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CONTENT

CHAPTER 1

GENERAL INTRODUCTION

1.1. Introduction

Sturgeons and paddle fishes (Acipenseriformes) are of high commercial value, are distributed in the northern hemisphere (Pikitch et al., 2005), and belong to one of the most ancient groups of Osteichthyes. Sturgeons are considered as living fossils for the fact that they have many biological and morphological features of ancient fishes. They are known to retain several primitive characteristics with the presence of an almost entire cartilaginous skeleton being a derived character rather than primitive (Finch, 1994; McPhail, 2007; Helfman et al., 2009). Sturgeon fossils date back to about 300 million years ago (mya) and it is presumed that they have been maintaining features for more than 200 million years (Dettlaff et al., 2012). This unique feature makes this phylogenetic group more interesting to research from the evolutionary point of view. Moreover, from economical point of view, these species are commercially important not only for caviar production but also for meat and isinglass (Zhang et al., 2013).

Sturgeons are late-maturing fishes with distinctive characteristics. They possess a heterocercal caudal fin similar to that of sharks, their body is spindle like covered by scaleless smooth skin and armored with five rows of lateral scutes. They are easily identifiable by their elongated bodies, flattened rostra, distinctive scutes and barbels, and elongated upper tail lobes. The dorsal part of the body has a varied coloring ranging from grey, brown, dark blue to close to black, the coloring becomes less intense on the ventral side. Several species of the reported sturgeons can grow to very large size with their length ranging about 2–3.5 m (7–12 feet) (Chebanov and Galich, 2011).

1.1.1. Classification of sturgeons

Sturgeons are characterized by a highly cartilaginous skeleton, heterocercal tails and a well-developed rostrum with an inferior mouth (Sokolov and Berdichevskii, 1989). The classification of sturgeons in accordance with the traditional systematics is as follows: regnum Animalia, superphylum Vertebrata, subphylum Gnatosthomata, superclass Pisces, class Osteichtyes (bony fish), subclass Actinopterygii, superorder Chondrostei, and order Acipenseriformes. This group includes the genera *Acipenser, Huso, Scaphirhynchus*, and *Pseudoscaphirhynchus* from the family Acipenseridae (sturgeons), as well as two species *Psephurus gladius* and *Polyodon spathula* from the family Polyodontidae (paddlefishes) (Rochard et al., 1991; Bemis et al., 1997). Altogether, there are 27 species of sturgeons and paddle fishes in the world are found along the coast of the Pacific and Atlantic oceans, seas, inland lakes and rivers in northern hemisphere (Bemis et al., 1997) (Tab. 1).

Acipenseriformes are divided into three different groups on the basis of chromosomal numbers (Havelka et al., 2011; Symonová et al., 2013) (Tab. 2):

I.) Species having about 120 chromosomes (Palaeotetraploidy, 4n)
II.) Species having 240–270 chromosomes (Palaeooctoploidy, 8n)
III.) Species having 370 chromosomes (Palaeododecaploidy, 12n)

Table 1. *Sturgeon species and their distribution.*

Taxonomical Name	Common name / alternate common names	Distribution	Status in International Union for Conservation of Nature (IUCN-2015)
Huso dauricus	Kaluga/Great Siberian Sturgeon/ Huso sturgeon/ Manchurian Sturgeon/Siberian Great Sturgeon/ Siberian huso sturgeon	Asia	Critically Endangered
Huso Huso	Beluga/European Sturgeon/Giant Sturgeon/ Great Sturgeon/Russian Sturgeon	Asia and Europe	Critically Endangered
Acipenser baerii	Siberian Sturgeon/ Lena river sturgeon/ Long-nosed Siberian sturgeon	Asia	Endangered
Acipenser brevirostrum	Shortnose Sturgeon/Short-nosed little Sturgeon	North America	Vulnerable
Acipenser dabryanus	Yangtze Sturgeon/Dabry's Sturgeon/ River Sturgeon/Jangtze sturgeon	Asia	Critically Endangered
Acipenser fulvescens	Lake Sturgeon/Rock Sturgeon	North America	Least Concern
Acipenser gueldenstaedtii	Russian Sturgeon/Diamond Sturgeon/Azov-Black Sea Sturgeon/ Danube Sturgeon/Diamond back Sturgeon	Asia and Europe	Critically Endangered
Acipenser medirostris	Green Sturgeon/Barbel Sturgeon	North America	Near Threatened
Acipenser mikadoi	Sakhalin Sturgeon	Asia	Critically Endangered
Acipenser naccarii	Adriatic Sturgeon	Western Europe	Critically Endangered
Acipenser nudiventris	Ship Sturgeon/Bastard Sturgeon/ Fringebarbel Sturgeon/Spiny Sturgeon/Thorn Sturgeon	Asia and Europe	Critically Endangered
Acipenser oxyrinchus	Gulf Sturgeon	North America and Europe	Near Threatened
Acipenser persicus	Persian Sturgeon	Asia and Europe	Critically Endangered
Acipenser ruthenus	Sterlet/Albino Sterlet/Sterlet Sturgeon	Asia and Europe	Vulnerable
Acipenser schrenckii	Amur Sturgeon/Japanese Sturgeon	Asia	Critically Endangered
Acipenser sinensis	Chinese Sturgeon	Asia	Critically Endangered
Acipenser stellatus	Stellate Sturgeon/Star Sturgeon/ Starry Sturgeon/Stellatus/Sevruga	Asia and Europe	Critically Endangered
Acipenser sturio	Atlantic Sturgeon/Baltic Sturgeon/ Common Sturgeon/Sea Sturgeon	Western Europe	Critically Endangered
Acipenser transmontanus	White Sturgeon/Columbia Sturgeon/ Oregon Sturgeon/pacific Sturgeon/ Sacramento Sturgeon	North America	Least Concern

Pseudoscaphirhynchus fedtschenkoi	Syr Darya Sturgeon/Syr-Dar Shovelnose Sturgeon/Syr-Darya Shovelnose Sturgeon	Asia	Critically Endangered
Pseudoscaphirhynchus hermanni	Dwarf Sturgeon/little Amu-Darya Shovelnose/little Shovelnose Sturgeon/Small Amu-dar Shovelnose Sturgeon/Small Shovelnose Sturgeon	Asia	Critically Endangered
Pseudoscaphirhynchus kaufmanni	Amu Darya Sturgeon/Great Shovelnose Sturgeon	Asia	Critically Endangered
Scaphirhynchus albus	Pallid Shovelnose Sturgeon	North America	Endangered
Scaphirhynchus platorynchus	Shovelnose Sturgeon/Sand Sturgeon	North America	Vulnerable
Scaphirhynchus suttkusi	Alabama Sturgeon	North America	Critically Endangered

Note: The table is taken from http://www.pond-life.me.uk/sturgeon/specieslist. * The data has been taken from "The IUCN Red List of Threatened Species", http://www.iucnredlist.org/

Table 2. *Numbers of chromosomes of the different sturgeon species.*

Species	Chromosome number	Reference
Scaphirhynchus platorynchus	112	Ohno et al. (1969)
A. nudiventris	116 ± 4	Nowruzfashkhami et al. (2006)
	118±2	Sokolov and Vasil'ev (1989)
H. huso	116 ± 4	Fontana and Colombo (1974)
	118±2	Fontana et al. (1998)
A. sturio	116 ± 4	Fontana and Colombo (1974)
	121 ±3	Tagliavini et al. (1999)
A. ruthenus	118±2	Fontana et al. (1975)
	118 ± 4	Rab (1986)
	118±2	Birstein and Vasil'ev (1987)
A. stellatus	118±2	Birstein and Vasil'ev (1987)
	146 ±6	Chicca et al. (2002)
Polyodon spathula	120	Dingerkus and Howell (1976)
A. oxyrinchus	121 ±3	Fontana et al. (2008)
A. baerii	229 - 240	Fopp-Bayat et al. (2006)
	246 ±8	Fontana (1994)
	246 ± 10	Fontana et al. (1997)
	249 ±5	Vasil'ev et al. (1980)
A. naccarii	239 ±7	Fontana and Colombo (1974)
	246 ±8	Fontana (1994)
	248 ±4	Fontana et al. (1999)
A. transmontanus	246 ± 10	Fontana et al. (1997)
	248 ±8	Fontana (1994)
	256 ±6	Wang et al. (2003)
	271 ±2	Van Eenennaam et al. (1998)
A. mikadoi	247 ± 33	Vishnyakova et al. (2009)
	262 ±4	Vasil'ev et al. (2009)

Species	Chromosome number	Reference
A. gueldenstaedtii	249 ±2	Arefjev and Nikolaev (1991)
	250 ±8	Birstein and Vasil'ev (1987)
	258 ±4	Fontana et al. (1996)
A. medirostris	249 ±8	Van Eenennaam et al. (1999)
A. persicus	258 ±4	Nowruzfashkhami et al. (2000)
A. fulvescens	262 ±6	Fontana et al. (2004)
A. sinensis	264	Yu et al. (1987)
	264	Zhou et al. (2008)
H. dauricus	268 ±4	Vasil'ev et al. (2009)
A. brevirostrum	372	Kim et al. (2005)
	372 ±6	Fontana et al. (2008)

Source: Havelka et al., 2011

1.2. Sturgeon reproduction

Sturgeons migrate for reproduction and feeding. With regard to their life cycle, two migration models have been described (Bemis and Kynard, 1997) and are defined below:

1. **Diadromous:** The fish migrates from sea water to fresh water, and vice versa. The majority of the Acipenseriformes are diadromous species and exhibit two types of migration behavior:
   Anadromous – spends most of their life cycle in their feeding areas in the sea and migrate to fresh water areas for breeding. Some of the anadromous sturgeon species are *H. huso, A. oxyrinchus, A. naccarii, A. sinensis, A. nudiventris, A. stellatus* and *H. dauricus.*
   Amphidromous – spends most of their life cycle in fresh water – the breeding phase of their life cycle occurs in fresh water, while the feeding and growth phases occur during the migration to sea. *A. brevirostrum, A. dabryanus* and *A. schrenckii* are amphidromus species among the sturgeons.
2. **Potamodromous**: The fish migrates within the river/lake for short distance (McDowall, 1992). Some of the potamodromous sturgeon species are *Acipenser dabryanus, A. ruthenus, A. fulvescens, Scaphirhynchus albus, S. suttkusi* and *S. platorynchus.*

Sturgeons breed in any of the seasons depending on water temperature and water flow velocity. In addition, the distance covered by sturgeons in their migration from the feeding grounds in the sea to the breeding areas in the rivers varies. It is correlated with the fact that the existence of adequate breeding areas is essential for the success of breeding (Suciu, 2008; Garrido-Ramos et al., 2009).

1.3. Survival threats and actions for conservation in sturgeons

As mentioned before, there are 25 species of sturgeons and 2 species of paddle fishes found in the rivers and northern seas hemisphere (Bemis et al., 1997). However, at present, almost all sturgeon species are facing threat of extinction (Williot et al., 1997; Vasil'eva, 1999; Billard, 2002). According to IUCN-2015 (International Union for Conservation of Nature-2015) (Tab. 1), sturgeons are the critically endangered species than any other group of species. This is majorly due to improper management practices and over fishing. The commercial exploitation of sturgeon wild stocks escalated rapidly during the 20th century and caused decline in major sturgeon fisheries (Pikitch et al., 2005). Construction of dams and river modifications led to

loss of spawning grounds and prevented migration of sturgeons to sea, and sedimentation along river beds downstream the dams pose obstacles for young sturgeons to feed. Besides, water contamination with agricultural biocides and industrial wastes is also a major concern. Sturgeons are benthic feeders and are very sensitive to toxic pollutants in sediments and bioaccumulation of these pollutants has significant effect on the gonadal development and reproduction due to late puberty. However, among all, the key factor in their population decline is attributed to their meat and high commercial value of caviar (Billard, 2002; Ludwig, 2008; Ruiz-Rejón, 2009). Till 20th century, United States was represented as commanded exporter of black caviar, mainly roe of *A. oxyrinchus* (Birstein et al., 1997; Secor, 2002). In the early 20th century, Russia became the major caviar trading country for the whole century when rapid reduction of wild populations and establishment of conservation programs reduced the amount of legal catches (Taylor, 1997; Secor et al., 2000). Lately, the Caspian Sea nations like Iran, Kazakhstan, Russia, and to a lesser extent Azerbaijan and Turkmenistan, dominated the international trade in capture fisheries products.

Measures have been taken for the conservation of sturgeon species that require immediate and critical attention, which will ultimately improve sturgeons' culture for meat and caviar purpose along with increase in wild population. Regulation of international trade in Acipenseriformes by Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) is a major international action to protect these species from extinction, which imposed limited export and catch quotas since 1997 when all commercially utilized sturgeon species were listed in Annex II as species that may become threatened with extinction (Bronzi et al., 2011; Hoover, 1998; Raymakers and Hoover, 2002). Despite the strict regulations, illegal overexploitation still represents a huge threat to wild populations. On the other hand, the restrictions on the exploitation of wild stock been increasing the demands for aquaculture-produced black caviar in the current scenario.

For instance, Caspian Sea was once the richest resource for world's largest sturgeon fishery; however, recently sharp decline in catch has been reported that in-turn has led to absolute ban on commercial fishing (Pourkazemi, 2006). Despite outbreaks of certain diseases and hydroelectric projects, which are playing critical role in reducing Caspian Sea stocks, the greatest threat is found to be unreported and unregulated fishing beyond ban imposed on many species quotas allotted (Ruban and Khodorevskaya, 2011). A wide range of restocking strategies can be designed based on status and requirement of each species. Sufficient knowledge on biology and ecology, population dynamics and genetic structure of populations of sturgeons is essential for conservation purpose. Habitat restoration, artificial propagation and creating public awareness regarding conservation also comes up as an effective strategy. Farming systems for captive broodstock development and a combination of farming and stocking can act as complementary measure to ongoing conservation activities. Use of *ex-situ* conservation strategies and biotechnological approaches serve as valuable tool in assessing genetic structure and diversity of population thereby understanding the patterns of gene flow among the populations (Billard and Lecointre, 2001).

1.4 Genome evolution and polyploidy in sturgeons

Sturgeons are known for their polyploid origin with at least three independent genome duplication events taking place during their evolution and more genome duplication events are expected (Havelka et al., 2013). They possess a unique karyotype when compared to other vertebrates as they possess variable number of micro chromosomes (Billard and Lecointre, 2001; Fontana, 2002; Symonová et al., 2013). Especially in family Acipenseridae, number of chromosomes varies from ~ 120 to ~ 360 chromosomes. Also, increasing number of

chromosomes is then closely connected with increasing of DNA content in cell nuclei. Several scientists worked on ploidy levels of sturgeons using different genetic tools like karyotyping, fluorescent in situ hybridization (FISH), microsatellites (Serebryakova, 1972; Arefjev, 1983; Fontana, 1994; Fontana et al., 2008). The DNA content ranges from 2.44 pgDNA nucleus^{-1} in *H. huso* (Birstein et al., 1993) to 13.78 pgDNA nucleus^{-1} in *A. brevirostrum* (Hardie and Hebert, 2003). Type of polyploidy involved has a great influence in their evolutionary success. Auto-polyploidy involves instant duplication of genome of organism, whereas allopolyploidy involves hybridization of two species with distinct genomes and genome fails to restore its original ploidy level. Several evolutionary scenarios have been proposed in sturgeons to explain origin of their polyploidy with theories on lineage specific multiple genome duplications (Birstein et al., 1998; Ludwig et al., 2001) to that of a combination of auto and allopolyploidy (Fontana et al., 2008) and multiple hybridizations leading to allopolyploidization (Vasil'ev, 1999; Fontana, 2002). Ludwig et al. (2001) classified the sturgeon species with ~ 120 chromosomes as functional (recent) diploids, species with ~ 240–270 chromosomes as functional (recent) tetraploids and with ~ 370 chromosomes as functional (recent) hexaploids. It is also said that due to this unusual genetic structure of sturgeon, sturgeon species hybridize more easily than other fishes (Birstein et al., 1997; Ludwig et al., 2009). With respect to the evolution of the sturgeon genome through consecutive genome duplication, species with ~ 120 chromosomes are considered as basal and thus evolutionary the oldest group (Krieger et al., 2008). However, in the evolution of sturgeons not only duplication of genomes did play a significant role, from an evolutionary point of view quite recently, functional reduction also did play vital role (Ludwig et al., 2001; Havelka et al., 2013). Consequently, there still exist unclear views on individual ploidy levels of sturgeons in the available literature. While some consider them as evolutionary tetraploids, octoploids and dodecaploids (Ohno et al., 1969; Birstein and Vasil'ev, 1987; Birstein et al., 1993; Symonová et al., 2013), other authors divide sturgeons into functional diploids, tetraploids and hexaploids (Fontana, 1994; Ludwig et al., 2001; Fontana et al., 2008; Havelka et al., 2013).

1.5. Hybridization in sturgeons

Hybridization is the process in which mating of genetically differentiated individuals or groups is made or occurs, and this technique is widely adopted for exploiting hybrid vigor. It provides a substantial advantage in terms of increasing adaptive genetic variation (Grant and Grant, 1992). Hybridization is widely reported in fish and occurs naturally and induced artificially (Hubbs, 1955; Schwartz, 1981; Ludwig et al., 2008). Fishes hybridize more commonly than any other vertebrates, which is attributed by various factors (Campton, 1987; Allendorf and Waples, 1996) such as external fertilization, weak behavioral isolation mechanisms, unequal abundance of two parental species, competition for limited spawning habitat, decreasing habitat complexity and susceptibility to secondary contact between recently evolved forms (Campton, 1987).

The unusual genetic patterns allow sturgeons to hybridize and produce many interspecific and intergeneric hybrids (Havelka et al., 2013). More than 20 hybrids of sturgeons have been reported till date (Havelka et al., 2011). Sturgeons have high capacity for hybridization due to the fact that diploids can hybridize with diploids, tetraploids to tetraploids and diploids to tetraploids. Intercross between diploids and tetraploids produce triploids that are supposed to be sterile while other crosses yield fertile diploid or tetraploids individuals. Fertile hybrids on long term pose serious threat to genetic diversity of native population. Hybrids are often characterized by greater growth performance which eventually leads to competition for food and resources with native species and, often risking native species to the brink of extinction

(Ludwig et al., 2009). It was also demonstrated that hybridization is one among the most rapidly acting genetic threat to endangered populations, concerning extinction often occurring in less than five generations (Wolf et al., 2001). Hybrids are majorly resulted from intended release programs, and in other cases from habitat alterations or unintended escapees from hatchery (Birstein et al., 1998). More often, backcrosses with native specimens results in genetic clearance of nuclear genotypes, so that evidence for ancestral hybridization is often only detectable in mitochondrial DNA (Ludwig et al., 2001; Birstein et al., 2005). Additionally, the viability of the hybrids depends on the origin, genetic compatibility, relatedness, ploidy of the parental species. Also, the fertility of interspecific hybrids depends on the structure and compatibility of karyotypes in the parent species. Generally, in fishes, interspecific hybrids of distantly related parental species with varied chromosome number are usually sterile because their chromosomes cannot correctly pair during zygotene stage of meiosis prophase I and such impairment interferes with gonadal development and gametogenesis (Piferrer et al., 2009). It was always assumed that the hybrids formed between species originated from different ploidies are sterile. But recently, Vasil'ev et al. (2014) and Linhartová et al. (2017) revealed limited fertility of interspecific hybrid males produced between sturgeon species with odd ploidy.

1.5.1. Natural hybrids

Natural hybridization happens more frequently between closely related fish species and some amphibians than in other groups of vertebrates (Birstein, 2002). The main cause for the incidence of hybridization in sturgeon is overlap in breeding season and reduction of spawning sites due to anthropogenic intervention (Dudu et al., 2011). The likelihood of hybridization so much increases by changing the water flow, the water pollution and the erection of dams or migration obstacles. The change or interruption made in water flow reduced the number of spawning sites and, thus increased the number of various species of sturgeon in limited areas suitable for their spawning (Billard and Lecointre, 2001). An example is the Iron Gates dam on to Romania, which was built on the Danube river. The construction of this dam interrupted the migration of sturgeon species which previously migrated for the reproduction of the Black Sea upstream to Budapest, Vienna and Bavaria (Kynard et al., 2002; Paraschiv et al., 2006). Similarly, the destruction of most spawning grounds of Gaint sturgeon (*H. huso*) occurred after construction dam Volgograd in Russia (Barannikova et al., 1993). In China, almost all the natural spawning sites of endemic sturgeon species were destroyed while constructing Gorges reservoir on the Yangtze River (Wu et al., 2004).

