

# 4.

## Distribution and genetic diversity of sturgeons feeding in the marine area of the Danube Delta Biosphere Reserve

HOLOSTENCO Daniela<sup>1</sup>, ONĂRĂ Dalia Florentina<sup>1</sup>, SUCIU Radu<sup>1</sup>, HONȚ Ștefan<sup>1</sup>, PARASCHIV Marian<sup>1</sup>

<sup>1</sup> - Danube Delta National Institute for Research and Development: 165 Babadag street, Tulcea - 820112, Romania

Address of author responsible for correspondence: HOLOSTENCO Daniela – Danube Delta National Institute for Research and Development: 165 Babadag street, Tulcea - 820112, Romania; e-mail: [dana.holostenco@indd.tim.ro](mailto:dana.holostenco@indd.tim.ro)

**ABSTRACT.** The NW Black Sea basin supports sympatric population of beluga (*Huso huso*), stellate sturgeon (*Acipenser stellatus*) and Russian sturgeon (*A. gueldenstaedtii*). These ecologically similar fish exhibit varying life strategies during their extended juvenile period and appear to overlap at some stages within the marine coastal waters. The aim of the study was to assess the distribution and genetic diversity of sturgeons feeding in the Black Sea coastal area of the Danube Delta Biosphere Reserve (DDBR) during 2012. A total number of 76 sturgeons (70 stellate, 5 beluga and 1 Russian sturgeon) were captured during three fishing expeditions along the Romanian Black Sea coast of the DDBR using two fishing methods: active (pelagic trawling) and passive fishing (bottom set trammel nets). A high number of stellate sturgeons were captured in Sahalin and Zatoane areas highlighting their importance as feeding sites for all life stages of this species. Capturing of only 5 beluga sturgeon specimens suggested that coastal waters are less important feeding areas for this species. Genetic diversity of stellate and beluga sturgeons was assessed by PCR-RFLP of mtDNA *D-loop* region. Six out of ten composite haplotypes were detected in stellate sturgeons and two composite haplotypes in beluga sturgeons. Further studies on the dynamics of sturgeon population, on behavioural and ecological factors influencing the seasonal distribution are prerequisite for improved management and sustainable use of these resources of the Black Sea.

**Key words:** wild sturgeons, genetic diversity, essential habitats, sturgeon feeding, Black Sea

### INTRODUCTION

Sturgeons are very primitive bony fish inhabiting the earth for more than 200 million years ([12]; [23]). They have undergone little morphological change indicating a very slow evolution earning their status as “living fossils”. The group is distributed exclusively throughout the Northern Hemisphere, living in large rivers, lakes, coastal waters, and interior seas of Europe, Asia and North America [23]. Most of the sturgeon species are anadromous, given that males and females when sexually mature return from the sea to the river where they were born. The fish group has been highly appreciated during the time for their eggs, the caviar, an expensive and highly nutritive delicacy [10].

Dramatically increasing price of caviar during the last two decades due to high market demand resulted in increased fishing pressure on sturgeon stocks worldwide [27]. This, combined with dam construction, river channelization and other man made alteration on key habitats [3], pollution of water bodies, significantly reduced their abundance worldwide ([8]; [18]). The group continues to struggle to reach sustainable population levels despite bans on sturgeon fishing for most sturgeon species issued worldwide [16].

Six native sturgeon species have been commercially harvested in the Danube River for more than 2000 years: the anadromous species beluga sturgeon (*Huso huso*), Russian sturgeon (*Acipenser gueldenstaedtii*), stellate sturgeon (*A. stellatus*), and the European Atlantic sturgeon (*A. sturio*) and the river resident ship sturgeon (*A. nudiiventris*) and sterlet (*A. ruthenus*) ([3]; [4]; [5]; [6]; [19]).

The human population along the Danube took advantage of their spawning migration in the river and by mid of 19th century depleted their stocks, and even drove two sturgeon species on the brink of extinction ([3]; [5]; [6]; [37]). Nowadays only three anadromous sturgeon species inhabiting the NW Black Sea are still spawning in the Lower Danube River (LDR): beluga sturgeon (*Huso huso*), stellate sturgeon (*Acipenser stellatus*), Russian sturgeon (*Acipenser gueldenstaedtii*) and resident species, *Acipenser ruthenus*. All of them, excepting the sterlet (listed as vulnerable), are listed in the Red list of IUCN as critically endangered species (IUCN Red List) [43].

