https://doi.org/10.7427/DDI.24.04

4. A Glimpse of a Better Future for the Danube's Flagship Species - First Return of Stocked Sturgeons into the River

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Abstract: In the present study, 4 specimens of sturgeons from stocking-programmes was recorded, out of a total 34 Danube River upstream-migrants towards their spawning grounds. The catches were recorded over a period of 42 days of scientific fishing on an area of 50.23% of the Danube River width. Three out of four native species were recorded during scientific fishing: 4 *Huso huso, 27 A. stellatus* and 3 *A. gueldenstaedtii.* CWT reading showed that all four individuals from stocking-programmes were males, three *A. gueldenstaedtii* and one *A. stellatus*. The captured specimens represent not only the first registered individuals returning to their home river but also a real chance for restoration of the *A. gueldenstaedtii* population into the Danube River. To the best of our knowledge, this is the first time in the world when the return of stocked sturgeons to their home river is reported. Consequently, identifying the main factors which have enabled this is crucial and represents a real breakthrough in sturgeon conservation. Our data suggest that the stocking program success is more likely dependent on the presence of wild individuals from whom stocked individuals can "learn" the way to the spawning grounds, than on the intrinsic traits of the stocking programme. Therefore, there is a need for additional studies to further understand if this return is an isolated case or it is the beginning of a better future for Danube River sturgeon populations. More information is also needed to assess the contribution of the stocked individuals to spawning events.
Keywords: stocked sturgeons, supportive stocking, return to the river, Danube River, sturgeon conservation

INTRODUCTION

Sturgeons comprise a group of around 25 living fish species (Family Acipenseridae) with a history dating back to the Upper-Cretaceous (Near et al., 2012), and a wide distribution area across Eurasia and North America. Most species are completely or partially anadromous, spawning in fresh water and spending most of their life in marine or brackish waters. However, a small number of species has adapted over time to have an entirely freshwater lifecycle. Most of the sturgeons are caviar-producing species, a gastronomic delicacy with a history of more than two millennia. The increase in caviar demand in the last two centuries has been one of the factors that have driven the sturgeon species to a critical point in their existence. Overfishing, heavy poaching and habitat disruption on one side, and late maturation, long reproductive cycles, and their long migration routes to spawning grounds cut-off by dams, on the other side, have brought them under a severe threat. Nowadays, more than 85% of living sturgeon species are classified by the International Union for Conservation of Nature (IUCN) as at risk of extinction, making them one of the most critically endangered groups of species (Qiwei, 2010). Therefore, sturgeons are among the most valuable wildlife resources, in need of special conservation programs with long-term vision and effect.
The sharp demographic decline in sturgeon populations observed worldwide attracted the attention of researchers and governments and highlighted the need for urgent action to maintain sturgeon fisheries. Therefore, the story of sturgeon population recovery begins in the 1950s in Russia, when the first artificial

breeding attempts were made to increase the wild populations in the Caspian and Azov Sea (Secor et al., 2000). In other words, the supportive stocking and artificial propagation trend began in the Soviet Union, being later adopted by other Caspian countries such as Iran in the 1970s (Abdolhay and Tahori, 2006). Since the 1990s, China has implemented various recovery and management actions for the Chinese sturgeon (*A. dabryanus*) in the Yangtze River (Wu et al., 2014). Later on, in 2007 in Western Europe, France initiated the recovery programmes of European sturgeon (*Acipenser sturio*) in the Garonne River (Williot et al., 2009), after a training period on Siberian sturgeon (*A. baerii*) in Russia (Williot et al., 2002). The idea of first experimental stocking with Atlantic sturgeon (*A. oxyrinchus*), in the Oder River and its tributaries, started in 2006 (Wuertz et al., 2011) and was implemented in subsequent years. Soon after, a group of European countries followed the initiative of France and started restocking programs for both European sturgeon (*A. sturio*) in the Elbe River (Williot et al., 2009; Williot and Kirschbaum, 2011) and Adriatic sturgeon (*A. naccarii*) in the Po River (Boscari et al., 2014). Meanwhile, in 2008 an experimental restocking programme in the Elbe River was designed, using tagged individuals (T-bar tags and ultrasonic transmitters) originating from France, to investigate the use of habitats and migration routes under modified river conditions (Gessner, 2009). A second experimental restocking programme was deployed in the Oste River in 2009 (Gessner et al., 2011). In the same period, the Chinese authorities realized that management actions were not enough for Chinese sturgeon (*A. dabryanus*) recovery, and started a supportive stocking program in 2007 (Wu et al., 2014).