According to the morphological description, hybrids between many sturgeon species can survive in the wild and are frequently found in the rivers (Birstein et al., 1997) (Table 3). In natural conditions, sturgeons hybridize leading, sometimes, to fertile intergeneric or interspecific hybrids (Costache et al., 2012). According to Birstein (2002), by using genetic markers like species-specific PCR (Polymerase Chain Reaction) and Restriction Fragment Length Polymorphism (PCR-RFLP) and Random Amplified Polymorphic DNA (RAPD), the scientists have found the natural hybrids between female Russian sturgeon and male stellate and sterlet in the Volga River, and between the Russian or Persian (*A. persicus*) female sturgeon and male ship sturgeon (*A. nudiventris*) in the Caspian Sea. Due to difference in chromosome sets of their parental species, these hybrids were found to be sterile (DeSalle and Birstein, 1996; Birstein et al., 1998; Birstein, 2002). In addition, the natural hybrids between the Amur sturgeon (*A. schrenckii*) and Kaluga (*A. dauricus*) have been well-known, reproducing on the same spawning grounds (Vasil'ev et al., 2010). In the Black Sea and Lower Danube, eight microsatellite loci in combination with statistical analysis was done for detecting hybrids of

sterlet (*A. ruthenus*), Russian sturgeon (*A. gueldenstaedtii*), starry sturgeon (*A. stellatus*) and giant sturgeon (*H. huso*) (Dudu et al., 2011). Ludwig et al. (2009) have provided genetic evidence of hybridization between sterlet and Siberian sturgeon in the Danube River. Another report on hybridization of sturgeon species with different geographic distribution in Mississippi River and spawning habits was reported by Tranah et al. in 2004. They identified the hybrids between pallid *(S. albus*) and shovelnose (*S. platorhynchus*) sturgeon which were genetically intermediate to both the species. Microsatellite data and mitochondrial DNA were used in order to identify the hybrids exactly. Additionally, the hybrids were found to be piscivorous like the pallid sturgeon and not like the shovel nose sturgeon which feeds on benthic macro invertebrates. The farm escapees or accident or deliberate introduction of fertile hybrids to the natural waters would lead to serious genetic problems and in some cases would be a threat to the native populations (Jenneckens et al., 2000; Maury-Brachet et al., 2008). This resulted in increasing catches of Siberian sturgeon (*A. baerii*) in European rivers which strongly correlated with their increasing numbers in European aquaculture (Arndt et al., 2002; Holčík et al., 2006; Masár et al., 2006). Ludwig et al. (2009) firstly documented natural reproduction of Siberian sturgeon (*A. baerii*) outside their natural range in Europe. By using combination of mtDNA control region and seven microsatellite loci, they described several interspecific hybrids between exotic *A. baerii* and native *A. ruthenus*. As mentioned previously, the hybrids produced between parental species leading to even ploidy (for example: 4n × 4n, 4n × 8n, 8n × 12n, 8n × 8n, 12n × 12 n, etc.) should be fertile and hybrids formed from parental species leading to odd ploidy (for example: 6n × 4n, 10n × 4n, etc.) should be sterile. Some scientists discussed possible fertility of sturgeon hybrids having intermediate DNA content referring to recent triploids (3n) or evolutionary hexaploids (6n) (Flajšhans and Vajcová, 2000). Using flow cytometry, these authors detected evolutionary pentaploids (5n) and evolutionary heptaploids (7n) individuals among sturgeon aquaculture stocks and they suggested that these individuals might have originated from hybridization of evolutionary hexaploid specimen (6n) with evolutionary tetraploid specimen (4n) and evolutionary hexaploid specimen (6n) with evolutionary octoploid specimen (8n) respectively. Recent observation on the gamete development of the sturgeon hybrids originated from different ploidies revealed possibility of limited fertility in the males which is a major breakthrough in sturgeon gonadal development (Vasil'ev et al., 2014; Linhartová et al., 2017). Henceforth, it implies the general assumption of sterility of hybrid sturgeon with odd ploidy can be fallacious and therefore accidental escape of these hybrids from farms to wild should be seriously reconsidered.

Table 3. *Sturgeon Hybrids found in natural waters.*

River	Hybrids
Drainage area of the Caspian Sea	*A. ruthenus × A. stellatus*
	H. huso × A. gueldenstaedtii
	A. stellatus × A. ruthenus
	H. huso × A. ruthenus
	A. nudiventris × A. gueldenstaedtii
	H. huso × A. nudiventris
	A. gueldenstaedtii × A. ruthenus
	H. huso × A. stellatus
	A. gueldenstaedtii × A. stellatus
	H. huso × A. persicus
	A. gueldenstaedtii × A. persicus
	A. ruthenus × H. huso
	A. nudiventris × A. stellatus
	A. stellatus × A. nudiventris
Azov Sea drainage basin	*A. ruthenus × A. stellatus*
Black Sea Catchment	*A. ruthenus × A. stellatus*
	A. gueldenstaedtii × H. huso
	A. ruthenus × A. nudiventris
	A. stellatus × H. huso
	A. stellatus × A. ruthenus
	A. nudiventris × H. huso
	A. ruthenus × A. gueldenstaedtii
	H. huso × A. stellatus
	A. stellatus × A. gueldenstaedtii
	A. nudiventris × A. gueldenstaedtii
	A. sturio × A. gueldenstaedtii
	A. gueldenstaedtii × A. sturio
	A. gueldenstaedtii × A. nudiventris
Siberian river	*A. baerii × A. ruthenus*
	H. dauricus × A. schrenckii
Central Asia	*Pseudoscaphirhynchus Kaufmann × P. hermanni*
North America	*Scaphirhynchus albus × S. platorynchus*

Source: Birstein et al., 1997

1.5.2. Artificial hybrids

The first artificial hybridization of Acipenseridae has done between females of sterlet (*A. ruthenus*) and males of Russian sturgeon (*A. gueldenstaedtii*) and stellate sturgeon (*A. stellatus*) in 1869 (Nikoljukin, 1971). Russians began producing hybrids for aquaculture purpose, 'bester' the intergeneric hybrid of beluga (*H. huso*) and sterlet attaining large size and rapid growth was developed in 1952 but a decline in progeny survival was reported in

successive generations (Arefjev, 1997). Following this, many hybrids were produced viz bester × *H. huso*, bester × *A. ruthenus, H. huso* × *A. nudiventris, A. ruthenus* × *A. nudiventris, A. gueldenstaedtii* × *A. ruthenus, A. gueldenstaedtii* × *H. huso, H. huso* × *A. stellatus* and *A. stellatus* × *A. medirostris* (Krylova, 1999). In special laboratory conditions, the viability of different sturgeon interspecies hybrids can be higher than in the wild (Burtsev, 1997). Although these hybrids can mature and produce eggs, but all these hybrids were seen to perform in between both the parents and not best than the superior parent (Birstein, 2002). The potential rate of growth of hybrids was high (Ludwig et al., 2009), therefore they are wildly used in aquaculture and sport fishing (Havelka et al., 2011). As a rule, artificially produced hybrid female in the first generation with parental species having same ploidy level were fertile and produces the eggs, whereas hybrids in which the parental species originating from different ploidy were sterile (Arefjev, 1997). Some females from the second generation of artificially produced hybrids were found to be fertile in Bester (*H. huso* × *A. Ruthenus*) (Burtsev, 1997). Recently, hybrid between Kaluga sturgeon and Amur sturgeon exhibited better growth rate than its parental species and has become the most exploited for caviar production (Wei et al., 2011; Boscari et al., 2014). Hybrids produced from *A. sturio* and *A. oxyrinchus* showed a high level of heterozygosity (Tiedemann et al., 2007). Off lately, we (Shivaramu et al., 2019) made an effort in estimating on critical swimming speeds in hybrids of *A. ruthenus* originating from different populations. They found no significant difference in critical swimming speeds between hybrids.

Second generation (F2) hybrids

The second generation hybrids between beluga female (*H. huso*) and sterlet male (*A. ruthenus*) was first obtained in the Rogozhinsk sturgeon hatchery, located in the Don River delta in 1966 (Nikoljukin, 1971). The fish production indices of F2 hybrids (percentage of fertilized eggs, survival of young in reservoirs and ponds) proved in the majority of cases to be not lower, but at times even higher, than in the hybrid F1. Morphologically, both hybrid F1 and F2 took an intermediate position between the parental species. F2 performed better in traits like body coloration (Nikoljukin, 1971).

1.6. Consequences of hybridization

In general, hybrids are produced to exploit heterosis and the individuals are used for commercial purpose or they can be used to produce new breeds. Hybrids play a significant role for increasing the yield of several species of freshwater and marine fishes in aquaculture (Rahman et al., 2013). Several hybrids have been produced to increase growth rate and improve productivity through hybrid vigor. Hybridization is a potential tool to improve the valuable traits such as good flesh quality, disease resistance and increase environmental tolerances, better food conversion, take advantages of sexual dimorphism and increase harvesting rate in culture systems. In some cases, hybridization may lead to greater genetic diversity, increased fitness and greater adaptation to local environments (DeWet et al., 1983). But hybridization at all times does not have positive effects, it can have certain negative effects which are attributed by many factors.

The negative effects of hybridization may begin with the loss of genetic diversity through genetic assimilation of smaller population by a larger one (Cade, 1983). Genetic assimilation is common when small populations suffering bottleneck come in contact with larger or reproductively more successful population or species. Another commonly associated problem with it is outbreeding depression where the hybrid offspring have reduced reproductive

success and fertility than the parents (Allendorf and Luikart, 2009). The hybrids between genetically differentiated taxa often showed reduced fitness (Templeton, 1986). It is reported that certain salmonid fishes may be endangered due to outbreeding depression (Allendorf and Leary, 1988). The most commonly associated negative impacts with interspecific hybridization is genetic extinction where the fertile hybrids produced replace one or both the parental populations completely and eventually producing hybrid swarms. Hybrid swarm is referred to a population where all the individuals are hybrids to various degrees.

On the other hand intraspecific hybridization in the form of gene flow among populations has a several important effects. It is traditionally seen as a cohesive force which holds species together as a unit of evolution (Mayr, 1963). Ehrlich and Raven (1969) challenged that the amount of gene flow through intraspecific hybridization would be too low to act against the forces of genetic drift and local adaptation which may lead to differentiation. Allendorf and Luikart (2009) in contradiction to that have been able to prove that one migrant per individual per generation is sufficient to keep all the alleles present in all the populations and even one migrant per generation greatly increases the effective population size which is a prerequisite to prevent the accumulation of inbreeding depression among isolated population. Monson and Saddler (2010) documented this in zebrafish lines where they found that the colonies suffered increased incidence of inbreeding depression when they were crossed for several generations and they were able to demonstrate that hybridizing the inbred lines with the wild lines through a single generation of outcrossing provided outbreeding enhancement. They found a greater mating frequency when hybrid females were used for breeding rather than the inbred females. They also concluded that there is a greater genetic fitness in hybrids than the inbred lines.

Although, hybridization can have variable set of discrete and combined outcomes of genetic and environmental effects, the interbreeding, i.e. interspecific or intergeneric of sturgeon species has been well known in aquaculture as well as under natural conditions (Dudu et al., 2011; Costache et al., 2012; Havelka et al., 2013). In recent years, progress in artificial propagation technology has expedited the development of sturgeon aquaculture, producing artificial hybrids (Dudu et al., 2011). Furthermore, hybrids identification was done using only morphological characters taking into consideration that the hybrids inherit morphological features from the parental species and it is not reliable enough. Thus, only genetic studies can provide the necessary proof that nuclear genes from both parental species are present in the hybrid for hybrid identification.

1.7. Sturgeon Genetics

In the early 20th century, main areas of sturgeon research were addressing their biology and systematics as well as fisheries, while the more recent efforts have been increasingly focused on their farming and conservation (Billard and Lecointre, 2001; Jarić and Gessner, 2012). The correct identification of sturgeon species is very important for various reasons. Thus, the accurate identification of the species from which the various products, especially caviar, originate is required in order to avoid commercial frauds.

Genetics of sturgeon hybrids is one of problems that hybridization faces nowadays due to the relation between genetic threats to endangered population (Havelka et al., 2011). The increasing anthropogenic impact onto the natural environment of Eurasian sturgeon species and populations steadily resulted in an elevated incidence of natural hybrids and dramatically declining natural populations of pure species during recent decades and their lower chance to reproduce (Pikitch et al., 2005; Tsekov, 2008; Dudu et al., 2011).

1.7.1. Application of genetic markers in Genetic Management of sturgeons

Molecular markers are the powerful informational tools in hand which help us to reveal genetic relatedness among individuals, species and populations (Davey et al., 2011). Molecular markers have been used in many different aspects of genetic management in aquaculture. Their role in aquaculture has been extensively reviewed by many authors (Liu and Cordes, 2004; Maqsood and Ahmad, 2017). Molecular markers have been widely used for identification of strains and species, detection of inter and intraspecific hybridization, parentage and kinship analysis, assessment of parental contribution in mass spawning, estimation of effective population size and level of inbreeding, preventing inbreeding, mapping of quantitative trait loci (QTLs) and selective breeding. With regard to restocking programs, genetic markers have found a role in comparison of farmed strains and wild populations, choice of donor population, detection of genetic changes in hatchery reared fishes over generations and monitoring the impact of reared animals after release to the wild (Cross, 2000; Maqsood and Ahmad, 2017).

In last three decades, major advances are seen in the development of genetic markers. Wide range of markers (low, moderate or highly polymorphic) have developed. These include isozymes, mitochondrial DNA (mtDNA), restriction fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), microsatellites, expressed sequence tags (ESTs) and single nucleotide polymorphism (SNP). The markers have been classified into two categories. Type I are markers associated with genes of known function while Type II markers are associated with anonymous genomic segments. In aquaculture, the genetic markers were found useful in different aspects. Major properties that make one molecular marker more useful than others includes type and characteristics of the marker, ease and expense of application, and abundance in the genome and polymorphic information content (PIC) (Liu and Cordes, 2004).

In sturgeons, most of the molecular markers currently employed for genetic characterization and identification are based on mitochondrial DNA (mtDNA), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphic (AFLP), microsatellites (Zhang et al., 2013) and nuclear DNA markers (Boscari et al., 2014; Boscari et al., 2017; Havelka et al., 2017). In fishes, some mtDNA regions display a high degree of variability and therefore considered as a very useful tool for taxonomic analyses (Avise and Nelson, 1989; Meyer and Wilson, 1990). Although information on mtDNA sequences in sturgeons is restricted, research on other fish groups has provided a high number of universal primers, which can be used also on sturgeons. Slowly evolving regions, such as the rDNA ones, are employed for high level taxonomic characterizations, while fast evolving ones, such as control regions, are employed to discriminate closely related species or populations belonging to the same species (Fontana et al., 2001). However, heteroplasmy of control regions has been detected in several sturgeon species (Vodolazhskii et al., 2008), but suitability of this region is still dubious. Molecular markers such as single nucleotide polymorphisms (SNPs/ Nuclear marker) and amplified fragment polymorphisms (AFLPs) are additional genetic markers that may be useful for future studies to identify genetic patterns of neutral and adaptive variation in sturgeon. Although the variability of genome size among species, the ploidy status and the easiness hybridization is high, there are recent successful studies of genetic relationships and species identification in sturgeons based on nuclear genome (Boscari et al., 2017; Havelka et al., 2017).

Due to their salient characteristics (high polymorphism, high power of discrimination, codominant mendelian inheritance, etc.), nuclear markers like microsatellites have been proven to be very useful for identifying sturgeon hybrids taking into account that the alleles of an individual represent a combination of parental alleles. Several methods were proposed for identifying hybrid individuals (Campton and Utter, 1985; Miller, 2000; Chassaing et al.,

2011; Dudu et al., 2011). One of these methods for pure species and hybrid identification is based on the use of alleles that are present in one species only (diagnostic alleles) or of microsatellite loci that are fixed for alternate alleles in different species (diagnostic loci). Microsatellites can also be used to assess the genetic diversity between hybrids and purebreds, population structure and status, the origin and relatedness of individual fish and populations, assignment of captured progeny to families or populations, quantitative contribution to year classes, species identity and hybridization, forensics cases, and effects of conservation and management actions (Israel et al., 2004; Welsh et al., 2008; Bott et al., 2009).

In the case of sturgeons, the studies based on microsatellite markers analysis were initiated for the North American species (Börk et al., 2008). Considering the fact that microsatellites contain vital genetic information for the comparison of sturgeon populations, a number of research directions were especially aimed at the development of disomic microsatellites in the North American sturgeon species and paddlefish (May et al., 1997; McQuown et al., 2000; King et al., 2001; Heist et al., 2002; Henderson-Arzapalo and King, 2002; Welsh et al., 2008). The identification and characterization of new microsatellite loci in sturgeons is difficult due to the polyploidy of these fishes. Many potential microsatellites were eliminated from research studies since they were polysomic and they tend to complicate the interpretation of the inheritance mode and the intra and inter population genetic variation (Havelka et al., 2011). Nevertheless, for the North American species, researchers have developed a set of disomic loci and since then, the microsatellites have begun to be rapidly used in population genetics studies. Researchers have identified various levels of genetic diversity, population structures and stocks using different molecular markers in different sturgeon species (Welsh et al., 2003; Ludwig et al., 2009; Reinartz et al., 2011; Zhang et al., 2013).

There are no extensive studies determining the level of heterozygosity and comparison of phenotypic performances among the purebreds and hybrids of different types in sturgeons. Additionally, there are no reports on evaluation of introgression asymmetry through hybridization that is associated with extinction risks and fitness of hybrids across future generations in sturgeons yet, it is essential to fill the limited knowledge on genetic features and fitness fate of hybrids produced. Different mechanisms resulting from different types of hybridization can operate at the same time which can lead to heterosis or outbreeding depression. Determining which mechanism will be more prevalent in a particular generation of a hybrid originating from different populations or species or ploidy levels is demanding on which we would like to focus on in this thesis.

1.8. Aims of the thesis

The main aims of this study are as follows:

1. To estimate the critical swimming speeds of the intra-specific hybrids of Danube and Volga sterlets
2. To study and compare the genetic diversity and performance of intra-specific hybrids of Danube and Volga sterlets
3. To investigate the influence of interspecific hybridization on the growth and survival traits of sturgeon species with the same ploidy level (Russian sturgeon and Siberian sturgeon)
4. To determine the effect of interspecific hybridization on the growth and survival traits of sturgeon species with different ploidy level (Sterlet and Siberian sturgeon)

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CHAPTER 2

CRITICAL SWIMMING SPEED OF STERLET (*ACIPENSER RUTHENUS*): DOES INTRASPECIFIC HYBRIDIZATION AFFECT SWIMMING PERFORMANCE?

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Critical swimming speed of sterlet (*Acipenser ruthenus*): Does intraspecific hybridization affect swimming performance?

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Summary

We studied the effect of intraspecific hybridization on swimming performance in sterlet, hypothesizing that such hybridization increases the performance by inducing the hybrid vigor. A total of 12 purebred and hybrid crosses were reproduced from Danube (D) and Volga (V) populations of *Acipenser ruthenus*. Within each cross, one group of fish was exposed to temperature challenges mimicking the temperature variation in the natural environment during summer. Temperature challenges comprised a constant increase from 19°C to 24°C and then return to 19°C within 12 hr (dT<1°C/hr), and were carried out every third day over the experimental period of 20 days. As a control, fish from each cross were kept at a constant temperature of 19°C. Critical swimming speed (U_{crit}) was assessed on day 0 (29 days post hatch, dph), 10 (39 dph) and 20 (49 dph). The critical swimming speeds ranged from 5.12 cm/s (1.63 TL/s) to 16.44 cm/s (2.4 TL/s) during the experimental period (29–49 dph). There were no significant differences observed in U_{crit} between repeatedly temperature challenged and control groups, indicating that the temperature challenge did not alter the swimming performance. The critical swimming speed showed positive relationship with total body length. Comparing intraspecific hybrid crosses with purebred crosses, no significant difference in swimming performance was observed. It is thus concluded that swimming performance is a family specific trait. There is no indication that intraspecific hybridization affects swimming performance nor that close-to-natural temperature regimes increase swimming performance.

1 | INTRODUCTION

Currently, sturgeons (Acipenseridae) are among the most endangered fish species in the world (IUCN, 2011) and restoration attempts are underway worldwide. The production of hatchery fish from ex situ broodstocks for restocking are the key element of various programmes. However, hatchery rearing has a long-lasting effect on the fitness of fish and high post-release mortality of hatchery-reared juveniles has been observed (Sulak, Randall, & Clugston, 2014). Most of the mortalities occur immediately upon release, and is assumed to be mainly caused by a combination of post release starvation, predation and stress induced by facing changing environmental conditions in fishes as described in trout and salmon (Brown & Day, 2002; Støttrup, Sparrevohn, Modin, & Lehmann, 2002). Hence, it is essential to adapt hatchery technology and management strategies to improve fish fitness upon release.