Historically anadromous sturgeons had access to over 2,000 km of river habitats for spawning and nursery. The construction of Iron Gates dams at river km (Rkm) 943 in 1974 and at Rkm 864 in 1984 reduced dramatically their migration route [22] which for beluga it is documented to be as far as Bratislava (Rkm 1870), occasionally reached Austrian and even German stretch (Rkm 2320), and for Russian sturgeon upstream Bratislava (Rkm 1869). Population of stellate sturgeon was less affected by the dams construction from the historical migration route point of view, as the literature states that it was always rare in Middle and Upper Danube, occasionally ascending the river as far as Komarno [9].

Nevertheless, another major change that all sturgeon species of the Danube River are facing after the construction of dams is the altering of the riverbed structure downstream the dams where the essential habitats (wintering, spawning and nursery sites) are located. Therefore, location and recovery of current key habitats after dam constructions are essential tools for conservation and recovery of sturgeon stocks.

Weak or no fishing legislation in Romania until 2001 allowed the fishermen to deplete fish resources of the Danube River including sturgeons migrating for spawning in the river. Aiming to rehabilitate the depressed natural sturgeon populations spawning in the

LDR, in year 2006 the Romanian government issued a 10 year moratorium on commercial fishing of sturgeons and started implementing a large scale hatchery supplementation programme [19].

Despite the ten year sturgeon catch moratorium in Romania (since 2006), the ban of sturgeon fishery in Serbia in year 2009 and in Bulgaria (since 2011) with an extension of 4 years in year 2012 [37], the fishery management activities implemented in the four countries sharing sturgeon stocks of the LDR region, as required by CITES resolution Rez.12.7 (rev. COP 14) and agreed upon in the Regional Strategy [42], are still very poorly coordinated.

Other issues that sturgeon are facing nowadays, after ban implementations in countries along Danube are poaching and illegal trade of caviar which exist to a greater extent in the LDR. Investigations made by TRAFFIC and WWF shed light and well documented persisting sturgeon catches and a still flourishing black market for caviar and sturgeon products in the LDR, thus drawing attention on the importance of this facet of the sturgeon issue ([27]; [37]).

Aiming to conserve one of the last mainstays of these globally threatened fishes, the sturgeon population of the Black Sea spawning in the LDR, the Sturgeon Research Group within the Danube Delta National Institute have been carried out surveys to investigate sturgeon ecology during the last 19 years.

Some data have been achieved, in terms of essential habitats such as spawning habitats for beluga and sterlet sturgeon. Beluga sturgeon and sterlet share the same spawning grounds, in the same period of time as demonstrated by capturing sturgeon larvae at Rkm 310 (in years 2004 and 2007) and Rkm 100 (in years 2008 and 2010). Larvae were identified by PCR-RFLP method as being sterlet and beluga sturgeons (D. Onăra, unpublished data) [32].

For stellate sturgeon one spawning site was spotted on Borcea branch of Danube river at Km 57 – 55 using acoustic telemetry [26]. In year 2011 a group of stellate sturgeon was identified spawning in late June, in water temperature of 24-25 °C ([26]; [39]), although literature data indicates as spawning period April-May [33] and May-June at temperature between 17 and 23°C [36].

Combining acoustic telemetry techniques in 2011 ([26]; [39]) essential wintering site used by beluga sturgeon was identified on Bala branch at Rkm 7.7 and Rkm 5.4.

Nursery area for YOY was spotted in year 2000 by SRG at Rkm 123 and since then the spawning success and the recruitment of YOY for all four sturgeon species have been monitored at that particular location.

Significant information on sturgeon spawning behaviour showed up while monitoring the YOY at Rkm 123. For instance for beluga sturgeon spawning event take time one time per year and is water level and temperature depending [31], while for stellate sturgeon there are successive spawning events during one year and are not water level and temperature depending (D. Holostenco, unpublished data - [24]).

Populations within a sturgeon species, can differ in respect of timing of migrations, time spent in the river, and the spawning sites location. Protecting the genetic diversity of a species requires an understanding of these complexities, including specific life-stage habitat requirements, and appropriate management of any harvest regimes accordingly [29]. Genetic characterisation of a population became an essential tool for conservation and restoration programmes and to better evaluate the population status and the effects of management actions [1]. For fisheries management it is essential the knowledge of stock structure because failure to recognize existing subunits of an exploited stock may lead to overexploitation and, therefore, depletion of local populations or stocks ([11]; [21]). Identifying the genetic structure of a population, different groups / subpopulations that should be managed separately is crucial for improvement of sturgeon management in the LDR and the NW Black Sea.

