Turk. J. Fish.& Aquat. Sci. 23(1), TRJFAS20325
https://doi.org/10.4194/TRJFAS20325

Turkish Journal of FISHERIES and AQUATIC SCIENCES

REVIEW

Conservation of Vulnerable Sterlet Sturgeon, *Acipenser ruthenus* Using Artificial Gynogenesis

Mohammad Hassanzadeh Saber[1,*]

[1]Genetics & Biotechnology Department, International Sturgeon Research Institute, Agricultural Research Education and Extension Organization (AREEO), Rasht, Iran

How to cite

Hassanzadeh Saber, M. (2022). Conservation of Vulnerable Sterlet Sturgeon, *Acipenser ruthenus* Using Artificial Gynogenesis. *Turkish Journal of Fisheries and Aquatic Sciences*, *23(1)*, *TRJFAS*20325. https://doi.org/10.4194/TRJFAS20325

Article History
Received 16 August 2022
Accepted 05 August 2022
First Online 09 September 2022

Corresponding Author
Tel.: +981334500974
E-mail: saber.merag@gmail.com

Keywords
Sterlet sturgeon
Gynogenesis
Heterologous sperm
Conservation

Abstract

Sterlet sturgeon has been identified as a vulnerable species in the IUCN criteria, which is currently lack in most propagation and rehabilitation centers of Iran. This study was conducted with the aim of creating male sterlet sturgeon using gyneogenesis induction without using semen of this sturgeon. Siberian sturgeon, Acipenser baerii sperm was used in this study. To inactivate the sperm DNA, 473 µW/cm^2 UV irradiation was used for 90 seconds to maintain sperm motility for the fertility of the ovum. For diploidization of activated eggs, 34°C heat shock was used for 2 minutes, 15 minutes after activation. In this case, fertilization and hatching rate in the gynogenetic progeny were 59.5% and 41.6%, respectively. Sterlet sturgeon gynogenetic progeny was confirmed using microsatellite markers (AFU68 and AFUG9), which had only maternal heritability. Gynogenetic progeny was analyzed by histology of gonads at the age of 17 months. The results showed that both male and female sexes of sterlet sturgeon were created, but these progenies had different sex ratios.

Introduction

Sterlet, *Acipenser ruthenus* belongs to the smallest, freshwater species of Acipenseridae. Under aquaculture conditions, this species reaches sexual maturity at approximately 3–6 years old, which is the earliest among all the sturgeons. It has originally inhabited the rivers flowing into the Caspian, Black, Baltic, White, Barents, and Kara Seas, and the Sea of Azov (Fopp-Bayat & Ocalewicz, 2015). Currently, all the sturgeon species, including sterlet, are highly endangered due to water pollution, construction of the dams, and overfishing, and they are on the Red List of Threatened Species IUCN (Gessner et al., 2010).

Some traits including freshwater habitat, small size, and relatively early sexual maturation make the sterlet good model species in studies concerning genome manipulations in the sturgeons (Fopp-Bayat & Ocalewicz, 2015).

The shortage of male broods in most of the sturgeon stocks rehabilitation centers of Iran may have contributed to the lack of propagation and conservation of these valuable species (according to personal communications from the deputy director of the Iranian Fisheries Organization). Genetic manipulations, especially meiotic gynogenesis, can restore endangered species. Artificially induced gynogenesis includes inactivation of the paternal DNA in spermatozoa, then the eggs were activated by the spermatozoa with inactivated DNA, and finally, exposition of the activated eggs for the temperature or pressure shock inhibiting releasing of the second polar body (meiotic gynogenesis) or suppressing the first mitotic cleavage (mitotic gynogenesis) (Komen & Thorgaard, 2007). Gynogenesis plays an important role in conserving the maternal genome when only female gametes (eggs) are available (Pandian & Koteeswaran, 1998; Omoto *et al.*, 2005; Saber *et al.,* 2014). In this case, the development

of the egg (embryogenesis) occurs with a non-irradiated or irradiated heterologous spermatozoon (Peruzzi & Chatain, 2000). The advantages of using heterologous sperm in gynogenesis induction are to reduce the possibility of contamination associated with the use of homologous sperm (Pandian, 2011) or photoreactivation (Ijiri & Egami, 1980, Dorafshan *et al.,* 2006). On the other hand, the use of the UV-irradiated heterologous sperm provides the possibility that, if UV-irradiation penetration is not sufficient to destroy the sperm DNA, it remains healthy, which, in the case of hybrid production, can be a suitable phenotype marker to detect gynogenetic from non- gynogenetic progenies (Pandian, 2011).

Application of the motile spermatozoa with UV-inactivated nuclear genome for the eggs activation results in the development of haploid gynogenetic individuals. This technique has been applied in the researchs concerning the sex-determination system in fish, providing homozygous fish lines and fish for the breeding programs (Arai, 2001; Devlin & Nagahama, 2002). Gynogenesis has been induced in some sturgeon species (VanEenennaam *et al*., 1996; Flynn *et al*., 2006; Fopp-Bayat, 2007; Fopp-Bayat *et al*., 2007; HassanzadehSaber *et al*., 2008; Saber *et al*., 2014) to obtain offspring with the only maternal inheritance that can be used as gamete donors for the production of all-female stocks. In sturgeons, females are heterogametic (WZ genotype) (VanEenennaam *et al*., 1999; Omoto *et al*., 2005; Flynn *et al*., 2006; Fopp-Bayat, 2010; Saber & Hallajian, 2014; Fopp-Bayat *et al*., 2018) and gynogenesis induction can be supposed to generate 'super females' (WW genotype) that subsequently crossed with the normal males (ZZ genotype) to give all-female offspring. On the other hand, gynogenesis induction in the sturgeon with a female heterogamety sex-determination system and the presence of both W and Z zygotes in female breeders could exclude from extinct in the wild (EW) sturgeon species to create both female and male sexes (Omoto *et al*., 2005; Komen & Thorgaard, 2007; Grunina *et al*., 2011; Zou *et al*., 2011; Saber *et al*., 2014; Nowosad *et al*. 2015).

The aim of this study was to establish the sex of male sterlet sturgeon using gynogenesis induction through heterologous sperm (Siberian sturgeon) to protect this valuable species. On the other hand, the heterologous sperm of Siberian sturgeon can be used as a model to protect other sturgeons by inducing gynogenesis. Also, both males and females can be produced with only one female breeder.

