

Influence of interspecific hybridization on fitness-related traits in Siberian sturgeon and Russian sturgeon

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Citation: Shivaramu S., Vuong D.T., Havelka M., Šachlová H., Lebeda I., Kašpar V., Flajšhans M. (2019): Influence of interspecific hybridization on fitness-related traits in Siberian sturgeon and Russian sturgeon. Czech J. Anim. Sci., 64: 78–88.

Abstract: Polyploidy in sturgeons makes them highly susceptible to interspecific hybridization, and these interspecific hybrids have been described in nature as well as in captivity. Nevertheless, the fitness-related traits between sturgeon hybrids and pure species have been poorly compared as yet. In the present study, we compared the reproductive parameters such as fertilization rate and hatching rate, growth traits and genetic polymorphism in the artificially produced hybrids of the Siberian sturgeon (*Acipenser baerii*) and Russian sturgeon (*A. gueldenstaedtii*) with their purebreds. Fertilization and hatching rates were found to be significantly higher in Siberian sturgeon (♀) × Russian sturgeon (♂) hybrid group compared to purebreds. The highest cumulative survival rate was determined in purebred groups until 151 days post-hatch (dph); however, this trend changed and Russian sturgeon purebred showed the lowest cumulative survival rate (0.21%) by 913 dph. Similarly, the lowest average body weight was recorded in Russian sturgeon purebred group (264 g). In contrast, the highest average body weight was recorded in Russian sturgeon (♀) × Siberian sturgeon (♂) hybrids (435.3 g) and the highest cumulative survival rate was recorded in Siberian sturgeon (♀) × Russian sturgeon (♂) hybrids (12.32%) by 913 dph. No significant differences were found at heterozygosity levels among studied crosses. Our results showed that studied sturgeon hybrids had higher survival and growth if compared with the purebreds under provided hatchery conditions.

Keywords: Acipenseriformes; aquaculture; hybrid; growth traits; heterosis

Sturgeons (Acipenseriformes) are among the ancient and primitive fish groups of bony fishes which are extensively distributed in the Northern Hemisphere. However, their populations have rapidly declined throughout their range because of overharvest, pollution, habitat destruction and hydro constructions (Billard and Lecointre 2000; Pikitch

et al. 2005; Jaric and Gessner 2011). Currently, the International Union for Conservation of Nature (IUCN) lists 16 out of 27 species of sturgeons as critically endangered and these species are at the verge of extinction (Ludwig et al. 2009). In the past two decades, studies on biology and genetics of sturgeons have increased since their survival in

Supported by the Ministry of Education, Youth and Sports of the Czech Republic – Projects CENAKVA and Biodiverzita (CZ.02.1.01/0.0/0.0/16_025/0007370), by the Czech Science Foundation (GA CR projects No. 14-02940S and No. 18-09323S), and by the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 642893.

<https://doi.org/10.17221/165/2018-CJAS>

the wild is on the brink of extinction and they are considered as flagship species for aquaculture due to high commercial value for their caviar (Fontana et al. 2008; Dettlaff et al. 2012; Zhang et al. 2013).

Hybridization is a process in which offspring inherit restructured parental genes obtained by mating individuals of different genotypes, which is believed to have evolutionary significance in the process of speciation (Abbott et al. 2013). Such production of hybrids may generally have superior or preferred characteristics as compared to both parents leading to heterosis at the individual level and high genetic variance for several phenotypic traits at the species level which is the key breeding goal (Facon et al. 2008). Hybridization may preserve maximum genetic diversity and lead to novel adaptations, allowing higher fitness in a rapidly changing environment from an evolutionary perspective (Arnold 1997). One undesirable consequence of hybridization events might be reduced viability of the offspring because of outbreeding depression and should be seriously considered. In nature, hybridization occurs widely in fish compared to other vertebrates in nature (Allendorf and Waples 1996; Rahman et al. 2013). Factors that contribute to the high incidence of hybridization in natural waters are external fertilization, weak behavioural isolation mechanisms, an unequal abundance of two parental species, decreasing habitat complexity and eventually competition for spawning habitats (Campton 1987). Many interspecific and intergeneric hybrids among closely related fish species like cyprinids and catfishes are produced to exploit desirable traits like improved growth rate, flesh quality, and disease resistance through heterosis. Thus, interspecific hybridization as a potential tool to improve productivity in aquaculture has been widely studied among various fish species (Chevassus 1983; Bartley et al. 2001; Rahman et al. 2013).

Sturgeons are evolutionary polyploids inherently linked with at least three independent genome duplication events during their evolution (Havelka et al. 2013). They show a remarkable susceptibility to hybridize under natural conditions (Ludwig et al. 2009) and in artificial propagation (Zhang et al. 2013). The production of hybrids in sturgeon aquaculture has rapidly increased over last two decades because they are considered to perform better than pure species (Bronzi et al. 1999; Pikitch et al. 2005; Zhang et al. 2013). One clear example of the aforementioned phenomenon can be seen in the case of hybrid between Kaluga sturgeon (*Huso*

dauricus) and Amur sturgeon (*Acipenser schrenckii*). This hybrid exhibited better viability and growth than its parental species and has recently become the most exploited for caviar production (Wei et al. 2011; Boscari et al. 2014). Glogowski et al. (2002) compared the growth characteristics of juvenile hybrids between Siberian sturgeon (*A. baerii*) and Russian sturgeon ($\text{♀} \times \text{♂}$ *A. gueldenstaedtii*). Likewise, Arefjev (1999) documented the performance of the reciprocal hybrids of Russian sturgeon and Siberian sturgeon (*A. gueldenstaedtii* $\text{♀} \times \text{♂}$ *A. baerii*) which displayed high levels of variability. Contrarily, Billard and Lecointre (2000) reported that sturgeon hybrids usually do not perform better than the best parents since the superiority of sturgeon hybrids has not yet been demonstrated clearly. Ludwig (2006) stated that sturgeon hybrids may perform worse than either parental species due to outbreeding depression resulting from interspecific crosses. The tendency for hybridization and producing viable offspring is strongly influenced by various factors such as evolutionary polyploidy, genetic structure, gene flow pattern, gamete compatibility and similar reproductive behaviours of the parental species (Rahman et al. 2013). Still, extrinsic factors like culture systems, environmental parameters, stress associated with handling may equally influence the viability of hybrid offspring which has to be quantified. Furthermore, it is essential to study the genetic status and fitness characteristics of sturgeon hybrids since they can naturally occur in wild or as accidental escapees from aquaculture (Maury-Brachet et al. 2008), and would have a significant impact on the genetic integrity of wild populations (Ludwig et al. 2009). Despite their widespread use in aquaculture, sturgeon hybrids are not yet extensively studied. Therefore it is necessary to evaluate the hybrid performance in comparison to pure parental species to understand the influence of hybridization on the genotypes and phenotypes of first-generation hybrids.