Within the area of conservation genetics, molecular markers play an important role in helping us understanding sturgeons at many different levels. Information about intraspecific genetic variability is required to identify populations that should be managed as distinct stocks or provide information about populations that may be suitable genetic sources for restocking of depleted areas [23].

Recent data on screening the genetic variability at mtDNA D-loop region in stellate and beluga sturgeon spawning in the LDR (D. Onăra, unpublished data [25]; [32]) found 10 and 4 maternal lineage for stellate and beluga sturgeon respectively.

As populations can differ on the timing of migrations into river systems, the two separate groups of autumn and spring migrants documented for beluga sturgeon, Russian sturgeon and stellate sturgeon, need to be managed separately. Moreover, the existence of successive spawning events for stellate sturgeon in LDR was identified based on mitochondrial markers (D. Holostenco, unpublished data).

The population status of Russian sturgeon assessed by estimation of annual spawning success during the last ten years and recording of legally captured sturgeons in Romania during 2002 – 2005 indicates a severe decline of stocks. Due to the very limited number of adults migrating for spawning, the population size sharply declined in the last decades [39].

Despite the increasing progress on identifying and describing the essential habitats (spawning, feeding and wintering sites) and genetic structure of the sturgeon populations in the LDR, limited data is available regarding the ecology of the sturgeons in the Black Sea. Although sturgeons spawn in fresh water, the greatest part of their lifespan occurs in the sea.

Literature data indicates that juveniles of the anadromous sturgeon species of LDR undertake downstream migration during their first summer (year), feeding close to the river mouth before moving to the sea [2].

This study is the first attempt aimed to investigate the distribution and the genetic diversity of sturgeon juveniles feeding in the in the marine area of the Danube Delta Biosphere Reserve.

## MATERIALS AND METHODS

Three fishing expeditions were organised by the Sturgeon Research Group (SRG), within the Danube Delta National Institute, in April, July and September, in year 2012. Two fishing methods were used: active (demersal trawling during the day) and passive (bottom set trammel nets of 95 x 3.5 m and different mesh size: 30, 32 and 34 mm during the night) fishing for capturing sturgeon specimens in 10 areas along the Romanian Black Sea coastal waters in year 2012. The ten areas of the Black Sea that were investigated in this study are presented in Fig. 8. Fish were captured at different water depth. Stellate sturgeon were captured at 3.5-12.5 m, Russian sturgeon at 4.5 - 4.7 m and beluga sturgeon at 3.1 - 6.5 m water depth.

All turgeon specimens were measured, photographed, and fin clips were sampled for DNA analysis. The sturgeon juveniles were tagged with Floy Fingerling Tag (FFT), while adults and sub-adults with Floy T- Barr Tag (FTBT), before releasing back in the Black Sea.

70 stellate sturgeon, 5 beluga sturgeon and 1 Russian sturgeon were captured and sampled in the ten locations in the Black Sea during the three fishing expedition. Number of specimens, period of capture and total weight of fish are presented in Table 1.

**Table 1.**  
Data base of sturgeon captured in the 10 areas of the Black Sea coastal waters. Sturgeon species, number and total weight (TW) of fish are illustrated.

Season	<i>Acipenser stellatus</i>						<i>Huso huso</i>		<i>A. gueldenstaedti</i>		Total
	spring (22 <sup>nd</sup> - 29 <sup>th</sup> April)		summer (19 <sup>th</sup> July)		fall (25 <sup>th</sup> - 28 <sup>th</sup> September)		summer (19 <sup>th</sup> July)		summer (19 <sup>th</sup> July)		
Fishing area	No. of fish	TW (g)	No. of fish	TW (g)	No. of fish	TW (g)	No. of fish	TW (g)	No. of fish	TW (g)	No. of fish
1-Vadu	1	360	-	-	-	-	-	-	-	-	1
2-Chituc	1	270	-	-	-	-	-	-	-	-	1
3-Periboina	-	-	-	-	1	153	-	-	-	-	1
4-Portita	-	-	-	-	-	-	-	-	-	-	0
5-Periteasca	-	-	-	-	-	-	-	-	-	-	0
6-Perisor	-	-	-	-	3	0.65;1;3	-	-	-	-	3
7-Zaton	2	290; 1000	2	315; 1900	14	-	-	-	-	-	18
8-Sacalin	-	-	33	54.5 - 95	9	-	1	247	1	494	44
9-Sfantu Gheorghe	1	420	-	-	-	-	1	19	-	-	2
10-Sulina	1	220	-	-	2	-	1	100000	-	-	4
Total number	6		35		29		3		1		74

Genomic DNA was extracted from fin clips using phenol chloroform protocols according to [40]. The D-loop gene region was screened for variability by Polymorphism Chain Reaction – Restriction Fragment Length Polymorphism (PCR-RFLP) using primers and conditions in (D. Holostenco, unpublished data - [24]; [25]).

