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17. Management applications of genetic structure of anadromous sturgeon populations in the Lower Danube River (LDR), Romania

ONĂRĂ Dalia Florentina[1], HOLOSTENCO Daniela[1], SUCIU Radu[1]

[1] - Danube Delta National Institute for Research and Development: 165 Babadag street, Tulcea - 820112, Romania

Address of author responsible for correspondence: **ONĂRĂ Dalia Florentina** – Danube Delta National Institute for Research and Development: 165 Babadag street, Tulcea - 820112, Romania; e-mail: dalia@indd.tim.ro

ABSTRACT. During the last decades, the over-exploitation of sturgeon stocks for caviar production simultaneously with severe habitat deteriorations has led to drastic declines in the natural populations of the Danube River. As a result of (i) decrease of sturgeon catches from 37.5 tons in year 2002 to 11.8 tons in year 2005, (ii) disrupted age class structure of sturgeon adult cohorts in years 2003 and 2004, and (iii) lack or low recruitment in the period 2001 – 2004, in 2005 the Romanian Government started the Supportive Stocking Program of Lower Danube River with hatchery-produced young sturgeons in Romania. Subsequently, in 2006 the commercial sturgeon fishing in Romania was banned for a 10-year period. Genetic investigations were undertaken as an attempt to assess the genetic variability of the sturgeon brood fish, captured from the wild, used in two aquaculture facilities in Romania for obtaining juveniles for supportive stocking of LDR with young sturgeons produced by artificial propagation in year 2007. Our data indicate strong genetic diversity in case of stellate sturgeon and lack of diversity within the batch of beluga sturgeon brood fish captured in 2007, analyzed in the current study. Specific measures that could improve the management plan of sturgeon brood fish in the Romanian part of LDR in the light of recent FAO guidelines regarding the sturgeon hatchery practices and management for release were suggested.

Key words: sturgeons, management, artificial propagation, spawning, brood fish, Lower Danube River, Romania

INTRODUCTION

Sturgeons (Acipenseridae) and paddlefishes (Polyodontidae) are members of the order Acipenseriformes, a group of approximately 27 extant species distributed throughout North America and Eurasia that exist since 200 million years (from the Lower Jurassic) ([***3***]; [***5***]; [***6***]; [***7***]; [***8***]). Most of the sturgeon species are threatened or endangered (IUCN 2008). In this respect, Danube sturgeon species such as the great sturgeon or beluga (*Huso huso*), the Russian sturgeon (*Acipenser gueldenstaedtii*), the stellate or starry sturgeon or sevruga (*A. stellatus*), the freshwater sterlet (*A. ruthenus*), do not represent an exception.

These sturgeon species, considered relict fish species, are present in the ecosystems of the Black Sea, Caspian Sea and Azov Sea, due to shared inhabitancy of their ancestors, during the Pliocene, of the Paratethys (Sarmatic Sea) (**Fig. 1**) ([***4***]; [***12***]; [***39***]).

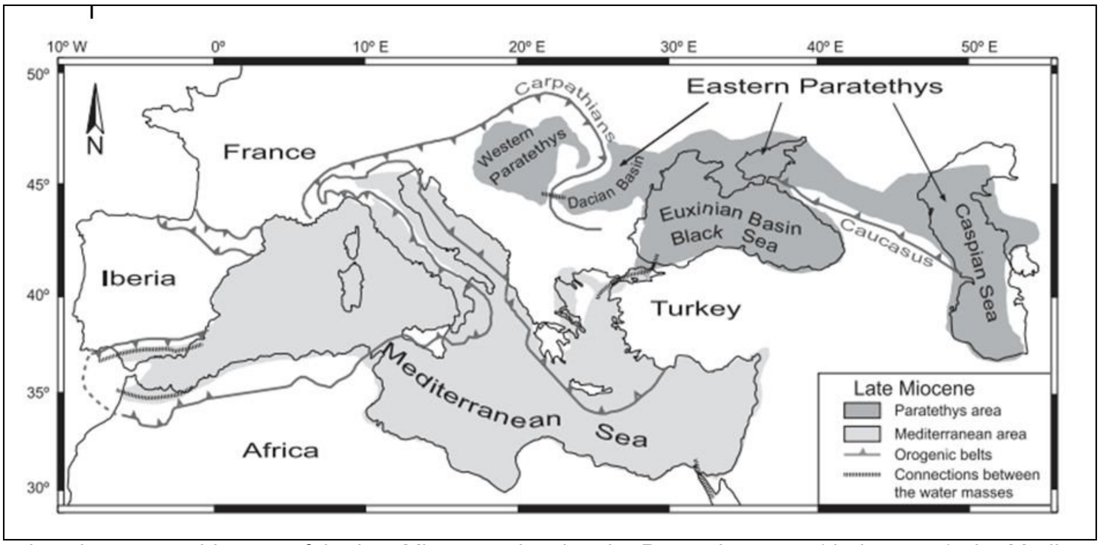

Fig. 1. Schematic paleogeographic map of the late Miocene, showing the Paratethys area (darker gray), the Mediterranean (lighter gray) and the present-day land contribution [***39***].

Most sturgeon species are anadromous meaning that even they spend the largest part of their lives in the marine environment, they need every several years to migrate into their home rivers to reproduce. Especially the winters races of beluga (*Huso huso*) and Russian sturgeon (*A. gueldenstaedtii*), were migrating from the Black Sea into the Danube, to the middle and sometimes even the upper Danube and larger tributaries ([***3***]; [***16***]).