Swimming performance is often assessed as critical swimming speed, a variable that plays a pivotal role in determining the potential of an individual to escape predators and is linked to the metabolic capacity of the individual fish (Brett, 1964). Also, swimming performance is important with regard to the design of fish ways that enable fish to bypass migratory obstacles such as dams. Modulation of swimming capacity due to environmental conditions such as temperature (Adams & Parsons, 1998; Jones, Kiceniuk, & Bamford,

1974; Taylor, Egginton, & Taylor, 1996) or salinity (Nelson, Tang, & Boutilier, 1996; Plaut, 2001) has been reported. Since metabolism and body shape (Nicoletto, 1991; Plaut, 2001) influence swimming capacity, genotypic contributions including numerous morphological and physiological traits can be assumed as the consequence of a fine-tuned, concerted evolution of the physiological and morphological systems involved. In sticklebacks *Gasterosteus aculeatus*, for example, genetic factors have a substantial influence on burst swimming performance which is correlated to enzyme activities (Garenc, Silversides, & Guderley, 1998). Interbreeding of broodstocks derived from distinct populations may result in a rearrangement of coevolved, fine-tuned genes affecting the overall swimming performance compared to purebred fish. The genetic changes resulting from breeding programs are hence likely to influence how a particular individual performs. Here, family specific effects on swimming performance in Lake trout *Salvelinus namaycush* and tropical reef fish have been reported (Green & McCormick, 2005; Humphrey, 2011). However very few studies have been conducted to explore to what extent genetic effects can explain the variation in performance within and among populations.

Sturgeons hybridize more easily than other fishes and even inter-specific hybridization is regularly observed in the wild (Dudu et al., 2011; Ludwig, Lippold, Debus, & Reinartz, 2009), though intra-specific hybridization is limited in the natural environment due to a strong homing effect. In aquaculture, intra-specific hybridization is a regular strategy to produce hybrids between different populations. Such production of hybrids generally causes heterosis at the individual level and/or high genetic variance for several phenotypic traits at the population level, leading to improved fitness (Facon, Pointier, Jarne, Sarda, & David, 2008), which is highly profitable for commercial sturgeon farming (Lutz, 1997). During the past 60 years, the number of inter-specific hybrids in sturgeon aquaculture has increased mainly due to better performance compared to the parent species, which is known as hybrid vigor (Bronzi, Rosenthal, Arlati, & Williot, 1999; Rieseberg, Sinervo, Linder, Ungerer, & Arias, 1996). For example the bester, a hybrid of beluga female and sterlet male, has a faster growth performance than its parental species (Arefjev, 1999). Conversely, however, Billard and Lecointre (2000) reported that sturgeon hybrids like *A. gueldenstaedtii* × *H. huso* or *H. huso* × *A. stellatus* did not perform better than the best parents, and there has been no demonstration of general superiority in fitness-related traits of sturgeon hybrids from past studies. Interbreeding for traits like growth rate and increased reproductive success (Su, Liljedahl, & Gall, 1999) is beneficial from a farmer's perspective, but these benefits may not relate to the fitness for restocking programmes. It is therefore necessary to study the impact of hybridization on those traits that may influence post-release performance in the wild.

Sterlet (*A. ruthenus*) is a relatively small-sized early maturing freshwater species which is found in the Eurasian rivers (Holcik, 1989). Sterlet has undergone a significant decline in the wild and only few wild populations remain intact (Birstein, 1993; Jarić et al., 2011). The Danube and the Volga, which flow into the Black Sea and the Caspian Sea, have established a complete genetic isolation in the Pliocene, some 5 million years ago (Esin, Esin, & Yanko-Hombach, 2016). Still, some hybridization due to human aquaculture activities have occurred. After a decline of stocks in the upper Danube in previous centuries, the reintroduction programs were carried out by using remaining specimens from middle and lower Danube and, non-native stocks from neighboring river systems. The Danube river sterlet showed signs of separation among its sub-populations in their stretches whereas the Volga showed a weak level of substructuring in its subpopulation (Rienartz, Lippold, Lieckfeldt, & Ludwig, 2011). Recently, Reinartz et al. (2011) observed that 23% of non-native sterlet in the wild population of Danube sterlet possessed a partial or complete Volga genotype. Investigation of genetic patterns within and between populations is considered as a prerequisite for a successful recovery program. Restocking with non-native stock can eventually lead to loss of gene complexes causing outbreeding depression and consequently threatening the fitness of future generations (Ludwig, 2006). Therefore, attention must be directed to ensure the genetic integrity of wild populations.

In this study, we hypothesized that intraspecific hybridization and subsequent recombination affects the fine-tuned, complex trait of swimming performance. Therefore, we assessed the swimming capacity of 12 purebred and hybrid crosses reproduced from Danube (D) and Volga (V) breeders which were confirmed for population divergence between each other by using a set of microsatellites. In addition, we challenged fish from each cross with temperature fluctuations to study the effect on their swimming performance.

2 | MATERIALS AND METHODS

2.1 | Reproduction

A total of 12 purebred and hybrid crosses were produced from Danube and Volga populations of sterlet. To achieve this, 12 males and 12 females were propagated from each population, which revealed discriminatory alleles in a set of microsatellite loci. Parental Volga and Danube broodstock originated from the Genetic Fisheries center (GFC), FFPW USB, Vodňany and a fish hatchery operated by Rybnikarstvi, Pohořelice, Czech Republic, respectively. Upon acclimatization at 14°C for 7 days in 5 m^3 re-circulating indoor tanks, final maturation was induced by hormonal treatment (intramuscular injection of carp pituitary suspension: 4 mg/kg in males and, 0.5 mg/kg priming dose followed by 4.5 mg/kg after 12 hr in females). Ovulated eggs were collected after micro-incision of the oviduct according to protocols from Štěch, Linhart, Shelton, and Mims (1999). The mean number of eggs/g in the individual females was 90 (11.15 *SD*) in Volga sterlet females and 92.5 (13.76 *SD*) in Danube sterlet females. Sperm was collected from the seminal duct using a plastic catheter of 5 mm diameter and transferred into a 100 ml tissue culture flask (Gela, Rodina, & Linhart, 2008). Sperm was stored at 4°C until sperm volume and motility were assessed. Only those males with more than 70% motility were used for fertilization.

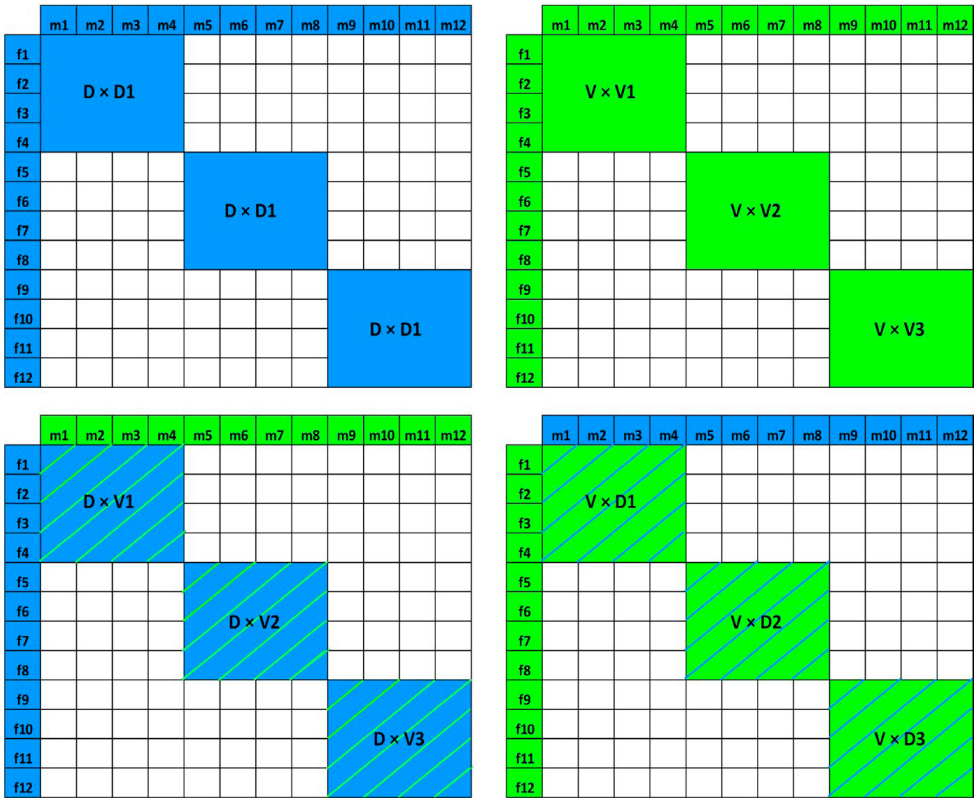

FIGURE 1 Reproduction of 12 male and 12 female sterlet from the Danube (D, blue) and the Volga (V, green) population, providing three groups of purebred Volga and Danube families (V1-V3, D1-D3) and six groups intraspecific hybrids

2.2 | Concerted artificial propagation

In order to establish hybrid and purebred crosses, a coordinated and controlled propagation of broodstock was organized at Vodňany and Pohořelice simultaneously (Figure 1). Milt from individual males of the Volga population was separately kept in an icebox at 4°C until transported to fertilize the Danube female eggs at the fish hatchery at Pohořelice from Vodňany. Three Danube (♀) × Danube (♂), three Danube (♀) × Volga (♂) crosses (Figure 1) were established and incubated at Pohořelice. Likewise, milt from individual males of the Danube population were separately stored and maintained at 4°C until transported to fertilize the Volga female eggs at the GFC of FFPW USB, Vodňany. 3 Volga (♀) × Volga (♂) and 3 Volga (♀) × Danube (♂) crosses were established. The fertilized eggs of the 6 established crosses at Pohořelice were transferred at the neurula stage to Vodňany, using plastic bags with water and an oxygen atmosphere at 15°C. The hatching and initial rearing of all the crosses was conducted in Vodňany.

2.3 | Fertilization and hatching

To establish each cross, an equal weight of 50 g of eggs was taken from four females. Eggs were pooled and divided into plastic beakers in 50 g aliquots. The plastic beakers with the aliquots were placed on an electronic shaker at a speed of 200 rpm and 10 mm deflection. In order to obtain equal genetic contribution from individual males, each aliquot was inseminated with 1.2 ml sperm from one of the four males per cross, and activated immediately by adding 200 ml dechlorinated water. Clay suspension was added to remove the egg stickiness. All aliquots per cross were pooled into a bowl and incubated on the shaker for 45 min followed by repeated washing with water in order to wash out the clay remnants, and then incubated in glass jar incubators in triplicates. During incubation, the glass jars were supplied with UV sterilized re-circulating tap water at 15.0°C, with an O_2 saturation of approximately 9 mg/L. To estimate the fertilization rate, approximately 100 eggs were randomly sampled from

each cross 6 hr post-fertilization, in triplicate. The live embryos were counted at the 2nd or 3rd cleavage division (Dettlaff, Ginsburg, & Schmalhausen, 2012). After two weeks, fish were transferred to the experimental facilities at the Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Berlin.

2.4 | Experimental setup

The experiment was carried out in a flow-through (1.5 V per h water exchange) exposure facility with climate control (Lutz et al., 2008). Maintenance of the rearing temperature was facilitated by a water bath setup (Monsees, Klatt, Kloas, & Wuertz, 2017). After ten days of acclimatization, around 40 fish were distributed to 9 L aquaria with an outflow (7 L rearing volume), providing a temperature control reared at a constant temperature of 19°C. One group per cross, assessed in replicate, was exposed to successive temperature challenges (5°C increase and return within 12 hr) every three days over a period of 20 days. Fish were fed by a commercial diet (Coppens Start Premium, 1 mm, 54% crude protein, 15% fat, 20.9 MJ/kg) to apparent saturation (approx. 4.5% body mass). Temperature, oxygen and pH were monitored daily (hourly during T challenge), and NO_2^--N and total ammonia nitrogen (TAN) every third day by the salicylate and the diazotization method using a Hach Lange DR 3900 photometer. Animal care and performance of experiments were in compliance with the EU Directive 2010/63/EU and approved by the national authorities (G0151/17, Landesamt für Gesundheit und Soziales, Berlin, Germany).

2.5 | Critical swimming speeds

Swimming performance was assessed as critical swimming speed U_{crit} at 19°C as described by Seebacher, Little, and James (2015). Eighteen juvenile fish (6 per replicate) per cross and treatment (control vs. T challenge) on day 0 (29 days post hatch i.e., dph), eight on day 10 (39 dph) and sixteen on day 20 (49 dph) were assessed individually. Prior to the assessment, feeding was stopped for 12 hr to limit energy allocation for digestion. After transfer to the transparent swimming tube (5 cm diameter, 20 cm length), fish was acclimatized for 15 min at 2.2 cm/s which was established by a line pump (Rule il500, 12 V DC, 1920 L/hr) and a variable power supply (Circuit Specialists). Real time flow-rate was measured by a DigiFlow 6710 M flow meter to control the velocity increase in steps of v_i (velocity increment) with t_i (time increment) respectively. Four fishes were assessed individually in different swimming tubes at a time. This was done to reduce the bias in the recorded U_{crit} between individuals in the treatment group due to long interval in the assessment. U_{crit} was recorded at the current velocity when fish were not able to maintain position against the current for 20 s. Subsequently, the velocity was reduced until the fish started swimming again, followed by an increase in velocity until the fish could no longer keep its position. Fishes that refused to swim at the initial, low flow rate were not considered for further assessment. Also, the fishes tested weren't reused for consecutive assessments. Body wet weight (g) and total body length (cm) were recorded and Fulton's condition factor was calculated to analyze the correlation with U_{crit} respectively.

Critical swimming speed (U_{crit}) was calculated according to Brett (1964):

$$U_{crit} = v_f + v_i * (t_f / t_i)$$

v_f is the highest velocity maintained for an entire interval; Vi is the increment in velocity (1.5 cm/s), t_f is the time the fish swam until exhaustion at the final speed; t_i is the time increment (60 s). The U_{crit} was converted to U_{crit} TL/s by dividing the swimming speed with the total body lengths (TL) in order to study the influence of size on the speed. The correlation of U_{crit} with TL was most significant.

Fulton's condition factor (K) was calculated following Froese (2006):

$$K = 100 * w/l^3$$

K is the condition factor (CF); w is the body weight of fish in g, TL is the total length of fish in cm.

2.6 | Statistical analysis

Statistical analysis was performed with the PRISM software package (version 4.03 GraphPad). Data were first analyzed for normal distribution using the Kolmogorov-Smirnov test. Multiple comparison of U_{crit} between the purebred and hybrid crosses was 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 on 0, 10 and 20 days separately. Differences between control and temperature-challenged group within a cross were analyzed by non-parametric Mann-Whitney rank sum test or parametric *t* test. Two-way ANOVA was carried out to analyze the effect of different temperature regimes and crosses on the U_{crit}. The significance was determined at alpha = 0.05. The temperature-challenged group was normalized to the respective control group by adopting a normalized response model. The relationship of body size with U_{crit} was analyzed by linear regression analysis.

3 | RESULTS

The average total length of the fishes were 3.08 cm (0.2 *SD*), 4.33 cm (0.53 *SD*) and 6.21 cm (0.65 *SD*) on 0 day, 10 day and 20 day respectively. The average wet weight of the fishes were 0.20 g (0.04 *SD*), 0.46 g (0.1 *SD*) and 1.3 g (0.35 *SD*) on 0 day, 10 day and 20 day respectively. The total length and body weight recorded did not significantly differ among the hybrid and purebred crosses (Two-way ANOVA; $F_{1,791} = 0.808$, $p > 0.05$).

U_{crit} (cm/s) increased over the experimental period of 20 days, correlated with the increasing TL of the fish (Figure 2). If U_{crit} was normalized to the TL of the respective fish, U_{crit} ranged between 0.63 to 4.7 TL/s during the experimental period (Figures 2and 3).

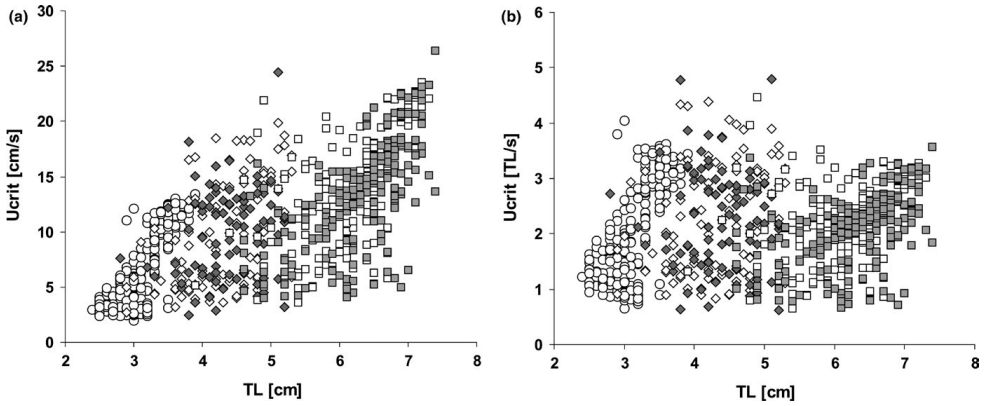

FIGURE 2 Swimming performance of sterlet (n = 792) reared at constant temperature (19°C, white) or exposed to regular temperature increases of 5°C within a day (dark), presented as (a) *Ucrit* [cm/s] or (b) *Ucrit* [TL/S]. Sampling was performed at 0 day (white circle), 10 days (diamond) and 20 days (square). *Ucrit*—critical swimming speed, TL: total length of the respective fish

There were no significant differences in U_{crit} (cm/s) or U_{crit} (TL/s) among crosses as well as between treatment groups at the respective time points i.e., 10 days (Two-way ANOVA; $F_{11,168}$ = 0.643, p > 0.05) and 20 days (Two-way ANOVA; $F_{11,360}$ = 1.118, p > 0.05) (Figure 3). Also, no significant differences between families were detected (ANOVA; $F_{11,204}$ = 0.292, p > 0.05). In all experimental groups a high individual variability was observed. U_{crit} (cm/s) increased over the experimental time, correlated to the increase of TL (Figure 3).

The U_{crit} values in the juvenile sterlet reared at constant temperature were not significantly different from those obtained for fish exposed to the temperature challenge (Figure 3; Table 1). After normalizing the values of U_{crit} between the crosses in the temperature challenged group and crosses in constant temperature respectively, they did not display a significant difference, but only 41% of the temperature challenged groups demonstrated a better swimming performance, and 59% revealed a decreased performance compared to the crosses reared at a constant temperature (Figure 4).