Materials and Methods

Sampling, Experimental Design, and Sexual Maturation

This study was conducted in April of 2017 at the Rearing Site of the International Sturgeon Research Institute of the Caspian Sea (Rasht, Iran). Before sampling gametes, sterlet breeders were anesthetized by clove powder and all efforts were made to minimize suffering. A female breeder of sterlet sturgeon at the age of 5 years with an average body weight of 1000 g and a male breeder of Siberian sturgeon at the age of 6 years with an average body weight of 7000 g (as heterologous) were isolated for providing eggs and spermatozoa to induce gynogenesis. The data on kinds of treatments, shock temperature, and its duration, fertilization, and hatching rate are summarized in Table 1. The experiment included a diploid control group, a hybrid control group (untreated sterlet sturgeon eggs and untreated Siberian sturgeon sperm), a haploid control group to determine the efficiency of UV inactivation of the sperm (untreated eggs and UV-irradiated sperm), a triploid control group to determine the efficacy of second polar body retention (heat-shocked eggs and untreated sperm), and a treatment designed to induce gynogenesis (heat-shocked eggs and UV-irradiated sperm). According to the temperature of the water (15°C), Ovulation in the female breeder (sterlet sturgeon) was induced using the synthetic GnRH (Samen Pharmaceutical Company, Mashhad, Iran) at a rate of 10 mg/kg BW in two steps, in the first stage, 20% and after 6 hours, the hormone remained was injected into the dorsal muscle. In the Siberian sturgeon, the GnRH injection was performed at a single stage (simultaneously with the second injection of the female) at a rate of 20 mg/kg BW. Then sperm and oocytes were respectively extracted from males and females (Pourdehghani *et al*., 2010).

Sperm DNA Destruction by UV-irradiation

After stripping out of sperm of male breeder (Siberian sturgeon), seminal fluid was separated by centrifuging (Labofuge 200, Heraeus, GmbH, Germany) twice at 5000 rpm for 5 min. According to Hassanzadeh Saber *et al*. (2008), for UV irradiation, 0.1 ml of fresh primary sperm sample was diluted with 0.9 ml of seminal fluid (1:9). Diluted sperm (1 ml) was spread on a Petri dish. The Petri dish was placed on a gently rotating platform (90 rpm) below a UV source, which provided an incident light intensity of 473 $\mu W/cm^2$ UV (UVG-54, UVP, Upland, CA, USA) for 90 s. The UV apparatus was covered with black plastic to contain the UV light and to prevent the photoreactivation of sperm DNA (Saber *et al*., 2014).

Sperm Motility Estimation

For sperm motility assessment, 10µl of milt was diluted with 500µl of water (activating medium) (Aramli *et al*., 2015). then, the sample of irradiated sperm and non-treated sperm sample were simultaneously placed on the glass slide and analyzed under the microscope to confirm the percentage of motility.

Heat Shock and Fertilization

After stripping out of eggs of the female breeder (sterlet), 9 ml of 16°C water from the incubation system was added to the irradiated sperm suspension and this mixture was immediately added to the ova. De-adhesion of the egg was conducted using NaCl–tannic acid solutions (Feledi *et al*., 2011). Eggs were approximately divided into five equal groups (10 g, ~900 eggs/each group). According to Dettlaff *et al*. (1993), the value of T_0 (duration of one mitotic cycle during the period of synchronous cleavage divisions) in *A. ruthenus* was 0.257, so at this temperature, the heat shock began 15 min after activation/fertilization in experiments. Eggs were transferred to boxes with perforated mesh and kept in water at 34°C for 2 min (according to Fopp-Bayat *et al*., 2007). Fertilized eggs were transferred to Weis incubators. To avoid photoreactivation, the sperm and embryos were stored in the dark for 24 h. Additionally, ova quality was checked by fertilization with the untreated sterlet sperm. Survival of the developing eggs and the viable fry was recorded at different developmental stages: fertilization (2 h after fertilization a.f) and hatching (6 days a.f.) at 16°C.

Larvae and Fry Rearing

Hatched larvae from each individual treatment were transferred to a special system of tanks (1 m in diameter and 10 cm in depth each), where they remained until sampling for microsatellite DNA analysis. Larval feeding was initiated using Artemia nauplii from 10 days post-hatching, followed by manufactured food containing 40% protein and 12% lipid (Faradaneh, Shahr-e-Kord, Iran) throughout the duration of the experiment (17 months).

Genetic Verification of Gynogenetic Progeny

Thirty fingerlings from putative gynogenetic groups and the same numbers from controls were randomly selected 3 months after hatching and their fin clips and two parental individuals were stored in 96% ethanol. Also, 10 specimens were randomly collected from the triploid treatment, and their DNA using microsatellite Afug9 marker was amplified. Genomic DNA for the amplification of two microsatellite markers [Afu68 (May *et al*., 1997) and Afug9 (Welsh & May 2006)] was extracted (Table 2) according to Hillis & Moritz (1990). The reaction mixture was prepared to a total volume of 25 µl with 100 n

g DNA template, 10× polymerase chain reaction (PCR) reaction buffer (20 mM Tris–HCl, 100 mM KCl, pH 8.0 at 25°C; 0.1 mM DDT, 50% Glycerol, 0.5% Tween 20, and 0.5% NP-40), 10 pM of each primer, 10 mM of each deoxynucleotide triphosphate (dNTP), 25 mM $MgCl_2$, and 500U Taq DNA Polymerase (Bio Flux, Tokyo, Japan). Double-distilled water was used to bring the reaction mixture to the desired final volume. Amplification was conducted with a Master cycler ep gradient thermal cycler (Eppendorf, Hamburg, Germany), with initial denaturation at 94°C for 3 min, followed by 30 amplification cycles (94°C, 30 s; 60– 62°C, 30 s; 72°C 30 s), and final elongation at 72°C for 5 min. Aliquots containing PCR products and reaction buffer were subjected to electrophoresis using a 6% polyacrylamide gel, and DNA bands were visualized by the silver staining method (Tegelström, 1986). Electrophoresis was conducted on an Amersham Pharmacia Biotech System, and the gel size was 18×16 cm. Amplified fragments were sized by comparing migration with a DNA standard, Gene Ruler™ 50 bp DNA Ladder (Fermentas). Specific microsatellite profiles for parents were noted and compared to those from experimental groups.

Table 1. The values of fertilization and hatching rates in haploid, gynogenetic, triploid and hybrid treatments

Treatment	UV irradiation time (Sec.)	Fertilization rate (%)	Hatching rate (%)
control	0	70.7±0.34	65.5±0.34
H90	90	52.1±0.52	1.1±0.11
G90	90	59.5±0.98	41.6±0.46
3N	0	67.8±0.8	47.9±0.63
Hybrid	0	72.3±1.3	57.7±0.34

Control: diploid control group; Hybrid: untreated sterlet sturgeon eggs and untreated Siberian sturgeon sperm; H90: a untreated eggs and UV-irradiated sperm; 3N: heat-shocked eggs and untreated sperm; and G90: heat-shocked eggs and UV-irradiated sperm

Table 2. Characterization of used microsatellite markers for verification of gynogenetic progeny

	Locus	GeneBank Accession No	Repeat motif	Primer sequence(5`-3`)	Sequenced clone size (bp)
1	*Afu-68*	U72739	(GATA)13	F: TTATTGGATGGTGTACCTAAAC R: AGCCCAACACGACAATATC	120
2	*Afug-9*	AF529447	(GATA)14(GA)2GATA(GA)2(GATA)6	F: ATTCCTCCAGCCGTATTATTA R: AAGCAGTTAGTTTATGTGGTTGTG	165

The Studied Morphological Parameters in Sterlet, Siberian Sturgeon and Their Hybrids

Morphometric characteristics such as total length, head length, snout length, mouth width were investigated in 10 hybrids and compared with the controls.