This study was designed to investigate the influence of interspecific hybridization on sturgeon fitness-related traits. The purebred and hybrid crosses of Russian sturgeon and Siberian sturgeon were produced by artificial propagation. Fitness-related characteristics such as reproductive features (fertilization rate and hatching rate), growth (average body weight), and cumulative survival were investigated and compared among hatchery produced hybrids and purebred groups. Heterosis effect for growth, cumulative survival, and specific growth rate in dif-

<https://doi.org/10.17221/165/2018-CJAS>

ferent rearing periods was also estimated. The level of genetic polymorphism among the groups was analyzed by a set of microsatellite markers.

MATERIAL AND METHODS

Ethics. The study was carried out at the Genetic Fisheries Center of the Faculty of Fisheries and Protection of Waters (FFPW) in Vodňany. The experimental protocol of the study underwent an ethical review process and was approved by the expert committee of the Institutional Animal Care and Use Committee (IACUC) at the University of South Bohemia (USB), according to the law on the protection of animals against cruelty (Act No. 246/1992 Coll., ref. number 16OZ15759/2013-17214). To decrease stress at the time of fish handling, the fish were anesthetized using clove oil during the biopsy.

Fish broodstock handling and breeding. Two *A. baerii* (♂S), three *A. gueldenstaedtii* males (♂R), two *A. baerii* (♀S) and two *A. gueldenstaedtii* females (♀R) originating from the genetic fisheries centre at the Faculty of Fisheries and Protection of Waters were used for the production of purebred and hybrid groups (Table 1). Fish were kept in controlled conditions in 5 m³ indoor tanks supplied with re-circulating water system at 15°C for 7 days prior to hormone stimulation. Fish were immersed in 0.07 ml/l clove oil anesthesia before handling. Spermiation was induced by injecting males intramuscularly with 4 mg/kg M_B carp pituitary powder (Rybníkářství Pohořelice, Czech Republic) in physiological saline 36 h before expected sperm collection. Sperm was collected in a 100 ml tissue culture flask by inserting a plastic catheter of 5 mm diameter into the seminal duct (Gela et al. 2008). Ovulation was induced in females with an initial injection of 0.5 mg/kg M_B carp pituitary suspension in physiological saline 42 h before expected ovulation, and a second injection after 12 h with 4.5 mg/kg M_B of the same suspension (Gela et al. 2008). Ovulated eggs were collected by microsurgical incision of oviducts following the procedure given by Stech et al. (1999); they were maintained in aerobic conditions prior to fertilization by storing under 16°C during the evaluation of gamete parameters like motility rate, egg counting, etc. The females with better quality of eggs were used for further artificial propagation considering the impacts of egg quality on the fertilization and hatching rate.

Table 1. Characteristics of broodfish of *Acipenser baerii* and *Acipenser gueldenstaedtii* including age, body weight (BW) and total length (L_T)

Species	Age (years)	BW (kg)	L _T (cm)
Males			
<i>A. baerii</i>	10	6.5	118
<i>A. baerii</i>	9	7	123
<i>A. gueldenstaedtii</i>	8	7	106
<i>A. gueldenstaedtii</i>	8	7.5	119
<i>A. gueldenstaedtii</i>	8	7	112
Females			
<i>A. baerii</i>	18	17	142
<i>A. baerii</i>	9	10	126
<i>A. gueldenstaedtii</i>	15	12	131
<i>A. gueldenstaedtii</i>	15	14	134

Evaluation of sperm parameters. Samples from individual male were stored separately in an icebox at 4°C and assessed for sperm volume, sperm density, and sperm motility according to Linhart et al. (2000). The samples with above 70% motility were used for fertilization.

Fertilization and hatching. By using factorial mating design, 4 crosses were produced, out of which 2 were purebred crosses: *A. gueldenstaedtii* purebred (R♀ × R♂), *A. baerii* purebred (S♀ × S♂), and the remaining 2 were hybrid crosses: *A. gueldenstaedtii* × *A. baerii* (R♀ × S♂) and *A. baerii* × *A. gueldenstaedtii* (S♀ × R♂) (Figure 1). In order to establish each cross, an equal number of eggs from females were pooled and placed in 50 g aliquot plastic beakers according to the number of males. These beakers were placed

