperm motility was examined under a microscope (500x) after activation with 120 mM NaCl solution and expressed as the percentage of motile spermatozoa (Łuczyński et al. 2007, 2022). Samples with less than 70% of motile spermatozoa were discarded.

European perch males were collected using trap-nets from the same lake as northern pike. They were handled and stimulated as was described by Szczerbowski et al. (2009). Sperm was collected by belly massaging, using syringes (Kucharczyk et al. 2021). Samples with contamination: blood or urine were excluded from further treatment (Kucharczyk et al. 2019a). Sperm motility was checked as described above. Samples with motility less than 80% was excluded from the experiment.

Experiment No. 1. Genetic inactivation of perch spermatozoa

Northern pike eggs were obtained from three females and sperm from three males. Sperm of European perch was obtained also from three males. Samples of sperm were pooled, both species separately, and northern pike eggs were divided into experimental groups of approximately 900-1,000 eggs (6 ml), each group was in triplicate. Control group of pike eggs C was inseminated with 50 µl of intact pike sperm and activated with 5 ml of tap water. Then the perch sperm was diluted (1:39) with immobilizing solution (NaCl 0.75%, KCl 0.6%) (Dabrowski et al. 2000). After sperm dilution the eggs of control group D were inseminated with a 0.5 ml of diluted perch sperm. Experimental samples of sperm, were placed on a Petri dishes (about 1.0 mm layer) for UV irradiation and were irradiated for 1, 2, 4, 6, 8, 10, 12, 14 or 16 min. The samples of sperm were agitated, the process was carried on a rocking table, with a cycle of about 1 s (Łuczyński et al. 2007). The radiation source was a 30 W Phillips germicidal UV tube (253.7 nm) with a radiation intensity of 4.3 W m^{-2} (Delta OMH HD 2102.1, equipped with a probe Delta OMH LP471UVC; Italy). The UV tube was switched on at 30 min before the onset of irradiation. After irradiation 0.5 ml sperm from each experimental batch was used to inseminate three separate group of eggs, then the mixed gametes were activated with 6 ml of tap water (14.0°C). After water hardening each group of eggs was transferred to small (approximately 100 ml) glass incubators with continuous water flow and incubated at 14°C (±0.5°C) until hatching. From the beginning of UV irradiation until the end of the first mitotic division of the developing eggs, the laboratory was illuminated only with a dim light to avoid sperm photoreactivation (Łuczyński et al. 2007).

Experiment No. 2. Application of a heat shock

In Experiment No. 2 northern pike eggs were taken from two females (A and B), and sperm from three males. European perch sperm was taken from four males. Samples of sperm were pooled (>70% and >80% motility for northern pike and European perch, respectively), both species separately, and northern pike eggs were divided into control and experimental groups of eggs (900-1.000 eggs, 6 ml), each in triplicate. Control groups of eggs from both females (CA and CB) were inseminated with intact pike sperm (50 μl) and activated with water (6 ml). Then European perch sperm was diluted 1:39 and UV irradiated for 6 min, as described in Experiment No. 1. Irradiated perch sperm was pooled, and the control groups of eggs not subjected to a heat shock (DIA and DIB) and the experimental groups of eggs were inseminated, with 0.5 ml of diluted and irradiated sperm each. The gametes were then kept in small plastic containers and were activated with 14.0°C water (time 0). Approximately 2 min after gamete activation each group of eggs was transferred into a plastic sieve and immersed in water at 14.0°C. The heat shock was accomplished by transferring sieves containing the eggs to the water bath with temperature controlled at 34.0°C (± 0.1). Eggs were subjected to high temperatures for 3 or 5 min, beginning 12 or 14 min after activation (Łuczyński et al. 2007). After the heat shock each group of eggs was transferred to a small glass incubators, as described in Experiment No. 1, and incubated at 14.0°C (± 0.5), until hatching. From the onset of UV irradiation, until the end of first mitotic division of eggs, the laboratory was illuminated only with dim light.