In the Danube River the first sturgeon stocking attempts were made in the 1990s, by both Romania and Bulgaria, using the Soviet model (Bacalbașa-Dobrovici and Patriche, 1999) and following the philosophy of "*put and take*" (Sigler and Sigler, 1990). Overall, in these two stocking campaigns more than one million individuals were released into the Lower Danube, especially Russian sturgeon (*A. gueldenstaedtii*) and Stellate Sturgeon (*A. stellatus*), without any evaluation of their survival (Vassilev, 2006). Shortly after, other stocking campaigns were conducted, but at a smaller scale. For example, in 1995 around 10,000 sturgeon juveniles were stocked in the St. Gheorghe branch of the Danube Delta (Suciu, 1995). Despite these stocking campaigns, the sturgeon populations from the Danube River still showed a sharp demographic decline and a strong disequilibrium in age structure (Paraschiv et al., 2006; Suciu, 2008; Suciu and Guti, 2012). Therefore, in 2005 Romania started the first conservation-driven sturgeon supportive stocking program, based on a white sturgeon recovery plan in the Kootenai River (Kincaid, 1993), which was adapted for the Danube River. Initially, the supportive stocking program was implemented for a five-year period, but no assessment was performed, neither during its lifetime nor after its completion. Later on, in 2013, a new two-year program was implemented to quantify the stocking efficiency and survival rate (Ionescu et al., 2017). In addition, during the evaluation stage another two supportive stocking campaigns were conducted for three anadromous sturgeon species (Cristea et al., 2016): Beluga sturgeon (*Huso huso*), Russian sturgeon (*A. gueldenstaedtii*) and Stellate Sturgeon (*A. stellatus*).

Despite the numerous and tremendous efforts taken worldwide to conserve remnant populations and improve their status, or even to reintroduce extinct sturgeon populations through supportive or restocking programmes, none of them have fulfilled their purpose completely. Until now, no programme has been able to report the active spawning or even the return of stocked sturgeons into a river. The present study analyzes the occurrence of the first stocked sturgeons back into the Danube River. Furthermore, it aims to assess the particular circumstances that have made this phenomenon possible. Is this first return of stocked sturgeons into the river dependent on the intrinsic traits of the supportive stocking programme or is it simply a matter of luck?

MATERIAL AND METHODS

Capturing adults

The adult individuals were captured during a period of 42 consecutive days (30 Mar – 11 May 2018) of scientific fishing over an area of 7 km along Danube River (between RKM 123 and 130), and ~350 m wide (~50 % of the width of the Danube River, ~ 2.2 km^2 surface area), by professional fishermen and using two boats equipped with benthic trawls (Figure 1). The origin of each captured individual was verified by reading Coded Wire Tags (CWT) using a T-Wand detector (Northwest Marine Tech Inc, WA, USA). Adult individuals without a CWT have wild origin, while the presence of a CWT indicates the stocked origin from one of the Romanian Sturgeon Supportive Stocking programmes (RSSSP), based on the tag's position (right or left pectoral fin). All captured individuals were subjected to a biometric survey and then released downstream of the capture site as soon as possible, with few exceptions. Captured individuals of Beluga sturgeon (*Huso huso*) were additionally used for controlled propagation, and then released back into the river, at the release site (20 km downstream, RKM 100).

Capturing young of the year (YOY)

Young of the year (YOY) recruitment is an accurate indicator of sturgeon species' active spawning and abundance in the Danube River. Since 2000, sturgeon YOY recruitment has been monitored annually using constant parameters, in terms of fishing tools and river area. The YOY presence and abundance was assessed at RKM 123 (Figure 1) on the Romanian side of the river downstream of Reni (Ukraine), using a 95 m long trammel net (20mm mesh size), that was drifted over 850 m of river bottom. The fishing site is actually a feeding ground, where young sturgeons stop to feed on benthic fauna during their migration towards the sea.