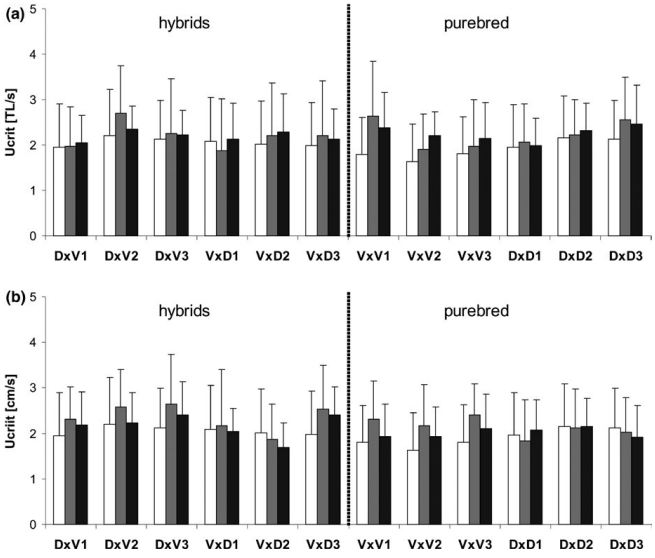

FIGURE 3 Swimming performance (*Ucrit* in cm/s) of juvenile sterlet reared (a) at constant temperature (19°C) or (b) exposed to regular temperature increases of 5°C within a day. Sampling was performed at 0 day (white bars), 10 days (grey bars) and 20 days (black bars). Significant differences in *Ucrit* over the experimental period of 20 days are indicated by different letters (Dunn's, p > 0.05). *Ucrit*—critical swimming speed, V: Volga population, D: Danube population

TABLE 1 Swimming performance (U_{crit} in cm/s expressed as mean ± standard deviation) of the juvenile sterlet from day 0 to 20 reared at constant temperature (C) and exposed to regular temperature increase of 5°C within a day (T)

	U_{crit}(cm/s)				
	Day 0	Day 10		Day 20	
Cross	C (*n* = 18)	C (*n* = 8)	T (*n* = 8)	C (*n* = 16)	T (*n* = 16)
Hybrid					
DxV1	6.59 ± 3.81	9.24 ± 4.97	9.98 ± 2.82	12.75 ± 4.54	13.37 ± 5.32
DxV2	6.97 ± 3.83	11.66 ± 4.14	11.70 ± 3.51	14.91 ± 4.20	13.98 ± 5.26
DxV3	6.98 ± 3.48	10.06 ± 5.39	12.15 ± 5.90	13.08 ± 3.80	16.44 ± 5.78
VxD1	6.47 ± 3.64	7.77 ± 4.37	9.44 ± 4.75	13.33 ± 5.51	12.79 ± 4.25
VxD2	6.61 ± 3.69	9.37 ± 5.64	7.41 ± 3.25	14.15 ± 5.22	11.04 ± 4.15
VxD3	6.29 ± 3.65	8.74 ± 5.38	10.55 ± 4.48	13.44 ± 4.88	14.56 ± 4.25
Purebred					
VxV1	5.54 ± 2.95	11.96 ± 6.63	10.11 ± 3.68	15.32 ± 5.66	12.78 ± 5.37
VxV2	5.13 ± 2.97	7.91 ± 4.33	9.53 ± 3.97	13.59 ± 3.61	12.20 ± 4.33
VxV3	5.71 ± 3.14	8.48 ± 5.22	10.97 ± 3.20	13.11 ± 5.22	13.74 ± 5.52
DxD1	6.33 ± 3.52	9.42 ± 4.86	7.83 ± 3.47	12.45 ± 4.84	12.81 ± 4.93
DxD2	6.75 ± 3.42	9.57 ± 4.20	9.27 ± 3.89	14.41 ± 4.19	13.72 ± 4.96
DxD3	6.85 ± 3.23	11.35 ± 3.94	8.90 ± 3.50	15.05 ± 5.37	12.38 ± 5.17

4 | DISCUSSION

Swimming performance is one among the important traits affecting the potential for survival of fishes, their ability to access habitats, avoid predators and acquire food (Plaut, 2001). Currently, information on the swimming capabilities of sturgeons is limited.

We found that the purebred and hybrid crosses did not exhibit any significant differences in U_{crit} over the experimental period of 20 days, which indicated no effect of intraspecific hybridization on U_{crit} at the juvenile stage. In other freshwater fish, it has been reported that parentage is a poor predictor of swimming performance in sockeye salmon (*Oncorhynchus nerka*) (Nadeau, Hinch, Pon, & Patterson, 2009), a characteristic indicated by our findings in sturgeon too. Still, in Threespine sticklebacks (*Gasterosteus aculeatus*) interfamily differences in burst swimming capacity have been recorded (Garenc, et al., 1998).

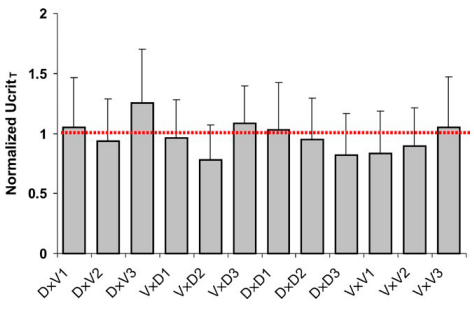

FIGURE 4 Swimming performance (*Ucrit*) of juvenile sterlet exposed to regular temperature fluctuatios of 10°C within 12 hr, carried out every 3 days and normalized to the respective group kept at constant temperatures at 19°C. The red dotted line represents the respective control at constant temperature. V: Volga population, D: Danube population

A wide array of physiological and biological characteristics have a significant impact on swimming ability, aside from genetic components. There have been several studies on swimming performance in juvenile sturgeons, often focusing on burst swimming though (Braaten, Elliott, Rhoten, Fuller, & McElroy, 2015; Cai et al., 2015; Jager et al., 2016). For example, Peake, McKinley, and Scruton (1997) reported that Lake sturgeon (*Acipenser fulvescens*) is incapable of high-speed burst swimming, as they do not show a change between prolonged and burst swim speeds as observed in other fish such as salmonids. Consequently, sturgeon rely on aerobic swimming (Kieffer, Arsenault, & Litvak, 2009), and exhibit rather low *Ucrit* at mean TL of 160 mm. As a consequence flow increment (Ui) is critical and needs to be chosen carefully. Here, we used a rather low increment of 1.4 cm/s. With respect to genetic components, the results of this study differed from previous studies which were designed to quantify the genetic effects on swimming performance of fish larvae (Humphrey, 2011). Whitefish and anemonefish larvae showed maternal effects on critical swimming speed. While whitefish larvae showed sire, dam and sire × dam effects on swimming ability (Kekalainen, Huuskonen, Tuomaala, & Kortet, 2010). There are several possible reasons why we observed no noticeable genetic effects of intraspecific hybridization on critical swimming capacity in this study. It may be due to low heterosis, which is not visible in the juvenile stage. It could also be due to the fact that the parents selected from the Danube and Volga populations had more similar genetic backgrounds (Ludwig et al., 2009) than expected.

The hybrid and purebred crosses demonstrated no apparent trend in the U_{crit} from day 0–20 with respect to the temperature

challenge. Still, we recorded that none of the crosses revealed a significant effect in their swimming performance under changing temperature. In fact, only 41% of the temperature challenged groups revealed a better swimming performance and 59% showed a decreased swimming performance when compared to the groups reared at the constant temperature. The overall swimming ability of the fish was not compromised indicating that temperature treatment as a main environmental stressors does reveal a consistent effect on swimming performance. We found that water temperature wasn't significantly influencing swimming performance whereas positive correlation of swimming performance with water temperature has been reported previously (Adams, Hoover, & Killgore, 1999; Boysen & Hoover, 2009). There was no significant increase in U_{crit} with changing temperature and the U_{crit} values differed significantly from day 0–20 among the crosses and between treatments, which is similar to results from an earlier study on adult shovelnose sturgeon (Hoover, Collins, Boysen, Katzenmeyer, & Killgore, 2011). Fish showed a notable relationship between body size and locomotion throughout the experiment, which can be compared with previous studies on juvenile sturgeons (Allen, Hodge, Werner, & Cech, 2006). The individual U_{crit} variation was very high among the crosses on 0, 10, and 20 days as stated in the studies by Adams and Parsons (1998) and Hoover et al. (2011).

The Volga and Danube rivers are the two largest river systems in Europe with varying water flow regimes in their ranges. The critical swimming speeds can have a marked impact on sturgeons' survival, which are known to be selective for specific water velocities in natural waters (Chan, Dibble, & Killgore, 1997; Stobutzki & Bellwood, 1994). Thus, the study of both the discrete and combined outcomes of genetic and environmental effects is essential towards a better understanding on the impacts of intraspecific hybridization and environmental challenges on sturgeon survival. In contrast to other traits (growth), there was no profound effects of hybrid vigor recorded and no negative effects of hybridization on swimming performance. Intraspecific hybridization may lead to greater genetic diversity, increased fitness and greater adaptation to local environments (DeWet, Fletcher, Hilu, & Harlan, 1983), which was not indicated in the present study during the early developmental stages. To our knowledge, this study brings first observation of the effect of intraspecific hybridization on swimming performance in sturgeons. Thus, it has an important implication for further studies to examine the possible genetic effects on critical swimming speed in hybrids during different developmental stages.

5 | CONCLUSIONS

Our study suggests that intraspecific hybridization has no detectable effects on the critical swimming capacity of sterlet. We found that neither constant nor fluctuating rearing temperatures do not significantly affect the critical swimming capacity of the purebred and hybrid crosses of sterlet. There is a scope for additional studies to elucidate the role of genetics on the swimming performance and other fitness-related traits of the inter-population hybrids intended for release programs.

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CONFLICT OF INTERESTS

No conflict of interest exists among the authors.

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CHAPTER 3

THE GENETIC ANALYSIS AND PERFORMANCE TESTS OF INTRASPECIFIC HYBRIDS OF DANUBE AND VOLGA STERLETS

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My share on this work was about 40 %.

THE GENETIC ANALYSIS AND PERFORMANCE TESTS OF INTRASPECIFIC HYBRIDS OF DANUBE AND VOLGA STERLETS

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ABSTRACT

We studied the effect of intraspecific hybridization on phenotypic traits such as average weight and cumulative survival in sterlet (*Acipenser ruthenus*) to verify if hybridization increases the performance by inducing the hybrid vigor. Sterlet stocks from Danube and Volga river basins were used to produce purebred and hybrid crosses considering them having low genetic divergence from one another as well as similar local adaptations. In order to establish hybrid and purebred crosses, parents which showed discriminatory alleles on at least one microsatellite locus were artificially propagated from each population respectively. Reciprocal crossing was also adopted to produce hybrid crosses to study the parental effect on genetic polymorphism and physiological fitness between reciprocal hybrids. The highest fertilization (81.73±2.16) followed by hatching rate (72.97±7.49) was recorded in Danube (♀) × Volga (♂) hybrid and the lowest fertilization (74.69±3.35) followed by hatching rate (41.10±8.3) was recorded in Volga purebred cross. The crosses were reared in indoor and outdoor intensive and recirculatory aquaculture system at different developmental stages and periodically checked for growth traits every two months after days post hatch (dph). The highest growth rate (144.9±59.5) was noted in the Danube × Volga hybrid and highest survival was observed in Volga × Danube hybrid on 504 dph. Likewise, the lowest growth rate (124.8±57.6) was found in the Volga purebred on 504 dph. With respect to genetic parameters, a set of 6 microsatellite loci was used. The mean number of alleles was significantly higher in Danube × Volga hybrid, whereas Volga purebred displayed least mean number of alleles. This suggests us that it's not only the hybrids but it is always necessary to take the position of the individual population in a hybridization matrix in to account. The level of genetic polymorphism was high in the Danube × Volga hybrid which was the probable cause for novel adaptation and heterosis, allowing higher fitness in changing environment. Our analysis revealed that the produced intraspecific hybrids performed better than the purebreds.

Keywords: *Hybrid; Sturgeon; Aquaculture; growth traits; Fitness*

1. Introduction

Acipenseriformes represent a very ancient group of fishes and known for source of one amongst the most expensive food products, viz. black caviar. There are 25 extant sturgeon species and 2 paddle fish species inhabiting exclusively in the northern hemisphere along the coast of the Pacific and Atlantic oceans, seas, inland lakes and rivers. Sturgeons represent one of the most highly imperilled groups of taxa, with a worldwide reduction in abundance and distributions common among most species (Birstein et al., 1997; Rosenthal et al., 2006). Most of the sturgeon species are on the brink of extinction (Birstein et al., 1997). The causes of the depletion of sturgeon include biological traits of populations (late sexual maturation and long intervals among periods of spawning) and anthropogenic effects (environmental

pollution destroying spawning habitats, human interventions preventing the migration of fish to their spawning grounds and overfishing).Therefore, the demand for captive breeding and aquaculture of sturgeons to produce caviar has increased, as wild stocks have become depleted or closed for legal harvest.

The sterlet (*Acipenser ruthenus*) is an Eurasian rheophile freshwater species inhabiting rivers flowing into the Caspian, Black, Azov, Baltic, White, Barents and Kara Seas (Berg, 1948). It is one of the common sturgeon species with relatively small size and short reproductive cycle found in Eurasian waters. The onset of sexual maturity occurs between 3–7 years in males and 5–12 years in females (Sokolov and Vasiliev, 1989; Fopp-Bayat et al., 2015). It is one among the important commercial fish with the features which have enabled breeders to obtain fast growing hybrids, for example the bester, a hybrid of the giant sturgeon (*Huso huso*) female and a sterlet male (Chebanov and Billard, 2001). Although this species is less important for caviar production, it is threatened according to the IUCN Red List (2013). Overfishing, pouching, habitat destruction and other anthropogenic causes lead to profound decline of this species in the Upper and Middle Danube River (Jarić and Gessner, 2011). Restocking activities are being undertaken along the stretches of Danube by using remaining specimens and non-native stocks from neighboring river systems in Romania and Hungary (Bloesch et al., 2006; Guti and Gaebele, 2009; Bloesch, 2016). Projects like STURGENE also targeted restocking actions in the lower Danube (Reinartz et al., 2016). Recently, the LIFE project "LIFE- Sterlet: Restoration of sterlet populations in the Austrian Danube" targeting restocking actions for sterlet in the Upper Danube has been started (Friedrich, 2018). It is pivotal to identify, protect, and restore the life cycle and habitats of sturgeons on the Danube River in order to combat additional habitat alterations and to mitigate existing deficiencies for successful restocking. Furthermore, Reinartz et al. (2011) revealed that wild population of Danube sterlet is already contaminated with about 23% of non-native sterlet carrying partial or complete Volga genotype which alerts the importance of genetic investigation of the stocks before using them for restocking purposes.

Genetic variability in the wild populations which are in the risk of extinction is considered as a key element with regard to their adaptability against the drastic climate change and anthropogenic pressures and hence could play a crucial role in the restocking success stocking (Drauch and Rhodes, 2007; Schreier et al., 2012; Friedrich, 2018). In sturgeons, many of the locally adapted populations have been decreased to a degree where genetic heterogeneity is extremely limited or where locally adapted populations have become extinct (Gessner et al., 2011). Investigation of genetic patterns within and between populations is considered as a prerequisite for a successful recovery program (Reinartz et al., 2011; Friedrich, 2018). As such the question of the proper source for recovery stocking is becoming an urgent question for the initial phase of the recovery programs (Boscari et al., 2014). One potential option to tackle this question is the increase of genetic heterogeneity through intraspecific hybridization. Unlike interspecific hybridization, intraspecific hybridization (hybridization within species) often produces viable and fertile individuals; however, the fitness of offspring can be higher (heterosis) or lower (outbreeding depression) than that of their parental populations. Production of intraspecific hybrids between different populations is an efficient strategy in aquaculture. The most widely recognized short-term benefits of intraspecific hybrids is hybrid vigor or heterosis which is exhibited in terms of the superiority in phenotypic traits of the hybrids over their parental stocks (Facon et al., 2008; Lipmann and Zamir, 2007; Rius and Darling, 2014). This phenomena can have lot of beneficial impact on fitness traits ranging from benefits that are completely independent of selective environment to those that are specific to the novel environment due to mixing of populations. One likely option for restocking could be the mixing fish from different isolated populations to increase the genetic

diversity and allow future selection based upon the natural pressures in the natural waters (Friedrich, 2018). Besides, heterosis or neutral outcomes are not considered detrimental to conservation efforts, henceforth the focus of selecting candidate populations should be on the prevention of outbreeding depression. Outbreeding depression is considered as a likely outcome when the chosen breeding populations are with fixed chromosomal differences which lived in different environments for over 20 generations and not had any gene flow for over 500 years (Frankham et al., 2011). In order to avoid the outbreeding depression when more than one population is being used in restocking, it is best to choose parental populations that have low genetic divergence from one another as well as similar local adaptations (Edmands, 1999, 2007; Audet et al., 2017). So for, there are no studies documented on evaluation of the implications of intraspecific hybridization in a controlled hatchery environment that is consistent with current hatchery practices in sturgeons. Such intraspecific hybridizations due to the heterosis effect at the individual level for several phenotypic traits at the species level was evaluated in our present study using model species, the sterlet. Additionally, a wide genetic analysis of the intraspecific hybrids using set of microsatellite loci was conducted.

2. Materials and methods

This experiment was conducted at the Genetic Fisheries Center, Faculty of Fisheries and Protection of Waters in Vodňany, Czech Republic.

Sampling for Population divergence studies

We collected tissue sample (fin clip) from 100 fishes from hatchery stocks originated from Danube (Rybnikarstvi, Pohořelice) and Volga (Genetic Fisheries Center, Vodňany) respectively and stored in 96% ethanol. The tissue samples was further used for microsatellite genotyping. The fishes (12 in numbers) which expressed discriminatory alleles in at least 1–2 microsatellite loci from Danube and Volga stocks respectively, were used for subsequent production of hybrid and purebreds.

Fish broodstock handling and hormonal induction

We produced 12 purebred and hybrid families from Danube and Volga population of sterlet. For this purpose, 12 males and 12 females were artificially propagated from each population which expressed discriminatory alleles and highest genetic diversity in set of microsatellite loci. The mean body mass of the selected broodstocks from Danube and Volga populations for artificial propagation are mentioned in table 1. Parental Volga was from genetic fisheries center, FFPW, Vodňany and Danube broodstock was originated from a fish hatchery operated by Rybnikarstvi, Pohořelice, The Czech Republic respectively. The handling of broodstock, final maturation by hormonal induction of the broodstock and evaluation of gamete quality was conducted as elaborated in Shivaramu et al. (2019a) (Chapter 2).

Table 1. *Mean body mass (MB) of the selected broodstock in Volga and Danube population.*

Population	Mean body mass ± SD (kg)
Volga males	1.41 ± 0.284
Volga females	1.58 ± 0.543
Danube males	1.77 ± 0.494
Danube females	1.85 ± 0.455

Concerted artificial Propagation, Fertilization and Hatching

In order to establish hybrid and purebred families, we organized a concerted artificial propagation of broodstock at Vodňany and Pohořelice simultaneously following the methodology described by Shivaramu et al. (2019a) (Chapter 2). The fertilization scheme and incubation of fertilized eggs under regulated environmental conditions was done as mentioned in Shivaramu et al. (2019a) (Chapter 2). The fertilization rate and hatching rate was evaluated as per the protocol described by Dettlaff et al. (1993). The 45 swim-up larvae from each group was collected and preserved in 96% ethanol after hatching for molecular analysis.

Rearing conditions

The rearing of fishes was carried out according to standard aquaculture hatchery protocols by using recirculatory aquaculture (RAS) and intensive culture system. RAS is a rapidly growing technology in fish farming which reuses the water in culture system by the use of mechanical and biological filters. Larval rearing is the difficult phase in sturgeon rearing. Fry survival depends on culture system and a complete nutritional program with given attention to diet formulation, feeding schedule and food preference. After complete removal of their yolk sac, the larvae were firstly fed with the diced sludge worms (*Tubifex tubifex*) for two weeks and then shifted to co-feeding (combination of diced sludge worms and formulated dry feed). After 4 weeks, the larvae completely fed with dry formulated feed. The various formulated feeds fed to the fishes throughout the experiment were from Alltech Coppens, the Netherlands. Around 30% of mortality was recorded in the fish during the shift to fry formulated feed from live fish feed. After initial 3 months of rearing in indoor troughs, the groups were separately reared with the initial stocking density of around 7 kg/m^3 in indoor circular tanks which were moved to outdoor tanks after 229 dph for communal rearing. The fishes were implanted by Individual Passive Integrated Transponder (PIT) tags (134.2 kHz; AEG Comp., Germany) subcutaneously after 175 dph. The different size ranges of feeds were used for different developmental stages of fishes (Tab. 2).

Table 2. *Summary table containing details on proximate composition of the different feed fed to fishes at different size ranges.*

Fish weight (g)	Rearing system	Days post hatch	Life stage	Feed	Feed size (mm)	Protein (%)	Fat (%)	Crude fibre (%)	Ash (%)	Total P (%)
0.2–0.5	Indoor trough	1–11	Larvae	Alltech Coppens® Advance	0.2–0.5	56	15	0.1	12	1.99
0.5–1.5	Indoor trough	1–29	Fry	Alltech Coppens® Advance	0.5–0.8	56	15	0.1	12.0	1.99

1.5–5.0	Indoor circular tanks	29–58	Early fingerling	Alltech Coppens® Start Premium	1.0	54	15	0.3	10.3	1.73
5.0–10	Indoor circular tanks	58–85	Fingerling	Alltech Coppens® Start Premium	1.0/1.5	54	15	0.3	10.3	1.73
10–50	Indoor circular tanks	85–175	Early juvenile	Alltech Coppens® Alevin	2.0	54	15	1.1	9.0	1.32
50–100	Indoor circular tanks	229–325	Early juvenile	Alltech Coppens® Alevin	2.0	54	15	1.1	9.0	1.32
100–200	Outdoor circular tanks	386–504	Early Juvenile	Alltech Coppens® Supreme-15	3.0	49	10	1.5	7.9	1.27

Performance tests

The fishes of all groups were periodically checked for average weight and cumulative survival on 77, 175, 229, 325, 386 and 504 dph.