Data analysis

The efficiency of genetic inactivation of spermatozoa (UV irradiation) was evaluated based on the hatching percentage of normally developed, diploid and abnormally developed, haploid larvae (Łuczyński et al. 2007) which was validated previously with ploidy determination using flow cytometry (Lin et al. 2001). In Experiment No. 2 the effect of the heat shock treatment on the survival and ploidy level was examined based on hatching percentage of gynogenetic diploid and haploid larvae. The normality of data distribution, expressed as means±SD, was determined with Statistica 13.1 (StatSoft, Inc., Tulsa, OK, USA). The data were normally distributed (Shapiro–Wilk test), and the variances were homogenous (Levene's test). Differences in means among groups for each variable were assessed with ANOVA and Tukey's multiple range tests for group comparisons (values were significant at $p < 0.05$).

Results

Experiment No. 1. Genetic inactivation of perch spermatozoa

The hatching rate of eggs inseminated with intact northern pike sperm (control group C) was 52.6±7.3% (Fig. 1). Insemination of eggs with diluted but not irradiated perch sperm (control group D) resulted in no development and massive egg abortion. Insemination of eggs with sperm subjected to 1 min irradiation (group 1) resulted in 11.9% of haploid larvae and only 0.79% of diploid larvae. Irradiation lasting 4 min (1032 J m^{-2}) resulted in obtaining only haploid embryos (Fig. 1). The highest rate of haploid larvae (29.9 ± 0.85%) was noted in the group inseminated with perch sperm irradiated for 6 min (1548 J m^{-2}). No viable embryos were observed in groups of eggs inseminated with sperm irradiated for more than 10 minutes (2580 J m^{-2}).

Experiment No. 2. Application of the heat shock

The heat shock applied 12 or 14 min after gamete activation, for 3 or 5 min at 34.0°C, resulted in obtaining of gynogenetic specimen due to retention of the

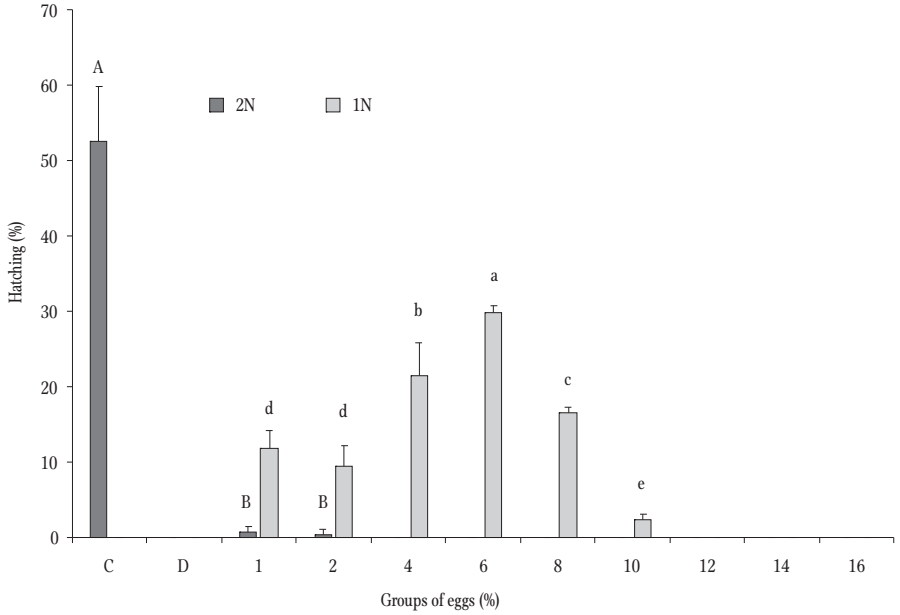

Figure 1. Hatchability (in%; mean ± SD) of diploid (2n) and haploid (1n) embryos of northern pike (*E. lucius*) in experiment 1, where genetically inactivated UV sperm of European perch (*P. fluviatilis*) was used for fertilization of the research samples. Descriptions of the groups are provided in the Material and methods chapter. Groups marked with the same letter index do not differ statistically: 2n – capital letters; 1n – lowercase letters.