The beluga sturgeon (*Huso huso*), the biggest freshwater fish, has the longest upstream migration, that historically was present in the middle Danube and even upstream, up to Bavaria ([***2***]; [***16***]). By damming the river at the Iron Gates I in 1974 (RKm 942) and

Iron Gates II in 1984 (RKm 863), reduced more than half of their migration way [***2***] and dramatically affected the natural spawning of this species. Therefore, the decrease of population recruitment resulted in extinction of the long-migratory races that occurred at the end of the XX^{th} century [***37***].

Two biological 'races' have been described for the beluga sturgeon: the vernal / spring and the autumn / winter race. The spawning sites are located in areas with gravel and crevices on the river bed, and with depths of 6 - 20 m. The males reach sexual maturity at 10 - 13 years and the females at 13 - 15 years, having a body length of about 2 m [***29***].

The Russian / Danube sturgeon (*A. gueldenstaedtii*) has a wide morphologic variability [***1***]. Thus, over 100 years ago, Antipa described three rostrum morphs (*typica*, *longirostris* and *acutirostris*) and two varieties, *armored form* that has the skin surface covered with small snowflake-like denticles and *naked form*. Despite the low number of Russian sturgeon that nowadays migrate for spawning in the Danube River, all these morphs and varieties were recently observed (**Fig. 2**) [***13***].

Fig. 2. Head ventral view of hatchery - obtained Russian sturgeon YOY lanched in the Danube River in 2003.
Note. From left, the rostrum morphs: longirostris, acutirostris and typica (© foto credit: R. Suciu) ([***1***]; [***13***]).

Russian sturgeon is usually 100 - 200 cm long and weighs about 20 - 30 kg; however, it may reach a maximum of 236 cm and 115 kg. In Danube basin, Russian sturgeon first matures at 11 - 13 years for males, and 12 - 16 years for females, with a generation length of 33 years. As all sturgeons, Russian sturgeon does not spawn every year, but once in 2 - 5 years, in April - June, in 8 - 20 m deep water, on gravel, sand or clay [***29***].

Stellate sturgeon (*Acipenser stellatus*) spans its life in the sea, coastal and estuarine zones, where it forages on clay sand bottoms, as well as intensively in middle and upper water layers. Spawning occurs in the main course of large and deep rivers, in strong-current habitats, on stone or gravel bottoms. In Danube it is also known to spawn on river thresholds, on sand or sandy clay ([***1***]; [***31***]).

Stellate sturgeon first matures at 5 - 6 years for males, and 7 - 10 years for females, spawning every 3 - 4 years. Literature data indicates as spawning period April - May [***29***] and May - June at temperatures between 17°C and 23°C [***32***] for Danube stellate sturgeon and April - September for Caspian stellate sturgeon ([***27***]; [***31***]). Recent evidences indicate the latest stellate sturgeon spawning event in the end of June (in year 2011), on Borcea Branch of the Danube River, in water temperature of 24°C - 25°C [***18***].

During the last decades, the over-exploitation of sturgeon stocks for caviar production simultaneously with severe habitat deteriorations has led to drastic declines in the natural populations of the Danube River. On the Lower Danube River (LDR), in Romania, catches of beluga sturgeons decreased from an average of 250 metric tonnes/year during 1950 - 1965 to only 8.4 tonnes in year 2005 [***37***]. Hereupon, IUCN in 1997 listed all commercially used sturgeon species world-wide in Annex II of the CITES regulations, and it was required internationally agreed quotas for trade in order to promote protection [***9***].

Since 2003, CITES issued export quotas only for the caviar originated from the sturgeons included in the on-line data base of catches in order to encourage fishermen to respect the new legislation. A strong indicator of the detrimental effects of fishing was the lack of adult sturgeons migrating in the river to spawn [***34***]. As a result, in Romania in 2006 the commercial sturgeon fishing was banned for a 10-year period by the joint Order of Minister of Environment and Water Management and Minister of Agriculture, Forests and Rural Development on conservation of wild sturgeon populations and development of sturgeon aquaculture in Romania (Order No. 262/330/2006) [***42***].

Hatchery supplementation program is one of the approaches used for restoring of endangered sturgeon stocks. Artificial propagation procedures have been developed for Danube sturgeon species and there are several facilities along the Danube involved in the program ([***32***]; [***37***]).

To preserve the genetic diversity, a minimum effective number (N_e) of 100 brood fish / generation interval should be used for artificial propagation, (N_e) 14 in stellate sturgeon, 12 in Russian / Danube sturgeon and 7 in beluga sturgeon.

In order to achieve N_e = 100 and an inbreeding rate / generation ΔF max = 0,50 % ([***23***]; [***30***]), the hatchery-obtained progeny for the stocking were produced by artificial propagation of an effective number of brood fish captured alive from the wild, as foreseen in Annex A to the Regional Strategy [***41***].

For supportive stocking of Lower Danube with young sturgeons produced by artificial propagation using the maximum number of brood fish captured from the wild, eggs from each female were divided into a number of batches according to the number of males available and fertilized separately with sperm from each male. The offspring were reared in aquaculture to a TL ≥ 10 cm and tagged prior stocking using coded wire tags (CWT) by Northwest Marine Technologies as presented elsewhere ([***30***]; [***37***]).

