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3. Overview of the Romanian Sturgeon Supportive Stocking Programme in the Lower Danube River System

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Astract: Due to the worldwide decline of the sturgeon population, stocking programmes were developed for many of these species. The critically endangered anadromous sturgeon populations inhabiting the NW Black Sea and spawning in the Lower Danube River are not an exception. Despite no knowledge of the genetic diversity of the remnant populations, the Romanian supportive stocking programme started in 2005, along with the fishing ban in 2006. These actions were aimed to restore the wild sturgeon populations, spawning in the Lower Danube River. This paper presents and discusses the sturgeon supportive stocking programme implemented in Romania and its achievements. Our screening revealed that the minimum effective number of breeders (N_e) could not be achieved for beluga sturgeon (***Huso huso)*** in 2006 and for Russian sturgeon (***Acipenser gueldenstaedtii***) throughout the entire programme due to the lack of spawners in the wild. Evaluation of the supportive stocking programme success revealed a good survival rate and adaptation of fish stocked to the wild conditions in the river and sea. It showed that two of the species, beluga and stellate sturgeon (***A. stellatus***) still spawn naturally in the wild, and their populations are in a good genetic shape, so far, having in mind the habitat decline due to the dams constructions on the river and the post genetic bottleneck recovery. Russian sturgeon population do not exhibits any improvement sign so far. The lack of its spawning events in the wild is still noticed, as the majority of fish captured during the evaluation are of hatchery origin, first generation from wild spawners. Therefore, for beluga and stellate sturgeon, the conservation measures should be revised based on the genetic structure of the spawners and a well-defined breeding schemes. For Russian sturgeon the supportive stocking programme should continue, being highly demanded in order to produce a continuum age structured population. The crucial moment for sturgeon conservation will be when hatchery reared specimens return in the river as adults and participate in the spawning events, an event which should be expected within the next years. Overall, the return of the reared individuals in the river as adults and their participation in the spawning events is expected to produce a real change in the Danube sturgeon species fate with a long-term ripple effect.

Keywords: wild sturgeons, hatchery origin sturgeons, Lower Danube River, NW Black Sea, supportive stocking

INTRODUCTION

Although stock enhancement has been viewed as a positive tool for fisheries management for over 100 years (*Homarus sp*., since 1850s) (Molony et al., 2005), it is considered the most controversial approach towards restoring depleted fish populations and maintaining a sustainable fishery (Lorenzen, 2005). According to the Food and Agriculture Organization of the United Nations [FAO] (De Silva & Funge-Smith, 2005) the term has been used to describe most types of stocking, aiming to increase the size of fishable stock. When discussing about stock enhancement most people uses the term of restocking / rebuilding, as the production and release of hatchery-reared fish into an area where the species historically occurred, but are now rare or extinct, or where a fishery has declined or collapsed (Molony et al., 2005).

Initial decline in sturgeon populations worldwide was primarily due to the increasing of commercial harvest (Rochard et al., 1990; Auer, 2005). Subsequently it overlapped with the construction of dams blocking their historical access to upstream spawning grounds and altering conditions of their essential habitats (Billard & Lecointre, 2001; Secor et al., 2002). Furthermore, deforestation, diversion of watercourses, gravel and sand extraction also negatively influenced the spawning, physiology and food availability of sturgeon populations left in rivers (Pikitch et al., 2005). Poaching is and will remain a continuous threat for all sturgeon populations as spawners are vulnerable to being caught while migrating upstream the river systems to the spawning grounds (Auer, 2005).

Depletion of worldwide wild sturgeon populations led to an increasing use of restocking with hatchery produced juveniles at the beginning of 1990's. Hatcheries were used to produce millions of fish, either to create or to support a fishery or to mitigate for the impact caused by the habitat loss. These programmes that release millions of fish into a natural basin (Chebanov et al., 2002) without any knowledge about the essential habitats availability required for different life stages and a prior genetic screening of the remaining wild populations is now considered controversial (Secor et al., 2000; Secor et al., 2002; Ryabova et al., 2006).