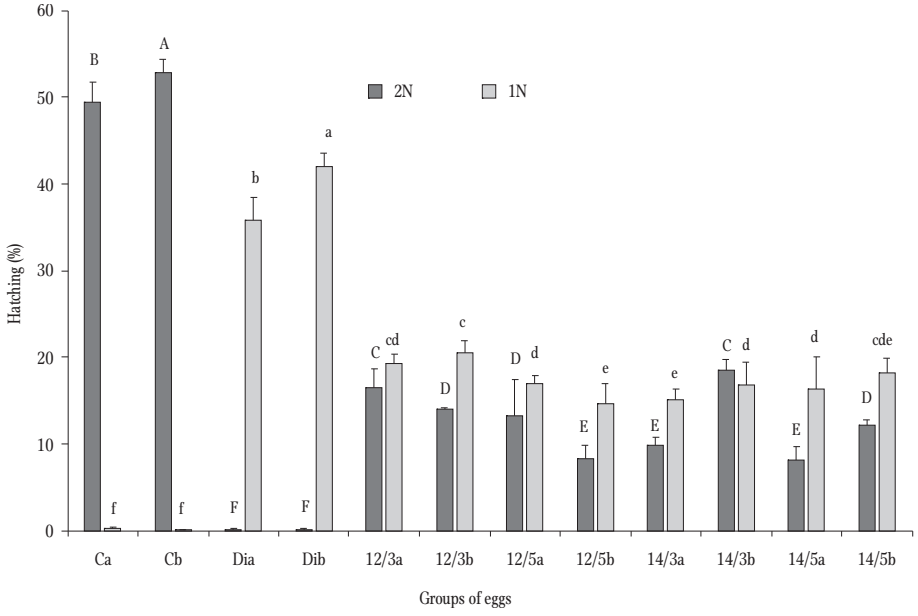

Figure 2. Hatchability (in %; mean ± SD) of diploid (2n) and haploid (1n) gynogenetic embryos of northern pike (*E. lucius*) in experiment 2, involved using genetically inactivated by UV sperm of European perch (*P. fluviatilis*) in treated groups. Descriptions of the groups are provided in the Material and methods chapter. Groups marked with the same letter index do not differ statistically: 2n – capital letters; 1n – lowercase letters.

second polar body in all experimental groups (Fig. 2). However, the results for corresponding treatments were significantly different with the eggs from females A and B. Heat shock applied 14 min after gamete activation and lasting 3 min was the most efficient and resulted in 18.5 ± 1.3% of gynogenetic larvae for female B (Fig. 2). Heat shock applied 12 min after gamete activation, lasting 3 min was also

effective in the case of female A, resulting in obtaining of 16.5 ± 2.1% gynogenetic specimen. The least effective was shock applied at 14 min, lasting 5 min (8.15 ± 1.48%) and at 12 min after gametes activation for 5 min (8.23 ± 1.51%), for female A and B, respectively. The eggs from females A and B were good quality, since the hatching percentages in these groups were 49.4 ± 2.4% and 52.9 ± 1.5%, respectively. The use of diluted and irradiated perch sperm resulted in decrease of hatching rate by approximately 14% and 10%, for female A and B, respectively.

Discussion

The results of Experiment 1 and 2 revealed that European perch sperm can be an effective and convenient tool for inducting northern pike gynogenesis. The results observed in group D (and our unpublished data) confirmed, that activation of pike eggs with intact (diluted but not irradiated) perch sperm resulted in 100% mortality and no embryonic development was observed. The use of UV-irradiated perch sperm resulted in relatively high percentage of developing haploid embryos (up to almost 30%, compared to approximately 53% diploids in group C), but the process of genetic inactivation of perch sperm should be standardized in the future. A similar situation was observed during research on the induction of gynogenesis in muskellunge (*Esox masquinongy* Mitchill) (Dabrowski et al. 2000). Muskellunge eggs inseminated with intact yellow perch sperm produced no viable embryos, whereas insemination with UV-irradiated sperm resulted in the development of haploid muskellunge embryos. These authors also tried to use rainbow trout (*Oncorhynchus mykiss* (Walbaum)) sperm, both untreated and UV-irradiated, but no gastrulation was observed. Activation of northern pike eggs was also possible using UV-irradiated rainbow trout sperm. The survival up to 47% to gastrula stage was observed after irradiation lasting for 4 minutes (Chourrout 1982).