Histological Verification of Experimental Progeny

Sex identification was performed on 30 gynogenetic sterlet at 17 months of age. Individual fish was put into the rectangular plastic container (400-L volume) filled with fresh 15–19°C flowing water and 150 mg/L clove powder for sedating the fish. The fish were then placed on a table for examination. A 20-25 mm anterior-posterior incision was made with a sharp scalpel (No. 10 straight blade) through the ventral area of the body wall. The surgical incision site was located as described by Chapman (1989) for white sturgeon *Acipenser transmontanus*. Then, a small biopsy sample of the gonad (5-8 mm) was collected by fine forceps. In this case, forceps were carefully inserted into the body cavity until reaching the gonad. The tissue samples were then cut by sharp scissors and fixed in Bouin's solution for the next histological assay. Then after 48 h, samples were embedded in paraffin following a schedule to put them into alcohol series for dehydration and clearing. The blocks were sectioned with a thickness of 6 micrometers and stained with hematoxylin and eosin for histological examination (Amiri *et al*., 1996; Van Eenennaam & Doroshov, 1998; Raji & Norouzi, 2010). Sex identification and evaluations of sexual maturity were done by observing the prepared samples under a light microscope according to Amiri *et al*. (1996).

Statistical Analysis

All data on the fertilization and hatching rate of the gynogenetic and control in the experiments were analyzed using SPSS software and one-way analysis of variance (ANOVA) with Tukey's honestly significant difference (HSD) test and were expressed as the mean ± S.E. Analysis of variance (ANOVA) was used to determine significant differences between groups by SPSS20.0. When differences were significant, the Duncan test was used for comparison. Effects were considered as significant at $P<0.05$.

Results

Sperm Motility, Fertilization and Hatching Rates, and Heat Shock Effects on Diploidization

Siberian sturgeon sperm had 80±1.66% forward motility with a duration of 318 sec and density of 1.56×10^9 ml (Table 3). After exposure of the same sperm to UV radiation for 90 seconds, its motility was reduced to 50±4.72%, which was significantly different from that of the control ($P<0.05$).

The fertilization and hatching rates in the diploid control group were 70.7% and 65.5%, respectively, while these rates in the hybrid control group were 72.3±1.3% and 57.7±0.34%, respectively. In the haploid group, the UV-irradiated sperm were combined with the sterlet sturgeon eggs to indicate that the sperm lacked genetic activity. The fertilization and hatching rates in this group were 52.1% and 1.1%, respectively. The hatching rate was very low in the haploid group, and it also showed "haploid syndrome" that died quickly, therefore, the ploidy of these progenies could not be determined. Mortality of haploid larvae indicated UV irradiation effects on inactivation of heterologous sperm DNA. Unlike the haploid group, the fertilization rate was 59.5±0.98% ($P<0.05$) and the hatching rate was 41.6±0.46% ($P<0.05$) in the diploid gynogenetic treatment (G_{90}). This indicated that heat shock was very effective in the retention of the second polar body and diploidization in the gynogen group, as shown in the triploid treatment, in which the fertilization rate was 67.8±0.8% and the hatching rate was 47.9±0.63% ($P<0.05$). The mean fertilization and hatching rates of sterlet sturgeon in different treatments are summarized in Table 1 and Figure 1.

Genetic Verification of Gynogenetic Progenies and Their Parents

The results of the microsatellite DNA analysis showed that there was no genetic contribution from the paternal genome in the gynogenetic group. Figure 2 shows the allelic patterns in gynogenetic progeny that have been compared with their maternal genome (sterlet) and paternal genome (Siberian sturgeon) using the Afu68 marker. This marker was monomorphic in the gynogenetic group and an allele of 140 bp was observed in those groups of fish. The 140-bp allele was

Table 3. Observed parameters of heterologous Siberian sturgeon sperm

	Observed parameter	Value
1	Motility percentage (%)	80
2	Motility duration (Sec.)	318
3	pH	8.03
4	Osmolality (Osm/kg)	152
5	Sample volume (ml)	120
6	Sperm density ($\times10^9$ sp/ml)	1.56
7	Spermatocrit (%)	13.33

characteristic of the maternal genome of the gynogenetic offspring, while the male (sperm donor) exhibited alleles of a pair of 168 and 161 bp and another pair of 126 and 121 bp.

Figure 3 shows the allelic patterns in gynogenetic progeny that have been compared with their maternal genome (sterlet) and paternal genome (Siberian sturgeon) using the Afug9 marker. This marker was monomorphic in the gynogenetic group and alleles of a pair of 167 and 147 bp were observed. These alleles were characteristic of the maternal alleles of the gynogenetic offspring, while the male (sperm donor) exhibited alleles of a pair of 190 and 173 bp and another pair of 142 and 124 bp. The inherited alleles among all

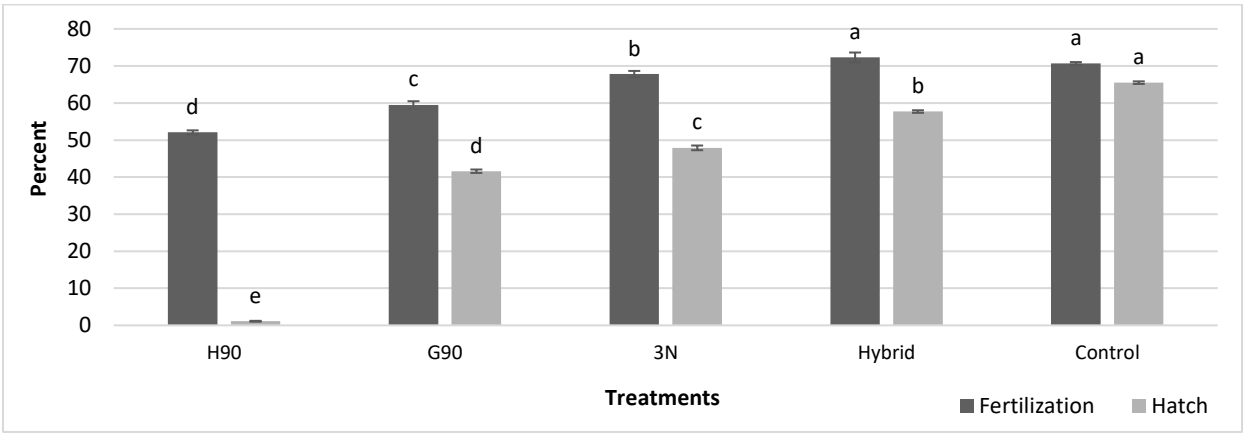

Figure 1. Comparison of fertilization and hatching rates in haploid, gynogenetic, triploid and hybrid treatments (Control: diploid control; Hybrid: female sterlet sturgeon and male Siberian sturgeon; H90: Haploid; 3N: Triploid; and G90: gynogenetic progeny).