The current study takes into discussion the case of sturgeon brood fish captured in Lower Danube River (LDR) in year 2007 and shared by six Romanian aquaculture facilities, for artificial propagation purposes, to obtain both juveniles for the Romanian Supportive Stocking Programme and offspring for aquaculture.

The aim of this study was to assess the genetic variability of the sturgeon brood fish, captured from the wild, used in two aquaculture facilities in Romania for obtaining juveniles for supportive stocking of LDR with young sturgeons produced by artificial propagation in year 2007 and to suggest specific measures that could improve the management plan of sturgeon brood fish in the Romanian part of LDR in the light of recent FAO guidelines regarding the sturgeon hatchery practices and management for release [***11***].

MATERIALS AND METHODS

Sturgeon brood fish data

Special permits were issued by Romanian National Agency for Fisheries and Aquaculture (NAFA) for capturing alive the sturgeon brood fish needed for artificial propagation. Legally captured adult sturgeons were individually marked by NAFA fishery inspectors using PIT (Passive Integrated Transponder) tags and subsequently released back to the Danube River after the artificial propagation procedure.

The records of NAFA Romania regarding the sturgeon brood fish captured in 2007 used in the current study were accessed online: http://sturgeons.ddni.ro/sturgeons/index.php?option=com_content&view=article&id=127&Itemid=159

Records of anadromous sturgeon species captured in year 2007, according to NAFA database, are summarized in **Table 1**.

Table 1.

NAFA records on anadromous sturgeon brood fish captured in year 2007 for artificial propagation.

Species	Gender	No of individuals	Weight (Kg)	Total weight (Kg)
Beluga sturgeon	females	6	132 ± 52	792.0
	males	11	72 ± 18	796.0
	Total	**17**	**93 ± 44**	**1588.0**
Russian sturgeon	females	1	40	40
	males	5	10.8 ± 1.2	54.2
	Total	**6**	**15.7 ± 12.0**	**94.2**
Stellate sturgeon	females	30	8.0 ± 2.0	241.4
	males	59	5.9 ± 1.1	350.8
	Total	**89**	6.7 ± 1.8	**592.2**

Sturgeon samples

Fin clip samples were collected in April - May 2007 from individuals available in two aquaculture facilities located along Lower Danube River and preserved in 99% ethanol for further molecular analysis.

30 samples from live sturgeon brood stock were successfully analyzed (**Table 2**).

DNA isolation

Genomic DNA was isolated from fin clips by Proteinase K digestion and purification using phenol: chloroform as described in literature [**38**]. The DNA concentration and quality were assessed spectrophotometrically at 260 nm and electrophoretically in 0.8 % agarose gel.

Polymerase chain reaction (PCR)

PCR amplifications were performed in GeneAmp PCR System 9700 (Applied Biosystems) termocycler in 50 ml reaction volume containing 1U of GoTaq polymerase (Promega), 10 ml 5x buffer, 200 µM dNTP, 100 µM of each primer and 200 ng DNA. Reaction volume was filled with sterile deionized water.

A. ND 5/6 mtDNA region

To amplify *ND 5/6 mtDNA region* (2.5 kb) were used the primers [**14**]:
Sturg5aF: 5'- ACC CCC TTA TCG TAT CCC TCA ACC -3'
Sturg5aR: 5'- AGC TTC GAA CCC TGC CCC TTA TTT -3'.

PCR reaction was carried out under the following conditions: a denaturing step for 5 min at 94 °C, followed by 35 cycles of 94 °C for 1 min, 67 °C for 1 min, 72 °C for 1 min and a final extension at 72 ºC for 7 min.

B. Cytochrome B (CytB) mtDNA region

To amplify *CytB mtDNA region* (1.4 kb) were used the primers [***26***]:
cytb-for1 (5'-CGTTGTHWTTCAACTAYARRAAC-3')
cytb-rev1 (5'-CTTCGGTTTACAAGACCG-3')

PCR reaction was carried out under the following conditions: a denaturing step for 5 min at 94 °C, followed by 35 cycles of 94 °C for 1 min, 50 °C for 1 min, 72 °C for 1 min and a final extension at 72 ºC for 7 min.

PCR products were electrophoresed in 0.8% agarose gel in 0.5 x TBE buffer and visualized with ethidium bromide luminescence.

RFLP

Five restriction enzymes that were previously selected for polymorphism analysis of sturgeon species of the Danube River [***14***], were applied in the current study: *Hae*III (Promega R6171)*, Alu*I (Promega R628), *Dde*I (Promega R6295), *Rsa*I (Promega R4374) and *Hinf*I (Promega R6201).

Enzymatic digestions were performed overnight at temperatures recommended by the manufacturer in final volume of 10 µl containing 1U of restriction enzyme. Samples subjected to digestion were separated by horizontal electrophoresis in 2.5% agarose gel in 0.5 x Tris buffer / boric acid / EDTA. DNA was visualized under UV light (302 nm) after reaction with ethidium bromide.

Statistical analysis

Significance of differences between weight of females and males within sturgeon species was determined using Statistica 7 software (StatSoft, Inc. 1984 - 2004) by Student’s t-test. P-values less than 0.05 were accepted as significant.