Morphological measurements, age and statistics

Both adult and YOY sturgeons were subjected to biometric measurements: total length – TL; standard length – SL; total weight – TW; and sex identification, when possible.
Age estimation of the tagged individuals was done using the appropriate form of the von Bertalanffy growth curve (Fabens, 1965) in the context of inferring age (*t*) from length (*l*) using equation 1 (Kirkwood, 1983)

$$t = t_0 - \frac{1}{k}\ln(1 - \frac{l}{L_\infty})$$

where *t* is the estimated age, t_0 is the theoretical age at length zero, *k* is the instantaneous growth coefficient, *l* is total length of the individual, and L_∞ is the asymptotic length. For the Stellate sturgeon it was assumed that t_0 = -10, *k* = 0.051 and L_∞ = 193.5, based on Ceapa et al. (2002) reported data, calculated for wild Stellate sturgeon from the Danube River. To estimate the age of tagged Russian sturgeon individuals it was assumed that t_0 = -4.5, *k* = 0.054, and L_∞ = 214, based on Tavakoli et al. (2018) reported data, calculated for wild Russian sturgeon from the Caspian Sea.
The Length - Weight relationships and their 95% confidence interval were calculated based on $W=aTL^b$ formula. Length-Weight parameter values for Russian sturgeon were *a* = 0.0068 [min 0.0039; max 0.0097] and *b* = 2.98 [min 2.88; max 3.056], and for Stellate sturgeon *a* = 0.0191 [min 0.0104; max 0.034] and *b* = 2.72 [min 2.584; max 2.792], retrieved from FishBase (Froese andPauly, 2018).