Therefore a conservative approach towards restoring depleted population by stocking came later and asked for prior knowledge on the species biology and ecology as well as on the baseline genetic status of the populations involved before any actions in the wild basins (Hallerman, 2003). In addition, the status of essential habitats in the river required by different life stages is needed to be assessed. Otherwise stocking can have no effect or even worse, have undesired effect on the long term population viability in the wild (Miller & Kapuscinski, 2003).

Once forming substantial populations in the Danube River basin and the adjacent NW Black Sea the sturgeons declined dramatically in the region. From the six historical native Danube sturgeon species that inhabited or partly migrated as far as Regensburg, in Germany (Antipa, 1909; Bacalbaşa-Dobrovici, 1997; Bloesch et al., 2006; Lenhardt et al., 2006), only four of them are still occurring: Russian sturgeon (*A. gueldenstaedti)*, sterlet sturgeon (*A. ruthenus)*, stellate sturgeon (*A. stellatus)*, and beluga sturgeon (*H. huso*). Four were anadromous (*H. huso, A. gueldenstaedtii, A. stellatus, A. sturio*), while the other two were fresh water resident species (*A. ruthenus* and *A. nudiventris*). Nowadays, three of the anadromous sturgeon species (*H. huso, A. stellatus,* and *A. gueldenstaedtii*) are still spawning in the Lower Danube River (LDR), being listed as critically endangered by IUCN Red list. Since 1997, the fourth anadromous species, Atlantic sturgeon (*A. sturio)*, has been reported as possibly extinct (Bacalbaşa-Dobrovici, 1997), and later declared for good, extinct in the Black Sea basin (Bloesch et al., 2006; Suciu, 2008; Gesner et al., 2010). In the case of the two freshwater resident species, nowadays just one is still occurring. Sterlet sturgeon (*A. ruthenus*) is still spawning in the river and is currently listed as vulnerable, while the other one, the ship sturgeon *(A. nudiventris*), was initially considered to have an unclear status (Bloesch et al., 2006), and later listed as "*possibly Extinct in Romania"* by the IUCN Red List (Gesner et al., 2010).

First threats affecting the remaining sturgeon populations was historical overexploitation and habitat reduction. The construction of dams at Iron Gate I and II in 1960s and Gabčíkovo in 1970s, inland navigation development (Kottelat et al., 2010) and flood protection, accelerated their decline (Bacalbaşa-Dobrovici, 1997; Birstein et al., 1997; Bloesch et al., 2006; Guti, 2006). Beside these, the lack of fishery regulation enforcement for almost 11 years in Romania after the fall of the Communist Regime, in 1989, accelerated their decline (Suciu, 2008). Poaching was and is still an existing threat (Birstein et al., 1997; Bloesch et al., 2006; Holčík et al., 2006) as a lack of law enforcement still exists (Reinartz et al., 2012; Ludwig et al., 2015) within the basin and the wild caviar is still demanded on the black market. In addition to these extrinsic threats (overfishing, poaching and habitat reduction), another factor that shaped their rapid decline and their very slow rehabilitation was and is their own longevity. The sturgeon are long life-span species (over 100 years), with a late maturing cycle, between 3-5 years for *A. ruthenus* and 13-15 years for *H. huso*. Besides that, sturgeon species do not spawn every year, since, after a spawning event the gonads require between 2 and 5 years to ripe again. Therefore, the extrinsic threats has started the sturgeons decline while their own intrinsic traits sharped it, making the restocking programs an urgently required tool to overcome the near extinction-driven status.
Attempts of stocking the Danube with hatchery reared sturgeons go back in the 1990's (Bacalbașa-Dobrovici & Patriche, 1999) following that time philosophy "put and take" (Sigler & Sigler, 1990), more than one million larvae and juveniles, especially Russian and stellate sturgeons and to a lesser extent beluga and sterlet sturgeons (Vassilev, 2006; Smederevac-Lalić et al., 2011) were released in the LDR

by Romania and Bulgaria. However, only this mass releasing without any prior investigation of the status of the remnant populations and habitat availability turned out to have no positive effect (Vassilev, 2006; Vecsei et al., 2007; Smederevac-Lalić et al., 2011).