### Data analysis

Mitochondrial haplotype frequencies of stellate sturgeons were assessed using the Excel add-in programme "Haplotype Analysis" [14] and MS Excel.

## RESULTS

70 stellate sturgeon, 5 beluga sturgeon and 1 Russian sturgeon were captures during the three expedition in 8 of the 10 area investigated by active and passive fishing in the Romanian coastal area of the Black Sea (Fig. 1).

Distribution of total length vs total weight was calculated for stellate sturgeon (N=70) captured in the coastal area of the Black Sea and is represented in Fig. 2.

$GT (g) = 0.0002 TL^{3.5696}$  describes the regression ( $R^2 = 0.9624$ ) between length and weight for stellate sturgeon. The low number of beluga sturgeon captured in the study did not allow this analysis.

### Genetic diversity of sturgeon captured in the coastal water of the Black Sea

#### Beluga sturgeon

Two restriction enzymes were used to assess the polymorphism of *mtDNA D-loop region* in beluga sturgeon: *BsrSI* (Promega R7241) and *BspHI* (NEB R0517). 2 composite haplotypes (*BsrSI*, *BspHI*) were identified: haplo-1 (AB) and haplo-3 (BA) despite the low number of beluga specimens (N=3) captured in this study.

Previous studies (D. Onăra, unpublished data - [30]; [32]) identified 4 composite haloptyes D-loop (*BsrSI*, *BspHI*) for beluga sturgeon population of the LDR during 2004 – 2010 (N=275), haplotype I being the most frequent one (57.82%) and the other three haplotypes with frequency between 17.82 % and 8.73%.