Table 2.

Data base of analyzed samples showing the species, individual PIT tag, gender, place and date of capture, available in aquaculture facilities A and B located along LDR in April – May 2007.

Sample ID	PIT Tag No.	Sturgeon species	Gender	Age	Date of capture	Aquacult. facility	Location [D. km]	TW (Kg)
7 4 1	4658410	beluga	F	adult	31 March 2007	A	Isaccea RKm 100	142
7 4 2	4698732	beluga	F	adult	01 April 2007	A	Isaccea RKm 100	110
7 4 3	476970	beluga	M	adult	08 Feb. 2007	A	Cotu Pisicii	104
7 4 4	4618324	beluga	M	adult	18 March 2007	A	Cotu Pisicii	43
7 4 12	4699616	beluga	M	adult	04 April 2007	A	Isaccea RKm 100	87
7 4 13	4659088	beluga	M	adult	03 April 2007	A	Isaccea RKm 100	64
7 4 14	4597024	beluga	M	adult	05 April 2007	A	Isaccea RKm 100	71
7 4 5	4701886	stellate	F	adult	01 April 2007	A	Cotu Pisicii	8
7 4 6	4604150	stellate	F	adult	07 April 2007	A	Isaccea RKm 100	10
7 4 7	470855	Russian sturgeon	M	adult	08 April 2007	A	Isaccea RKm 100	9
7 4 9	380599	stellate	M	adult	16 April 2007	A	Isaccea RKm 100	7
7 4 15	4678038	stellate	M	adult	04 April 2007	A	Isaccea RKm 100	4.5
7 4 16	4691818	stellate	M	adult	05 April 2007	A	Isaccea RKm 100	5.5
7 4 17	4656117	stellate	F	adult	03 April 2007	A	Isaccea RKm 100	6
7 4 18	4684140	stellate	M	adult	04 April 2007	A	Isaccea RKm 100	5
7 4 19	4679296	stellate	M	adult	05 April 2007	A	Isaccea RKm 100	4
7 6 10	4687626	stellate	M	adult	03 May 2007	B	Isaccea RKm 100	No data
7 6 12	4689008	stellate	F	adult	16 May 2007	B	RKm 120-134	5
7 5 1	4615678	beluga	M	adult	26 April 2007	B	RKm 120-134	60
7 5 2	4602734	stellate	M	adult	10 April 2007	B	RKm 120-134	5
7 5 3	4690888	stellate	M	adult	10 April 2007	B	RKm 120-134	6.5
7 5 4	4654434	stellate	M	adult	10 April 2007	B	RKm 120-134	6
7 5 6	384174	stellate	F	adult	Not available	B	RKm 120-134	No data
7 5 7	4607462	stellate	F	adult	11 April 2007	B	RKm 120-134	10
7 5 8	457563	stellate	M	adult	Not available	B	RKm 120-134	No data
7 5 9	4614658	stellate	M	adult	06 April 2007	B	RKm 120-134	6
7 5 10	4680410	stellate	M	adult	19 April 2007	B	RKm 120-134	6
7 5 11	477065	stellate	M	adult	Not available	B	RKm 120-134	No data
7 5 12	476054	stellate	M	adult	Not available	B	RKm 120-134	No data
7 5 13	4606228	stellate	M	adult	13 April 2005	B	RKm 120-134	5
7 5 14	4684575	stellate	M	adult	10 April 2007	B	RKm 120-134	5.5

RESULTS

Significant differences between weight (Kg) of genders were observed in beluga sturgeon (*p*=0.005), stellate sturgeon (p<0.0001) and Russian sturgeon (*p*<0.0001) (**Table 1**).

Data analysis did not show a correlation between gender of adult sturgeons and their capture date (r^2 = 0.0053) in any of the three anadromous species.

Several studies indicate that sturgeon males arrive first at the spawning site ([***10***]; [***28***]). Analysis of data recorded by NAFA could not establish a correlation between the capture date and gender of brood fish legally captured in year 2007. Nevertheless, as the adults were captured in April and early May during their migration, and not at their spawning sites, this fact should have had influenced the result. Therefore, the brood fish captured should have been a mixture of seasonal races, spring and autumn migrants.

Summarizing data available online in NAFA database revealed that minimum effective number (N_e) of 100 brood fish / generation interval was fulfilled in stellate sturgeon in four out of the six aquaculture facilities (**Table 3**). In case of beluga sturgeon, only one out of the six aquaculture facilities fulfilled the N_e, although the number of females was 2 instead of minimum 3 as recommended in Annex 1 of the joint Order of Minister of Environment and Water Management and Minister of Agriculture, Forests and Rural Development on conservation of wild sturgeon populations and development of sturgeon aquaculture in Romania (Order No. 262/330/2006) [***42***].

Table 3.

Summary of data available online in NAFA database showing the species, number and gender of adult wild sturgeons that were captured for artificial propagation purposes, by six aquaculture facilities (A-F) in spring 2007

Aquaculture facility	Beluga sturgeon			Stellate sturgeon			Russian sturgeon		
	♀	♂	♀+♂	♀	♂	♀+♂	♀	♂	♀+♂
A	2	5	**7**	7	10	**17**	0	1	1
B	2	1	3	8	14	**22**	0	1	1
C	1	4	5	5	12	**17**	1	1	2
D	0	1	1	5	4	9	0	0	0
E	0	0	0	1	8	9	0	0	0
F	1	0	1	4	11	**15**	0	2	2

Note. bold numbers of ♀+♂ stand for acceptable / correct N_e according to Annex 1 of Order No. 262/330/2006 [***42***].