Molecular analysis

Whole genomic DNA was extracted using Nucleo Spin® Tissue kit (MACHEREY-NAGEL GmbH & Co. KG, Germany) from the tissue samples (finclips for population divergence study and swim-up larvae for heterozygosity among groups). Six microsatellite markers viz., AciG 35 (Börk et al., 2008), AfuG 135 (Welsh et al., 2003), Aox 45 (King et al., 2001), Spl 101, Spl 163 and Spl 173 (McQuown et al., 2000) were used for amplification. The PCR amplification was carried out according to the procedure reported by Havelka et al. (2013). Microsatellite fragment analysis was conducted on 3500 ABI Genetic Analyzer (Applied Biosystems, USA) using GeneScan LIZ 600 size standard (Applied Biosystems), and genotypes were scored in Genemapper 4.1 software (Applied Biosystems, USA). The mean number of effective alleles (N_A), gene diversities of each locus, the fixation index (F), pairwise population differentiation (G_{ST}), D_A genetic distance, expected (H_e) and observed (H_o) heterozygosities between Danube and Volga populations were calculated using GeneAlex (Peakall and Smouse, 2006).

The mean number of alleles (N_a), expected (H_e) and observed (H_o) heterozygosities in each hybrid and purebred cross were used to access the level of polymorphism among analyzed crosses was calculated using GeneAlex (Peakall and Smouse, 2006).

Statistical analysis

Statistical analysis was performed with the Statistica 13 (STATISTICA advanced, module STATISTICA Multivariate Exploratory Technique; Statsoft). The data was first analyzed for normal distribution using the Kolmogorov-Smirnov test. Multiple comparison was carried out by one-way ANOVA with the significance of $\alpha = 0.05$ and Tukey's post-hoc (parametric data) or Kruskal-Wallis and Dunn's post-hoc (non-parametric data) test for fertilization and hatching rate. Differences in survival was evaluated using Pearson's Chi-square test on the surface significance of $\alpha = 0.05$. Statistical significance of differences in the average weights between individual groups was tested by analysis of variance ANOVA with the significance of $\alpha = 0.05$.

The significance of differences in N_A, H_o and H_e among the Danube and Volga populations (also the hybrid and purebred crosses) was tested with a one-way analysis of variance (ANOVA) with the significance of $\alpha = 0.05$.

3. Results and discussion

Population genetic analysis

The gene diversity was found to be highest in Danube population for locus Aox 45 (0.882) (Tab. 3). The overall gene diversity among all the loci was high in Danube population when compared to Volga population. The observed heterozygosity followed by expected heterozygosity was found highest in Danube population (Tab. 4). The pairwise G_{ST} and D_A matrix value between Danuve and Volga populations were 0.136 and 0.549 respectively. These significant values showed that the Danube and Volga stocks are moderately genetically differentiated. The fixation index values of Danube and Volga populations are mentioned in the table 3. The genetic variation among Danube and Volga stocks was on an optimal level. It is highly essential to track the genetic condition of the broodstock constantly. The selection of broodstock containing spawning individuals characterized by high genetic variation is done when the genetic diversity values reaches critical level. In such instances, selection of spawning pairs based on genetic profiles is highly required (Kaczmarczyk and Fopp-Bayat, 2013).

Table 3. *Gene diversity (Nei, 1987) per locus and population.*

Locus	Danube	Volga
Spl 163	0.811	0.772
Spl 101	0.743	0.831
Spl 173	0.589	0.650
AfuG 135	0.605	0.763
Aox 45	0.882	0.578
AciG 35	0.722	0.602

Table 4. *Summary statistics of the genetic variation among Danube and Volga Population. N_a = Mean no of alleles; H_e = expected heterozygosity; H_0 = observed heterozygosity; F = fixation index. * shows the group with significant difference at P < 0.05*

Locus	H_o	H_o SD	H_e	H_e SD	N_a	F
Danube	0.7346*	0.0133	0.7231	0.0482	5.7*	0.165
Volga	0.6862*	0.0287	0.7018	0.0197	5.2*	0.132

Performance of the purebreds and hybrids

Generally hybrids are formed in aquaculture to increase fitness, which is manifested by the improvement of many physiological and utility properties in comparison with parental species (Bartley et al., 2001; Shivaramu et al., 2019b). It is also essential to know the fitness characteristics of the hybrids which are produced in the wild. The genetic and physiological fate of the hybrids formed in wild due to accidental aquaculture escapees and use of non-native species or stock for restocking purposes are not yet extensively studied.

The observed values of fertilization rate and hatching rate of the groups are given in figure 1. The highest fertilization (81.73±2.16) followed by hatching rate (72.97±7.49) was recorded in Danube × Volga hybrid. The lowest fertilization rate (74.69±3.35) followed by hatching rate (41.10±8.3) was recorded in Volga purebred. The fertilization rate was significantly different between Danube × Volga hybrid and Volga purebred whereas recorded hatching rate was significantly different between Danube purebred and Volga purebred. Although the fertilization rate was found to be highest in Danube × Volga hybrid, we recorded decreased hatching rate in this group during the process of hatching. The reproductive features in many interspecific hybrids of sturgeons are widely studied although studies on intraspecific hybrids aren't well documented (Memis et al., 2009; Chebanov and Galich, 2011; Shivaramu et al., 2019b). However, in aquaculture, reproduction pointers are rather complementary indicator, while growth is probably one of the most desirable properties (Bartley et al., 2001).

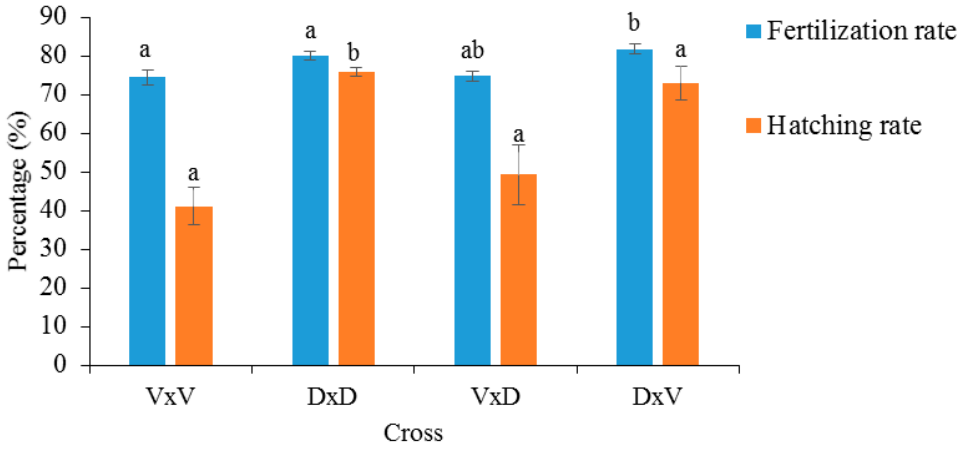

Figure 1. *Observed values of fertilization rate and hatching rate among the analyzed artificially bred hybrid and purebred groups of Volga and Danube sterlet populations. Columns with the same alphabetic superscript did not differ significantly at P < 0.05.*

The observed average weight and cumulative survival of the groups during all the assessments are shown in figure 2. The highest average weight (144.9±59.5) was noted in the Danube × Volga (Fig. 2) hybrid and highest survival was observed in Volga × Danube hybrid on 504 dph (Fig. 3). The lowest growth rate (124.8±57.6) was found in the Volga purebred on 504 dph. The lowest cumulative survival was found in Danube × Volga hybrid surprisingly. It could be due to combined effect of genetic and environmental factors. This suggests us that it is not only the hybrid vigor itself but it also depends on parental position in the hybridization matrix which is noteworthy. Many authors studied the notable effect of parental position in hybridization matrix in cat fishes and carps whose outcomes are comparable with our results (Gjerde et al., 2002; Panase and Mengumphan, 2015; Liu et al., 2017). The Danube × Volga hybrid recorded highest average weight in most of the assessment periods which might be caused by maternal effect. Additionally, females may have a stronger influence than males on the phenotypic expression of many traits of the offspring (Liu et al., 2017). The maternal effects may occur due to the mother's nuclear and extra nuclear genes and environmental factors (Falconer and Mackay, 1996). Overall, the hybrids performed better than parental

stocks in our study. Our results holds good with the various sturgeon hybrids that have been produced to increase growth rate and improve productivity through hybrid vigor (Glogowski et al., 2002; Wei et al., 2011; Zhang et al., 2013; Boscari et al., 2014; Shivaramu et al., 2019b).

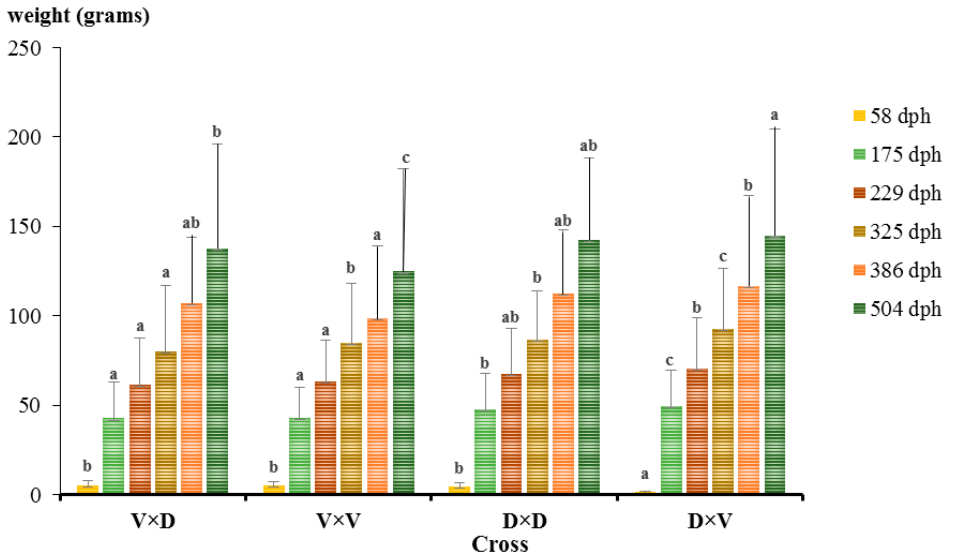

Figure 2. *Observed values of average weight among the analyzed artificially bred hybrid and purebred groups of Volga and Danube sterlet populations. Columns with the same alphabetic superscript did not differ significantly at P < 0.05.*

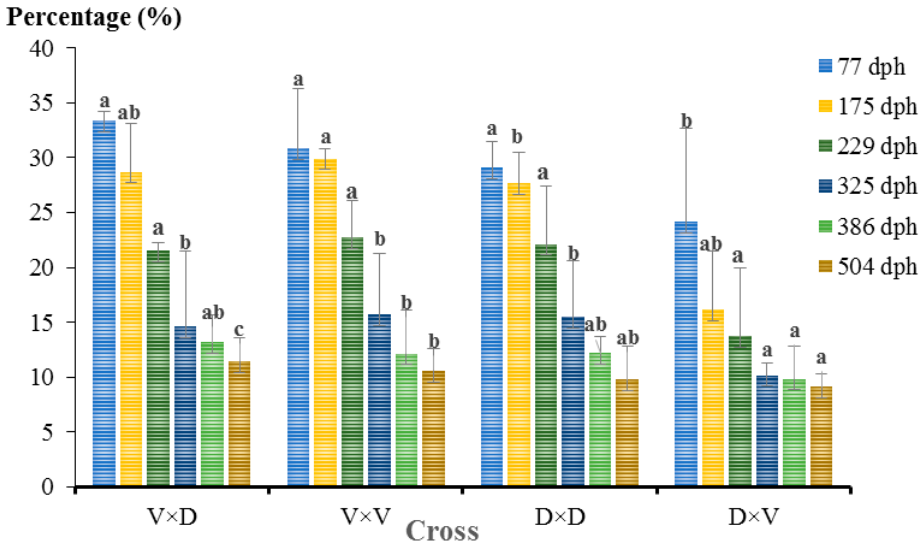

Figure 3. *Observed values of cumulative survival among the analyzed artificially bred hybrid and purebred groups of Volga and Danube sterlet populations. Columns with the same alphabetic superscript did not differ significantly at P < 0.05.*

Molecular analysis

The mean number of alleles was significantly higher in Danube × Volga hybrid, whereas Volga purebred displayed least mean number of alleles (Tab. 5). The observed (0.6962±0.0498) and expected (0.7589±0.0685) heterozygosities was high in the Danube × Volga hybrid (Tab. 5) which might be the probable cause for novel adaptation and heterosis, allowing higher fitness in changing environment (Arnold, 1997). Our analysis revealed that the produced intraspecific hybrids performed better than the purebreds which can be correlated with our previous results from interspecific hybrids of different sturgeon species (Shivaramu et al., 2019b). The significant competitive risk for wild populations due to intraspecific hybrids can be way less dangerous than interspecific hybrids since the level of genetic polymorphism and occurrence of the rare alleles is comparatively less. Also, they share same number of chromosomes and there is no chance of production of sterile hybrids. Nevertheless, there can be risks associated with outbreeding depression in future generations which should be equally considered while selecting the non-native stocks (Edmands, 2007). Henceforth, the hybrids produced in the present study can be exploited over the purebred of Danube and Volga socks in commercial aquaculture farming for increase growth rate, improve productivity through hybrid vigor and transfer desirable traits. The hybrids can be used as potential candidates in closed common environment experiments to study their fate in chosen closed wild conditions. Researchers studied the implications of stocking multiple strains of Atlantic salmon and assessed the correlation between genetic and reproductive quality in their broodstock (Audet et al., 2017). Although their results showed no record of outbreeding depression in the F1 generation, they recommended for future research on fate of f2 back crosses and subsequent generations could be more interesting.

Table 5. *Summary statistics of the genetic variation among the analyzed artificially bred purebred and hybrid crosses of Volga and Danube sterlet populations. N_a = Mean no of alleles; H_e = expected heterozygosity; H_0 = observed heterozygosity. * shows the group with significant difference at P < 0.05*

Locus	H_0	H_0 SD	H_e	H_e SD	N_a	N_a SD
D×D	0.6553	0.0442	0.7536	0.0482	4.5	1.44
V×V	0.5919*	0.0311	0.6250*	0.0327	4.3*	1.52
D×V	0.6962*	0.0498	0.7589*	0.0685	4.6*	1.65
V×D	0.6398	0.0523	0.7462	0.0279	4.5	1.28

Conclusion

We observed that one (D×V) of the intraspecific hybrids performed better than purebreds of Volga and Danube sterlets in all the assessment periods with respect to average weight, and its reciprocal (V×D) hybrid survived highest when compared to purebreds. The genetic diversity was significantly higher in one of the hybrid (D×V). Our results indicated D×V hybrid can be a potential hybrid for achieving better growth. Altogether, the hybrids can be used over purebreds for commercial aquaculture purposes. Future research focusing on investigation of the adult fecundity and reproductive fitness (gamete quality and survival) of F1 hybrids, as well as potential study of fitness-related traits in F2, backcrosses and subsequent generations should be undertaken. This can eventually provide insights on fate of intraspecific hybrids in sturgeon which could serve as a potential evidence for future conservation efforts.

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CHAPTER 4

INFLUENCE OF INTERSPECIFIC HYBRIDIZATION ON FITNESS-RELATED TRAITS IN SIBERIAN STURGEON AND RUSSIAN STURGEON

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

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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

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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 (♀ × *A. gueldenstaedtii* ♂). Likewise, Arefjev (1999) documented the performance of the reciprocal hybrids of Russian sturgeon and Siberian sturgeon (*A. gueldenstaedtii* ♀ × *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-

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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 m^3 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			
A. baerii	10	6.5	118
A. baerii	9	7	123
A. gueldenstaedtii	8	7	106
A. gueldenstaedtii	8	7.5	119
A. gueldenstaedtii	8	7	112
Females			
A. baerii	18	17	142
A. baerii	9	10	126
A. gueldenstaedtii	15	12	131
A. gueldenstaedtii	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

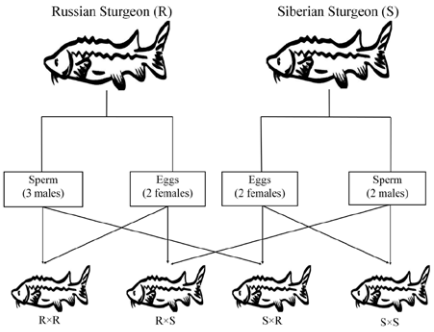

Figure 1. Schematic diagram showing the establishment of different crosses

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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 O_2. 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 m^3 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 m^3 indoor tanks with the average temperature of 22°C for separate group nursing. Initial stocking density was 7 kg/m^3 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 m^3 indoor circular tanks at 4°C without feeding. After wintering, fish were held in 3.5 m^3 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/m^3, 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 purebred 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):

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

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-

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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 ± 7.8%) followed by R × S (92.8 ± 5.35%). The lowest values of fertilization rate were recorded for the R × R purebred where the fertilization value (85.2 ± 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 ± 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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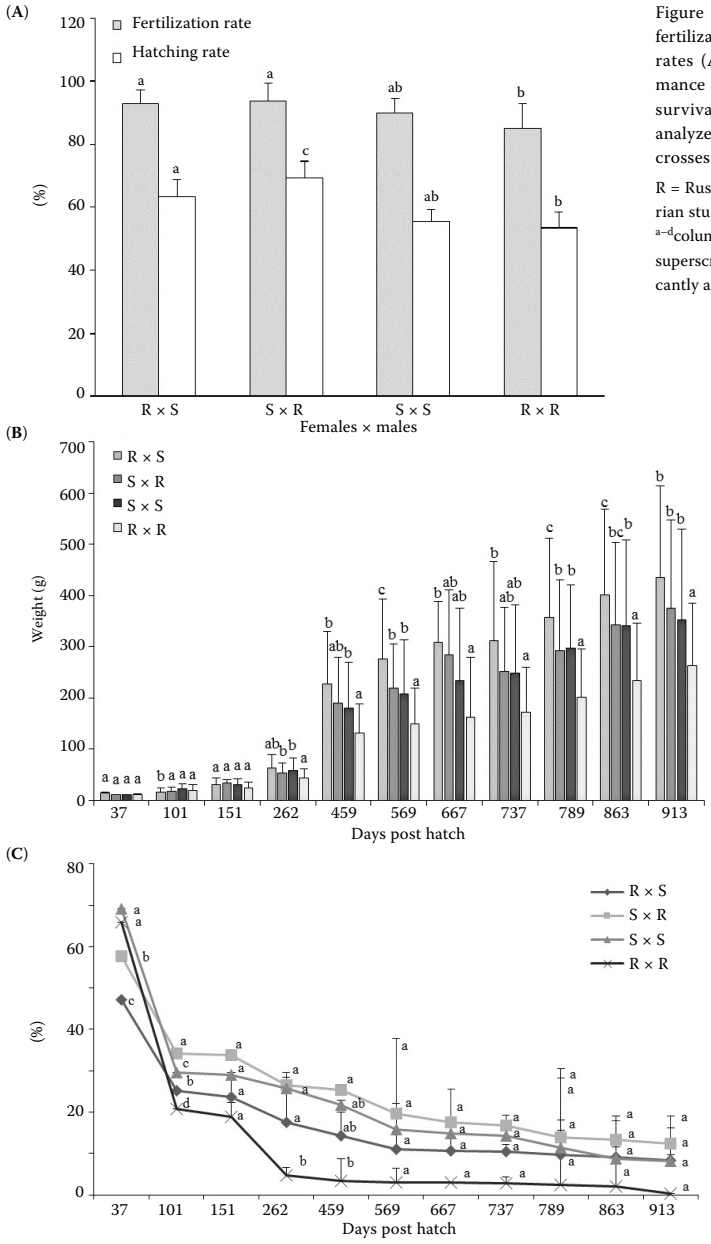

Figure 2. Observed values of fertilization rates and hatching rates (**A**), of growth performance (**B**), and of cumulative survival rate (**C**) among the analyzed hybrid and purebred crosses

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 (Szczepkowski 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

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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 × S and S × 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 (Jenneckens 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.