In 2001, all the countries along the Danube and Black Sea basins were mandated by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) to assess the status of their sturgeon populations (Suciu, 2008) in order to establish catch quotas for all countries and species within the basins. Therefore, starting from 2002 the CITES demands were implemented and the status of sturgeon populations in the LDR has been assessed yearly by the Danube Delta National Institute for Research and Development (DDNIRD) as CITES Scientific Authority for Acipenseriformes in Romania (Suciu, 2008). The annual survey of the Danube sturgeon populations highlighted a strong disequilibrium in age structure for all three extant anadromous species. In the beginning, the assessment revealed an unequal contribution of reproductive age class to the entire population age structure, due to overfishing which is acting as an active age structure filter. The beluga and Russian sturgeons exhibited a lack of "*first time spawners*" (Paraschiv et al., 2006; Suciu et al., 2007; Suciu, 2008; Suciu & Guti, 2012), due to a highly effective overfishing, of the caviar producing young adults. While, for the stellate sturgeon, the annual reproduction was mainly the contribution of the "*first time spawners*" (Ceapa et al., 2002; Paraschiv et al., 2006; Suciu et al., 2007; Suciu, 2008; Suciu & Guti, 2012), due to the depletion of older adults through overfishing. Since 2005 a very low recruitment from natural spawning was recorded for Russian and stellate sturgeon and for beluga since 2006 (Paraschiv et al., 2006; Suciu, 2008; Suciu & Guti, 2012). Overall, the strong disequilibrium in age structure highlighted in annual surveys, over multiples years, alongside the observed decrease of sturgeons commercial catches in Romania from 37.5 tons in 2003 to only 11.8 tons in 2005 (Navodaru et al., 1999) conducted Romania to outline a programme for conserving and restoring wild sturgeon populations in the LDR (Suciu, 2008).

Measures as temporary ban on commercial fishing to reduce pressure on wild populations, aquaculture development to meet caviar market demand, and artificial support through stocking the Danube with hatchery produced juveniles in order to help to rebuild natural age class structure were actions required for their recovery and conservation. Therefore, Romania banned the commercial sturgeon fishing in 2006, and started a small scale supportive stocking programme through a joint ministerial order (Suciu, 2008). According to the fishing ban the sturgeon moratorium will be in place for at least ten years to compensate the effects of intensive fishing on the age class structure of wild sturgeon populations in Romania (Suciu, 2008).

The aim of this paper is to present and discuss the Romanian sturgeon supportive stocking Programme since 2005 and its most important achievements, based on both scientific and official sources. Most of the data presented and discussed in the paper have been provided by the Danube Delta National Institute for Research and Development (DDNIRD).

OVERVIEW AND DISCUSSIONS

The first attempts of sturgeon stocking the LDR in the 90's was not a conservation-oriented action, and not at all a standing program due to its purpose philosophy "*put and take*" (Sigler & Sigler, 1990), and the lack of long-term planning. The first conservation-driven Romanian sturgeon supportive stocking programme has been implemented since 2005, for an initial period of 5 years. Following the principles of Kincaid's recovery plan for white sturgeon in the Kootenai River (Kincaid, 1993) and adapted for sturgeon populations life cycle in the LDR basin, the Romanian Ministry of Agriculture, Forests and Rural Development and the Ministry of Environment and Water Management released methodological outlines through a joint Ministerial Order. According to the order, some prerequisites were required prior to release fish in the river (Textbox 1).