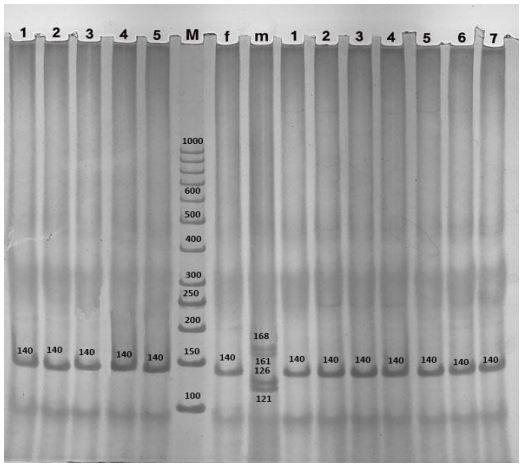

Figure 2. Allelic pattern of maternal (sterlet: f), paternal (Siberian sturgeon: m) and gynogenetic progeny using microsatellite marker *Afu68.*

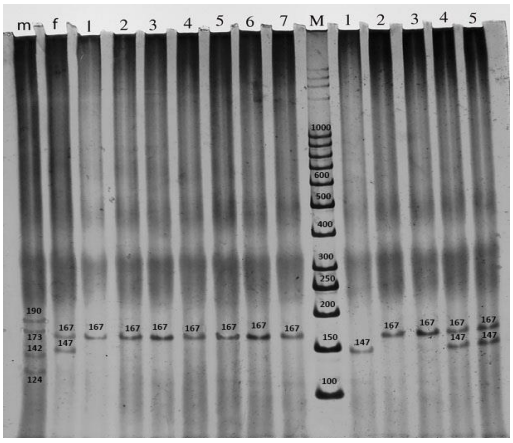

Figure 3. Allelic pattern of maternal (sterlet: f), paternal (Siberian sturgeon: m) and gynogenetic progeny using microsatellite marker *Afug9.*

gynogenetic progenies recognized all of the maternal patterns at 167, 147, and 167/147 bp which means no genetic contribution from the paternal genome in gynogenetic progenies.

Analysis of triploid progenies and their parents using Afug9 microsatellite marker showed that the maternal locus has two alleles of 167 and 147 bp, but the paternal loci have two alleles of 190 and 173 bp and two other alleles of 142 and 124 bp. This locus could verify the triploid treatment because all offspring have inherited both alleles of the maternal locus as well as two alleles of one paternal locus, but 3 specimens have inherited the paternal locus of 142 and 124 bp, and remained specimens have inherited other locus of the father. Therefore, all specimens of triploid treatment have four alleles wherein two alleles belonged to the mother and the other two alleles are related to the father. The observed alleles at microsatellite loci (Afu68 and Afug9) in the gynogenetic and triploid progeny of sterlet are summarized in Table 4.

Comparison of Phenotypic Characteristics of Sterlet, Siberian Sturgeon, and Their Hybrids

There was no significant difference in head length and mouth width between the hybrids and Siberian sturgeon ($P>0.05$), but there were significant differences in total length and snout length of hybrids, Siberian sturgeon and sterlet ($P<0.05$). In general, the hybrids are more similar to Siberian sturgeon (Figure. 4).

Sex Identification in the Gynogenetic Progeny

Sex identification on 30 specimens of gynogenetic diploid of sterlet showed that 23 (76.6%) specimens were female (stage I containing oogonia). The remaining 7 (23.3%) samples have been recognized as male and their gonads contained spermatogonia (Figure. 5). In the first year, sterlet fingerlings of all groups in this study were kept outdoors without thermal control and their growth rate was very low compared to similar ones kept indoors with temperature control.

Discussion

In this study, heterologous inactivated sperm (Siberian sturgeon) was used to induce gynogenesis, which is recommended in sturgeons due to deficiency of homologous or poor sperm. As Meng et al. (2016) have suggested, if homologous sperm quantity and quality are low, heterologous inactivated sperm can be used. The role of genetically inactivated heterologous sperm in the induction of gynogenesis is the ability to distinguish gynogenetic offspring from non-gynogenetic or hybrids, as some authors have suggested (Cabrita *et al*., 2008; Lebeda *et al*., 2018).

Table 4. Observed alleles at microsatellite loci: Afu-68 and Afug-9 in gynogenetic progeny of sterlet *Acipenser ruthenus* L. and their parents

The observed alleles (bp)				
Loci	♂ Siberian sturgeon	♀ Sterlet	Gynogenetic	Triploid
Afu-68	168/161/126/121	140/140	140/140	-
			-	-
Afug-9	190/173/142/124	167/147	167/147	190/173/167/147
			147/147	167/147/142/124
			167/167	190/173/167/167
			-	190/173/147/147
			-	167/167/142/124
			-	147/147/142/124

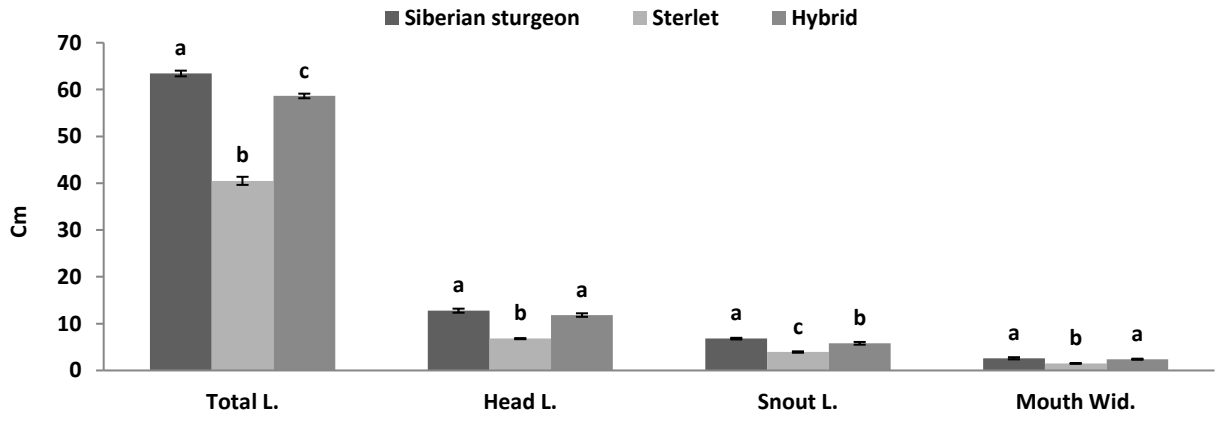

Figure 4. Comparison of the morphometric characteristics of Siberian sturgeon, sterlet and their hybrid.