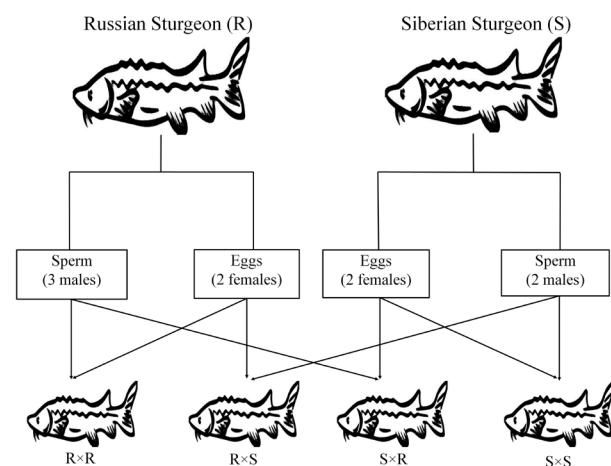


Figure 1. Schematic diagram showing the establishment of different crosses

<https://doi.org/10.17221/165/2018-CJAS>

on an electronic shaker at a speed of 200 rpm and 10 mm deflection. Each aliquot was inseminated with 1.5 ml sperm collected from one of the two/three males per cross separately and spermatozoa were activated immediately by adding 200 ml dechlorinated water. Clay suspension was added 3 min after fertilization to remove egg stickiness, all aliquots of the respective crosses were pooled into a bowl and left on the shaking table for 45 min, and were then subjected to repeated washing with water to remove clay remnants. The fertilized eggs were incubated in Kannengieter incubation jars. During incubation, the Kannengieter flasks were supplied with UV sterilized re-circulating tap water at 15°C, 9 mg/l O₂. To estimate the fertilization rate, around 100 eggs were randomly sampled in triplicate from each cross after 6 h post-fertilization, and the live embryos were counted at the 2nd or 3rd cleavage division. The larvae started to hatch after 4–5 days of incubation, and hatching rate was determined following the protocol of Linhart et al. (2006) and few larvae per each cross were sampled for subsequent molecular analyses.

Fish rearing conditions. The larvae of each cross were initially reared in separate 0.3 m³ indoor tanks. After yolk sac absorption, larvae were shifted to exogenous feeding on diced sludge worms (*Tubifex tubifex*) for two weeks. Progeny of each cross was moved after 100 days of initial rearing to separate 3.5 m³ indoor tanks with the average temperature of 22°C for separate group nursing. Initial stocking density was 7 kg/m³ and larvae were fed *ad libitum* with a formulated commercial feed (Coppens® Start Premium; Coppens International B.V., the Netherlands) containing 54% protein, 15% fat, 1% crude fibre, and 9.4% ash. On day 101 post-hatch (dph) juveniles were colour-marked with Visible Implant Elastomers (Northwest Marine Technology Inc., USA) on the inner ventral side of the rostrum to indicate group origin. These colour-marked fishes were identified and an equal number from each group were stocked for communal rearing in triplicates with identical environmental conditions such as aeration, continuous partial water exchange, feeding rate, and photoperiod. Fishes were implanted by Individual Passive Integrated Transponder (PIT) tags (134.2 kHz; AEG Comp., Germany) subcutaneously after a year. After the second summer, the juveniles were transferred for overwintering in 4 m³ indoor circular tanks at 4°C without feeding. After wintering, fish were held in 3.5 m³ outdoor circular tanks with an average

temperature of 22°C and were fed on daily commercial diet of 4% of total fish biomass (Coppens® Supreme-10 containing 49% protein, 10% fat, 0.8% crude fibre, and 7.9% ash) in the subsequent seasons. The fish were reared in outdoor earthen ponds with an initial stocking density of 25 kg/m³, and fed daily at 4% of total fish biomass the aforementioned commercial diet for last 6 months of assessment period (from 789 dph to 913 dph).

Measurement of growth traits performance of fishes. Fishes were weighed and assessed for periodic growth and survival rates on 10, 37, 101, 151, 262, 459, 569, 667, 737, 789, 863 and 913 dph. To determine the body weight (wet weight), each individual was weighed on weighing balance to 0.1 g and average values were calculated.

Estimation of average heterosis and specific growth rate. Heterosis was estimated for growth and survival traits of hybrid crosses (R × S and S × R) by using average body weight values for growth and survival values from the periodic assessment of pure-bred crosses (R × R and S × S). The heterosis effect was calculated by using the formula given below for both these traits as described by Zheng et al. (2006).

$$\text{Average heterosis} = [(F1 - MP)/MP] \times 100$$

where:

F1 = value of hybrid

MP = mean value of two parents

Specific growth rate (SGR) is defined as the percentage daily weight gain related/proportional to the average weight for the reference period. The SGR was estimated using the below-mentioned formula (Lugert et al. 2016):

$$\text{SGR} = (\ln W_f - \ln W_i \times 100)/t$$

where:

W_f = final weight

W_i = initial weight

t = time (days) between W_f and W_i

Molecular analysis. Sturgeons are supposed to be of allopolyploid origin with at least three independent genome duplication events (Vasilev 2009). The levels of heterozygosity and genetic polymorphism among analyzed crosses were investigated at several microsatellite loci according to McQuown et al. (2000) and Welsh et al. (2003), and analyzed as described by Havelka et al. (2013). To achieve this, tissue samples (fin clips) from the 24 swim-up lar-

vae from each experimental group were collected and stored in 96% molecular grade ethanol after hatching. Whole genomic DNA was extracted using the Nucleo Spin[®] Tissue kit (Macherey-Nagel GmbH & Co. KG, Germany). In total, 13 microsatellite markers were initially tested for amplification. From 11 successfully amplified markers, following 8 markers were chosen for subsequent analyses based on their level of polymorphism: AciG 35 (Bork et al. 2008), AfuG 54, AfuG 135 (Welsh et al. 2003), Aox 45 (King et al. 2001), Spl 101, Spl 105, Spl 163 and Spl 173 (McQuown et al. 2000). The PCR amplification was achieved using the protocol described by Havelka et al. (2013). The fragment analysis of microsatellites was carried out on 3500 ABI Genetic Analyzer (Applied Biosystems, USA) using GeneScan LIZ 600 size standard (Applied Biosystems). The genotypes were scored in Genemapper 4.1 software (Applied Biosystems). Mean number of alleles (N_A), expected (H_E) and observed (H_O) heterozygosities were calculated with TETRASAT software (Markwith et al. 2006). The mean number of alleles present in each family was also used to access the level of polymorphism among analyzed crosses.

Statistical analysis. Data were first analyzed for normal distribution using the Kolmogorov–Smirnov test. Multiple comparisons were carried out by one-way ANOVA and Tukey’s post-hoc (parametric data) or Kruskal–Wallis and Dunn’s post-hoc (non-parametric data) test to detect the differences in fertilization, hatching, and growth rate among the crosses. Differences in survival were evaluated using Pearson’s Chi-square test. The significance of differences in N_A , H_E and H_O among the crosses was tested using one-way ANOVA. As data were not normally distributed even after transformation, non-parametric statistics, i.e., Kruskal–Wallis test followed by post-hoc comparisons of mean ranks of all crosses (Siegel and Castellan 1988) was applied. The statistical analysis was performed with the STATISTICA software (Version 13.2) at $P < 0.05$.