Textbox 1 Excerpt from joint Ministerial Order 330/ 262/2006 regarding sturgeon restocking and / or supportive stocking

Chapter III – Sturgeon acvaculture development, article 5:

- Annually, by 15 November, at the recommendation of the Scientific Authority for Acipenseriforms, the National Agency for Fisheries and Aquaculture establishes the number of breeders of each sturgeon species to be admitted for fishing, this number will be regionally negotiated and transmitted to the CITES Secretariat by 30 November.

- Is mandatory to obtain sturgeon juveniles for restocking and / or supportive stocking by capturing a minimum number of breeders, according to Appendix 1 to this order.

- Adults will be captured by non-destructive fishing methods using a special authorization issued by National Agency for Fishing and Aquaculture.

- It is mandatory to use artificial reproduction methods which ensure the survival of breeders;

- All the adults used as broodstock in the hatchery as well as the juveniles will be tagged prior to release in the river. Passive Integrated Transponder (PIT) will be used for adults and Coded wire tags (CWT) for the juveniles

- The juveniles will be raised until 10cm total length in the hatchery

Appendix 1

Minimum effective number of sturgeon breeders per generation (N_e) (from a population or sub-population) which should be used in artificial breeding programs for restocking and / or supportive stocking the Danube river is recommended to be inferred in order to ensure an effective population size per generation $N_{e(GEN)}$ of 100 and a maximum inbreeding rate per generation (ΔFmax) of 0.5%.

Therefore,

- *The recommended minimum effective number (Ne) of breeders is 14 for stellate sturgeon, 12 for Russian sturgeon and 7 for beluga sturgeon. The numbers were inferred in accordance with the species generation interval.*
- *The eggs from each female will be split in batches according to the number of males available. Then they will be fertilized with sperm from each male and obtained offspring will be raised separately.*

The Romanian sturgeon restocking programme was funded by National Agency for Fishery and Aquaculture in Romania and lasted until 2009. The programme ran for the three anadromous sturgeon species still naturally spawning in the river and to a lesser extent for the fresh water resident species, sterlet (*A. ruthenus*) (Table 1). All the adults used as broodstock within the programme were captured in the LDR based on special permits. They were tagged with Passive Integrated Transponder (PIT) to avoid a second use in the programme and were released after controlled propagation in the river where they have been captured. A non-destructive artificial reproduction procedure was used, and a fertilization scheme to maximize the genetic diversity was applied. All the hatchery juveniles were raised to a total length of ~10cm to enlarge the success of tagging. This size also assures a good survival rate in wild, minimizing the predation risk (Suciu, pers. comm.). Juveniles were marked with coded wire tag (CWT) in the right or left pectoral fin prior to releasing (Table 1) except those released in 2009 when only 10% were tagged. Tagging is a useful tool for the subsequent evaluation of the survival rate and adaptation of stocked animals at wild conditions in the river and sea, and thereby the stocking success. The return of stocked fish in the river as first time spawners can also be detected. After tagging, all hatchery reared juveniles were released in the same year of hatching at locations close to the sites where their parents were captured. They will undertake downstream migration towards the Black Sea in the same year of stocking as wild conspecifics migrate to the sea in the same year of hatching.

Some requirements of the Programme could not be achieved in different years mainly due to the low number of spawners captured in the Danube River. For instance, analyzing the data presented in the table 1, the N_e of breeders could not be achieved for beluga sturgeon in 2006 and for Russian sturgeon within the whole duration of the programme due to the lack of spawners in the wild. Even when the number of breeders was achieved (i.e. in 2007) for beluga sturgeon only 1 male and 1 female were artificially propagated due to the lack of coordination and planning of hatchery-producing offspring at that time (Onără et al., 2013). The sturgeon autumn migration in the river was not targeted by the programme as the fishing permits were issued only for spring spawning migration (Suciu et al., 2007).