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CHAPTER 5

THE HETEROSIS ESTIMATES FOR GROWTH AND SURVIVAL TRAITS IN STURGEON PUREBREDS AND INTERSPECIFIC HYBRIDS

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THE HETEROSIS ESTIMATES FOR GROWTH AND SURVIVAL TRAITS IN STURGEON PUREBREDS AND INTERSPECIFIC HYBRIDS

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ABSTRACT

The aim of the present study was to estimate and compare the growth and survival traits of the hybrids and purebreds produced by crossing the Siberian sturgeon (Acipenser baerii) and sterlet (*Acipenser ruthenus*) in order to determine the heterosis effect in the F1 generation. We compared the breeding conditions, growth traits and genetic polymorphism in the artificially produced hybrid crosses of sterlet and Siberian sturgeon with respect to their pure parental species in indoor and outdoor recirculatory aquaculture systems (RAS) at different developmental stages. Fertilization and hatching rates were found to be significantly higher in S×S purebred. The highest values of mean body weight was recorded in St×S hybrid (557.54±179.7 g) on 862 days post hatch (dph) while highest cumulative survival was recorded in S×S purebred (14.3%). The recorded cumulative survival and mean body weight were significantly lower in St×St purebred. The highest and positive heterosis was recorded for mean body weight of St×S hybrid (51.3% on 862 dph) throughout the assessment periods. The mean number of alleles was significantly higher in both the hybrid families compared to sterlet purebred. The significant differences were found at heterozygosity levels among studied crosses and St×St purebred displayed the lowest mean number of alleles. The studied sturgeon hybrids had higher mean body weight compared to their purebreds under hatchery conditions which can be potentially used in the sturgeon aquaculture to exploit the heterosis.

Keywords: *Hybrid; Sturgeon; Aquaculture; Polyploid; Fitness*

1. Introduction

Hybridization is a process in which offsprings inherit restructured parental genes, which are obtained by mating individuals of different genotypes originating from different populations and species (Zhang et al., 2013). The outcomes of hybridization can be highly diverse. There may be a generation of the novel multigene complex, which may lead to hybrid speciation, or more commonly introgressive hybridization is observed where genes of one species get incorporated to the genome of other species (Scribner et al., 2000). Several studies showed that fishes hybridize more commonly than any other vertebrates due to various factors such as external fertilization, weak isolation behavior, etc (Schwartz, 1981; Allendorf and Waples, 1996). It is often used as one of the most common breeding practices in aquaculture where heterosis can be manifested by crossing distinct genotypes which possibly leads to increased fitness of the offspring (Chevassus, 1983; Hulata, 2001). Heterosis or hybrid vigor is defined as hybrid with enhanced viability and developmental rates compared with its parents (Shull, 1948). The increased fitness may be phenotypically observed in terms of higher growth rates, higher viability, greater flexibility of adaptation and attaining faster sexual maturity.

Henceforth, this method have proved its potential in aquaculture and attracted the attention of breeding programs of commercially important fish species like common carp. However, it is also associated with some disadvantages attributed to intrinsic or environmentally mediated incompatibilities. In sturgeons, accidental release or escapees from hatcheries to wild have posed a great threat on genetic integrity of the wild stocks in Volga and Danube rivers (Jenneckens et al., 2000; Ludwig et al., 2009; Reinartz et al., 2011).

Sturgeons are one of the most ancient group of fishes that originated during the Jurassic period and have survived various mass extinction events (Bemis et al., 1997). These fishes are diadromous or some being resident to freshwater, found in the temperate waters of the northern hemisphere. Sturgeons show very late maturation starting from 5 to more than 30 years and are characterized by long reproductive cycles ranging between 2 to more than 10 years (Pikitch et al., 2005). Sturgeons faced a dramatic decline in their abundance in the last decades due to overfishing and poaching along with habitat destruction (through dam constructions, water pollution, anthropogenic activities). Most of the species are at the risk of extinction according to the Red List of Threatened Species under the International Union for Conservation of Nature (IUCN) (Fontana et al., 2008; Zhang et al., 2013).

Sturgeons are evolutionary polyploids with at least three independent genome duplication events during their evolution (Havelka et al., 2011). Billard and Lecointre (2001) highlighted that polyploidization might be the probable cause for sturgeons being highly susceptible for interspecific hybridization. It is also evident that sturgeon species share a close genetic relationship, evolutionary age and very similar reproductive features, which strongly influences the development of hybrids in natural waters. Various interspecific and intergeneric sturgeon hybrids are reported in nature (Birstein et al., 1997). Presently, all acipenseriformes can be divided in to three groups based on chromosome number, DNA content, and nucleus/cell size. (a) species with karyotypes comprising about 120 chromosomes; (b) species with 240 to 270 chromosomes; they are conventionally referred to as 250-chromosomes species; (c) species with around 370 chromosomes (Vasil'ev, 2009; Havelka et al., 2011). The genome size ranges from 2.44 pgDNA nucleus^{-1} in *Huso huso* (Birstein et al., 1993) to 13.78 pgDNA nucleus^{-1} in *Acipenser brevirostrum* (Hardie and Hebert, 2003). Two scales of Acipenseriformes ploidy have been proposed: (a) the "evolutionary scale": tetraploid species (~120 chromosomes), octoploid (~250 chromosomes), and dodecaploid (~370 chromosomes) species (Birstein and Bemis, 1997); and (b) the "contemporary/ functional scale": diploid (~120 chromosomes), tetraploid (~250 chromosomes), and hexaploid (~370 chromosomes) species (Ludwig et al., 2001; Symonová et al., 2013).

Many sturgeon hybrids are produced and their heterosis has been widely exploited in aquaculture over the last few decades (Bronzi et al., 1999; Tranah et al., 2004). Some authors investigated the growth characteristics of juvenile hybrids between Siberian sturgeon (*A. baerii*) and Russian sturgeon (*A. gueldenstaedtii*) and their reciprocal hybrids (Glogowski et al., 2002; Arefjev, 1999). In another study, hybrids of beluga female with sterlet male grew faster than the sterlet (*A. ruthenus*) and attained sexual maturity earlier than beluga (*H. huso*) (Burtsev, 1997). Conversely, reciprocal hybrids performed poorly (Steffens et al., 1990). This hybrid also produced high-quality caviar at a younger age as compared to pure beluga, which led to the expansion of hybrid cultivation using different sturgeon species (Boscari et al., 2014). It is reported that the sturgeon hybrids produced from the species of same ploidy showed better reproductive success and fitness in the second generation compared to hybrids produced from species of different ploidies (Nikoljukin, 1971). Recently, we (Shivaramu et al., 2019) reported that hybrids between Siberian and Russian sturgeon displayed better survival and growth rate. The hybrids produced from species of different ploidies were even completely sterile (Arefjev, 1999). The influence of hybridization on the

fitness-related traits has been quite extensively studied in fishes (Fleming et al., 2000; McGinnity et al., 2003). Despite their high affinity to hybridize (Birstein et al., 1997), sturgeon hybrids are poorly studied with respect to their fitness abilities. Therefore, it is essential to investigate the genetic variability and fitness traits of sturgeon hybrids in comparison to purebreds given their importance in aquaculture.

In the present study, the diploid species sterlet and tetraploid species Siberian sturgeon were used to produce hybrids and purebred crosses. The objectives were (i) to examine and compare the fitness traits such as reproductive features (fertilization rate and hatching rate), mean body weight, and cumulative survival among produced hybrids and purebred groups, (ii) to estimate the heterosis for the mean body weight and cumulative survival traits among the hybrids in recirculatory aquaculture (RAS) and intensive culture system. The level of genetic polymorphism among the groups was analysed by a set of microsatellite loci to study if they are attributing for the fitness of the respective crosses.

2. Materials and methods

Maintenance of brood stock and artificial propagation

The protocol of the study was carried out according to the Animal Research Committee of the FFPW. The fish were maintained in accordance with the principles of animal welfare act of Czech Republic and laboratory animal care in compliance with the law on the protection of animals against cruelty (Act no. 246/1992 Coll., ref. number 16OZ15759/2013-17214). All surgery was performed under clove oil anesthetic immersion (0.07 ml l^{-1}) and all efforts were made to minimize suffering during biopsy.

The broodstocks used in this study was obtained from the Genetic Fisheries Center of the Faculty of Fisheries and Protection of Waters, Vodňany, Czech Republic. In order to establish hybrid and purebred crosses, three male and three female *A. baerii* (S) and *A. ruthenus* (St) were used. The fish were acclimatized at 14°C for 7 days in 5 m^3 indoor tanks arranged as re-circulating water system. To induce Spermiation, males were administered an intramuscular injection of carp pituitary extract (CPE) at 4 mg kg^{-1} body weight in both species. Milt was collected 48 hours after injection from the urogenital papilla by using a plastic catheter of 5 mm diameter into a 100 ml tissue culture flask in order to avoid any contamination by mucus, water or feces. Milt was collected before stripping the females and stored in an icebox 4°C. Later on, the sperm volume and sperm motility were assessed following the Linhart et al. (2000). The samples with above 70% motility were used for fertilization. Ovulation was induced with CPE by an initial injection of 0.5 mg kg^{-1} body weight and a second injection of 4.5 mg kg^{-1} body weight, 12h after the first injection in females of both species (Gela et al., 2008). Ovulated eggs were collected after micro-surgical incision of the oviduct according to Štěch et al. (1999) and stored in aerobic conditions at 16°C until sperm parameters were analyzed.

Fertilization and hatching

The experimental set up included four groups of fishes in which 2 were purebred crosses: *A. baerii* purebred (S♀×S♂), *A. ruthenus* (St♀×St♂), and other 2 were hybrid crosses: *A. baerii* × *A. ruthenus* (S♀×St♂) and *A. ruthenus* × *A. baerii* (St♀×S♂). An equal weight of 50g of eggs was taken from three females to establish each cross. The eggs were pooled and divided into plastic containers of 50g aliquots respectively. Each aliquot was inseminated with 1.5 ml of sperm from one of three males and activated immediately by adding 200 ml of dechlorinated

water and fertilized separately by keeping the aliquots on an orbital shaker maintained at a speed of 200 rpm and 10 mm deflection for 2 minutes. The aliquots were separately fertilized by individual males in order to avoid sperm competition between different males and to balance the genetic contribution from the individual male. After fertilization, all aliquots per cross were pooled and washed with water, immersed in a desticking solution of fine clay suspension (20 g l^{-1}) and left on shaker (Gela et al., 2008). The fertilized eggs of each cross were shifted to Kannengieter incubation flask (supplied with UV sterilized re-circulating tap water at 15.0 °C, with an O_2 saturation of 9 mg l^{-1}) in triplicates after washing out the clay remnants for incubation. The fertilization rate was estimated after 6 hours using 100 eggs sampled randomly in triplicates. The live embryos were counted at the 2nd or 3rd cleavage division (4 or 8 blastomeres) according to Dettlaff et al. (1993). The dead eggs and egg clumps were regularly removed by siphoning. The larvae were counted and few were sampled for subsequent molecular analyses. Larvae sampled for molecular analyses were stored in 96% molecular grade ethanol after hatching. The larvae which hatched out in 5-7 days were separated from dead shells and malformed larvae to avoid the fungal infection and consequent larval mortalities. The newly hatched larvae weren't fed on natural or artificial feed as they rely on the food resource within their yolk-sac for the first 7-9 days depending on temperature.

Fish exogenous feeding, nursery and grow-out rearing conditions

The larvae of individual cross were initially reared in 0.3 m^3 separate indoor nursery tanks. The first feeding was done by diced sludge worms (*Tubifex tubifex*) for 1 week and larvae were co-fed till they were fully weaned for about 14-21 days and shifted to complete dry feeding *ad libitum* with a formulated commercial feed (Alltech Coppens® Start Premium; the Netherlands) containing 54% protein, 15% fat, 1% crude fiber, and 9.4% ash. The fishes of each cross were transferred to 3.5 m^3 separate indoor tanks maintained in the average temperature of 22ºC for separate group-nursing after 100 days post hatch (dph) with an initial stocking density of 7 kg/m^3. The Individual Passive Integrated Transponder (PIT) tags (134.2 kHz; AEG Comp., Germany) were implanted to the fishes subcutaneously on 294 dph. An equal number of juveniles from each group were stocked for communal rearing in replicates with ideal and uniform environmental conditions like aeration, water exchange, feeding rate, temperature etc. The juveniles were transferred for overwintering in 4 m^3 indoor circular tanks at 4ºC without feeding after the second summer. After wintering, the fish were held in 3.5 m^3 outdoor circular tanks with an average temperature of 22°C and were fed on daily commercial diet of 4% of total fish biomass (Alltech Coppens® Supreme-10 containing 49% of protein, 10% of fat, 0.8% of crude fibre, and 7.9% of ash) in the subsequent seasons. During the last 6 months of assessment period i.e., 704 to 862 dph, the fishes were maintained with the stocking density of 25 kg/m^3 in outdoor earthen ponds and fed daily at 4% of total fish biomass (Tab. 1).

Table 1. *Summary table describing fishes reared in different rearing systems at different developmental stages.*

Days post hatch	Fish weight (gm)	Life stage	Rearing system	Feed	Rearing type
1–22	0.2–0.5	Larvae	Indoor trough	Alltech Coppens® Advance	Separate
22–73	5–10	Fingerlings	Indoor trough	Coppens® Start Premium	Separate
73–294	50–150	Early juvenile	Indoor circular tank	Alltech Coppens® Supreme-10	Separate
294–438	100–300	Early juvenile	Indoor circular tank	Alltech Coppens® Supreme-10	Communal
438–704	180–400	Juvenile	Outdoor circular tank	Alltech Coppens® Supreme-10	Communal
704–862	200–600	Juvenile	Outdoor earthen pond	Alltech Coppens® Supreme-10	Communal

Assessment of fishes for growth traits and estimation of mid-parental/ average heterosis

Fishes were periodically weighed and checked for mean body weight and cumulative survival rates on 73, 168, 294, 379, 438, 561, 704, 789 and 862 dph. The fishes with lost tags were excluded from the analysis. We estimated heterosis for the mean body weight and cumulative survival traits of hybrid crosses (S×St and St×S) by using mean body weight and cumulative survival values from the periodic assessment of purebred crosses (S×S and St×St) according to Falconer and Mackay (1996).

Average heterosis = [(F1 – MP) / MP] x 100

Where, F1 = value of F1, MP = mean value of two parents (purebred crosses)

Sampling and Molecular analysis

The genetic polymorphism and heterozygosity level among analysed families were investigated at several microsatellite loci according to McQuown et al. (2000) and Welsh et al. (2003). DNA extraction was carried out using Nucleo Spin® Tissue kit (MACHEREY-NAGEL GmbH & Co. KG, Germany) from the tissue samples (fin clips) from each experimental group. Six microsatellite markers *viz.,* AciG 35 (Börk et al., 2008), AfuG 135 (Welsh et al., 2003), Aox 45 (King et al., 2001), Spl 101, Spl 163 and Spl 173 (McQuown et al., 2000) were used for amplification. The PCR amplification was carried out according to the protocol narrated by Havelka et al. (2013). Microsatellite fragment analysis with the accurate sizing of alleles was performed on 3500 ABI Genetic Analyzer (Applied Biosystems, USA) using GeneScan LIZ 600 size standard (Applied Biosystems), and genotypes were scored in Genemapper 4.1 software (Applied Biosystems, USA). The mean number of alleles (N_a) expected (H_e) and observed (H_o) heterozygosities in each cross was used to access the level of polymorphism among analyzed crosses which were estimated in GeneAlex software (Peakall and Smouse, 2006). The factorial correspondence analysis (FCA) was performed to visualize genetic relationships among crosses based on the multilocus genotypes in GENETIX 4.04 software (Belkhir et al., 2004).

Data analysis

All data were analyzed using Statistica software package (Version 13.2). Statistical significance was tested at 95% confidence level. Residuals were tested for normality 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 for mean body weight, fertilization and hatching rate. Differences in cumulative survival were evaluated using Pearson's Chi-square test. The significance of differences in N_a among the crosses was tested with a one-way analysis of variance (ANOVA).

3. Results and discussion

The scope for sturgeon aquaculture has been increasing due to the recent expansion of caviar luxury consumers that paved a way for the production of sturgeon hybrids over past decades (Bronzi et al., 1999). In aquaculture, hybrids are formed to increase fitness, which is manifested by the improved physiological and genetic properties compared to parental species (Bartley et al., 2001; Liu et al., 2017). However, some authors found no significant difference in the fitness traits of some fishes (Van der Sluijs et al., 2008). The sturgeon hybrids are also produced in hatcheries to shorten the sexual maturity of the late maturing species by crossing with faster maturing species like sterlet since the matured purebreds are unavailable in sufficient numbers during breeding seasons.

Fertilization and hatching

In this study, we found that the reproductive features like fertilization and hatching rates were significantly higher in S×S purebred (85.6±4.3% and 73.3±5.7% respectively). Both the hybrids displayed intermediate values of fertilization rate and hatching rate. The lowest values of fertilization rate and hatching rate were recorded in St×St purebred (Fig. 1). Chebanov and Galich (2011) documented in their sturgeon breeding manual that sterlet is generally more susceptible to incubation and hatching. The reproductive indicators are considered to be complementary indicators, while growth is probably one of the most driving and desirable indicators in aquaculture (Bartley et al., 2001).

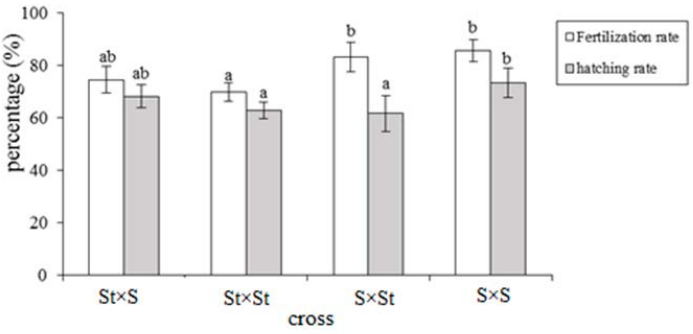

Figure 1. *Observed values of fertilization rates and hatching rates among the analyzed hybrid and purebred families of Siberian sturgeon and Sterlet. Columns with the same alphabetic superscript did not differ significantly at P < 0.05.*

Mean body weight and cumulative survival

We investigated growth performances of the purebreds and crossbreds in intensive culture systems at periodic intervals by assessing mean body weight and cumulative survival rate. The highest recorded mean body weight in St×S hybrid was 557.54±179.7 on 862 dph. This growth trend remained the same in most of the assessment periods from 73 dph to 862 dph (Fig. 2). However, the reciprocal hybrid group S×St showed an intermediate growth rate between their parental species. These results indicated greater growth performance of the St×S hybrid. The lowest mean body weights were recorded in sterlet purebred. The growth trend remained the same irrespective of solitary and communal rearing periods from 73 dph to 862 dph. Our findings are in agreement with the results from other studies where hybrids grew faster than the purebreds in sturgeons and other fishes too (Barulin et al., 2008; Su et al., 2013; Liu et al., 2017). In addition, the significance of heterosis for growth rate and other traits such as survival and disease resistance among crosses of wild and domesticated common carp (*Cyprinus carpio*) have also been well documented which parallels our current study (Hulata, 1995; Liu et al., 2017). Coming to the survival trait, cumulative survival rate was significantly higher in S×S purebred among all the assessment periods. The hybrids showed intermediate cumulative survival rate between the purebreds. The St×St purebred displayed the lowest cumulative survival in all the assessment periods (Fig. 3). This can be attributed by several combined forces. Chikhatchev et al. (1981) reported around 30% mortality at the time of switching to active feeding in bester and hybrid between beluga and stellate sturgeon (*A. stellatus*) when compared to their parental species. Based on the performance results we obtained, the St×S hybrid can be potentially used for aquaculture practices along with Siberian sturgeon purebred. However, the mean body weight and cumulative survival observed was comparatively less from the previous studies since the groups were reared in confined rearing system. This can be caused by several factors like unfavorable rearing conditions, pooled stocking and environmental fluctuations, which might have hampered the overall fitness of the fishes.