RESULTS

Hatching, fertilization and cumulative survival rates. The highest value of fertilization rate was recorded in S × R hybrid ($93.6 \pm 7.8\%$) followed by R × S ($92.8 \pm 5.35\%$). The lowest values of fertiliza-

tion rate were recorded for the R × R purebred where the fertilization value ($85.2 \pm 11.23\%$) was 8.4 per cent points lower than the highest fertilization rate recorded in the S × R. Fertilization rates were significantly higher ($P < 0.05$) in hybrids of R × S and S × R if compared to purebreds of R × R (Figure 2A).

Hatching rate was the highest for S × R hybrid ($69.4 \pm 6.11\%$). The lowest hatching rate value was reported for the R × R purebred and was 15.8 per cent point lower compared to the highest recorded hatching rate in the S × R hybrids. Hatching rates were significantly higher ($P < 0.05$) in hybrids of S × R if compared to purebreds of R × R and S × S. The hatching rates significantly varied among all groups except the S × S purebred with R × R and R × S groups. Both fertilization and hatching rates were significantly higher in S × R hybrid group if compared to purebreds of R × R and S × S (Figure 2A).

At the beginning of the experiment, the highest cumulative survival was shown by the R × R (65.9%) and S × S (69.1%) purebred groups, but this trend changed after 37 dph. The R × R purebred showed the least cumulative survival in most assessment times. Significant differences ($P < 0.05$) in cumulative survival were noted for the S × R group in most periods checked; however, no significant difference was recorded in the R × R group. On 913 dph, the highest cumulative survival was recorded in the S × R group (12.3%), whereas the lowest survival rates were recorded in the R × R group (0.21%) (Figure 2C).

Body weight and specific growth rate. The highest values of average body weight were observed for the R × S hybrid (435.3 ± 179.35 g) and the lowest were observed for the R × R purebred (264 ± 121.9 g) on 913 dph. Significant growth differences ($P < 0.05$) were observed for the R × S hybrid at most of the assessment times; nevertheless, no significant differences in growth were reported in R × R (Figure 2B).

The fish were maintained in tanks and the first two assessments were not considered for estimating the SGR since the fishes were too small to measure accurately. The highest SGR was recorded during initial rearing in indoor circular tanks (101–262 dph) as compared to outdoor circular tanks and earthen ponds. The highest SGR recorded was in the R × S group (0.35), and the R × R group purebred showed the lowest SGR (0.28) among all assessment periods (Table 2).

Heterosis for survival and growth. Estimated heterosis was high in the survival trait of the S × R group in most of the assessment times except the

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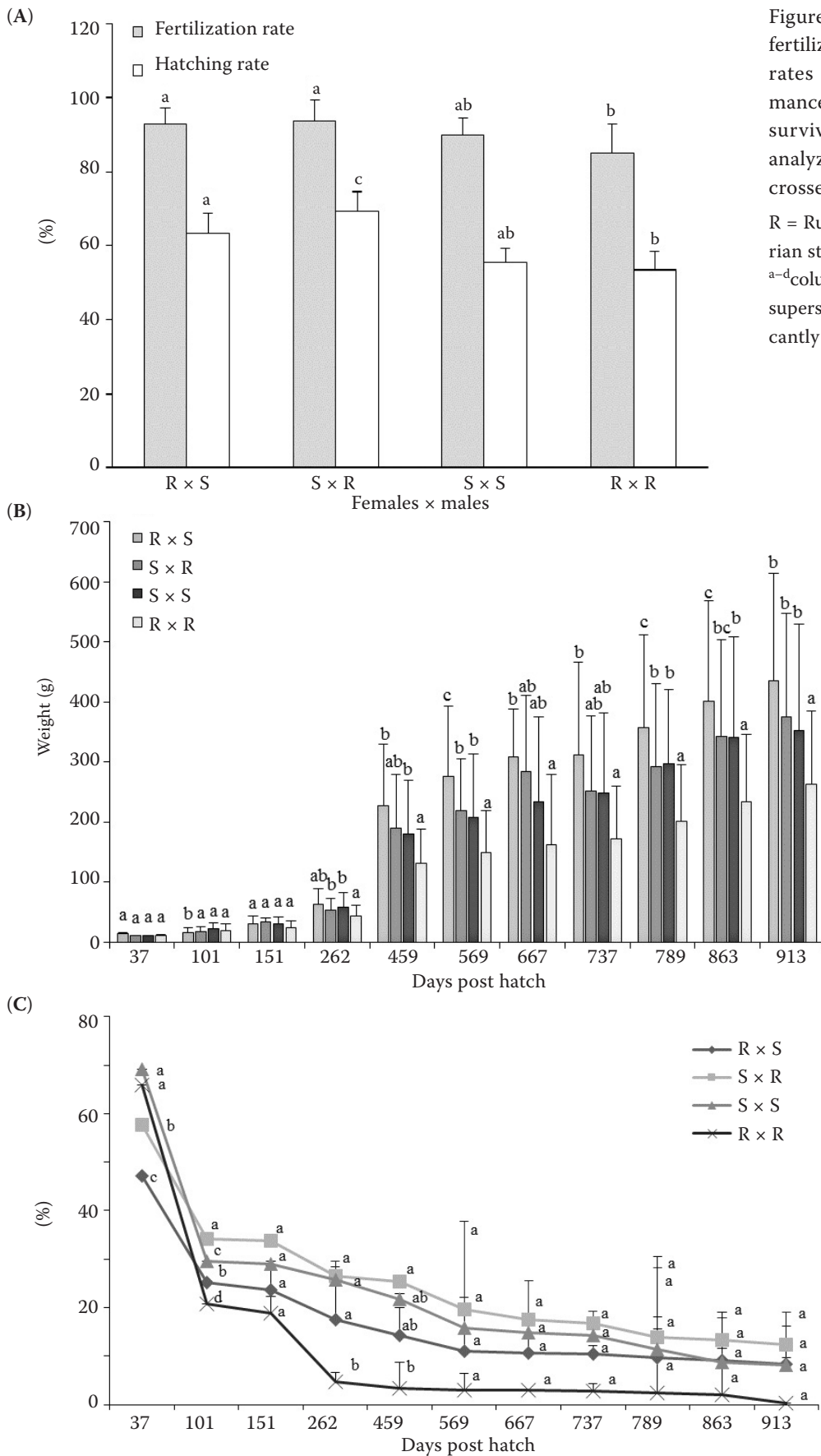