UV exposure to sperm for 90 seconds in order to genetically inactivate it, reduces sperm motility compared to the control in this study, the motility rate of UV-irradiated sperm reached 50% (control 80%). The decrease in sperm motility can be due to increased DNA breakage caused by UV-irradiation on sperm and its acrosome damage and then structural and functional destruction of sperm, as Li *et al*. (2000) reported that short-term exposure of sperm to UV irradiation (254 nm) caused the destruction of acrosome and flagellum. As the dose of UV increased, the damage of the acrosome became clearly visible until the majority of spermatozoa lost their flagella. Such huge damage made the spermatozoa immotile. Lebeda *et al*. (2014) reported that due to the influence of UV irradiation on the total volume of spermatozoa, the motility of spermatozoa decreases, which may indicate the inactivation of sperm DNA. The duration of sperm UV irradiation in this study was different from the previous study by Saber *et al*. (2014) (60 seconds), which may be due to the different sperm density of individual males in this species, As Lebeda *et al*. (2014) stated, different sperm densities in different species and even individual males can alter the duration of UV radiation.

The results of fertilization and hatching in the haploid group of sterlet showed that the fertilization rate decreased in this group compared to the control. Pruski *et al*. (2009) suggested that reduced motility in a UV-irradiated sperm could decrease fertility rates due to sperm structural changes. These changes are associated with the impact on sperm lipids, proteins, and DNA (Vernet *et al.*, 2004; Dietrich *et al.*, 2005). Peroxidation of the unsaturated fatty acids within the membrane and the attack of reactive oxygen species (ROS) on mitochondria could destabilize the sperm membrane and impair the mitochondrial energy metabolism, thereby affecting sperm motility and fertilizing capability (Pruski *et al.*, 2009).

The highest mortality rate in the haploid group, compared to the control, indicates the appropriate UV radiation and effective sperm DNA damage, as was reported by Saber *et al*. (2014). Olaniyi & Omitogun (2014) stated that decreased survival in haploid groups indicates spermatozoa genetic inactivation. A low number of hatched larvae in the haploid group displayed "haploid syndrome" that died very soon after hatching, as reported in many studies (VanEenennaam *et al*., 1996; Piferrer *et al*., 2004; Omoto *et al*., 2005; Fopp-Bayat *et al*., 2007; Xu *et al*., 2007; Hassanzadeh Saber *et al*., 2008; Saber *et al*., 2014; Fopp-Bayat & Ocalewicz, 2015; Pan *et al*., 2017).

Unlike the haploid group, the gynogenetic diploid group (G_{90}) had higher fertilization and hatching rates, indicating a positive effect of heat shock on successful retention of the second polar body in eggs, so that fertilization and hatching rates of eggs in the triploid group confirm this hypothesis. In this study, only one female breeder was used due to the low number of female sterlet breeders suitable for breeding. The quality of female breeders and their physiological condition especially synchronization during egg releasing is very important (Cassani & Caton, 1985; Varadaraj & Pandian, 1990).

The hybrids derived from the mating between female sterlet and male Siberian sturgeon in this study had similar phenotypic characteristics to the Siberian sturgeon (paternal parent) as observed in the study by Ronyai *et al*. (1991). The presence of hybrids in interspecific gynogenetic treatment could be a phenotypic marker for the identification of non-gynogenetic progeny in sturgeon (Saber *et al*., 2014).

In sturgeons, studies on the gynogenesis or chromosome set manipulations have some limitations (Fopp-Bayat *et al*., 2017). The number of sturgeon breeders is small and the fish reach sexual maturity relatively late. The survival of sturgeon individuals produced within chromosome set manipulations is extremely low. Thus, the number of eggs used for the gynogenetic experiments should be as high as possible. Finally, sterlets produce a small number of eggs, which is due to their small size (Fopp-Bayat et al., 2017). Also, gynogenesis induction increases the mortality rate,

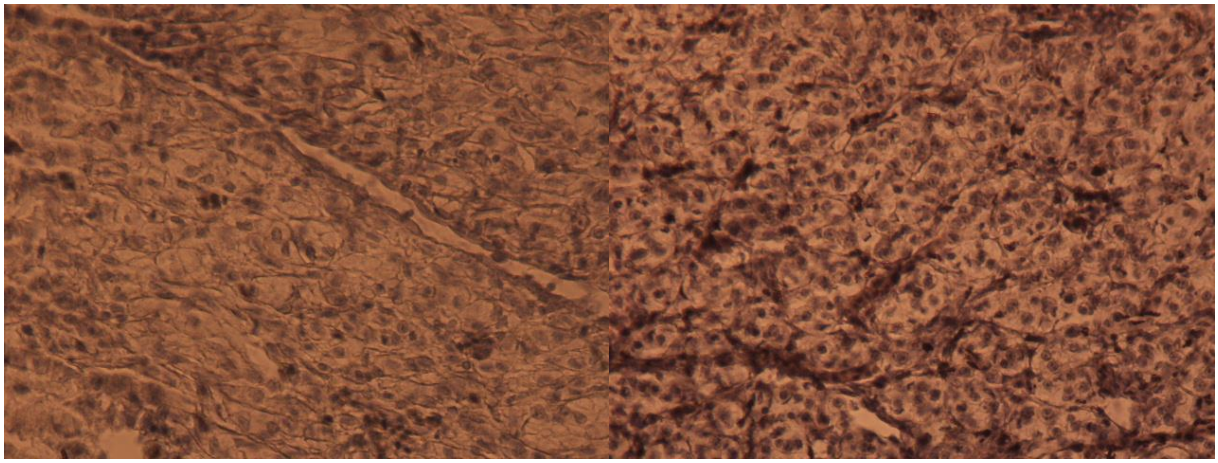

Figure 5. Histological illustrations of female (left) and male (right) gynogenetic sterlet gonads containing oogonia and spermatogonia, respectively

which is related to the severity of homozygosity and induction of shock during early developmental stages (Felip *et al*., 1999; Fopp-Bayat & Ocalewicz, 2015).

Microsatellite markers are the most appropriate method for confirming gynogenetic progeny and the genome heritability of parents and offspring (Fopp-Bayat, 2008) because they are abundant, codominant, have good transferability and high polymorphism in animals (Ma *et al*., 2018). In the present study, two microsatellite markers were used that both showed maternal inheritance in gynogenetic progeny. Hassanzadeh Saber *et al*. (2008) confirmed the allelic similarity in the gynogenetic progeny and the parent of the stellate sturgeon (*Acipenser stellatus*) using two microsatellite loci. Thorgaard *et al*. (1985) and Fopp-Bayat & Ocalewicz (2015) stated that only two or three loci are sufficient to confirm maternal inheritance. Maternal allelic inheritance without parental genome contribution in gynogenetic offspring of current study demonstrates the appropriate effect of UV irradiation on sperm and its genetic inactivation and diploidization by heat shock, as Fopp-Bayat & Ocalewicz (2015) have pointed to this issue.

