Danube beluga sturgeon monitoring: genetic population structure and migration patterns

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Introduction

Monitoring so called fishery independent characteristics of sturgeon populations (Suciu 2008) in the Lower Danube River (LDR), including genetic population structure and migration of adults and young of the year (YOY), was considered crucial for making progress in understanding the life cycle of these critically endangered flagship species of the Danube (Reinartz et al. 2012). Tracking movements and migration patterns of adult sturgeons in the LDR using acoustic telemetry dates back to 1998 - 2000 (Kynard et al. 2002) and was restarted at larger scale in 2011 (Hontz et al. 2012), while systematic monitoring of downstream migration of YOY sturgeons is conducted by the Sturgeon Research Group (SRG) of the Danube Delta National Institute (DDNI) since 2000 (Paraschiv et al. 2006; Paraschiv 2011; Rosten et al. 2012). Studies of genetic population structure of endangered sturgeon species in the LDR started in 1999 (Ferguson et al. 2000) but the existence of reproductively isolated groups / sub-populations and preliminary spatial distribution in the sea and the river was first described only in 2012 (Holostenco et al. 2012, 2013; Onără et al. 2014). This article is an overview of recent work and publications of the DDNI SRG on genetic diversity and migration patterns of beluga sturgeons in the LDR.

Genetic diversity of beluga sturgeons

To study genetic diversity of downstream migrating YOY beluga sturgeons born in the LDR during 2004 – 2012 we performed PCR-RFLP screening of the mtDNA control region in 300 samples using restriction enzymes *BsrI* and *BspHI* (Onără et al. 2014). This revealed the existence of four different haplotypes exhibiting variable frequencies *(Figure 1)* in both adults and YOY (76/44% in haplo-1, 3/9% in haplo-2, 9/24% in haplo-3, and 12/23% in haplo-4). However, no significant differences in haplotype frequencies (P = 0.5) could be distinguished among the adults while tentatively divided as spring and autumn migrants according to their capture date records.

The Neighbour Joining (NJ) tree *(Figure 2)* constructed using DISPAN programme suite based on Nei's genetic distance (DA) (Nei 1972) for mtDNA control region shows a genetic relationship between the YOY born in consecutive years (e.g. 2004–2005, 2009–2010). We explain this finding to be a consequence of gradual sexual maturation of females of the same year class spawning in successive years. This demonstrates that early life stages of beluga sturgeon migrate downstream in groups of genetically related individuals, as indicated by mean genetic distance between individuals in group YOY-04-B (D^2_{sh} = 0.000), while in the other groups of YOY this parameter shows moderate relationship between individuals as a result of some overlapping of groups.

These preliminary results of genetic variability of the YOY beluga sturgeons suggest abandoning the hypothesis that the beluga sturgeon population spawns in the LDR as a