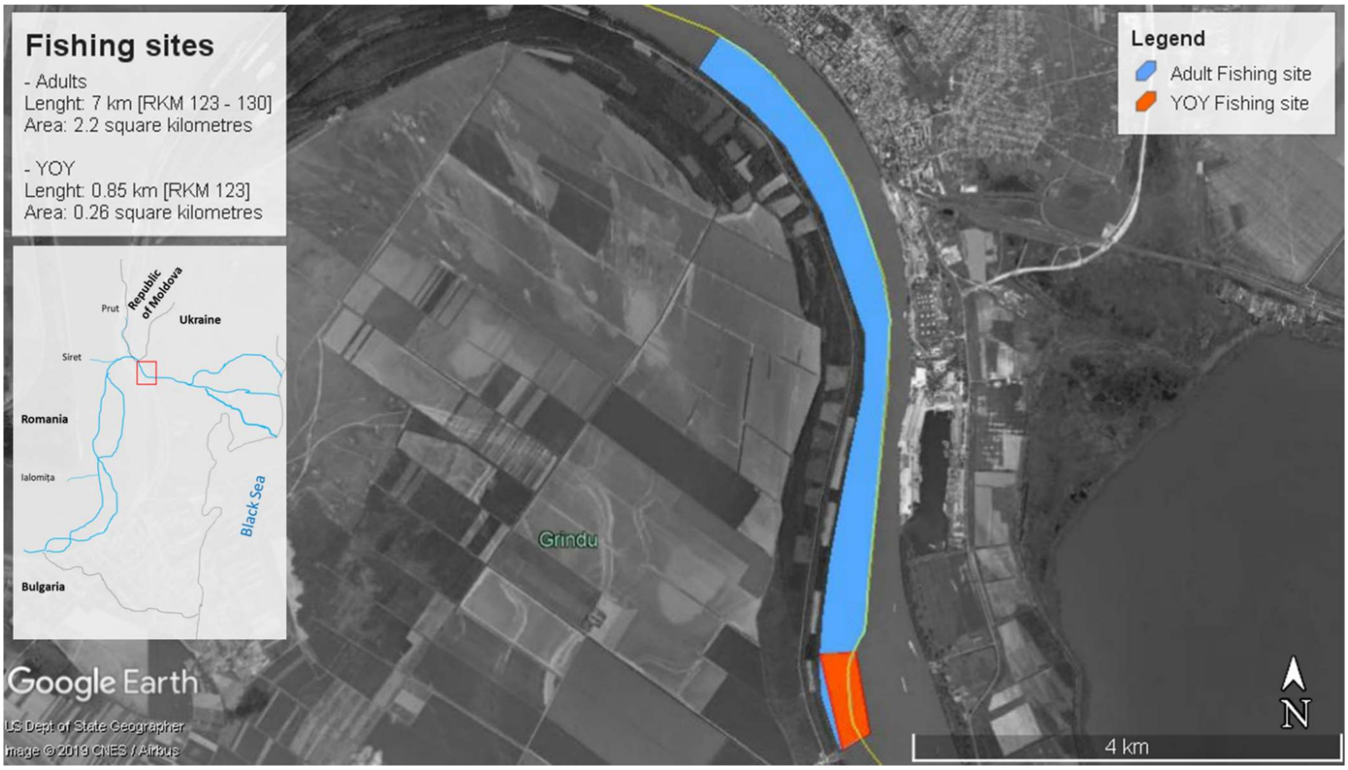


Figure 1 Fishing sites for adults and YOY along Danube River

Overview of Romanian Sturgeon Supportive Stocking Programmes (RSSSPs)

During 2005 - 2013 Romania conducted several sturgeons supportive stocking programmes, with three sturgeon species: Beluga sturgeon (*Huso huso*), Stellate sturgeon (*Acipenser stellatus*) and Russian sturgeon (*A. gueldenstaedtii*). For all three species, wild spawners were captured during spring spawning migration into the Danube River, in accordance with national regulations. The wild individuals, that were sexually mature, were reproduced under controlled conditions after hormonal stimulation. To maximize the broodstock genepool, the eggs of each female were evenly divided to the number of available males, to produce all possible genotypes. The offspring were farm-raised until their release into Danube River. Before releasing all YOY or juveniles were tagged in the right or left pectoral fin, using CWTs. Between 2005 and 2013 around 609 thousand wild sturgeon offspring were stocked into Danube River, using 6 release sites (Table 1).

Table 1 Overview of the f the Romanian Sturgeon Supportive Stocking programmes within 2005 and 2014

Species	Year of stocking	CWT position [a]	No of indv. stocked [b]	Age at stocking [c]	Release site(s)
H. huso	2006	LPF	12.5	6	Danube RKM 100 & Borcea Br. Km 40
	2007	RPF	15.1	7	Danube RKM 100
	2008	LPF	20	7	Borcea Br. Km 40
	2009	RPF	45	3	Borcea Br. Km 40
	2013	LPF	75.1	36	Danube RKM 630 & Borcea Br. Km 40
A. stellatus	2005	RPF	2.5	5	Danube RKM 153
	2005	DS2	5.5	5	Danube RKM 100 & Danube RKM 161
	2006	LPF	53.3	6	Danube RKM 161 & Borcea Br. Km 40
	2008	LPF	25	7	Borcea Br. Km 40
	2009	RPF	30	3	Borcea Br. Km 40
	2013	LPF	10	36	Danube RKM 630 & Danube RKM 310
	2013	LPF	3.2	12	Borcea Br. Km 40
	2014	LPF & RPF (1:1)	14.8	12	Danube RKM 100 & Borcea Br. Km 40
A. gueldenstaedtii	2005	RPF	2.5	16	Danube RKM 100
	2007	RPF	96.5	6.5	Danube RKM 630 & Borcea Br. Km 40
	2008	LPF	32.8	19	Danube RKM 630
	2009	RPF	50	3	Borcea Br. Km 40
	2013	LPF	5.1	36	Borcea Br. Km 40 & Sf. Gheorghe Br. Km 5
	2013	LPF	15.2	12	Danube RKM 630 & Borcea Br. Km 40
	2014	LPF & RPF (1:1)	94.8	12	Danube RKM 310 & Borcea Br. Km 40

[a] LPF – left pectoral fin, RPF – right pectoral fin; [b] thousands; [c] months, [d] 50% in LPF and 50% in RPF.

RESULTS AND DISCUSSION

Return of stocked sturgeons

During 42 consecutive days of scientific fishing in the Danube River 34 adult sturgeon individuals were captured: 4 Beluga sturgeons (2 females and 2 males), 27 Stellate sturgeons (10 females and 17 males), and 3 Russian sturgeon (all males). CWT reading revealed that 4 out of the 34 adult sturgeon individuals presented tags. Three of them were Russian sturgeons and one was a Stellate sturgeon.