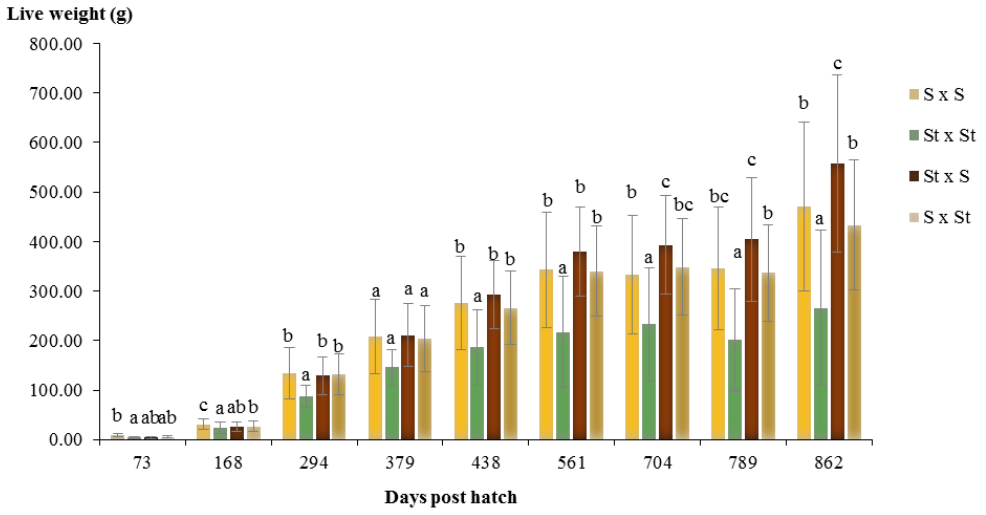

Figure 2. *Observed values of growth rate among the analyzed hybrid and purebred families of Siberian sturgeon and Sterlet. Columns with the same alphabetic superscript did not differ significantly at P < 0.05.*

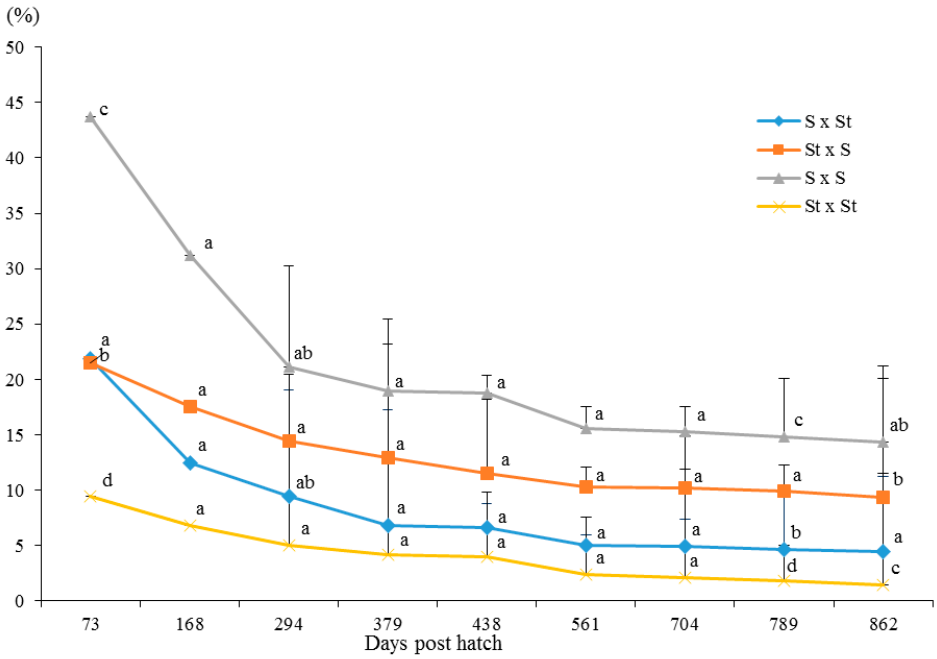

Figure 3. *Observed values of the cumulative survival rate among the analyzed hybrid and purebred families of Siberian sturgeon and Sterlet. Marks with the same alphabetic superscript did not differ significantly at P < 0.05.*

Heterosis of body weight and survival

The St×S hybrid displayed positive mid-parent heterosis for both mean body weight and cumulative survival traits in this study (Fig. 4). The estimates of heterosis for the weight traits were positive for both the hybrids (44.95% for St×S and 13.26% for S×St). Many authors reported similar results in interspecific hybrids of two cat fishes (*Clarias macrocephalus* and *Clarias gariepinus*), different strains of carp species like common carp (*Cyprinus carpio*) and Rohu carp (*Labeo rohita*) (Gjerde et al., 2002; Nielsen et al., 2010; Koolboon et al., 2014; Liu et al., 2017). However, the heterosis effect was comparatively low for mean body weight and negative for cumulative survival in S×St hybrid. This difference in performance can be possibly attributed by the mothering ability of the Siberian sturgeon in hybridization matrix which has been extensively studied in other species (Ibanez-Escriche et al., 2014).

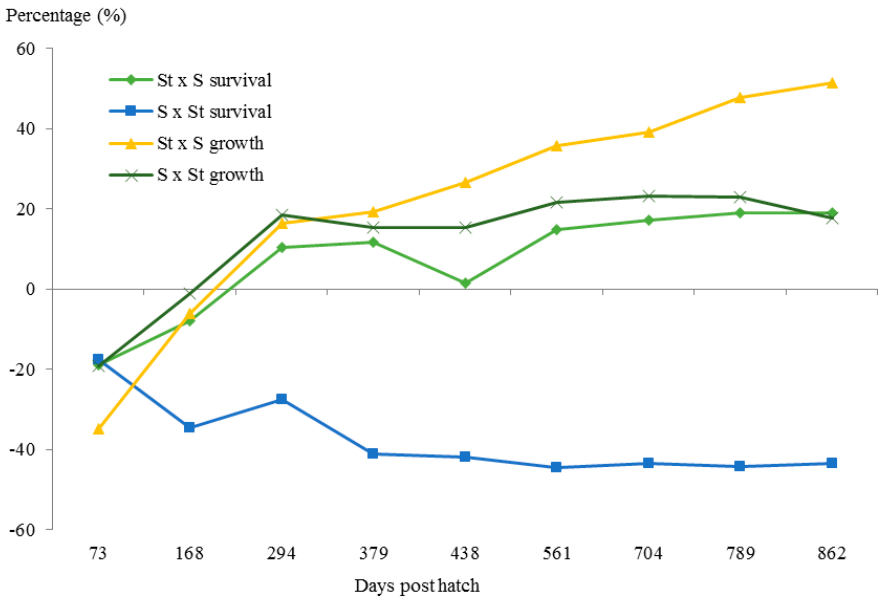

Figure 4. *Estimated values of heterosis among the analyzed hybrid families for growth and survival traits of Siberian sturgeon and Sterlet.*

Molecular analyses

The observed heterozygosity (Tab. 2) was found significantly highest in St × S hybrid (0.75) and least in St × St purebred (0.57). The mean number of alleles were significantly higher in both the hybrid families compared to sterlet purebred (Fig. 5). The St×St purebred and St×S hybrid family displayed a significant difference in the mean number of alleles. S×S purebred did not show any significant difference in the mean number of alleles as compared to hybrid families as well as St×St purebred. The genotypes data obtained from 6 microsatellite loci were run in a FCA test. The FCA grouped the purebred crosses in distinct clusters without any overlap. Both the hybrid clusters displayed intermediate position in between sterlet and Siberian sturgeon purebred clusters with very little overlap between each other (Fig. 6).

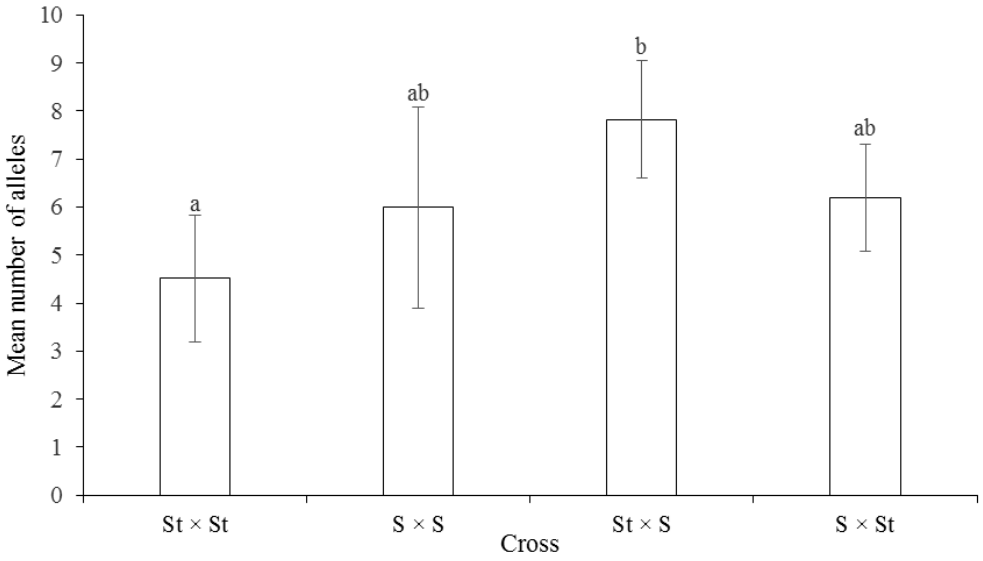

***Figure 5**. Observed values of mean number of alleles among the analyzed hybrid and purebred families of Siberian sturgeon and Sterlet. Columns with the same alphabetic superscript did not differ significantly at P < 0.05.*

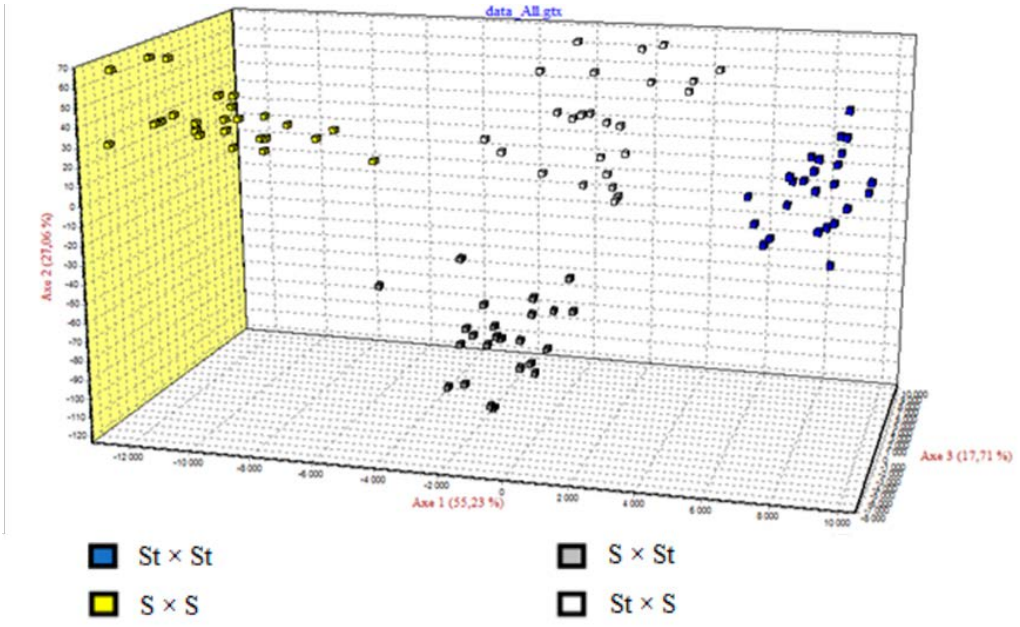

***Figure 6.** Factorial correspondence analysis (FCA) based on six microsatellite loci visualize genetic relationships among purebred and hybrid crosses. Sterlet purebred (blue squares), Siberian sturgeon purebred (Yellow squares), Siberian sturgeon × Sterlet (grey squares) and Sterlet × Siberian sturgeon (White squares).*

***Table 2**. Summary statistics of the genetic variation among the purebred and hybrid crosses of Siberian sturgeon and Sterlet. N_a = mean number of alleles; H_e = expected heterozygosity; H_0 = observed heterozygosity. * shows the group with significant difference at P < 0.05*

Cross	Loci typed	N_a	H_e	H_0
S × S	6	5.99±2.09	0.7317±0.0368	0.6818±0.0243
St × St	6	4.51±1.32*	0.6107±0.0414	0.5732±0.0387*
S × St	6	6.19±1.11	0.7658±0.0366	0.6893±0.0322
St × S	6	7.82±1.22*	0.7932±0.0285	0.7523±0.0351*

Interspecific hybridization may lead to increase in genetic polymorphism and heterozygosity levels of hybrid individuals (Bartley et al., 2001). This increase in the heterozygosity can add up to the improved growth and other features related to the fitness of hybrids (Leary et al., 1983; Reddy, 1999). The hybrid crosses had a higher mean number of alleles compare to sterlet purebred (since sterlet is recent diploid species with disomic inheritance) among the screened microsatellite loci. Siberian sturgeon purebred did not display a significant difference in the mean number of alleles compare to hybrid crosses. Interspecific hybridization probably served as the source of genetic variation in hybrid families compared to sterlet purebred. The Siberian sturgeon belongs to recent tetraploid group with tetrasomic inheritance, which historically should have more number of alleles. Higher mean number of alleles might have provided higher fitness to these hybrid crosses compared to the sterlet in terms of growth trait. However, we should also note that a wide array of physiological, biological and environmental components have a significant impact on fitness traits, aside from genetic components. Our results show that the produced interspecific hybrids can have considerable potential for aquaculture practices and selective breeding programs, especially to exploit their heterosis for mean body weight and cumulative survival. However, the development of hybrids should be done with caution as hybrids could alter the genetic integrity of the native species. In this current study, it can be assumed that the produced hybrids should be sterile since their parental species are originating from different ploidy level (Siberian sturgeon with 229-240 chromosomes and sterlet with 120 chromosomes). However, recently, Vasil'ev et al. (2014) confirmed fertility male hybrids of sterlet × Kaluga (*H. dauricus*) and Linhartová et al. (2017) found that males of sturgeon interspecific hybrids (produced between species originating from different ploidies) with their limited development of testes can be potentially fertile. The widespread introgression through restocking programs can ultimately lead to genomic extinction. The farmed escapees can be a major threat that needs critical measures to combat the biodiversity loss through outbreeding depression.

The cumulative survival and the mean body weight of interspecific hybrids and purebreds recorded in our experiment were comparatively lower than those reported in previous inferences. The lower performance of the fish crosses could be apparently attributed to suboptimal rearing environment. Similarly, fitness-related traits were influenced by unfavorable rearing conditions. We therefore infer that differences found in mean body weight and cumulative survival of purebreds and hybrids are of genetic origin solely. To the best of our knowledge, this is the first time to analyse and draw inferences of the effect of interspecific hybridization (species originating from different ploidies) on sturgeon fitness-related traits. Hence, the conception of this study to conventional sturgeon aquaculture should be done carefully and further studies are needed to elucidate links existing between hybridization and fitness-related traits.

4. Conclusion

Overall, we found that one of the inter-specific hybrids displayed the highest values of mean body weight compared to purebreds and its reciprocal hybrid in intensive culture system. Therefore, it suggests that the position of the individual species in a hybridization matrix is also one among the key elements in aquaculture production strategies when hybrids are produced to exploit the heterosis effect in aquaculture.

Acknowledgements

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CHAPTER 6

GENERAL DISCUSSION

Intraspecific hybridization

Thoughts on the importance and role of hybridization in evolution have changed over time. Some considered hybrids the 'raw materials' of evolution and a creative source of functional novelty (Arnold, 1997; Rieseberg and Wendel, 1993; Seehausen, 2004), while others argued hybridization was an evolutionary dead-end (Mayr, 1963). Unlike interspecific hybridization, intraspecific hybridization often produces fertile individuals; however, the fitness of offspring can vary when compared to their parental populations (Edmands et al., 1999, 2002, 2007; Frankham et al., 2002).

The first studies (Chapter 2 and 3) were focused on intraspecific hybridization and performances of intraspecific hybrids between Volga and Danube sterlet in terms of average weight, cumulative survival and critical swimming speeds were examined along with the estimation of genetic diversity. The Danube and the Volga rivers, which flow into the Black Sea and the Caspian Sea, have developed a complete genetic isolation in the Pliocene which is 5 million years ago (Esin et al., 2016). These two rivers are the two largest river systems in Europe with varying water flow regimes in their ranges (Shivaramu et al., 2019a; Chapter 3). The profound effects of hybrid vigor were recorded in terms of average weight and cumulative survival of produced hybrids whereas critical swimming speeds were not significantly affected. Intraspecific hybridization may lead to greater genetic diversity, increased fitness and greater adaptation to local environments (DeWet et al., 1983) indicated in this study can be noteworthy. Our study shows that produced intraspecific hybrids can be used in aquaculture over pure sterlet from Danube and Volga populations in order to exploit the genetic worth of two populations through heterosis on fitness-related traits. On the other hand, farm escapees from aquaculture to natural waters could back cross with the original parent population and consequently lead to deleterious effect on original net population genetic site. Due to the growth properties, hybrids could be more prominent in food competition and can access to limited food resources easily in the wild. However there may be cases where intraspecific hybridization may lead to rapid outbreeding depression which might be possible when the genetic divergence is more at the molecular level. Therefore populations that are genetically more similar at the molecular level and have shared a similar adaptive traits are found to be good for intraspecific hybridization (Allendorf and Luikart, 2009).

Furthermore, in certain cases the added genetic divergence between parental populations can lead to a mix of outbreeding depression and heterosis in different generations. For example, Edmands (1999) found that genetic divergence between distinct parental populations was correlated with hybrid vigour in the F1 generation and hybrid breakdown in the F2 and backcross generations. This delayed manifestation of outbreeding depression is the result of the return of deleterious homozygotic combinations between alleles that were present in the parental populations (Templeton, 1986; Edmands, 2007).

Interspecific hybridization

Interspecific hybrids are purposefully produced to increase the productivity of the aquaculture strains or for the sporting purpose (Lutz, 1997). Despite being interspecific hybridization often the tool used in aquaculture, its benefits, as well as its shortcomings, are often overlooked (Bartley et al., 2001). Due to the unusual configuration of their genome, sturgeons, all of which are polyploid, can cross more easily than other fish (Birstein et al., 1997) and this concern species with the same and/or different ploidy levels. Sturgeon evolution via genome duplication and functional reduction which is probably still an ongoing process.

In the next studies (Shivaramu et al., 2019b; Chapter 4), we focused on interspecific hybridization, fitness-related traits of the hybrids of Russian sturgeon and Siberian sturgeon and, compared with their parent species. Both Russian sturgeon and Siberian sturgeon are evolutionary octaploids or recent tetraploids. Also, these sturgeons are extensively reared along with other sturgeons in Europe for aquaculture purposes. As mentioned before, both the hybrids (reciprocal) performed better than both the parent species. One of the hybrid (Russian sturgeon × Siberian sturgeon) recorded best growth rate and its reciprocal hybrid recorded the best survival rate (Siberian sturgeon × Russian sturgeon). The genetic polymorphism was significantly higher in this particular hybrid compared to purebreds. This hybrid could also find its aquaculture application, although its fertility or sterility has not yet been confirmed experimentally (Arefjev, 1997). However, it can be assumed that both types of monitored hybrids should be fertile (Hochleithner, 2004; Chebanov and Galich, 2011), since it belongs to the group with the same ploidy level with 240–270 chromosomes (Birstein et al., 1993). The use of interspecific sturgeon hybrids in commercial sturgeon breeding proved to be, in many cases, more economically effective than the rearing of pure species (Burtsev, 1983). Likewise, in the third study, we conducted the performance test of the hybrids between sterlet and Siberian sturgeon in comparison to their purebreds. The sterlet is a recent diploid and Siberian sturgeon is a recent tetraploid sturgeon species. We were curious to find some significant impact of crossing different ploidies on the phenotypic traits due to the negative interaction which could occurred in the parental genes found at different loci of the intergeneric hybrid genome (Sheridan, 1981). Surprisingly, one of the hybrid (Sterlet × Siberian sturgeon) displayed highest growth rate and heterozygosity levels compared to purebreds and its reciprocal hybrid. Induction of allopolyploids over autopolyploids via hybridization can have set of advantages on increasing physiological fitness (Wang et al., 2006). It was also found that increasing number of alleles in polyploids could prevent loss of fitness via covering up of deleterious recessive mutations (Gu et al., 2003) which parallels with our experiment. Therefore, we conclude that it is always pivotal to consider the position of species or population in a hybridization matrix since its reciprocal cross can end up in contrary performance. Added to that, another major question related to sturgeon interspecific hybrids is their fertility or sterility. Generally in fish interspecific hybrids of distantly related parental species are usually sterile (Piferrer et al., 2009). But recently, Vasil'ev et al. (2014) confirmed fertilization ability of male hybrids of sterlet (*A. ruthenus*) × Kaluga (*H. dauricus*). Sterlet with 120 chromosomes and Kaluga with about 250–270 chromosomes are the species with varying ploidy. Likewise, Linhartová et al. (2017) found limited fertility of the male hybrids of sterlet and Siberian sturgeon with varying ploidy levels.