Figure 2. Observed values of fertilization rates and hatching rates (A), of growth performance (B), and of cumulative survival rate (C) among the analyzed hybrid and purebred crosses

R = Russian sturgeon, S = Siberian sturgeon

a–d columns/marks with the same superscript did not differ significantly at $P < 0.05$

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Table 2. Specific growth rate for each group in different rearing systems and overall periods assessment

Cross	Specific growth rate			
	indoor circular tanks (101–262 dph)	outdoor circular tanks (459–737 dph)	outdoor earthen ponds (789–913 dph)	overall (101–913 dph)
R × S	0.82	0.14	0.06	0.35
S × R	0.65	0.13	0.03	0.32
S × S	0.60	0.15	0.02	0.30
R × R	0.45	0.13	0.05	0.28

R = Russian sturgeon (*A. gueldenstaedtii*), S = Siberian sturgeon (*Acipenser baerii*), dph = days post hatch

Table 3. Average heterosis of survival and growth rate for hybrid groups over given periods

Days post hatch	Average heterosis			
	S × R growth	R × S growth	R × S survival	S × R survival
37	-4.17	25.00	-30.07	-14.67
101	-13.05	-20.67	0.16	35.96
151	22.59	12.21	-0.09	40.69
262	3.02	21.79	14.51	73.48
459	21.94	46.27	13.49	100.61
569	22.58	54.22	16.94	109.57
667	43.08	55.78	20.49	98.03
737	19.78	48.28	21.33	95.32
863	17.54	43.22	39.56	101.52
913	18.84	39.11	70.45	146.36

R = Russian sturgeon (*A. gueldenstaedtii*), S = Siberian sturgeon (*Acipenser baerii*)

Table 4. Summary statistics of the genetic variation among the purebred and hybrid crosses

Population	Sample size	Loci typed	N_A	H_E	H_O
S × S	24	8	5.31 ± 2.43	0.6816 ± 0.041	0.6278 ± 0.029
R × R	24	8	6.69 ± 2.02	0.7704 ± 0.023	0.6345 ± 0.031
R × S	24	8	5.92 ± 2.47	0.7321 ± 0.031	0.6604 ± 0.029
S × R	24	8	7.92 ± 2.66	0.7922 ± 0.027	0.6015 ± 0.03

R = Russian sturgeon (*A. gueldenstaedtii*), S = Siberian sturgeon (*Acipenser baerii*), N_A = mean number of alleles, H_E = expected heterozygosity, H_O = observed heterozygosity; values are means ± SD for all parameters

first two. Estimated heterosis was the highest in the 1st assessment (37 dph) and dropped during the 2nd assessment (101 dph), then eventually raised during next assessments for body weight trait in the R × S group. The lowest estimated heterosis was recorded for body weight trait in the S × R group in most of the periods checked (Table 3).

Genetic variability. Observed heterozygosity ranged from 0.6816 in S × S purebred to 0.7922 in S × R hybrid. No significant differences were observed in heterozygosity levels among analyzed crosses (Table 4). However, on the other hand, the mean

number of alleles per locus was significantly higher in the S × R hybrids, whereas the S × S purebreds showed a significantly lower mean number of alleles per locus. Interestingly, no other groups displayed significant differences in this parameter.

DISCUSSION

Interspecific hybridization might lead to an increase in genetic polymorphism and hence increase heterozygosity of hybrid individuals (deWet et al.

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1983). This increase in heterozygosity is considered as a major contribution to interspecific hybridization for growth indicators and other features related to the fitness of individuals (Scheerer and Thorgaard 1983; Reddy 1999) which will be discussed with the results obtained in the present study.

In our study, the R × R purebred group showed the lowest fertilization and hatching rates among the crosses. In aquaculture, reproductive indicators are considered to be complementary indicators, while survival and growth is probably one of the most driving and desirable indicators (Bartley et al. 2001). The highest cumulative survival was recorded in the S × R hybrid in most assessing periods. This could be associated with various intrinsic and extrinsic factors affecting the experimental groups between 10 to 913 dph. Once the larvae switches to formulated feeds, it is a very crucial period for the survival and growth traits, so the size of the feed should be seriously considered. If these conditions are not met, the larval size difference can result in cannibalism (Szczechowski et al. 2000). Memis et al. (2009) also found similar survival rates for Russian sturgeon purebreds, they observed the survival rate declined to 27% at 75 dph, and in our experiment survival rates recorded were 20.8% after 101 dph. The S × R hybrid group showed better survival rates throughout the experiment compared to the reciprocal hybrid R × S and S × S purebred, which can be discussed within many related studies. Safronov and Filipova (2000) compared the growth of the 1st and 2nd generation of Russian and Siberian sturgeon hybrids, and the hybrids produced by their reciprocal backcross with parental species, finding that hybrids formed by reciprocal backcross grew slower, but showed higher survival than hybrids and pure species. But the average growth and cumulative survival was lower when compared to the previous studies and it can be attributed to several factors like unfavourable rearing conditions, pooled stocking and environmental fluctuations.