All three captured Russian sturgeon specimens carrying CWT tags were males, having a total weight (TW) between 4.5 and 5.5 kg, and a total length (TL) ranging between 80 and 105 cm. Two of them were wearing tags in the left pectoral fin, and the third one in the right pectoral fin. Their age was estimated based on the von Bertalanffy growth curve, to 5 [t=4.95] years for one of them, and around 8 [t=7.67 and t=7.96] years for the other two. Both estimated age and CWT position were reliable tools for tracing the origins of the captured individuals back to one of the RSSSPs. Therefore, the 5-year-old Russian sturgeon was stocked during the 2014 RSSSP, at an age of one-year-old, in the Danube River (RKM 310) or Borcea Branch (Km 40). The other two were stocked during the 2013 RSSSP, at an age of three years old, in the Borcea Branch (Km 40) or Sf. Gheorghe Branch (Km 5). Overall, all three captured individuals presented good fitness, with values within the confidence interval range, close to the mean values (Figure 2A).

The Stellate sturgeon individual carrying a tag was a male at 5.8 kg TW and 130 cm TL. The estimated age was 12 years [t=11.84] and the position of the CWT was detected in the LPF. Both estimated age and CWT position indicated the 2006 RSSSP as the stocking program of its origin. In the 2006 RSSSP Stellate sturgeon were released into the Danube River (RKM 161) and Borcea Branch (Km 40) at an age of 6 months. In addition, another 26 Stellate sturgeon wild individuals (10 females and 16 males) were captured, with an estimated age ranging between 4 and 15 years. The length-weight relationship for all 27 individuals captured (wild and stocked) showed relatively good fitness, with values within the confidence interval range, but under the mean (Figure 2B).

In the Danube River, the last records of *A. gueldenstaedtii* offspring in the wild were in 2007 and 2010 (Suciu, 2008; Suciu and Guti, 2012). Therefore, any YOY recruitment observed since then, should be the result of an active spawning event of the stocked individuals. Young of the year (YOY) recruitment monitoring of *A. gueldenstaedtii* offspring did not identify any offspring in 2018. Consequently, the contribution of the stocked Russian sturgeon individuals to spawning events in the river could not be observed at this time.

On the other hand, for the Stellate sturgeon YOY recruitment was observed but the contribution of the stocked individuals to spawning events is uncertain. YOY recruitment for *A. stellatus* has been observed continuously since 2000. Therefore, we cannot assign these 2018 offspring to an active spawning event of the stocked individuals. At the same time, the hypothesis of active spawning events of the stocked individuals in the river cannot be refuted.