In current study the optimum UV-dose was 1548 J m^{-2} (6 min of irradiation) since it caused damage to the DNA of perch spermatozoa and resulted in the highest hatching rate. Only haploid embryos were observed in groups of eggs inseminated with UV-treated sperm, irradiated at a dose of 1032 J m^{-2} (4 min) resulted in obtaining. In our preliminary experiments (Łuczyński et al. 2022) European perch sperm was irradiated for 6 min, at a higher irradiation intensity (6.4 W m^{-2}), according to the UV-lamp manufacturer (Phillips). The calculated dose was as high as 2304 J m^{-2} but the real UV-intensity was probably lower, as in the present study we observed hatching rate as low as 2.32% in haploids, after irradiation for 10 min, at a similar dose (2580 J m^{-2}). Unfortunately, it is impossibile to compare the dose applied in this study with that applied to European perch sperm by Rougeot et al. (2005) since only data on irradiation duration is included in publication. Dabrowski et al. (2000) induced gynogenesis in muskellunge with yellow perch sperm, irradiated with UV at slightly higher intensity (5.2 W m^{-2}). Irradiation lasting for 4 min resulted in radiation dose of 1248 J m^{-2} (100% haploids), corresponds with the dose determined as optimal in the current study. However, Mallison et al. (1993) observed 100% haploidy in yellow perch at a radiation dose as low as 3240 erg mm^{-2}, while the optimum dose (highest hatching percentage) was within the range of 3240-4860 erg mm^{-2}. Similar doses to those in current study were also reported as optimal for rainbow trout (12000-18000 erg mm^{-2}) (Palti and Thorgaard 1997), common bream (*Abramis brama* (L.)) (1344-2112 J m^{-2}) (Kucharczyk et al. 1996), 1000-1500 J m^{-2} for white bass (*Morone chrysops* (Raf.)) (Gomelsky et al. 2000) or pond loach (*Misgurnus anguillicaudatus* (Cantor)) (12000 erg mm^{-2}) (Suzuki et al. 1985). Some authors reported lower doses, enabling genetic inactivation of rainbow trout sperm (3600 erg mm^{-2}) (Levanduski et al. 1990) or four species of Salmoniformes, including rainbow trout (2400-4800 erg mm^{-2}) (Onozato and Yamaha 1983). Other researchers also observed lower optimal UV-doses, such as 800 J m^{-2} for yellowtail tetra sperm (do Nascimento et al. 2020),

800 J m^{-2} (Pang et al. 2022) or 9630 erg mm^{-2} for common carp (*Cyprinus carpio* L.) sperm (Taniguchi et al. 1986). Therefore, it is difficult to compare the optimal UV-doses reported by many researchers since the process of genetic inactivation of spermatozoa has still not been well standardized with regard to, for example sperm dilution rates; final concentrations of spermatozoa per ml of irradiated dilution; thicknesses of irradiated layers of diluted sperm.