We noticed that the S × R hybrid group grew significantly faster than other groups from 37 dph which is comparable with the study by Barulin et al. (2008). They observed higher growth in terms of weight and length gain in the hybrid R × S compared to the purebreds of the R × R and S × S between 30–50 dph. The average weight of the hybrid R × S was 25% higher than the S × S purebred and 22% higher than the R × R purebred in their study,

whereas we recorded a 20% increase in the average weight of the R × S hybrid in comparison to the R × R purebred. Growth differences in terms of average body weight were not significant between the S × R hybrid and the S × S purebred, which contrasts the findings of Glogowski et al. (2002) who described better growth properties of the S × R hybrid compared to the S × S purebred. Moreover, a high standard deviation in the average weight of individuals was recorded in all experimental groups. This can be probably due to the stocking of uneven size ranges of the individuals leading to the competition for feed. Chebanov and Galich (2011) in their sturgeon rearing manual recommended that fish weighing between 0.2 and 0.3 g should be sorted into three size groups every 10 days in order to reduce competition. Sorting increases growth rates, reduces the size of individuals, improves the feed conversion ratio (FCR) and ultimately resolves the stress associated with competition for feed which should be considered in the future studies.

The hybrid crosses displayed positive average heterosis in most of the assessment times for growth and survival traits. Numerous studies have already been done in cyprinid species, some are consistent with our results (Nielsen et al. 2010; Liu et al. 2017). The crosses between different species increase heterozygosity, also reduce effects of recessive lethal genes which enhances the fitness resulting from heterosis (Whitlock et al. 2000). Besides, we found positive heterosis in S × R group for survival trait and intriguing same group displayed the lowest heterosis for body weight trait, but the R × S group recorded intermediate heterosis in the survival and body weight traits. This shift in the performance and heterosis effect could be because of parental position in the hybridization matrix and the level of genetic divergence between the two species. Furthermore, similar results were obtained in previous studies for commercially important aquaculture species. Wang and Xia (2002) revealed the positive relationship between heterosis in growth and genetic distances of interspecific hybrids and intraspecific crosses of Tilapia. In addition, Koolboon et al. (2014) studied the significant correlations between genetic distance and heterosis in catfish which was notably not expressed in the present study. Inheritance of genetic material from species to species through hybridization serves as a source of adaptive genetic

variation (Grant and Grant 1992). However, no significant increment of genetic polymorphism was observed in analyzed hybrid crosses compared to purebred crosses according to the results obtained by microsatellite genotyping. This can be because of the low number of the brood stock used for production of hybrids or particularly due to Siberian and Russian sturgeons are closely related species with a low level of genetic differentiation (Birstein et al. 2005). Besides, both species are recent tetraploids having the same number of gene copies in their genome, theoretically the same number of alleles per locus. Different mean number of alleles per locus between the $S \times S$ and $S \times R$ might be due to single locus variability rather than a sign of total genetic polymorphism among hybrids and purebred crosses in our study. The success of interspecific hybridization also depends on the effective population size, genetic structure and gene flow patterns of the parental species (Rahman et al. 2013) and influences the measurable phenotypes. Our data show that hybrid crosses expressed better fitness-related traits when compared to purebreds, thus suggesting that interspecific hybridization provides a survival advantage to sturgeons during their evolutionary period (Birstein et al. 1997). Nevertheless, the negative effects on maintaining genetic integrity and diversity should be seriously considered. The main disadvantage of using hybrids is that escapees from farms can mix with wild populations (Maury-Brachet et al. 2008) which can pose a threat to the genetic integrity of wild populations (Ludwig et al. 2009). Possible fertility of the hybrid may result in harm to wild populations through genomic introgression. This situation has already been evidently observed in some wild environments (Jennekens et al. 2000; Ludwig et al. 2009; Reinartz et al. 2011). Hence it is pivotal not to release artificially reproduced hybrids from hatcheries or introduce non-native stocks in restocking programs aimed to minimize the additional risk of extinction.

The growth and survival rate of the inter-specific hybrids and purebreds under this study were lower than those commonly reported. The overall performance of the fish crosses was probably negatively influenced by unfavourable rearing conditions. However, as these fish were reared in communal stock, examined fitness-related traits were equally affected by unfavourable rearing conditions. Hence, observed differences in growth (average body weight) and survival were most likely caused by

genetic origin (purebred vs hybrid). To the best of our knowledge, this study brings the first observation of the effect of hybridization on sturgeon fitness-related traits. Thus, it has an important implication for further studies of the phenomena, but any generalization of the results to sturgeon aquaculture should be done with precaution.

CONCLUSION

The current study showed that interspecific hybrids performed better than purebreds. With given importance of sturgeons in aquaculture, their performance in terms of reproducibility, growth, and survivability should be studied to broaden knowledge on sturgeon hybrids performance. We have also recorded significant differences in growth performance between the reciprocal hybrid crosses. Therefore, it is always necessary to consider the position of the individual species in a hybridization matrix.

REFERENCES

- Abbott R., Albach D., Ansell S., Arntzen J.W., Baird S.J., Bierne N., Boughman J., Brelsford A., Buerkle C.A., Buggs R., Butlin R.K., Dieckmann U., Eroukhmanoff F., Grill A., Cahan S.H., Hermansen J.S., Hewitt G., Hudson A.G., Jiggins C., Jones J., Keller B., Marczewski T., Mallet J., Martinez-Rodriguez P., Most M., Mullen S., Nichols R., Nolte A.W., Parisod C., Pfennig K., Rice A.M., Ritchie M.G., Seifert B., Smadja C.M., Stelkens R., Szymura J.M., Vainola R., Wolf J.B., Zinner D. (2013): Hybridization and speciation. *Journal of Evolutionary Biology*, 26, 229–246.
- Allendorf F.W., Waples R.S. (1996): Conservation and genetics of salmonid fishes. In: *Awise J.C. and Hamrick J.L. (eds): Conservation Genetics: Case Histories from Nature*. Springer, New York, USA, 238–280.
- Arefev V.A. (1999): Cytogenetics of inter-ploid hybridization of sturgeons. *Journal of Applied Ichthyology*, 15, 277.
- Arnold M.L. (1997): *Natural Hybridization and Evolution*. Oxford University Press, Oxford, UK.
- Bartley D.M., Rana K., Immink A.J. (2001): The use of inter-specific hybrids in aquaculture and fisheries. *Reviews in Fish Biology and Fisheries*, 10, 325–337.
- Barulin N.V., Mamedov R.A., Lashkevich A.I. (2008): Hybrid *Acipenser gueldenstaedtii* × *Acipenser baerii* a prospective object of sturgeon culture. In: *Proc. Internat. Research and Practice Conference: Strategy of Aquaculture Development under Current Conditions*. Minsk, USSR, 24, 46–51.