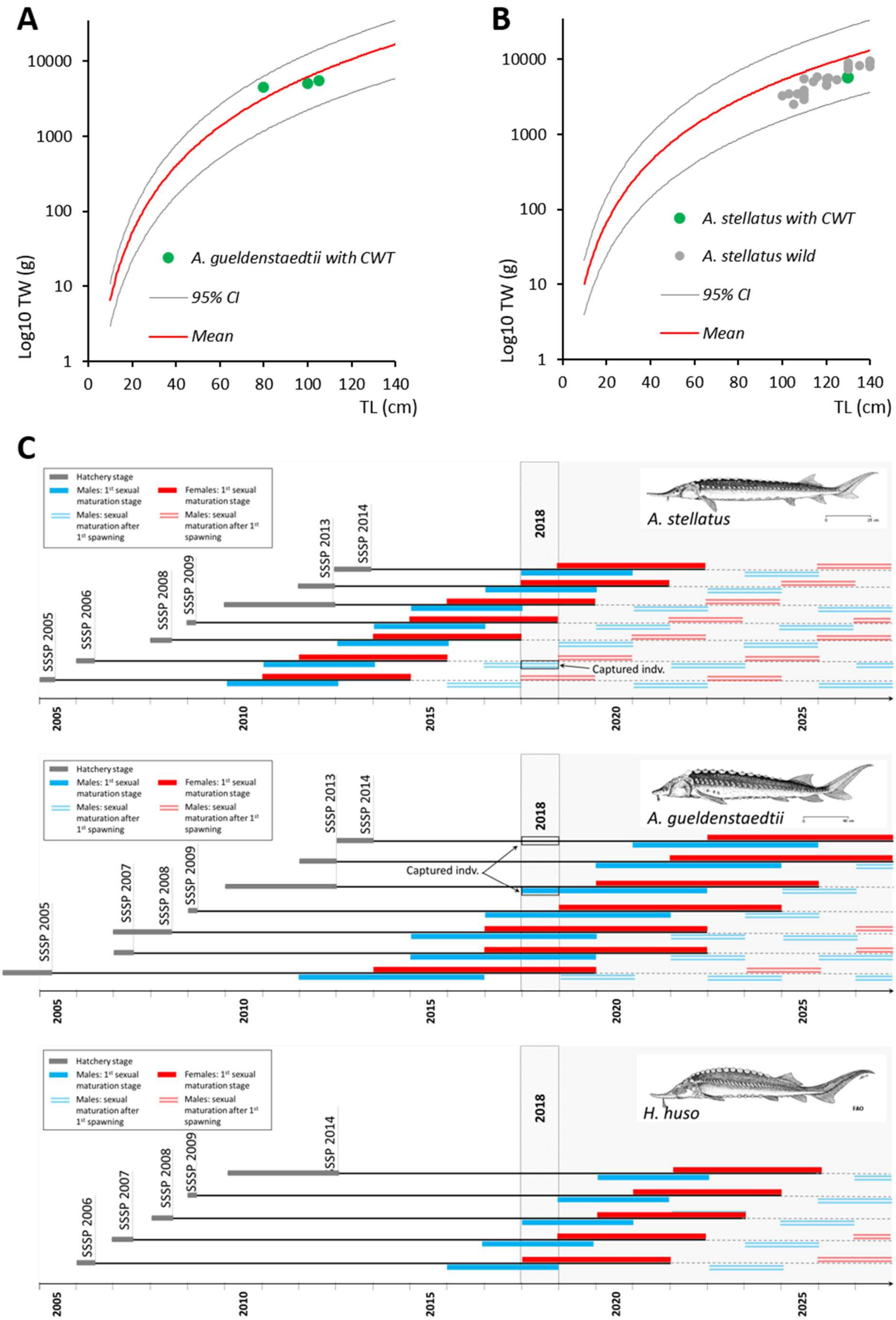
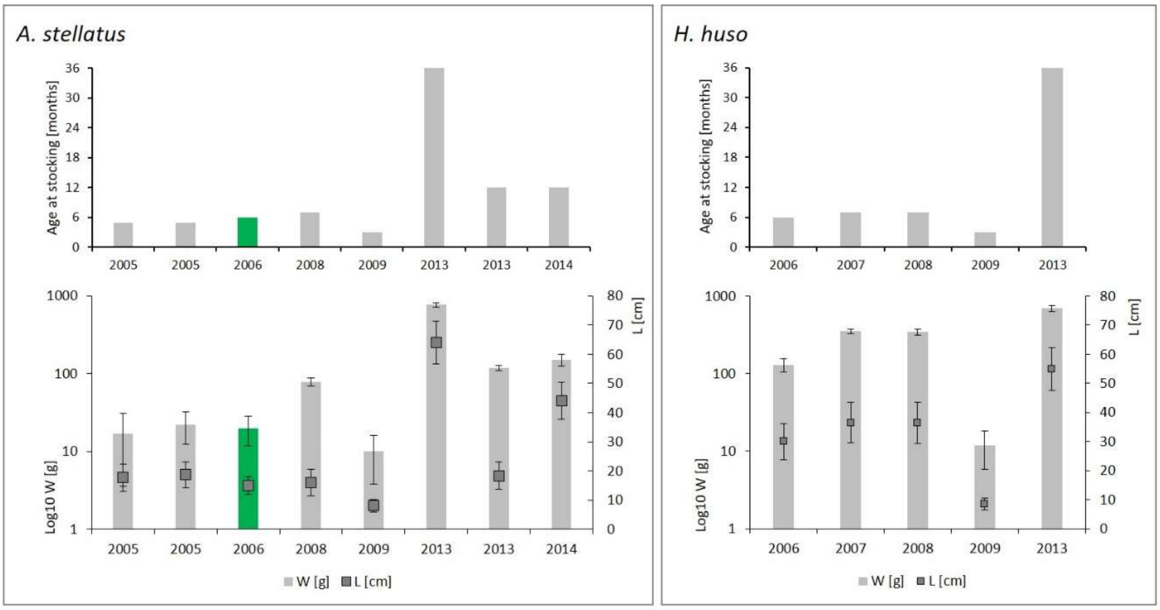