All the manipulations in the current study, beginning from the onset of UV-irradiation were conducted in dim light, to avoid sperm photoreactivation (Ijiri and Egami 1980, Kaastrup and Horlyck 1987, Cleaver 2003). A similar procedure was applied in our earlier experiments on northern pike gynogenesis (Łuczyński et al. 2007), and this is also used widely by other researchers on muskellunge (Lin and Dabrowski 1996, Garcia-Abiado et al. 2001), common carp (*C. carpio*) (Cherfas et al. 1990), common bream (*A. brama)* (Kucharczyk et al. 1996) or yellow tetra (*Astyanax altiparanae* Garutti & Britski) (do Nascimento et al. 2020). The reversal of the deleterious effects of UV-irradiation with visible light illumination was reported to a limited extent in rainbow trout (Dorafshan et al. 2006), but photoreactivation was not observed even after exposing UV-irradiated Caspian trout (*Salmo caspius* Kessler) sperm to visible light of a relatively high intensity (60 W, distance 30 cm, duration 10 min) (Dorafshan et al. 2014). In the present experiment the diluted sperm was in a thin layer, of approximately 1 mm depth. Palti and Thorgaard (1997) stated, that a sample layer of 0.97 mm appeared to allow effective spermatozoa genetic inactivation even without sperm agitation. In the present study and during earlier experiments on inducing gynogenesis in pike using northern pike sperm, the layer of diluted sperm did not exceed 1 mm to ensure that the spermatozoa were properly irradiated (Łuczyński et al. 2004, 2007). Similar depths of irradiated samples during the genetic inactivation of sperm are also reported for four *Salmoniformes* species (Onozato and Yamaha 1983), *C. carpio* (Taniguchi et al. 1986, Varadaraj 1990) and *Rhamdia sapo* (Quoy & Gaimard) (Valcarcel et al. 1994). One result of Experiment 1 was the typical „Hertwig effect", with low survival observed in groups of eggs inseminated with sperm irradiated at relatively low doses of UV-radiation and the paradoxical increase of survival observed with increasing doses of radiation. This effect is explained by irradiation at low doses and the incomplete genetic inactivation of sperm DNA that results in aneuploid embryos, the survival of which is lower than embryos, with complete DNA inactivation (Piferrer et al. 2004). The lowest hatching percentage in groups of pike eggs inseminated with UV-irradiated perch sperm was observed in those activated with sperm that were irradiated for 2 min (dose 516 J m^{-2}). During our earlier studies on gynogenesis in pike the lowest survival was observed when northern pike sperm was irradiated with a comparable dose of 768-1152 J m^{-2} (for 2 and 3 minutes) (Łuczyński et al. 1997, Łuczyński et al. 2007). The "Hertwig effect" was also observed by other researchers who reported the lowest survival rates after irradiating the sperm of four *Salmoniformes* species at doses of 600 erg mm^{-2} (Onozato and Yamaha 1983), C*arassius auratus* (L.) at 100-150 erg mm^{-2} (Fujioka 1993), *Plecoglossus altivelis* (Temminck & Schlegel) at 1605 erg mm^{-2} (Taniguchi et al. 1986), and white bass (*Morone chrysops* (Raf.)) at 100-150 erg mm^{-2} (Gomelsky et al. 2000).

Exclusively haploid embryos hatched from groups of eggs inseminated with European perch sperm UV-treated for at least 4 min (1032 J m^{-2}) in present study. Haploids were easily distinguished based on their pigmentation and the size and shape of the developing embryos, beginning at the eyed-embryo stage. Hatched haploids (Fig. 3a) showed the typical „haploid syndrome", that was recognizable as microcephalia and shortened and twisted body, compared to diploid larvae (Fig. 3b). This very characteristic type of body deformity in haploids is very different from the other types of body deformation described in fish embryos (Żarski et al. 2015, Nowosad et al. 2018, Nowosad and Kucharczyk 2019). Similar haploids of esocid fishes were also identified in muskellunge by Lin and Dabrowski (1996). Most of haploid northern pike

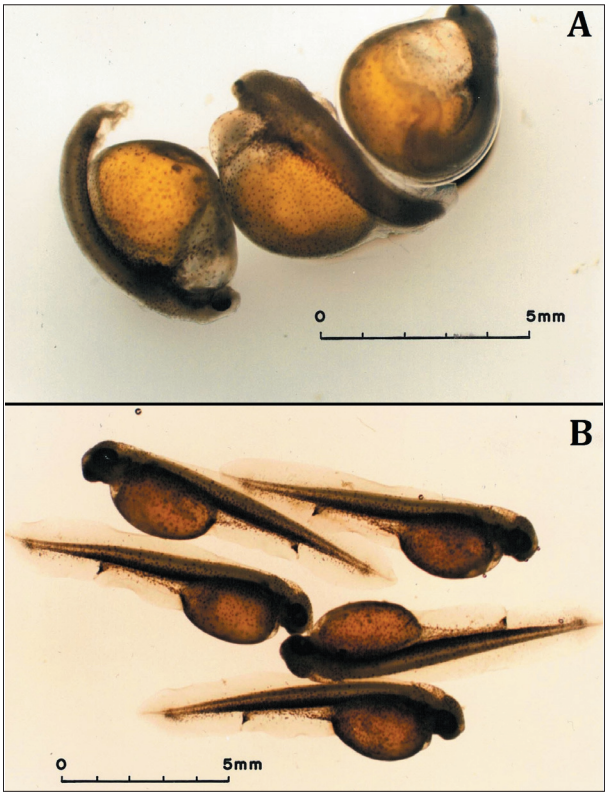

Figure 3. Embryos (larvae) of the northern pike (*E. lucius*) shortly after hatching: A - haploid embryos (1n); B – diploid embryos (2n) without body deformation.