<https://doi.org/10.17221/165/2018-CJAS>

- Billard R., Lecointre G. (2000): Biology and conservation of sturgeon and paddlefish. *Reviews in Fish Biology and Fisheries*, 10, 355–392.
- Birstein V.J., Hanner R., DeSalle R. (1997): Phylogeny of the Acipenseriformes: Cytogenetic and molecular approaches. In: Birstein V.J., Waldman J.R., Bemis W.E. (eds): *Sturgeon Biodiversity and Conservation*. Kluwer Academic Publishers, Dordrecht, the Netherlands, 127–155.
- Birstein V.J., Ruban G., Ludwig A., Doukakis P., DeSalle R. (2005): The enigmatic Caspian Sea Russian sturgeon: How many cryptic forms does it contain? *Systematics and Biodiversity*, 3, 203–218.
- Bork K., Drauch A., Israel J.A., Pedroia J., Rodzen J., May B. (2008): Development of new microsatellite primers for green sturgeon and white sturgeon. *Conservation Genetics*, 9, 973–979.
- Boscari E., Barmintseva A., Pujolar J.M., Doukakis P., Muge N., Congiu L. (2014): Species and hybrid identification of sturgeon caviar: A new molecular approach to detect illegal trade. *Molecular Ecology Resources*, 14, 489–498.
- Bronzi P., Rosenthal H., Arlati G., Williot P. (1999): A brief overview on the status and prospects of sturgeon farming in Western and Central Europe. *Journal of Applied Ichthyology*, 15, 224–227.
- Campton D.E. (1987): Natural hybridization and introgression in fishes: Methods of detection and interpretation. In: Ryman N. and Utter F.M. (eds): *Population Genetics and Fishery Management*. University of Washington Press, Seattle, USA, 161–192.
- Chebanov M.S., Galich E.V. (2011): *Sturgeon Hatchery Manual*. Available at <https://secure.wisconsinaquaculture.com/Docs/550.PDF> (accessed July 10, 2018).
- Chevassus B. (1983): Hybridization in fish. *Aquaculture*, 33, 245–262.
- Dettlaff T.A., Ginsburg A.S., Schmalhausen O.I. (2012): *Sturgeon fishes: Developmental biology and aquaculture*. Springer Science and Business Media.
- deWet J.M.J., Fletcher G.B., Hilu K.W., Harlan J.R. (1983): Origin of *Tripsacum andersonii* (Gramineae). *American Journal of Botany*, 70, 706–711.
- Facon B., Pointier J.P., Jarne P., Sarda V., David P. (2008): High genetic variance in life-history strategies within invasive populations by way of multiple introductions. *Current Biology*, 18, 363–367.
- Fontana F., Congiu L., Mudrak V.A., Quattro J.M., Smith T.I., Ware K., Doroshov S.I. (2008): Evidence of hexaploid karyotype in shortnose sturgeon. *Genome*, 51, 113–119.
- Gela D., Rodina M., Linhart O. (2008): The artificial reproduction of the sturgeons (Acipenser). *Methodology edition (Technology Series)*. Research Institute of Fish Culture and Hydrobiology, University of South Bohemia in České Budějovice, Vodňany, 78, 24. (in Czech)
- Glogowski J., Kolman R., Szczepkowski M., Horvath A., Urbanyi B., Sieczynski P., Rzemieniecki A., Domagala J., Demianowicz W., Kowalski R., Ciereszko A. (2002): Fertilization rate of Siberian sturgeon (*Acipenser baeri*, Brandt) milt cryopreserved with methanol. *Aquaculture*, 211, 367–373.
- Grant P.R., Grant R.B. (1992): Hybridization of bird species. *Science*, 256, 193–197.
- Havelka M., Hulak M., Bailie D.A., Prodohl P.A., Flajshans M. (2013): Extensive genome duplications in sturgeons: New evidence from microsatellite data. *Journal of Applied Ichthyology*, 29, 704–708.
- Jaric I., Gessner J. (2011): Analysis of publications on sturgeon research between 1996 and 2010. *Scientometrics*, 90, 715–735.
- Jenneckens I., Meyer J.N., Debus L., Pitra C., Ludwig A. (2000): Evidence of mitochondrial DNA clones of Siberian sturgeon, *Acipenser baerii*, within Russian sturgeon, *Acipenser gueldenstaedtii*, caught in the River Volga. *Ecology Letters*, 3, 503–508.
- King T.L., Lubinski B.A., Spidle A.P. (2001): Microsatellite DNA variation in Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*) and cross-species amplification in the Acipenseridae. *Conservation Genetics*, 2, 103–119.
- Koolboon U., Koonawootrittriron S., Kamolrat W., Nannakorn U. (2014): Effects of parental strains and heterosis of the hybrid between *Clarias macrocephalus* and *Clarias gariepinus*. *Aquaculture*, 424, 131–139.
- Linhart O., Rodina M., Cosson J. (2000): Cryopreservation of sperm in common carp *Cyprinus carpio*: Sperm motility and hatching success of embryos. *Cryobiology*, 41, 241–250.
- Linhart O., Rodina M., Flajshans M., Mavrodiev N., Nebesarova J., Gela D., Kocour M. (2006): Studies on sperm of diploid and triploid tench, *Tinca tinca* (L.). *Aquaculture International*, 14, 9–25.
- Liu X., Liang H., Li Z., Liang Y., Lu C., Li C., Chang Y., Zou G., Hu G. (2017): Performances of the hybrid between CyCa nucleocytoplasmic hybrid fish and scattered mirror carp in different culture environments. *Scientific Reports*, 7, 46329.
- Ludwig A. (2006): A sturgeon view on conservation genetics. *European Journal of Wildlife Research*, 52, 3–8.
- Ludwig A., Lippold S., Debus L., Reinartz R. (2009): First evidence of hybridization between endangered sterlets (*Acipenser ruthenus*) and exotic Siberian sturgeons (*Acipenser baerii*) in the Danube River. *Biological Invasions*, 11, 753–760.
- Lugert V., Thaller G., Tetens J., Schulz C., Krieter J. (2016): A review on fish growth calculation: Multiple functions