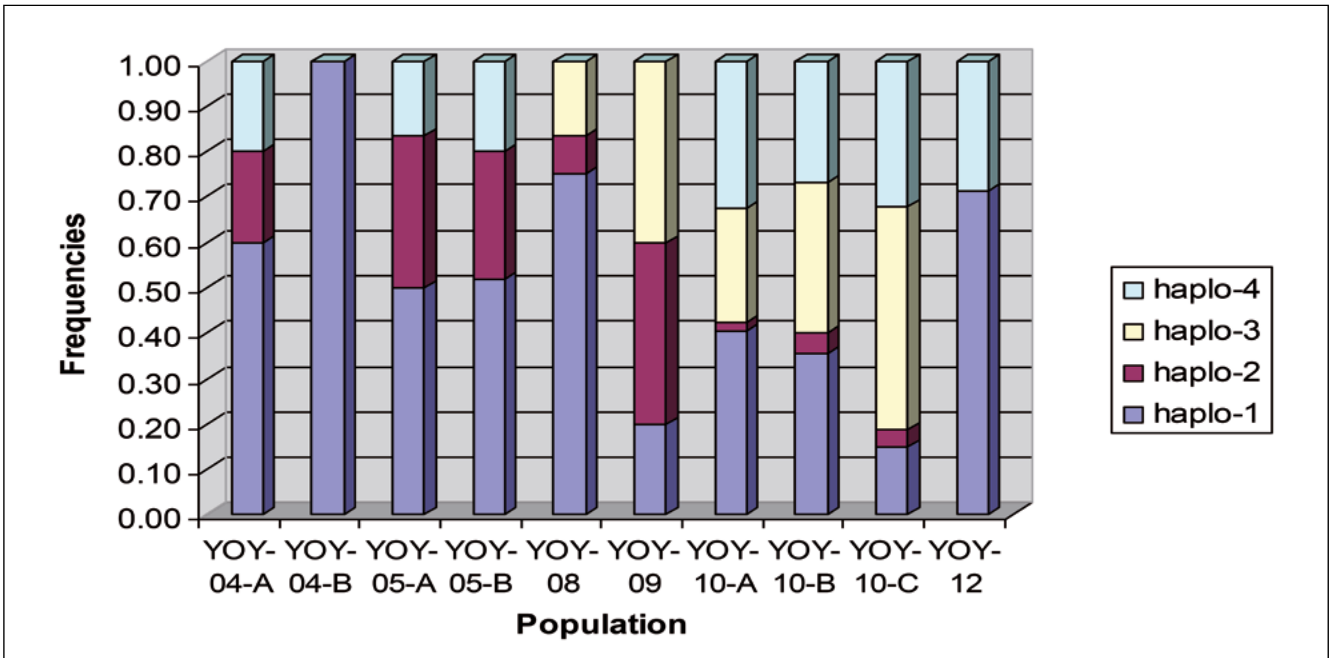

Figure 1. Haplotype frequencies of 10 beluga sturgeons YOY groups sampled in the LDR during 2004–2012 (Onără et al. 2014)

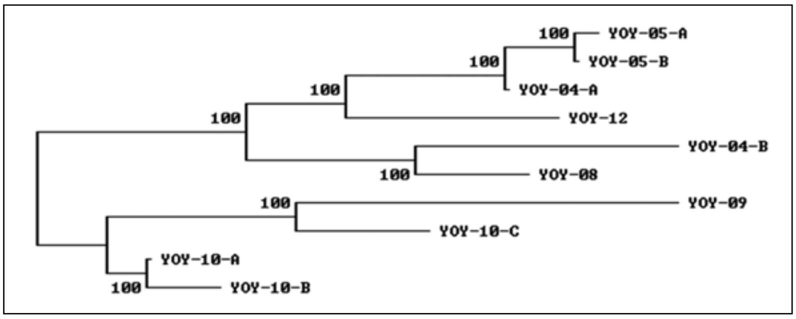

Figure 2. NJ dendrogram based on Nei's genetic distance (DA) (Nei 1972) of control region haplotypes in all 10 beluga sturgeon YOY groups. Bootstrap values are given at each node (Onără et al. 2014)

panmictic unit (Onără et al. 2014). Recently, by sequencing the enlarged control region of mtDNA in 300 YOY beluga sturgeons sampled during 2004–2015, we succeeded to demonstrate, with the cooperation of young geneticists of the University of Ferrara guided by Leonardo Congiu, that after 2006 the catch moratorium enabled a growing number of females to access spawning grounds in the LDR producing offspring. These findings at mtDNA level need to be confirmed by currently ongoing analyses at nuclear level, and if validated, they will indirectly show the positive effect of conservation measures implemented since 2006.

Migration patterns of adult and YOY sturgeons in the river

During ongoing acoustic telemetry studies of movements and behaviour of sturgeons arriving downstream of the Iron Gate 2 dams (rkm 863), we detected several beluga sturgeons carrying acoustic transmitters (Vemco, Canada) implanted by us (2012) and INCDPM (2013). Observations on three exemplary beluga sturgeon males, systematically dropping back after being tagged in the Borcea branch, show the extraordinary swimming capacity of these fish *(Table 1).*

Beluga male No.1 developed a ground speed of 41.5 km/day over a distance of 747 km, at a water temperature of 6.3 – 8 °C, needing only about 20 days from mid-March to early April to complete the journey from the sea to arrive at the Iron Gate 2 dams just in time for the spawning season.

So far we do not have a valid explanation for the behaviour of beluga male No. 2 which was recorded at Isaccea (rkm 100) in December 2013. It moved to the sea and returned two years later being recorded by the automatic receiver installed at Ruse (rkm 500) in November 2015. Then it completed the journey to the Iron Gate 2 dam in only 8 days moving upstream with a ground speed of 45.1 km/day, at a slowly decreasing water temperature of 10.6 to 8.6 °C. Finally, it returned slowly downstream (ground speed only 28 km/day) to the Borcea branch being recorded on December 14 by our receiver installed at km 15.