A
Log10 TW (g)
TL (cm)
A. gueldenstaedtii with CWT
95% CI
Mean
B
A. stellatus with CWT
A. stellatus wild
C
Hatchery stage
Males: 1st sexual maturation stage
Males: sexual maturation after 1st spawning
Females: 1st sexual maturation stage
Males: sexual maturation after 1st spawning
A. stellatus
A. gueldenstaedtii
H. huso
Captured indv.
2018
SSSP 2005
SSSP 2006
SSSP 2007
SSSP 2008
SSSP 2009
SSSP 2013
SSSP 2014
2005
2010
2015
2020
2025

Figure 2 Length – weight relationship of the captured individuals and RSSSPs outlines

RSSSPs: similarities and differences

Overall, the Romanian Sturgeon Supportive Stocking programmes exhibit a high degree of variability, in terms of stocking age, individuals' size (TW and TL), and even release sites. Due to lack of data regarding optimal parameters (age, size or distance to the sea) for stocking, a wide range of RSSSP variants were adopted to increase the likelihood of success. Therefore, sturgeon offspring were released at various ages, ranging between 3 months and 3 years old (Figure 2C). Likewise, the size of the individuals varied broadly, from 9-10 g at 10 cm, to 765 g at 64 cm (Figure 3). Besides that, release site distance to the Black Sea ranged between 5 km and 630 Km.



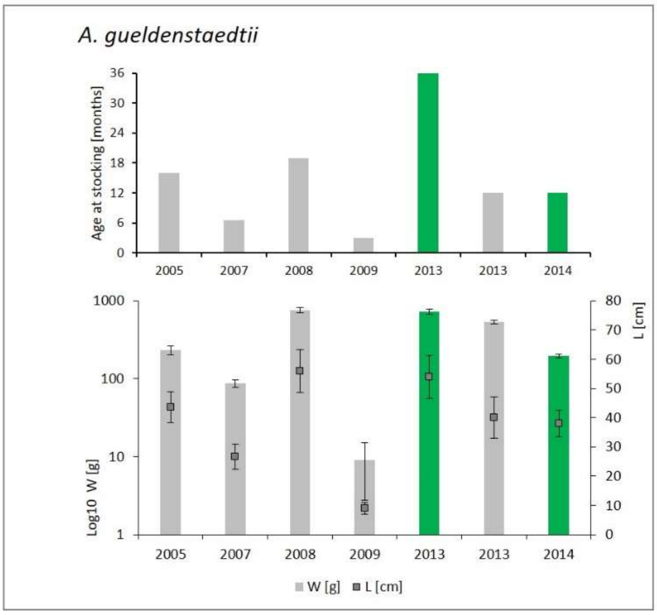


Figure 3 Age and mean length-weight relationship of the stocked individuals by species.

Since the 1950s, tremendous efforts have been made worldwide to restore sturgeon populations through numerous restocking or supportive stocking programmes, but until now none of them have been confirmed to have fulfilled their main goal. The crucial moment for all these programmes will come when hatchery-reared specimens return to their home river as adults and participate in spawning events.

To the best of our knowledge, this is the first report in the world on stocked sturgeons returning to their home river. Therefore, a big uncertainty presents itself: Is this first return of stocked sturgeons into their home river dependent on the intrinsic traits of the supportive stocking programme, or is it simply a matter of luck?