embryos hatched, but all the larvae died within 48 hours after hatching. Haploidy of „abnormal" larvae was previously also confirmed on the basis on cytological examinations (Kucharczyk et al. 1999).

Heat shock applied soon after egg insemination induced gynogenetic development in all experimental groups of northern pike eggs, submitted to a heat shock of 34.0°C, applied 12-14 min after activation, for 3 or 5 min. The percentage of gynogenetic larvae and survival in the experimental groups depended significantly on the source (maternal effect) and quality of the eggs, both in the current study and in earlier experiments on gynogenesis in pike (Łuczyński et al. 1997, 2007) or other species (Levanduski et al. 1990, Diaz et al. 1993). The timing of the application and the duration of the heat shock affected embryo survival and the percentage of gynogenetic larvae in heat-shocked groups of eggs. For female A eggs, the best results were obtained when using a heat shock at 12 min after egg activation, and lasted for 3 min (16.53% gynogenotes) and for 5 min (13.32% gynogenotes) (Fig. 2).

Treatments applied 15 min after egg activation were less effective, resulted in 9.77% and 8.15% gynogenotes, for shocks lasting for 3 and 5 min, respectively. The highest percentage of gynogenetic larvae was observed, when eggs from female B were submitted to a heat shock for 3 min, beginning at 14 and 12 min after egg activation, with 18.49% and 13.94% gynogenotes, respectively. Treatments of 5 min were less effective, with 12.10% and 8.23% gynogenotes when shocks were applied at 14 and 12 min after gamete activation, respectively. Relatively high-intensity heat shocks of 34.0°C, for 3 or 5 min, at 4-14 min after activation also resulted in high percentages of gynogenetic specimen (Łuczyński et al. 1997). Lin and Dabrowski (1996) obtained gynogenetic muskellunge larvae after applying relatively low-intensity heat shocks, within a broad range of parameters (28.0-30.0°C, starting 5-30 min after insemination, duration 5-30 min). The best results were obtained with a heat shock of 30.0°C for 8-10 min, beginning 20 min after gamete activation. Activating northern pike eggs with UV-irradiated European perch sperm and subjecting inseminated eggs to the thermal shock resulted in a relatively high percentage of gynogenetic larvae.

Conclusions

The conducted research has shown that it is possible to induce gynogenetic development in northern pike with the use of heterologous sperm from European perch. The obtained yields at the level of 16.5-18.5% of gynogenotes survival are relatively high and allow for the production of such offspring on an industrial scale. Interestingly, interspecies hybrids between pike (female) and perch (male) are non-viable. Additionally, it allows to obtain certainty as to the effectiveness of the applied methods in the production of gynogenetic offspring. This achievement allows for the future planning of the creation of pike gynogenotes on a semi-industrial and industrial

scale and the breeding of monosex female stocks only.

Declaration of competing interest. The authors declare no conflict of interest.

Data Availability Statement. The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy.

Informed Consent Statement. The managers of fishing districts obtained the consent of the respective marshal's offices to catch northern pike spawners during the closed season (1 March–30 April) for use in artificial reproduction to produce stocking material.

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Author contribution. M.J.Ł. and D.K. designed the study. M.J.Ł. and D.K. conducted the field investigations and provided data. M.J.Ł., J.N., D.K. managed the data base and did some of the analyses. J.N. conduced the statistical analyses. M.J.Ł., J.N., J.Ł. and D.K. did some of the data analyses and drafted the manuscript. All authors contributed to writing the manuscript and approved the final version that was submitted.

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