The Romanian programme started without any prior genetic knowledge of the remnant sturgeon populations in the LDR. Only in 2013 and 2014 based on mtDNA control region investigations, four haplotypes were identified for beluga sturgeon (Onără et al., 2014) and ten haplotypes for stellate sturgeon (Holostenco et al., 2013) populations of the LDR.

Table 1. Overview of sturgeon supportive stocking programme of Romania. Species, number of broodstock, number of fish stocked in different years and locations in the Lower Danube River, hatchery involved in the programme, period of stocking, place of CWT implanting

Year[1]	Species	No of wild parents used for artificial reproduction	Hatchery place	No. of juveniles stocked	Place of stocking (rkm)	Period of stocking	CWT insertion place
2005	*Acipenser gueldenstaedtii*	1 female and 1 male	Isaccea	2,588	Danube rkm100	mid September	Right pectoral fin
	Acipenser stellatus	14 (information regarding sex not available)	Brates and Isaccea	7,881	Danube rkm 100; 153; 161.5; Mm 72.7	mid September	Right pectoral fin / first dorsal scute
2006	*Acipenser stellatus*	14 (information regarding sex is not available)	Brateș and Tamădău& Isaccea	53,300	Danube rkm 161; Borcea branch km 39	beginning of December	Left pectoral fin
	Huso huso	2 females and 3 males	Tamădău and Isaccea	12,500	Danube rkm 100; Borcea branch km 39	beginning of December	Left pectoral fin
2007	*Acipenser gueldenstaedtii*	1 female and 3 males	Tamădău	86,500	Danube rkm 630	mid December	Right pectoral fin
				10,000	Borcea Branch rkm 39	mid December	Right pectoral fin
	Huso huso	2 females and 5 males	Isaccea	15,129	Dunăre, Mm 54, Isaccea	mid December	Right pectoral fin
2008	*Acipenser stellatus*	7 females and 7 males	Tămădău	25,000	Borcea Branch rkm 39	mid December	Left pectoral fin
	Huso huso	3 females and 5 males	Tămădău	20,000	Borcea Branch rkm 39	mid December	Left pectoral fin
	Acipenser gueldenstaedtii	1 female and 3 males	Ianca	32,800	Danube rkm 630	mid December	Left pectoral fin

2009	*Acipenser stellatus*	*Information N.A.*	Tămădău	30,000	Borcea Branch rkm 39	end of July	Right pectoral fin
	Huso huso	*Information N.A.*		45,000	Borcea Branch rkm 39	end of July	Right pectoral fin
	Acipenser gueldenstaedtii	*Information N.A.*		50,000	Borcea Branch rkm 39	end of July	Right pectoral fin
	Acipenser ruthenus	*Information N.A.*		35,000	Borcea Branch rkm 39	end of July	Right pectoral fin
Total (2005-2009)				**425,698**			

[1] Year of juveniles' production and stocking

For a conservation programme to have a positive effect the genetic diversity and structure of the remaining populations should be known in order to identify the management units (Kohlmann et al., 2017b) and further establish a broodstock and an appropriate breeding scheme (Kohlmann et al., 2017a). Recent research on sterlet sturgeon at both, mtDNA and nDNA level, revealed a significant number of maternal lines on mitochondrial data, and the presence of at least two ancestral populations with a high tendency to homogenize the gene pool at the nuclear level (Kohlmann K. et al., 2018). Also, an active genetic uniformity tendency induced by spatial contraction due to habitat fragmentation by dams has been observed (Friedrich et al., 2018; Kohlmann et al., 2018). An intensive gene flow between Middle and Lower Danube River was identified based on sequencing of 257bp D-loop fragment (mtDNA) for sterlet sturgeon population of the Danube River (Cvijanovic et al., 2015). Genetic studies are still ongoing in order to describe the actual status of the remnant populations that is required for the implementation of adequate conservation measures.