PCR-RFLP

Polymorphism was detected using *Hae*III*, Alu*I, *Dde*I, *Rsa*I and *Hinf*I in the two mtDNA regions *ND5/6* and *Cyt b* of stellate sturgeon (**Table 4**), while no differences among beluga sturgeon individuals were revealed.

PCR-RFLP haplotypes of *ND 5/6 mtDNA* (*Hae*III*)* of beluga sturgeon and stellate sturgeon individuals analyzed in this study (N = 30) are illustrated in **Fig. 3**.

RFLP analysis of 23 samples of stellate sturgeon resulted in 6 composite haplotypes of *ND5/6* region and 4 composite haplotypes of *Cyt b*.

The ND5/6 composite haplotypes showed different frequencies among stellate sturgeon males (N=17) and females (N=6) (**Fig. 4**). Therefore, in the female group only haplotypes I and III were identified, while males showed a higher genetic diversity.

The highest number of *ND5/6* haplotypes was noticed in individuals captured between 1st of April and 7th of April 2007, when all six composite haplotypes were present (**Fig. 5**).

Cyt b composite haplotypes, showed different frequencies among males (N=15) and females (N=6) of stellate sturgeon (**Fig. 6**). Thus, in the female group composite halopype I was the most frequent accounting for 71% of total stellate sturgeon samples, while the males showed a higher genetic diversity. However, due to the low number of stellate sturgeon females that could be analyzed in the current study, no statistic analysis could be performed.

Halopype I, the most frequent haplotype accounting for 71% of total stellate sturgeon samples was detected in individuals captured in the first half of April and on 16th of May 2007 (**Fig. 7**).

Rare *Cyt b* composite haplotypes such as II and IV were identified in two stellate sturgeon males that were used for artificial spawning in aquaculture facility B (**Table 3**).

Tabel 4.

Restriction enzymes used in RFLP analysis detecting polymorphism in stellate sturgeon.

Restriction enzyme	Recognition site	Polymorphism detected in mtDNA region
Hae III	5’-GG↓CC-3’	***ND 5/6***
Alu I	5’-AG↓CT-3’	***ND 5/6***
Dde I	5’-C↓TNA-3’	***ND 5/6***
Rsa I	5’-GT↓AC-3’	***ND 5/6, Cyt b***
*Hin*f I	5’-G↓ANTC-3’	***ND 5/6, Cyt b***

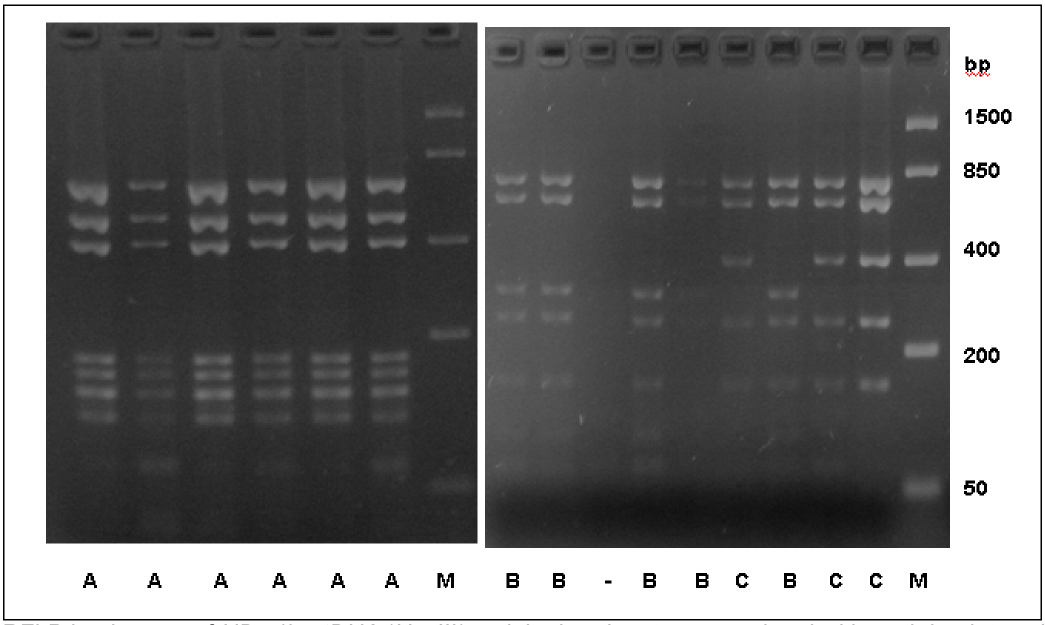

Fig. 3. PCR-RFLP haplotypes of *ND 5/6 mtDNA* (*Hae*III*)* and the band pattern associated with each haplotype in the beluga sturgeon (left) and stellate sturgeon (right)

Note. M represents the DNA FastRuler Low Range DNA Ladder, ready-to-use (Fermentas, SM 1103)