Normally, the sex ratio in sterlet is 1:1 (Fopp-Bayat *et al*., 2018) as in other sturgeons (Wuertz *et al*., 2018; Hurvitz *et al*., 2007; Doroshov *et al*., 1997). In this study, the gonad histological study of gynogenetic fish showed that 76.6% of the fish produced were female and only 23.4% were male, indicating a tendency of sex towards female and independent assorting the sex-determining gene of the centromere. VanEenennaam *et al*. (1999) suggested when the sex-determining factor is independently assorted of the centromere, about 67% heterozygotes may be produced and the value may go up to 88%.

Fopp-Bayat *et al*. (2018) introduced the gynogenetic sterlet sex-determination system as female heterogamy. In their study, 7% of male gynogenetic progeny were induced, whereas in the present study 23.4% of male progeny have been induced, which may depend on the distance the sex-determining gene of the centromere (Shelton & Mims, 2012; Arai & Fujimoto, 2018).

Most researchers believe that conservation of wild stocks and improvement of sturgeon aquaculture development can be accomplished through genome manipulation techniques (Omoto *et al*., 2005; Flynn *et al*., 2006; Fopp-Bayat, 2010; Grunina *et al*., 2011; Shelton & Mims, 2012; Saber & Hallajian, 2014; Fopp-Bayat *et al*., 2018). Therefore, manipulating the genome (induction of gynogenesis) in sterlet sturgeon with heterologous sperm could compensate for the problem of male shortage in this valuable species and save it from extinction.

Conclusion

This study demonstrated the role of inactivated heterologous sperm of Siberian sturgeon in producing sterlet gynogenetic progeny and other sturgeon. Therefore, it is possible to protect this species from extinction and to produce both males and females, only by having female breeders and the practical results of this study can be applied in fisheries management and sturgeon rehabilitation centers.

It is recommended that in future studies, sturgeon haploid progenies be diploidized with hydrostatic pressure shock. Genetically inactivated sperm of other fish (such as bony fish) could also be used to induce gynogenesis.

Ethical Statement

Not applicable

Funding Information

Not founding

Author Contribution

Conceptualization, Writing -review and editing, Data Curation, Formal Analysis, Investigation, Methodology, Visualization and Writing -original draft, Project Administration, Resources and Supervision

Conflict of Interest

Failure to declare 70 competing interests can result in immediate rejection of a manuscript.

Acknowledgements

The authors thank Cultivation and Biotechnology Departments of International Sturgeon Research Institute for their help during the experiment

References

Amiri, B. M., Maebayashi, M., Hara, A., Adachi, S. & Yamauchi, K. (1996). Ovarian development and serum sex steroid and vitellogenin profiles in the female cultured sturgeon hybrid, the bester. *Journal of Fish Biology,* 48, 1164-1178. https://doi.org/10.1111/j.1095-8649.1996.tb01812.x

Arai, K. (2001). Genetic improvement of aquaculture finfish species by chromosome manipulation techniques in Japan. *Reproductive Biotechnology in Finfish Aquaculture,* 197, 205-228. https://doi.org/10.1016/B978-0-444-50913-0.50013-8

Arai, K. & Fujimoto, T. (2018). Chromosome manipulation techniques and applications to aquaculture. In H. P. Wang, F. Piferrer, S. L. Chen & Z. G. Shen (Eds.), *Sex Control in Aquaculture,* (pp. 137-162). John Wiley & Sons Ltd.

Aramli, M. S., Azarin, H. & Farsi, P. (2015). Motility parameters, adenosine triphosphate content and oxidative stress indices of sterlet (*Acipenser ruthenus*) sperm after 6 days of storage. *Aquaculture Research*, 48, 719-724. doi: 10.1111/are.12855.

Cabrita, E., Robles, V. & Herráez, M. P. (2008). *Methods in reproductive aquaculture: marine and freshwater species.* CRC Press., https://doi.org/10.1201/9780849380549

Cassani, J. R. & Caton, W. E. (1985). Induced triploidy in grass carp (*Ctenopharyngodon idella*). *Aquaculture,* 46, 37-44. https://doi.org/10.1016/0044-8486(85)90173-5

Chapman, F. A. (1989). Sexual maturation and reproductive parameters of wild and domestic stocks of white sturgeon (*Acipenser transmontanus*). Ph.D. Dissertation, University of California, Davis. P: 200.

Dettlaff, T. A., Ginsburg, A. S. & Schmalhausen, O. I. (1993). *Sturgeon Fishes Developmental Biology and Aquaculture*. Springer-Verlag Berlin Heidelberg.

Devlin, R. H. & Nagahama, Y. (2002). Sex determination and sex differentiation in fish: an overview of genetic, physiological, and environmental influences. *Aquaculture*, 208 (3-4), 191-364. doi.org/10.1016/S0044-8486(02)00057-1

Dietrich, G. J., Szpyrka, A., Wojtczak, M., Dobosz, S., Goryczko, K. & Ciereszko, A. (2005). Effects of UV irradiation and hydrogen peroxide on DNA fragmentation, motility and fertilizing ability of rainbow trout (*Oncorhynchus mykiss*) spermatozoa. *Theriogenology*, *64*(8), 1809-1822. DOI: 10.1016/j.theriogenology.2005.04.010

Dorafshan, S., Kalbassi, M. R., Pourkazemi, M. & Mojazi Amiri, B. (2006). Optimization of UV irradiation for production of gynogenetic rainbow trout, *Oncorhynchus mykiss*: emphasising Hertwig effect and photoreactivation. *Iranian Journal of Fisheries Sciences*, 6 (1), (19-34).

Doroshov, S. I., Moberg, G. P. & VanEenennaam, J. P. (1997). Observations on the reproductive cycle of cultured White sturgeon (*Acipenser transmontanus*). *Environmental Biology of Fishes,* 48, 265–278.

Feledi, T., Kucska, B. & Ronyai, A. (2011). Effect of different fertilization and egg de-adhesion methods on the artificial propagation of Siberian sturgeon. *Archives of Polish Fisheries,* 19, 119–122. DOI 10.2478/v10086-011-0014-7

Felip, A., Piferrer, F., Carrillo, M. & Zanuy, S. (1999). The relationship between the effects of UV light and thermal shock on gametes and the viability of early developmental stages in a marine teleost fish, the sea bass (*Dicentrarchus labrax* L.). *Heredity,* 83, 387–397. DOI: 10.1038/sj.hdy.6886030

Flynn, S. R., Matsuoka, M., Reith, M., Martin-Robichaud, D. J. & Benfey T. J. (2006). Gynogenesis and sex determination in shortnose sturgeon (*Acipenser brevirostrum* Lesuere). *Aquaculture,* 253, 721–727. DOI: 10.1016/j.aquaculture.2005.09.016

Fopp-Bayat, D. (2007). Verification of meiotic gynogenesis in Siberian sturgeon (*Acipenser baeri* Brandt) using microsatellite DNA and cytogenetical markers. *Journal of Fish Biology*, 71, 478-485. https://doi.org/10.1111/j.1095-8649.2007.01704.x

Fopp-Bayat, D. (2008). Inheritance of microsatellite loci in polyploid Siberian sturgeon (*Acipenser baeri* Brandt) based on uniparental haploids. *Aquaculture Research,* 39, 1787-1792. DOI: 10.1111/j.1365-2109.2008.02059.x

Fopp-Bayat, D. (2010). Meiotic gynogenesis revealed not homogametic female sex determination system in Siberian sturgeon (*Acipenser baeri* Brandt). *Aquaculture,* 305, 174–177. DOI: 10.1016/j.aquaculture.2010.04.011

Fopp-Bayat, D., Ocalewicz, K., Kucinski, M., Jankun, M. & Laczynska, B. (2017). Disturbances in the ploidy level in the gynogenetic starlet (*Acipenser ruthenus*). *Journal of Applied Genetics,* 58, 373-380. DOI 10.1007/s13353-017-0389-2.