Beluga male No.3 was returning from upstream after the spring spawning season (late April – early May 2012). After being tagged on May 24, 2012 in the Borcea branch at a water temperature of 18.5 °C, it moved further downstream, in the beginning slowly (35 km/day) to the entrance of the Caleia branch (rkm 195), and then very fast (87.7 km/day), being recorded after 26 hours at Isaccea (rkm 100), on 29 May 2012. It returned to the river two years later during the fall migration being recorded at rkm 100 on 1 November 2014 and 8 days later at km 9.8 on Bala branch, upstream of the submerged sill (Déak & Matei 2015). It then stayed over winter somewhere upstream of the entrance to the Bala branch (rkm 345) and arrived next year for the spawning season (7–26 April 2015) in the vicinity of the Iron Gate 2 dams, being recorded by our automatic receiver installed

Species (fish code)	sex	Date of tagging	Location of releasing	History of recordings	Short description of u/s and d/s migration	Dist. [km]	Average ground speed [km/day]
1.Huso huso (13730/31)	male	Nov. 2013**	Borcea Branch≈ Danube River km 300	rkm 100 18 Nov. 2013 rkm 100 15 Mar. 2014 rkm 847 02 April 2014 rkm 860 05 April 2014 rkm 847 08 April 2014 rkm 100 26 April 2014	Moved d/s after tagging; returned next year in the spring; moved 747 km u/s in 18 days; moved d/s (747 km in 18 days) after spawning	747 23 23 747	u/s 41.5 u/s 7.7 d/s 7.6 d/s 41.5
2.Huso huso (13682/83)	male	Nov. 26, 2013*	Borcea Branch≈ Danube River km 300	rkm 100 01 Dec. 2013 B km 9.8 12 Nov. 2015* rkm 500 15 Nov. 2015 rkm 861 23 Nov. 2015 B km 15 14 Dec. 2015	After tagging moved d/s rkm 100; returned u/s in fall 2015 and arrived at Iron Gate 2 in Nov.; returned d/s in Dec.	361 588	u/s 45.1 d/s 28.0
3.Huso huso (14335/36)	male	May 24, 2012*	Borcea Branch≈ Danube River km 300	rkm 300 25 May 2012 rkm 195 28 May 2012 rkm 100 29 May 2012 rkm 100 01 Nov. 2014 B km 9.8 08 Nov 2014* rkm 847 07 Apr. 2015 rkm 847 26 Apr. 2015	Moved d/s after tagging and left the Danube River; returned to the river in fall of 2014; passed on Bala* branch and was wintering in the River u/s rkm 345; continued migration during 2015; arrived at rkm 847	95 219	d/s 87.7 u/s 27.5

**Déak & Matei (2015); ** INCDPM (2013)*

Table 1. Upstream (u/s) and downstream (d/s) migration of adult sturgeons recorded with acoustic telemetry (a few representative fish)

Origin	N	Age (month)	TW (g)	SL (cm)	Distance (km/days)	Ground speed (km/day ; m/sec)	Route
Wild	20	1.5	27	13.5	165/2	82.5 ; 0.95	Reni (rkm 123) → St. George (km 5)
Stocked / aquaculture	26	3.5	131	24	327/5	65.4 ; 0.75	Stelnica (rkm 300) → St. George (km 5)
Stocked / aquaculture		3.5	154	26	144/2	72.0 ; 0.83	Isaccea (rkm 102) → Sf. George (km 5)
Stocked / aquaculture *	100	18	460	48	655/10	65.5 ; 0.75	Ercsi / downstream of Budapest (rkm 1615) → Tekija / Serbia (rkm 960)

** Data by courtesy of Miklos Pannonhalmi / Water Directorate Györ, Hungary and Mirjana Lenhardt / IMSI Belgrade / Serbia*

Table 2. Downstream migration swimming speed of wild and stocked YOY beluga sturgeon (Huso huso) as recorded by Vemco acoustic telemetry transmitters and receivers (Rosten et al. 2011)

Origin	Releasing location	N	Chilia Branch (%)	St. George Branch (%)	Unknown (%)	Total
Wild	Reni rkm 123	20	2.2	26.1	15.2	43.5
Stocked	Borcea Branch km 40	13	15.2	4.4	8.7	28.3
Stocked	Isaccea rkm 102	13	23.9	2.2	2.2	28.3
		46	41.3	32.6	26.1	100

Table 3. Route taken by YOY beluga sturgeons as recorded by acoustic telemetry transmitters (June – August 2010) (Rosten et al. 2011)

at Pristol (rkm 847), while arriving, and 19 day later, passing downstream.

All three beluga sturgeons recorded by us arriving at Iron Gate 2 dams belong to the group of fall migrants. The migration pattern of these few fish confirms the hypothesis that beluga sturgeon males which enter the river during the fall migration season are long distance migrants. They stay over winter in the river (downstream of rkm 100 or upstream of rkm 345) and continue their migration in spring to the Iron Gate 2 dam.