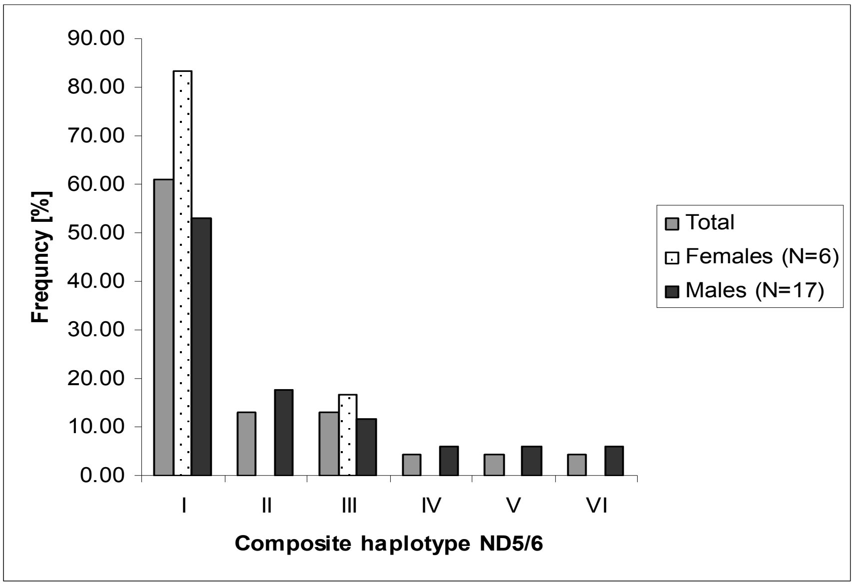

Fig. 4. Frequency of composite haplotypes within the stellate sturgeon brood fish.

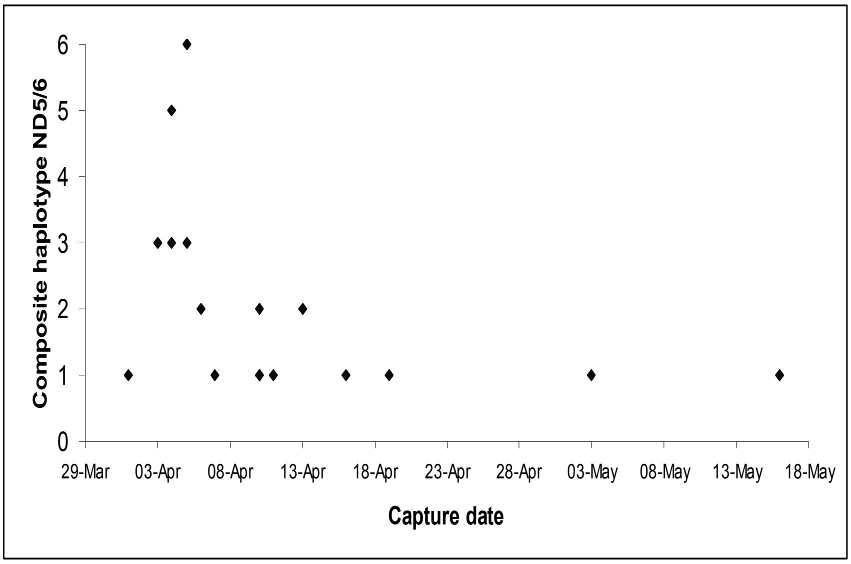

Fig. 5. Temporal distribution of ND5/6 composite haplotypes within the stellate sturgeon brood fish

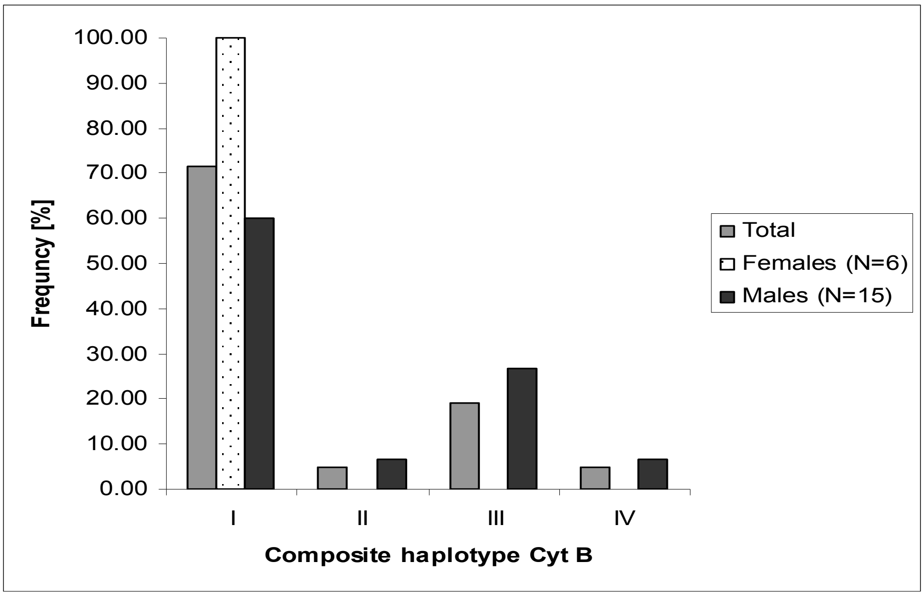

Fig. 6. Frequency of *Cyt b* composite haplotypes within the stellate sturgeon brood fish