Present scenario of sturgeon hybrids in aquaculture systems as well as in open waters

Generally hybrids are formed in aquaculture to increase fitness, which is manifested by the improvement of many physiological and utility properties in comparison with parental species (Bartley et al., 2001; Kocour et al., 2011). Hybridization may lead to an increase in polymorphism and hence increases the heterozygosity of hybrid individuals (Bartley et al., 2001). Moreover, the increase of heterozygosity itself is considered to be the major contribution of interspecific hybridization for growth indicators and other features related to fitness of individuals (Leary et al., 1983; Scheerer and Thorgaard, 1983; Reddy, 1999). However, some fish hybrids recorded no significant difference in traceability of survival, growth and feed conversion ratio (Siegwarth and Summerfelt, 1993; Van der Sluijs et al., 2008).

Additionally, the production of hybrids brings not only positive but also negative ones. The main disadvantage of hybrids in aquaculture is that they can escape from farms (Maury-Brachet et al., 2008) and can be genetically contaminating the wild population (Ludwig et al., 2009; Reinartz et al., 2011). This fact is very important for sturgeons seriously, especially with regard to the possible fertility of different types of hybrids (Vasil'ev et al., 2014; Linhartová et al., 2017), which can make the problem even more pronounced. And the types of hybrids that are being watched may pose such a threat. Their escape from aquaculture to natural waters could occur to their back-crossing with the original parent species and consequently to the extrusion of the original net population of the site. Due to the growth properties, hybrids could be more prominent in food competition and would easily for access to limited food resources. In addition, it can be assumed that this situation is already taking place in natural conditions, especially with regard to the findings of the haplotypes of Siberian sturgeon in the Volga River Basin, where they regularly also breeds sturgeon (Jenneckens et al., 2000). Likewise, Reinartz et al. (2011) documented genetic contamination of the sterlet population in Danube river by partial or complete genotypes originated from sterlet of Volga river. Henceforth, attention must be given in order to ensure the genetic integrity of the wild species and populations. Initiation of multidisplinary project including longterm multitasks involving genetic, physiological and ecological monitoring can eventually save the sturgeon species (Friedrich, 2018).

Scope of molecular markers in genetic investigations of sturgeon hybrids

The use of modern biotechnology in aquaculture and fisheries is comparatively new when compared to animal science. However, biotechnology has employed a significant role aquaculture, genetic improvement and conservation of several fish species, especially the salmonids and sturgeons (Shah et al., 2017).

The present scenario with sturgeons genome is complicated because they can create autopolyploids in every generation and can hybridize interspecifically or intergenerically in the groups of species of the same ploidy level, as well as between groups of species of different ploidy levels, whilst almost all autopolyploids and some hybrids can be morphologically indistinguishable from pure species. Molecular approaches are the essential tool for not only hybrid identification but also estimating genetic diversity among groups and undertake population genetic analysis. A wide array of molecular markers like mitochondrial DNA (mtDNA) (Jenneckens et al., 2000), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphic (AFLP) (Congiu et al., 2001), microsatellites (Dudu et al., 2011; Zhang et al., 2013) and nuclear DNA markers (Boscari et al., 2014; Boscari et al., 2017; Havelka et al., 2017) have been successfully used in species identification, genetic divergences studies and assess genetic polymorphism among sturgeon hybrids. We used microsatellite for estimating the genetic polymorphism among the hybrids and purebred due to their advantages (high polymorphism, high power of discrimination, codominant Mendelian inheritance, etc.). In all the three experiments (Chapter2, 3, 4 and 5), the genetic diversity and level of heterozygosity was found to be significantly higher in at least one hybrid when compared to their purebred species or population. Our results can be governed by transmission of genes from one species/ population into another through the interspecific/intraspecific hybridization provide a substantial advantage in terms of increasing adaptive genetic variation (Grant and Grant, 1992). In evolutionary terms, interspecific hybridization can contribute to preserve genetic diversity. This may lead to new mechanisms adaptation enabling greater survival rate and increasing the fitness of the resulting hybrids (Arnold, 1997). Polyploidization associated with interspecific hybridization probably gave evolutionary advantage to sturgeons and contributed

to their survival until today (Birstein et al., 1997). Additionally, interspecific hybridization is ongoing with sturgeon process (e.g. Birstein et al., 1997; Flajšhans and Vajcová 2000; Ludwig et al., 2009; Dudu et al., 2011) which may theoretically result in increased levels of fitness in the resulting hybrids. Nevertheless, the phenotypic variance of a quantitative trait is always regulated by genetic variance, environmental variance and genetic-environmental interaction variance (Tave, 1993; Shah et al., 2017).

Recommendation for future research directions and implications for sturgeon fisheries management

We would suggest to channelize the research into following multiple lanes in order to have greater visibility of the different types of hybridization associated detrimental effects on wild populations. Additionally, the sturgeons are rapidly decreasing in their numbers due to various reasons and hence much of research (like genetic, population dynamics, physiological, and ecological studies) should be directed in the lane for saving them.

a) Hybridization: Hybridization experiments among different species and stocks should be conducted in order to investigate the success rate of reciprocal crossing and the Fl hybrids fertility fate of both the male and female's position in hybridization matrix. This should be repeated for a wide number of populations to verify the results of gamete compatibilities among different populations for exploitation of hybrid vigor for aquaculture programs. The same data can be used for conservation based project while selecting non-native specimens for release programs.
b) Spawning activities: Records of the species spawning in the wild should be undertaken along with the environmental surveys to compare the conditions under which interbreeding does or does not occur. These observations may also be used in combination with other methods, e.g. mtDNA, microsatellite loci, nuclear DNA, to assess aspects of mating behavior where exactly the hybridization is occurring in natural waters.
c) Population dynamics and autoecology studies: More studies on the behavior of different sturgeon species in different ecological niches should be extensively studied. By doing so, the biological and environmental factors which are driving the population growth and age can be revealed which can serve as potent information for future restoration projects.
d) Performance testing: Studies to compare the performance of hybrids to their parent species should be undertaken. This can include checking the set of physiological traits to evaluate the effects of varying experimental environments, e.g. Body weight, survival, meristic characters, swimming abilities of the hybrids and parent species.
e) Investigation of wild population in natural waters: Studying wild populations of hybrids are pivotal in order to identify parent species and hybrids using genetic markers, detect niche overlaps with extensive feeding and varied diet studies, compare performances like growth, condition factors, mortality and fecundity. Once all of the above mentioned areas are extensively studied and identified, the possible ameliorative management options are achievable to combat the hybridization-associated problems in the natural waters.
f) Restoration: There is an urgent act of restoration of the migratory routes, breeding sites and spawning habitats in the natural ecosystem in order to reduce the development of hybrids. Construction of dams can lead to the genetic separation between upstream and downstream stocks in a long run, henceforth the population substructuring should be combated by building the fish passes. The combination of *in-situ* and *ex-*

situ conservation measures can serve the restoration purpose of sturgeon stocks in wild. *In-situ* measures target the preservation of the complete sturgeon life cycle as well as protection of its genetic diversity in its natural habitat. The *ex-situ* measures targets establishment of captive living gene banks by rearing juveniles in an off-site hatchery and releasing them into the wild at a later stage. This process will stabilize and strengthens the wild populations which eventually save the wild stock from extinction.

g) Restocking programs: Restocking became most used tool to sustain the endangered sturgeon populations in the last decade. These release programs can eventually lead to outbreeding between the wild and hatchery reared/ non-native stocks which can be a potential threat for native gene pool. Outbreeding often increases the genetic distance between the wild stock and released fishes and leads to subsequent biodiversity loss. Henceforth, population genetic analysis of the broodstock used for artificial propogation in order to produce specimens for restocking purposes should be conducted and the specimens which are genetically close (autochthonous) to the wild stock should be used as broodstock for production of specimens for intended release programmes. The use of non-native stocks (allochthonous) for restocking purposes should be ceased since it can lead to genomic extinction of the native stock in the long term.
h) Public awareness: The dissemination of the present scenario of sturgeon's stock and consequences of the ongoing pouching should be given to the local fishermen. Also, the increased awareness should be raised in stakeholders, hatchery managers and general public to combat the accidental or intentional release of the hatchery raised hybrids and, farmed escapees from hatcheries and floodplains into the open waters.

Conclusion

The experiments were focused on tracking fitness-related features interspecific and intraspecific hybrids of sturgeons and their comparison with the characteristics of pure parental species. From the obtained results show that interspecific and intraspecific hybrids can have considerable potential for aquaculture breeds, especially with a view to increasing their survival rate and further improving growth properties. On the other hand, it was, in this work, confirmed that improvements in fitness-related properties of sturgeon hybrids cannot be taken all alone. It is always necessary to take into account not only the production of hybrid but also the position of individual species and population in a hybridization matrix. With respect to the growth properties of the monitored hybrids, these may hybrids in the event of their release into natural waters, present a significant competitive risk for wild populations of pure sturgeon species. The results can be utilized to exploit the heterosis in aquaculture purposes for the production of quality caviar and fillets. To our knowledge, this study brings first observation of the effect of different types of hybridization on sturgeon fitness-related traits. Due to the easy hybridization of sturgeons in aquaculture conditions, as well as in natural waters, presents a considerable potential for this issue for further scientific research aimed at using different types of hybrids in aquaculture, as well as their impact on pure species in natural water. Also, any generalization of the obtained results of this thesis to standardized sturgeon aquaculture should be done with precaution.

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Shivaramu, S., Santo, C.E., Kašpar, V., Bierbach, D., Gessner, J., Rodina, M., Gela, D., Flajšhans, M., Wuertz, S., 2019a. Critical swimming speed of sterlet (*Acipenser ruthenus*): Does intraspecific hybridization affect swimming performance?. J. Appl. Ichthyol. 35(1), 217–225.

Shivaramu, S., Vuong, D.T., Havelka, M., Šachlová, H., Lebeda, I., Kašpar, V., Flajšhans, M., 2019b. Influence of interspecific hybridization on fitness-related traits in Siberian sturgeon and Russian sturgeon. Czech J. Anim. Sci. 64(2), 78–88.

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ENGLISH SUMMARY

Hybridization of sturgeons

Sahana Shivaramu

Sturgeons (order Acipenseriformes) are supposed to be one of the oldest groups of vertebrates still living on our planet. Their evolutionary age, the variation in their diadromous migration, life history, highly profitable black caviar market and the wide public attention due to their near extinction status make sturgeons and paddlefishes one of the most valued and interesting group of fishes. Also, the demand for captive breeding and aquaculture of sturgeons to produce caviar has increased, as wild stocks have become depleted or closed for legal harvest. Moreover, their polyploid ancestry makes them very susceptible for hybridization phenomena. These interspecific and intergeneric hybrids have been already described in nature as well as in captivity. Nevertheless, the fitness of sturgeon hybrids between populations, between species originating from same ploidy level and between species bearing different ploidy level in comparison with pure species has not been extensively studied yet even though it can have significant impact on sturgeon aquaculture production.

The present study focused on different types of hybridization and their impacts on compare reproductive features (fertilization rate and hatching rate) and, fitness-related traits such as growth rate, cumulative survival rate, critical swimming speeds and specific growth rate in sturgeon, as well as level of heterozygosity among sturgeon hybrids and purebreds using set of microsatellite loci.

Production of intraspecific hybrids between different populations is one of the effective strategy in aquaculture in order to obtain better offspring. Additionally, it is pivotal to monitor the genetic condition of the stocks before producing hybrids. The population genetic analysis of Danube and Volga population of sterlet showed that both the populations are genetically divergent. Additionally it revealed the gene diversity and level of heterozygosity was high in Danube population compared to Volga population. The intraspecific hybrids produced between Danube and Volga sterlet were subjected to performance tests. The significant effects of hybrid vigor were recorded in terms of average weight and cumulative survival of produced hybrids as well as reproductive features like fertilization rate and hatching rate. The results from critical swimming speed in changing temperatures assessments revealed no significant differences among the purebred and hybrid crosses which indicated no effect of intraspecific hybridization on critical swimming speed in juvenile stage. The mean number of alleles and level of heterozygosity was significantly higher in one of the reciprocal hybrid. This suggests us that it is not just production of hybrids but it is always necessary to take the position of the individual population in a hybridization matrix.

Detailed investigation of fitness-related traits (growth rate and cumulative survival) in purebreds of Russian sturgeon and Siberian sturgeon and their interspecific hybrids were undertaken. Russian sturgeon and Siberian sturgeon belongs to same ploidy level (Recent tetraploids). Our results revealed both the hybrids (reciprocal) performed better than both the parent species in one of the fitness-related traits examined. One of the hybrid (Russian sturgeon × Siberian sturgeon) recorded best growth rate and its reciprocal hybrid recorded the best survival rate (Siberian sturgeon × Russian sturgeon). Although no significant difference was observed in the heterozygosity levels among the purebred and hybrid crosses, the mean number of alleles was significantly higher in Siberian sturgeon × Russian sturgeon hybrid.

Experimental interbreeding of sturgeon species (sterlet and Siberian sturgeon) bearing different ploidy levels and chromosome numbers, followed by molecular investigation

of obtained progeny and examination of fitness-related traits revealed one of the hybrid performed better than purebreds. Additionally the heterozygosity levels were significantly high in one of the hybrid. The general rule of the negative interaction which could occur between the parental genes with different ploidy and its apparent impact on the intergeneric hybrid genome was not supported in our study.

Therefore, given the evidences and observations provided by this study in controlled conditions, the above mentioned intraspecific and interspecific hybrids could be suggested for intensive aquaculture and might be also suitable for polyculture stocks. Finally, breeding of sturgeon hybrids might be seen as an effective and promising alternative to pure species in sturgeon aquaculture, but suitable kind of hybrid must be selected in order to exploit the maximum level of heterosis. With respect to the growth properties of the monitored hybrids, these hybrids may present a significant competitive risk for wild populations of pure sturgeon species, in the event of their release into wild. Added to that, due to the easy hybridization of sturgeons in aquaculture conditions, as well as in natural waters, presents a considerable potential for this issue for further scientific research aimed at using different types of hybrids in aquaculture, as well as their impact on pure species in natural waters.

CZECH SUMMARY

Hybridizace jeseterů

Sahana Shivaramu

Jeseteři (řád Acipenseriformes) jsou bráni jako jednou z nejstarších skupin obratlovců žijících na naší planetě. Jejich evoluční věk, variabilita, diadromní migrace, historie života, vysoce ziskový trh s černým kaviárem a široká pozornost veřejnosti díky jejich blízkému stavu vyhynutí činí z jeseterů a veslonosů jednu z nejcennějších a nejzajímavějších skupin ryb. Zvýšila se také poptávka po chovu a akvakultuře jeseterů k produkci kaviáru, protože počet divokých populací se snížil nebo byl ukončen legální odlov. Navíc je jejich polyploidní původ činí velmi citlivými na hybridizační jevy. Tito mezidruhoví a mezigenerační hybridi byli již popsáni v přírodě i v zajetí. Nicméně způsobilost hybridů jeseterů mezi populacemi, mezi druhy pocházejícími ze stejné úrovně ploidie a mezi druhy nesoucími různou úroveň ploidie ve srovnání s čistými druhy, nebyla dosud rozsáhle studována, i když může mít významný dopad na produkci jeseterů z akvakultury.

Tato studie se zaměřila na různé typy hybridizace a jejich dopady na porovnávané reprodukční rysy (oplozenost a líhnivost) a rysy související s fitness, jako je rychlost růstu, míra kumulativního přežití, kritické rychlosti plavání a specifická rychlost růstu jeseterů a jejich úroveň heterozygozity mezi čistými plemeny a hybridy pomocí souboru mikrosatelitních lokusů.

Produkce vnitrodruhových hybridů mezi různými populacemi je jednou z účinných strategií v akvakultuře pro zisk lepšího potomstva. Kromě toho je klíčové sledovat genetický stav populace před produkcí hybridů. Populační genetická analýza populace jesetera malého z Dunaje a Volhy ukázala, že obě populace jsou geneticky odlišné. Navíc se ukázalo, že genová diverzita a úroveň heterozygozity je v populaci Dunaje ve srovnání s populací Volhy vysoká. Intraspecifičtí hybridi vzniklí mezi Dunajskou a Volhovskou populací jesetera malého byli podrobeni užitkovým testům. Významné účinky hybridní zdatnosti byly zaznamenány vzhledem k průměrné hmotnosti a kumulativnímu přežití produkovaných hybridů, jakož i v reprodukčních vlastnostech, jako je míra oplozenosti a líhnovosti. Výsledky kritické rychlosti plavání při měnících se teplotách neprokázaly žádné významné rozdíly mezi čistými plemeny a hybridy, které navíc neprokázaly žádný účinek vnitrodruhové hybridizace na kritickou rychlost plavání v juvenilním stadiu. Průměrný počet alel a úroveň heterozygozity byla významně vyšší u jednoho z reciprokých hybridů. To naznačuje, že se nejedná pouze o produkci hybridů, ale vždy je nutné zaujmout pozici individuální populace v hybridizační matrici.

Bylo provedeno podrobné zkoumání vlastností souvisejících s kondicí (růstová rychlost a kumulativní přežití) u čistých plemen jesetera ruského a sibiřského a jejich mezidruhových hybridů. Naše výsledky ukázaly, že oba hybridi (i reciproční) byly lepší než oba mateřské druhy v jednom ze zkoumaných rysů. Jeden z hybridů (jeseter ruský × jeseter sibiřský) zaznamenal nejlepší tempo růstu a jeho vzájemný hybrid zaznamenal nejlepší míru přežití (jeseter sibiřský × jeseter ruský). Ačkoli nebyl pozorován významný rozdíl v hladinách heterozygotnosti mezi čistými plemeny a hybridy, průměrný počet alel byl významně vyšší u hybrida jesetera sibiřského × jesetera ruského.

Experimentální křížení druhů jeseterů (jesetera sibiřského a malého) s rozdílnými hladinami ploidií a počtem chromozomů, následované molekulárním ověřením získaných potomků a vyšetřením vlastností souvisejících s fitness odhalily, že jeden z hybridů byl lepší než čisté plemeno. Hladiny heterozygotnosti byly navíc významně vysoké u jednoho z hybridů. Obecné pravidlo negativní interakce, ke které mohlo dojít mezi rodičovskými geny s různou ploidií a jeho zjevným dopadem na mezigenerační hybridní genom, nebylo v naší studii podpořeno.

Vzhledem k důkazům a pozorováním, které tato studie poskytuje za kontrolovaných podmínek, by výše uvedení vnitrodruhoví a mezidruhoví hybridi mohli být navrženi pro intenzivní akvakulturu a mohli by být také vhodní pro polykulturní populace. Chov hybridů jeseterů by mohl být považován za účinnou a slibnou alternativu k čistým druhům v akvakultuře, ale musí být vybrán vhodný druh hybrida, aby bylo možné využít jeho maximální úroveň heterózy. Pokud jde o růstové vlastnosti sledovaných hybridů, mohou tito hybridi v případě jejich uvolnění do volné vody představovat významné konkurenční riziko pro divoké populace čistých druhů jeseterů. Kromě toho, vzhledem k snadné hybridizaci jeseterovitých ryb v akvakultuře, stejně tak i ve volných vodách, představuje tento problém značný potenciál pro další vědecký výzkum zaměřený na využívání různých typů hybridů v akvakultuře, jakož i jejich dopad na čistá plemena ve volné vodě.

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LIST OF PUBLICATIONS

Peer-reviewed journals with IF

Shivaramu, S., Santo, C.E., Kašpar, V., Bierbach, D., Gessner, J., Rodina, M., Gela, D., Flajšhans, M., Wuertz, S., 2019. Critical swimming speed of sterlet (*Acipenser ruthenus*): Does intraspecific hybridization affect swimming performance? Journal of Applied Ichthyology 35: 217–225 (IF 2018 = 0.877)

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 Journal of Animal Science 64: 78-88. (IF 2018 = 1.008)

Abstracts and conference proceedings

Shivaramu, S., Lebeda, I., Kašpar, V., Flajšhans, M., 2018. The influence of intraspecific hybridization on fitness traits in sterlet (*Acipenser ruthenus*). In Book of abstracts "Sustaining iconic diadromous fishes: The potential and pitfalls of cultivation", 17–20 June, 2018, Arendal, Norway.

Shivaramu, S., Vuong, D.T., Havelka, M., Šachlová, H., Lebeda, I., Flajšhans, M., 2017. Genetic parameters for growth and survival traits in sturgeon purebred and hybrid families. In Book of abstracts "Aquaculture Europe 2017", 17–20, October, 2017, Dubrovnik, Croatia.

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Shivaramu, S., Vuong, D.T., Havelka, M., Šachlova, H., Lebada, I., Kašpar, V., Flajšhans, M., 2017. Genetic parameters for growth and survival traits in sturgeon purebred and hybrid families. Aquaculture Europe 2017, Dubrovnik, 17–20 October, 2017, Dubrovnik, Croatia.	2017
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