Characteristics of downstream migration and route taken on the delta branches by YOY beluga sturgeons of wild (N = 34) and aquaculture (N = 26) origin was investigated during June – August 2010. After tagging with small acoustic transmitters (Thelma Biotel, Norway) they were released to the river at the monitoring site of rkm 123 / Reni (the wild YOY), on Borcea branch at km 40 / Stelnica, and rkm 102 / Isaccea (the YOY stocked from aquaculture). Average ground speed developed by YOY beluga varied from 82.5 km/day in the fish of wild origin to 65.4 – 72 km/day in those of aquaculture origin *(Table 2)*. Noteworthy is the finding that most (26%) of tagged wild YOY moved downstream on the St. George branch, while most of the stocked YOY released from Borcea km 40 (15%) and Isaccea rkm 102 (24%) moved on the Chilia branch *(Table 3)*.

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References

Déak G, Matei M (2015): Methods, techniques and monitoring results regarding the sturgeon migration on Lower Danube. http://www.afdj.ro/sites/default/files/prezentari/presentation_incdpm_deak_bern_convention_0.pdf

Ferguson A, Suciu R, Prodöhl P, Hynes R (2000): Genetic population structure of endangered sturgeon species of Lower Danube. Royal Society Joint Projects with Central / Eastern Europe and the former Soviet Union, Final report, August 2000: 15 pp

Holostenco D, Onara D, Taflan E, Suciu R (2012): Genetic diversity of adult stellate sturgeons captured in the Lower Danube River during 1998–2011. Book of Abstracts, 39th IAD Conference. Szentendre, Hungary: 44

Holostenco D, Onără D, Suciu R, Honț S, Paraschiv M (2013): Distribution and genetic diversity of sturgeons feeding in the marine area of the Danube Delta Biosphere Reserve, Romania. Scientific Annals of the Danube Delta Institute vol. 19: 25–34

Hontz S, Iani M, Paraschiv M, Cristea A, Tănase B, Bâdiliță A M, Deák G, Suciu R (2012): Acoustic telemetry study of movements of adult sturgeon in the Lower Danube River (Km 175 – 375) during 2011. Book of Abstracts, 39th IAD Conference, Szentendre, Hungary: 45

INCDPM (2013): Monitoring of Environmental Impact of the Works for Improvement of the Navigation Conditions on the Danube between Călăraşi and Braila, km 375 - km 175. http://afdj.ro/en/content/romomed

Kynard B, Suciu R, Horgan M (2002): Migration and habitats of diadromous Danube River sturgeons in Romania: 1998–2000. Journal of Applied Ichthyology 18: 529–535

Nei M (1972): Genetic distance between populations. American Naturalist 106: 283–292

Onără D, Holostenco D, Paraschiv M, Suciu R (2014): Preliminary genetic variability of Lower Danube River young of the year (YOY) beluga sturgeon *Huso huso* (Linnaeus, 1758) using mtDNA markers. Journal of Applied Ichthyology 30: 1286–1289

Paraschiv M, Suciu R, Suciu M (2006): Present state of sturgeon stocks in the lower Danube River, Romania. In: Proceedings 36th International Conference of IAD, Vienna: 152 – 158

Paraschiv M (2011): Downstream migration of young of the year (YOY) of sturgeons in the Lower Danube River. PhD thesis, Lower Danube University of Galatz: 115 pp

Reinartz R, Bloesch J, Sandu C, Suciu R, Lenhardt M, Guti G, Jahrl J (2012): Sturgeon conservation in the Danube River Basin: How to implement the Sturgeon Action Plan 2005. Limnological Reports 39, Proceedings of 39th IAD Conference, Szentendre, Hungary: 101–107

Rosten C, Suciu R, Hawley K, Holostenco D, Onara D, Paraschiv M, Iani M, Kristensen T, Taflan E (2011): Downstream movement of YOY beluga sturgeons in the Lower Danube River. International Conference on Conservation, Recovery and Sustainable use of Danube River Sturgeons March 29–April 2, 2011, Tulcea, Romania, (PP oral presentation, 17 slides)

Rosten CM, Onara D, Hawley K, Suciu R (2012): The status of Danube beluga sturgeon (Huso huso): Past, present and future. Vann, Oslo, 04: 523–534

Suciu R (2008): Sturgeons of the NW Black Sea and the Lower Danube River Countries. Case study, International Expert Workshop on CITES Non-Detriment Findings, Cancun / Mexico, November 17–22, 2008, 27 pp