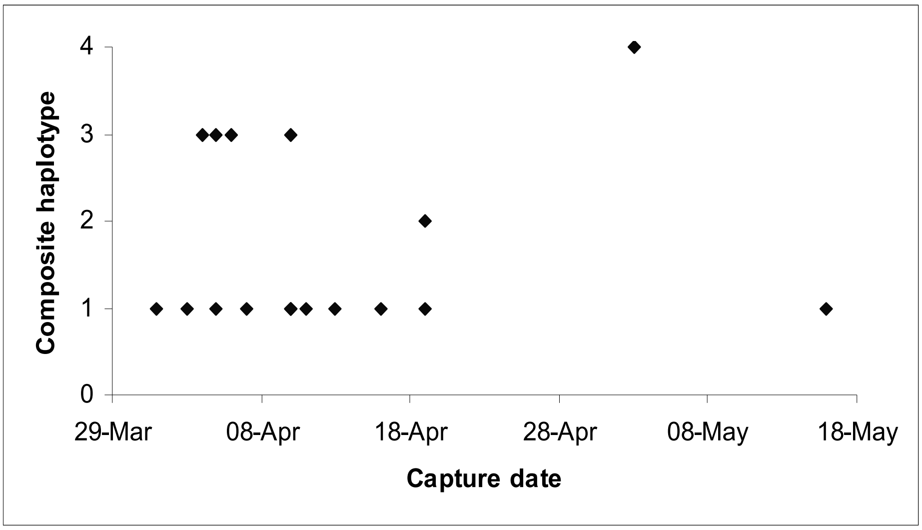

Fig. 7. Temporal distribution of *Cyt b* composite haplotypes within the stellate sturgeon brood fish.

DISCUSSIONS

The critical issue in the stocking programs is to estimate how genetic diversity will be preserved if hatcheries have access to only limited number of brood fish. FAO Code (1995) [***40***] mentions in Article 9.3.5 that "*States should, where appropriate, promote research and, when feasible, the development of culture techniques for endangered species to protect, rehabilitate and enhance their stocks, taking into account the critical need to conserve genetic diversity of endangered species*."

Therefore, genetic investigations were undertaken as an attempt to assess the genetic variability of the sturgeon brood fish available in two aquaculture facilities in spring of year 2007. We had access for sampling to only 30 out of the 89 recorded individuals in NAFA online database.

Our results based on PCR-RFLP of mtDNA regions *ND5/6* and *Cyt b* indicate a strong genetic diversity in case of stellate sturgeon and lack of diversity within the batch of beluga sturgeon brood fish captured in 2007, analyzed in the current study.

According to NAFA online database, only 5 Russian sturgeon adults, a female and four males, were captured in year 2007 and these fish were captured by three different aquaculture facilities. Therefore, only one of the six aquaculture facilities could perform artificial propagation in this most endangered species. Within the batch of brood fish analyzed in this study, only one male of Russian sturgeon was sampled and analyzed.

Although the minimum effective number (N_e) of 100 brood fish / generation interval for Russian sturgeon is 12, lack of sufficient brood fish conducted to obtaining progeny from only one pair of individuals. The case of Russian sturgeon in year 2007 is an example of weakness in management regulations of natural resources in Romania. Three Russian sturgeon males that migrated for spawning in LDR were captured and could not be used in artificial propagations due to lacking co-ordination and planning of hatchery-producing progeny at that season.

As a result of the very low number of individuals legally captured for hatchery-producing progeny from endangered populations such as *H. huso* and *A. gueldenstaedtii* in the LDR (**Table 4**), the minimum effective number (N_e) of 100 brood fish / generation interval could not be fulfilled for any of these two sturgeon species in year 2007. The low number of brood fish is a consequence of dramatic decrease of population / stock size during the last decades and still existing illegal fishing and there is a clear risk that genetic diversity decreases within the entire population.

Recent investigations made by TRAFFIC and WWF documented persisting sturgeon catches and a still flourishing black market for caviar and sturgeon products in the LDR, pointing out the size of illegal sturgeon fishing and caviar trading in Romania and Bulgaria and drawing attention to the insufficient enforcement. These illegal catches are a huge threat to the last remaining sturgeon stocks in the Danube River, and the highly commendable catch moratoria in Romania and Bulgaria will not be successful if poaching cannot be reduced significantly ([***20***]; [***22***]).

It has been recognized that stocking of hatchery-produced progeny may include the risk of altering the genetic structure of the natural stocks while also producing juveniles not fit for survival in nature. Therefore, to serve the specific release purposes, the entire culture process and the strategy for conservation culture must be critically reassessed to adjust the design of rehabilitation programmes and subsequently the design of hatcheries and the mode of their operation [***11***].

One of the most important questions that can be addressed using genetics is that of where the sturgeons spawn. The first step in answering this question is to identify through genetic analysis reproductively isolated populations or stocks of a sturgeon species. Population differentiation can be caused by differences in timing or geographic location of spawning and can occur in the absence of any physical barriers separating populations [***28***].

A scheme needs to be created for protecting the species as a whole, by knowing the number of distinct sub-populations or stocks, to ensure that individual populations persist and the evolutionary and ecological potential of a species is preserved [***28***]. However, it is unlikely that such groups / stocks are completely reproductively isolated. The probability of crossing within these groups is higher than the probability of intergroup crossing, giving the chance of their potential distinctions in the frequencies of certain alleles. It also cannot be excluded that they have different adaptations depending on the time of reproduction [***33***]. The MSc thesis of D. Holostenco [***17***], using mtDNA markers, indicated the existence of at least 10 genetically distinct groups of stellate sturgeons, still spawning in the Lower Danube River.