Fopp-Bayat, D., Hliwa, P. & Ocalewicz, K. (2018). Presence of gynogenetic males suggests a female heterogamety in sterlet (*Acipenser ruthenus* L.). *Animal Reproduction Science,* 189, 110-118. https://doi.org/10.1016/j.anireprosci.2017.12.014.

Fopp-Bayat, D., Kolman, R. & Woznicki, P. (2007). Induction of meiotic gynogenesis in sterlet (*Acipenser ruthenus*). *Aquaculture,* 264, 54–58. DOI: 10.1016/j.aquaculture.2006.12.006

Fopp-Bayat, D. & Ocalewicz, K. (2015). Activation of the albino sterlet (*Acipenser ruthenus*) eggs by UV-irradiated bester hybrid spermatozoa to provide gynogenetic progeny. *Reproduction in Domestic Animals,* 50, 554-559. DOI: 10.1111/rda.12521

Gessner, J., Freyhof, J. & Kottelat, M. (2010). *Acipenser gueldenstaedtii*. IUCN 2012. IUCN Red List of Threatened Species. Version 2012.2. www.iucnredlist.org

Grunina, A. S., Skoblina, M. N., Recoubratsky, A. V., Kovalev, K. V., Barmintseva, A. E. & Goncharov, B. F. (2011). Obtaining gynogenetic progeny of Siberian sturgeon (*Acipenser baerii*) using eggs matured and ovulated in vitro. *Journal of Applied Ichthyology,* 27, 701–705. DOI: 10.1111/j.1439-0426.2010.01643.x

Hassanzadeh Saber, M. H., Baradaran Noveiri, S., Pourkazemi, M. & Yarmohammadi, M. (2008). Induction of gynogenesis in stellate sturgeon (*Acipenser stellatus* Pallas 1771) and its verification using microsatellite markers. *Aquaculture Research,* 39, 1183-1787. DOI: 10.1111/j.1365-2109.2008.02015.x

Hillis, D. M. & Moritz, C. (1990). Molecular taxonomi. Sinauer Associate, Inc. Publishers. Massachusetts.

Hurvitz, A., Jackson, K., Degani, G. & Levavi-Sivan, B. (2007). Use of endoscopy for gender and ovarian stage determinations in Russian sturgeon (*Acipenser gueldenstaedtii*) grown in aquaculture. *Aquaculture,* 270, 158–166. DOI: 10.1016/j.aquaculture.2007.05.020

Ijiri, K. & Egami, N. (1980). Hertwig effect causes by UV irradiation of sperm of *Oryzias latipes* (Teleost) and its photoreactivation. *Mutation Research,* 69, 241–248. https://doi.org/10.1016/0027-5107(80)90089-5

Komen, H. & Thorgaard, G. H. (2007). Androgenesis, gynogenesis and production of clones in fishes: A review. *Aquaculture,* 269, 150-173. http://dx.doi.org/10.1016/j.aquaculture.2007.05.009

Lebeda, I., Dzyuba, B., Rodina, M. & Flajshans, M. (2014). Optimization of sperm irradiation protocol for induced gynogenesis in Siberian sturgeon (*Acipenser baerii*). *Aquaculture International,* 22, 485-495. doi: 10.1007/s10499-013-9658-1.

Lebeda, I., Steinbach, Ch. & Flajshans, M. (2018). Flow cytometry for assessing the efficacy of interspecific gynogenesis induction in sturgeon. *Journal of Fish Biology,* 92(6), 1819-1831. DOI: 10.1111/jfb.13623

Li, Q., Osada, M., Kashihara, M., Hirohashi, K. & Kijima, A. (2000). Effects of ultraviolet irradiation on genetical inactivation and morphological structure of sperm of the Japanese scallop (*Patinopecten yessoensis*). *Aquaculture,* 186, 233–42. DOI: 10.1016/s0044-8486(99)00384-1

Ma, H. Y., Chen, S. L. & Ji, X. S. (2018). Sex-Specific Markers, Gynogenesis, and Sex Control in Spotted Halibut. In H. P. Wang, F. Piferrer, S. L. Chen & Z. G. Shen (Eds.), *Sex*

Control in Aquaculture, (pp. 631-643). John Wiley & Sons Ltd. https://doi.org/10.1002/9781119127291.ch32

May, B., Charles, C., Krueger, C. & Kincaid, L. (1997). Genetic variation at Microsatellite loci in sturgeon. Primer sequence homology in *Acipenser* and *Scaphirhynchus*. *Canadian Journal of Fisheries and Aquatic Science,* 54, 1542-1547. https://doi.org/10.1139/f97-061

Meng, Z., Liu, X., Liu, B., Hu, P., Ji, Y., Yang, Z., Zhang, H. Liu, X. & Lei, J. (2016). Induction of mitotic gynogenesis in turbot (*Scophthalmus maximus)*. *Aquaculture,* 451, 429-435. http://dx.doi.org/10.1016/j.aquaculture.2015.09.010

Nowosad, J., Kucharczyk, D., Targonáska, K. & Kujawa, R. (2015). Comparison of temperature shock timing to induced artificial mitotic gynogenesis and androgenesis in common tench. *Aquaculture International,* 23, 45–53. DOI 10.1007/s10499-014-9796-0

Olaniyi, W. A. & Omitogun, O. G. (2014). Monosex fish production in fisheries management and its potentials for catfish aquaculture in Nigeria. In M. Behnassi et al. (Eds.) *Vulnerability of Agriculture, Water and Fisheries to Climate Change,* (pp. 301-312). Springer Science + Business Media Dordrecht 2014. DOI 10.1007/978-94-017-8962-2_19,

Omoto, N., Maebayashi, M., Adachi, S., Arai, K. & Yamauchi, K. (2005). Sex ratios of triploids and gynogenetic diploids induced in the hybrid sturgeon, the bester (*Huso huso* female × *Acipenser ruthenus* male). *Aquaculture,* 245, 39–47. DOI: 10.1016/j.aquaculture.2004.12.004

Pan, Z. J., Zhu, C. K., Wang, H., Chang, G. L., Ding, H. Y., Qiang, X. G. & Yu, X. S. (2017). Induction of meiotic gynogenesis in bagrid catfish (*Pseudobagrus ussuriensis*) with homologous sperm and its confirmation for female homogamety. *Aquaculture Research,* 48, 5659-5665. DOI: 10.1111/are.13388.