In the present study, the capture of 4 sturgeon individuals with stocking-programme origins, out of 34 Danube River upstream-migrants towards their spawning grounds, was recorded. The captures occurred during a period of 42 consecutive days of scientific fishing over an area of 50.23% of the river width. During the scientific fishing, 3 out of 4 native species were recorded: Beluga sturgeons (2 females and 2 males), Stellate sturgeons (10 females and 17 males), and Russian sturgeons (3 males). CWT reading revealed that all 4 individuals with stocking programmes origins were males, three Russian sturgeons and one Stellate sturgeon. Therefore, two expected questions emerge: Why so few? Why are all the captured stocked individuals' males? The number of stocked sturgeons caught seems to be quite low - only 4 out of 34 (11.76%). However, to explain why so few were caught we need to take into account two main factors: (*i*) the capture likelihood of the fishing area; and (*ii*) each sturgeon species' abundance. The fishing site for the adults was just a 7 km-long section of the Danube River (between RKM 120 and 127), and ~400 m wide (50.23 % of the fishing area, between RKM 120 and 127). In addition, only two boats were used, for 10 hours per day, to capture these individuals. Therefore, if we assume that sturgeons migrate upstream continuously, regardless of day or night, based on fishing conditions and effort deployed, only around 21% of the upstream migrants could be captured. Besides that, the abundance of each sturgeon species should also be taken into account. The Stellate sturgeon wild population in the Danube river has shown an increase in annual recruitment of offspring in the last years. Accordingly, the Stellate sturgeon exhibits naturally occurring spawning events, and thus a greater number of breeders migrating upstream towards spawning grounds. In this particular case, even though only one Stellate sturgeon individual presented a CWT out of 27 (representing 3.7%) was captured, a low proportion of stocked individuals should be expected due to wild population abundance. On the other hand, the Russian sturgeon case is very different, because 3 individuals were captured and all of them had been stocked. The Russian sturgeon population in the Danube River is almost extinct, the last records of naturally occurring spawning events were in 2007 and 2010 (Suciu, 2008; Suciu and Guti, 2012). Hence, the captured individuals represent not only the first recorded stocked individuals returning to their home river, but also a real chance for the restoration of the Russian sturgeon population into the Danube River. In other words, this is a game-changing moment for sturgeon restocking or supportive stocking programmes and for the fate of their target populations. Consequently, identifying the specific stocking programme traits which have produced this result is crucial and represents a real breakthrough in sturgeon conservation.

Based on analysis of the RSSSPs outlines, we failed to identify specific traits which have made possible the returns of the stocked individuals into their home river. As was presented above, the RSSSPs showed a high degree of variability in terms of stocking age, individual size, and even release sites. Moreover, the traits of the returned stocked individuals do not seem to follow any rule regarding the conditions of the restocking program. The age at stocking was 6 months for the Stellate sturgeon, and between 12 and 36 months for the Russian sturgeons. In addition, the fitness of the individuals varied broadly. Finally, neither the stocking density nor stocking sites seemed to influence supportive stocking success. A possible factor which influenced the returns of these individuals may be the presence of wild individuals. Social interactions have been proved to shape the timing of spawning migrations in anadromous fish such as salmons (Berdahl et al., 2017). Therefore, the presence of the returning wild spawners, which tend to swim upstream in small groups following females, could have influenced the stocked individuals to migrate through group social interaction. In several studies, "spawn-curious" immature sturgeons have previously been reported (Beamesderfer et al., 1995; Heironimus et al., 2015; Schulze, 2017). Such individuals migrate instinctively upstream with the mature ones. Our data also showed immature individuals migrating upstream with the adults - one stocked Russian sturgeon (5 years old) and 9 wild Stellate sturgeons (from 4 to 6 years old). Group behavior seems to play an important role in young sturgeons' upstream migration. Sturgeon males seem to be more susceptible to social interaction than females, regarding the upstream spawning migration, and therein lies a possible answer to why all captured stocked individuals were males. Likewise, the presence of wild individuals could have more weight in defining success likelihood than the intrinsic traits of the supportive stocking programs.

CONCLUSIONS

To the best of our knowledge, this is the first time in the world when the return of stocked sturgeons to their home river is reported. This is a game-changing moment for sturgeon restocking or supportive stocking programmes and for the fate of their populations. Consequently, identifying the main factors which have enabled this is crucial and represents a real breakthrough in sturgeon conservation. Our data suggest that the stocking program success is more likely dependent on the presence of wild individuals from whom stocked individuals can "learn" the way to the spawning grounds, than on the intrinsic traits of the stocking programme. Therefore, there is a need for additional studies to further understand if this return is an isolated case or it is the beginning of a better future for sturgeon populations in the Danube River. More information is also needed to assess the contribution of stocked individuals to spawning events.

ACKNOWLEDGMENTS

These findings were possible because of bycatch events in our ongoing ERA-Net COFASP genomics project „Whole genome-based improvement of two marine aquaculture sturgeon species (Acipenser oxyrinchus and Huso huso)", - STURGEoNOMICS."

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Received 06.12.2018

Revised 23.04.2019