<https://doi.org/10.17221/165/2018-CJAS>

- in fish production and their specific application. Reviews in Aquaculture, 8, 30–42.
- Markwith S.H., Stewart D.J., Dyer J.L. (2006): TETRASAT: A program for the population analysis of allotetraploid microsatellite data. Molecular Ecology Notes, 6, 586–589.
- Maury-Brachet R., Rochard E., Durrieu G., Boudou A. (2008): The “storm of the century” (December 1999) and the incidental escape of Siberian sturgeons (*Acipenser baeri*) in the Gironde estuary (SW France): An original bioindicator for metal contamination. Environmental Science and Pollution Research, 15, 89–94.
- McQuown E.C., Sloss B.L., Sheehan R.J., Rodzen J., Tranah G.J., May B. (2000): Microsatellite analysis of genetic variation in sturgeon: New primer sequences for Scaphirhynchus and *Acipenser*. Transactions of the American Fisheries Society, 129, 1380–1388.
- Memis D., Ercan E., Celikkale M.S., Timur M., Zarkua Z. (2009): Growth and survival rate of Russian sturgeon (*Acipenser gueldenstaedtii*) larvae from fertilized eggs to artificial feeding. Turkish Journal of Fisheries and Aquatic Sciences, 9, 47–52.
- Nielsen H.M., Odegard J., Olesen I., Gjerde B., Ardo L., Jeney G., Jeney Z. (2010): Genetic analysis of common carp (*Cyprinus carpio*) strains: I. genetic parameters and heterosis for growth traits and survival. Aquaculture, 304, 14–21.
- Pikitch E.K., Doukakis P., Lauck L., Chakrabarty P., Erickson D.L. (2005): Status, trends and management of sturgeon and paddlefish fisheries. Fish and Fisheries, 6, 233–265.
- Rahman M.A., Arshad A., Marimuthu K., Ara R., Amin S.M.N. (2013): Inter-specific hybridization and its potential for aquaculture of fin fishes. Asian Journal of Animal and Veterinary Advances, 8, 139–153.
- Reddy P.V.G.K. (1999): Genetic resources of Indian major carps. FAO Fisheries Technical Paper No. 387.
- Reinartz R., Lippold S., Lieckfeldt D., Ludwig A. (2011): Population genetic analyses of *Acipenser ruthenus* as a prerequisite for the conservation of the uppermost Danube population. Journal of Applied Ichthyology, 27, 477–483.
- Safronov A.S., Filipova O.P. (2000): Experiment on rearing the hybrid of Russian (*Acipenser gueldenstaedti* Br.) × Siberian (*Acipenser baeri* Br.) sturgeon in the warm water fish farm in the Vologda region. In: Book of Abstracts of the International Conference: Sturgeons on the Threshold of the XXIst Century. Astrakhan, USSR, 11–15. (in Russian)
- Scheerer P.D., Thorgaard G.H. (1983): Increased survival in salmonid hybrids by induced triploidy. Canadian Journal of Fisheries and Aquatic Sciences, 40, 2040–2044.
- Siegel S.N., Castellan J. (1988): Nonparametric Statistics for the Behavioral Sciences. McGraw-Hill, New York, USA.
- Stech L., Linhart O., Shelton W.L., Mims S.D. (1999): Minimally invasive surgical removal of ovulated eggs of paddlefish (*Polyodon spathula*). Aquaculture International, 7, 129–133.
- Szczepkowski M., Kolman R., Szczepkowska B. (2000): A comparison of selected morphometric characteristics of the juveniles of Siberian sturgeon (*Acipenser baeri* Brandt) and its hybrid with Russian sturgeon (*Acipenser Gueldenstaedti* Brandt). Archives of Polish Fisheries, 8, 193–204.
- Vasilev V.P. (2009): Mechanisms of polyploid evolution in fish: Polyploidy in sturgeons. In: Carmona R., Domezain A., Gallego M.G., Hernando J.A., Rodríguez F., Ruiz-Rejón M. (eds): Biology, Conservation and Sustainable Development of Sturgeons. Springer, Dordrecht, the Netherlands, 97–117.
- Wang J., Xia D. (2002): Studies on fish heterosis with DNA fingerprinting. Aquaculture Research, 33, 941–947.
- Wei Q.W., Zou Y., Li P., Li L. (2011): Sturgeon aquaculture in China: Progress, strategies and prospects assessed on the basis of nation-wide surveys (2007–2009). Journal of Applied Ichthyology, 27, 162–168.
- Welsh A.B., Blumberg M., May B. (2003): Identification of microsatellite loci in lake sturgeon, *Acipenser fulvescens*, and their variability in green sturgeon, *A. medirostris*. Molecular Ecology Notes, 3, 47–55.
- Whitlock M.C., Ingvarsson P.K., Hatceld T. (2000): Local drift load and the heterosis of interconnected populations. Heredity, 84, 452–457.
- Zhang X., Wu W., Li L., Ma X., Chen J. (2013): Genetic variation and relationships of seven sturgeon species and ten interspecific hybrids. Genetics Selection Evolution, 45, 21.
- Zheng H., Zhang G., Guo X., Liu X. (2006): Heterosis between two stocks of the bay scallop, *Argopecten irradians irradians* Lamarck (1819). Journal of Shellfish Research, 25, 807–812.

Received: 2018–09–06

Accepted: 2018–11–21