Pandian, T. J. (2011). *Sex determination in fish*. CRC Press.

Pandian, T. A. & Koteeswaran, R. (1998). Ploidy induction and sex control in fish. *Hydrobiologia*, 384(1-3), 167-243. DOI: 10.1023/A:1003332526659

Peruzzi, S. & Chatain, B. (2000). Pressure and cold shock induction of meiotic gynogenesis and triploidy in the European sea bass (*Dicentrarchus labrax* L.): relative efficiency of methods and parental variability. *Aquaculture,* 189, 23–37. https://doi.org/10.1016/S0044-8486(00)00355-0

Piferrer, F., Cal, R. M., Gómez, C., Álvarez-Blázquez, B., Castro, J. & Martínez, P. (2004). Induction of gynogenesis in the turbot (*Scophthalmus maximus*): Effects of UV irradiation on sperm motility, the Hertwig effect and viability during the first 6 months of age. *Aquaculture,* 238(1-4), 403-419. https://doi:10.1016/j.aquaculture.2004.05.009

Pourdehghani, M., Kazemi, R. & Pourkazemi, M. (2010). Propagation and cultivation of sterlet sturgeon as ornamental fish in Iran. The first Conference of Iranian Ornamental Fishes, Tehran, Iran.

Pruski, A. M., Nahon, S., Escande, M. L. & Charles, F. (2009). Ultraviolet radiation induces structural and chromatin damage in Mediterraneansea-urchin spermatozoa. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis,* 673(1), 67-73. https://doi.org/10.1016/j.mrgentox.2008.11.013

Raji, A. R. & Norouzi, E. (2010). Histological and histochemical study on the alimentary canal in Walking catfish (*Claris batrachus*) and piranha (*Serrasalmus nattereri*). *Iranian Journal of Veterinary Research,* 14, 65-68.

Ronyai, A., Ruttkay, A., Varadi, L. & Peteri, A. (1991) Growth comparative trial of fingerlings of sterlet (*Acipenser ruthenus* L.) and that of its hybrid with male Siberian sturgeon (*Acipenser baerii* B.) Acipenser (October 3–6, 1989 Bordeaux, France). P. Williot (Ed.), (pp. 417–421).

Saber, M. H. & Hallajian, A. (2014). Study of sex determination system in ship sturgeon (*Acipenser nudiventris*) using meiotic gynogenesis. *Aquaculture International,* 22, 273–279. https://doi.org/10.1007/s10499-013-9676-z

Saber, M. H., Noveiri, S. B., Pourkazemi, M., Yazdani, M., Ghoroghi, A., Bahmani, M., Pourdehghani, M., Chakmehdouz, F., Yarmohammadi, M. & Nowruzfashkhami, M. (2014). Induction of meiotic gynogenesis in ship sturgeon (*Acipenser nudiventris*) using UV-irradiated heterologous sperm. *Journal of Applied Genetics,* 55, 223–229. DOI 10.1007/s13353-013-0188-3

Shelton, W. L. & Mims, S. D. (2012). Evidence for female heterogametic sex determination in paddlefish (*Polyodon spathula*) based on gynogenesis. *Aquaculture,* 356-357, 116-118. DOI: 10.1016/j.aquaculture.2012.05.029

Tegelström, H. (1986). Mitochondrial DNA in natural populations: an improved routine for the screening of genetic variation based on sensitive silver staining. *Electrophoresis,* 7, 226–229. https://doi.org/10.1002/elps.1150070508

Thorgaard, G. H., Scheerer, P. D. & Parsons, J. E. (1985). Residual paternal inheritance in gynogenetic rainbow trout (*Salmo gairdneri*): implications for gene transfer. *Theoretical and Applied Genetics,* 71, 119–121. DOI: 10.1007/bf00278262

VanEenennaam, J. P. & Doroshov, S. I. (1998). Effects of age and body size on gonadal development in Atlantic sturgeon (*Acipenser oxyrinchus* Mitchill). *Journal of Fish Biology,* 53, 624- 637. https://doi.org/10.1111/j.1095-8649.1998.tb01006.x

VanEenennaam, A. L., VanEenennaam, J. P. V., Medrano, J. F. & Dorshov, S. I. (1996). Rapid verification of meiotic gynogenesis and polploidy in with sturgeon (*Acipenser transmontanus* Richardson). *Aquaculture,* 147, 177-189. https://doi.org/10.1016/S0044-8486(96)01369-5

VanEenennaam, A. L., VanEenennaam, J. P., Medrano, J. F. & Doroshov, S. I. (1999). Evidence of female heterogametic genetic sex determination in white sturgeon. *Journal of Heredity,* 90, 231–233. https://doi.org/10.1093/jhered/90.1.231

Varadaraj, K. (1990). Production of diploid (*Oreachromis mossambicus)* gynogenes using heterologous sperm of (*Cyprinus carpio*). *Indian Journal of Experimental Biology,* 28, 701-705.

Varadaraj, K. & Panadian, T. J. (1990). Production of all female sterile triploid (*Oreochromis mossambicus*). *Aquaculture,* 84, 117-123. DOI: 10.1016/0044-8486(90)90342-k

Vernet, P., Aitken, R. J. & Drevet, J. R. (2004) Antioxidant strategies in the epididymis. Molecular *and* Cellular Endocrinology, 216, 31-39. DOI: 10.1016/j.mce.2003.10.069

Welsh, A. & May, B. (2006). Development and standardization of disomic microsatellite markers for lake sturgeon genetic studies. *Journal of Applied Ichthyology,* 22(5), 337–344.

https://doi:10.1111/j.1439-0426.2006.00814.x

Wuertz, S., Güralp, H., Pšenička, M. & Chebanov, M. (2018). Sex determination in sturgeon. In H. P. Wang, F. Piferrer, S. L. Chen & Z. G. Shen (Eds.), *Sex Control in Aquaculture,* (pp. 645-667). John Wiley & Sons Ltd.

Xu, H., You, F., Yan, B. L. & Zhang, P. (2007). Effects of ultra-violet irradiation on sperm motility and diploid gynogenesis induction in large yellow croaker (*Pseudosciaena crocea*) undergoing cold shock. *Aquaculture International,* 15, 371–382. https://doi.org/10.1007/s10499-007-9101-6

Zou, Y. C., Wei, Q. W. & Pan, G. B. (2011). Induction of meiotic gynogenesis in paddlefish (*Polyodon spathula*) and its confirmation using microsatellite markers. *Journal of Appllied Ichthyology,* 27, 496–500. doi: 10.1111/j.1439-0426.2011.01681.x