In addition, the stocking efficiency of the Romanian supportive stocking programme (2005-2009) was not assessed during its lifetime or after the completion, due to the lack of funding. Post stocking evaluation is urgently required as to analyze whether the stocking strategy was adequate or there is a need to be modified / adjusted. Also, a reevaluation of a stocked river basin after a period of time is needed in order to decide if stocking is still necessary, should be readjusted or even stopped (Cowx, 1998; Cowx, 1999).

Only in 2013, Romania, through Fishery Operational Programme, funded a two years study aiming to evaluate the stocking efficiency in the Black Sea. The project was run in collaboration with Bulgaria, Serbia, Ukraine, Georgia and Turkey, countries sharing the same sturgeon stocks in the Black Sea. Positive results were registered regarding the survival rate and adaptation of the stocked individuals at wild conditions, and about their distribution along the NW Black Sea coast (Ionescu et al., 2017).The stocked individuals seems to mimic with a high fidelity the behavior of the wild ones. In addition, the same study revealed that stellate and beluga sturgeon populations are in a good shape as most of the juveniles captured were of wild origin, meaning that the wild spawning is still occurring naturally in a significant rate. They successfully spawn in the LDR and their populations are in a good genetic shape, showing a post-bottleneck recovery. Therefore, the sturgeon fishing ban and the supportive stocking programme had a visible positive effect on their rehabilitation, at least for two of the species, stellate and beluga sturgeon. For the Russian sturgeon population, the status is somehow opposite. Russian sturgeon population is still facing a sharp demographic decline due to overfishing during 1990 – 2000 (Suciu, 2008). The stocking evaluation results showed that the majority of the fish captured during the project were from the supportive stocking programme. The genetic status of wild population is affected by the lack of spawning in the wild since 2006 (Paraschiv et al., 2006). Even if, both, fishing ban and supportive stocking programme have not showed any visible signs of improving so far, their contribution to population recovery is still running due to the presence of the juveniles, which in the next years will be able to spawn, slowly reestablishing the Russian sturgeon population. Therefore, the research team recommended a prolongation of the fishing ban for all the sturgeon species, and a revision of conservation programme for beluga and stellate sturgeon, as well as a continuation of the supportive stocking for Russian sturgeon population that is still in a critical status(Ionescu et al., 2017).
Overall, despite the tremendous efforts made on numerous restocking programmes worldwide in order to recover the sturgeon populations, the long-term effect is still uncertain. A turning point for the

conservation of these critically endangered species will be when hatchery origin fish will return to the rivers and participate in spawning events.

CONCLUSIONS

Although still controversial the supportive stocking programmes along with other conservation measures have been used as important tools for restoring depauperated fish populations in the world. The Romanian supportive stocking programme for sturgeons was outlined based on critically endangered wild populations still spawning in the LDR.

Our overview showed that the programme respected mostly the protocol stipulated in the law with some exceptions regarding the assurance of the minimum effective number of broodstock for Russian sturgeon for the whole duration of the programme.

The evaluation of the programme revealed a good survival rate and adaptation of the hatchery produced sturgeons stocked in the LDR to the wild conditions in the river and sea as well as the presence of wild juveniles of beluga and stellate sturgeon in the Black Sea, meaning that these two species still manage to spawn in the LDR.

In our opinion, the Romanian sturgeon supportive stocking programme along with sturgeon fishery ban contributes to the recovery of all still spawning sturgeon populations in the river. The most affected until now seems to be the Russian sturgeon, and based on the two years evaluation, we believe that the supportive stocking will slightly improve its status due to the presence of juveniles which will be able to spawn after several years thus reestablishing the population. The populations of other two species, beluga and stellate sturgeon, are in a better state with spawning still occurring in the river. Nevertheless, the real game changing moment in sturgeon conservation will be when the stocked individuals and their offspring will return in river to spawn in the next years to rebuild self-sustaining populations.

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