There are several problems to face during the human mediated species recovery, such as gene flow, inbreeding, outbreeding, mixing the seasonal sturgeon races and homing of anadromous species. Despite these problems, to prevent extinction of some sturgeon species, risks needs to be taken and appropriate solutions could be envisaged. Those risks might be diminished by development of a brood stock management plan, based on biological aspect such as ecological and genetic features of the sturgeon stocks of the Danube River, economical and administrative aspects.

For example, during 1960 - 2000, in the Northern Caspian Sea, up to 100 million juveniles of *A. gueldenstaedtii*, *A. stellatus*, and *H. huso* were released annually for stabilizing caviar production ([***24***]; [***25***]). Meantime, the genotypic structures of these populations significantly changed. According to Jenneckens et al., 2000 [***21***], about 33% of Volga Russian sturgeon, *A. gueldenstaedtii,* possesses mitochondrial haplotypes originating from the Siberian sturgeon, *A. baerii*. Hybrids between these both species were highly viable and release or escape of *A. baerii* and / or hybrids could have resulted in the baerii-like mitochondrial DNA lineages found in Russian sturgeon [***25***]. Another viable hybrid is the cross between *H. huso* and *A. ruthenus*, so-called bester, which became widely spread in aquaculture.

Inbreeding occurs when individuals sharing one or more very recent common ancestors do reproduce together. Therefore, mating of such individuals tends to concentrate identical alleles in descendents, and, if continued, in the whole population / species [***25***]. The release of high numbers of genetically closely related specimens can have negative consequences for the genetic structure of the native populations, because the influence of inbreeding depends on the effective population size (number of adults effectively having offspring in each year).

Outbreeding depression represents the loss in fitness of individuals resulting from crosses between genetically distant individuals and can be caused by different mechanisms. The most important is the “dilution” of locally adapted genotypes following hybridization between populations that have diverged because of natural selection and which have become fixed for different alleles. For example, outbreeding depressions played an important role in the 6 artificial translocation attempts of stellate sturgeon, *A. stellatus*, from the Caspian Sea into the Sea of Azov. As consequence of their limited adaptation to the new environmental conditions, their body weight was significantly less than the body weight of the native Azov stocks [***25***].

A possible genetic differentiation of stellate sturgeon sub-populations was observed in LDR [***18***] and Volga River [***33***]. The question regarding genetic differentiation of stellate sturgeon is very important for practical fish rearing: if a species is monotypic, the legally captured spawners of the autumn / winter race can be crossed with the representatives of spring groups. However, if the species is not monotypic, the intergroup crosses may irremediably damage the genetic structure of population. Meanwhile, autumn / winter spawners are used in hatcheries at an ever increasing rate due to a lack of spawners of the late spring group. In addition, the winter spawners are crossed with spring spawners [***33***].

Recent data regarding stellate sturgeon spawning in LDR identified a late migrating group that spawns on Borcea branch in the end of June [***19***]. As the stellate sturgeon brood fish for artificial propagation in year 2007 were captured in April and beginning of May, the late migrating group was not represented within the Danube River supportive stocking programme.

Although not so clear as in stellate sturgeon of the LDR, recent data suggests the existence of reproductively isolated beluga sturgeon groups in LDR (unpublished data), and therefore the hatchery-produced progeny by inter-group crosses may cause mid (or long) term damage to the genetic structure of population.

An additional risk for release programs that needs to be pointed out is effect of homing in anadromous species. To date, only limited data is available for sturgeon ([***34***]; [***36***]). Sturgeons return to their natal river to spawn and it is this behaviour that creates genetic structure and separate (sub-)populations, although for some species there is admittedly little genetic data to evaluate natal philopatry [***10***].

Taking the homing objective into account, recent FAO guidelines for hatchery practices and management for release emphasise that hatchery stock rehabilitation programmes should consider it highly desirable to hold pre-larvae and specifically post-larvae not in any other foreign water source (including groundwater) but use the intended home river as the water source. Therefore, the prime source should be river water from the release site, despite economic and other operational considerations (e.g. favourable and pathogen-free groundwater and constant temperature, identical with the incubation temperature) [***11***].

The sad history of European Atlantic sturgeon, which is practically extirpated in most of European rivers, demonstrated that preventive actions should be taken at the early warning signs, such as decreased reproduction and changes in population structure. Conservation efforts should address to fishery management, as well as to protection and improvement of spawning and rearing habitats for sturgeons [***15***].

CONCLUSIONS

In conclusion, guidelines based on the molecular genetic data need to be developed with an aim to ensure conservation of genetic diversity of LDR sturgeon species in the wild. More specifically, appropriate guidelines should provide recommendations for:

1. **Wild fisheries management and stock enhancement strategies**. For that reason, effects of hatchery-produced progeny on wild fish and potential problems associated with mixing between stocks need to be minimized; management units (reproductively isolated sturgeon groups) should be described and used as a basis of conservation work.

2. **Aquaculture management**. Consider issues relating to broodstock genetics to avoid inbreeding and associated problems, and possible genetic alteration in captivity.

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