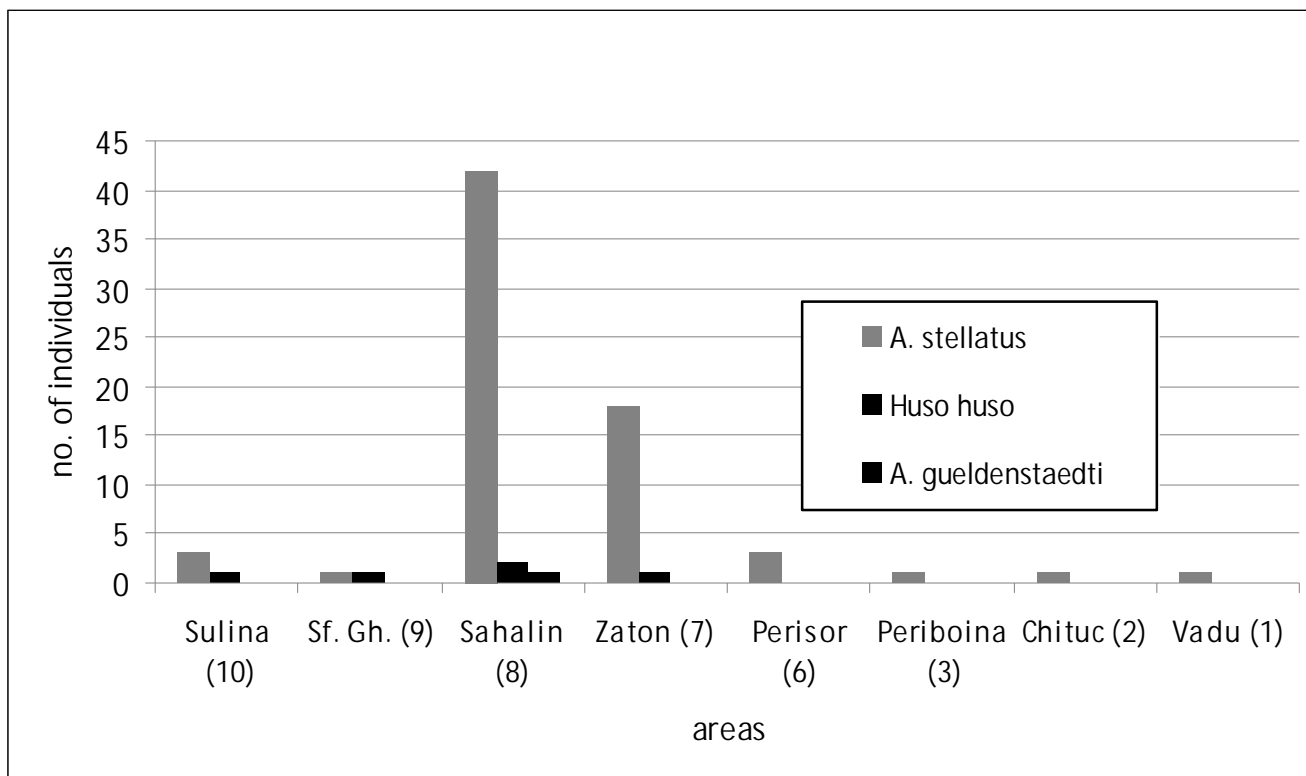


Fig. 1. Distribution of sturgeon specimens (N=76) captured in the 8 out of 10 locations in the Romanian coastal area of the Black Sea during April – September 2012.

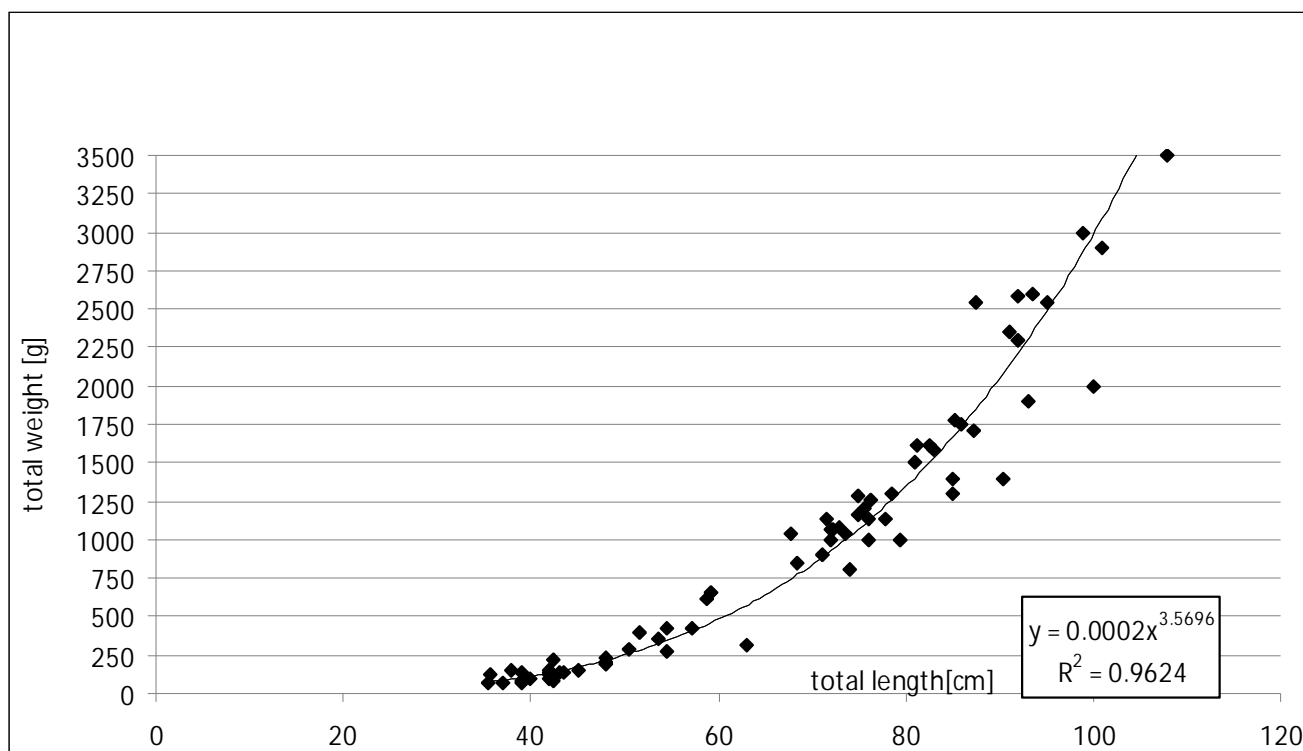
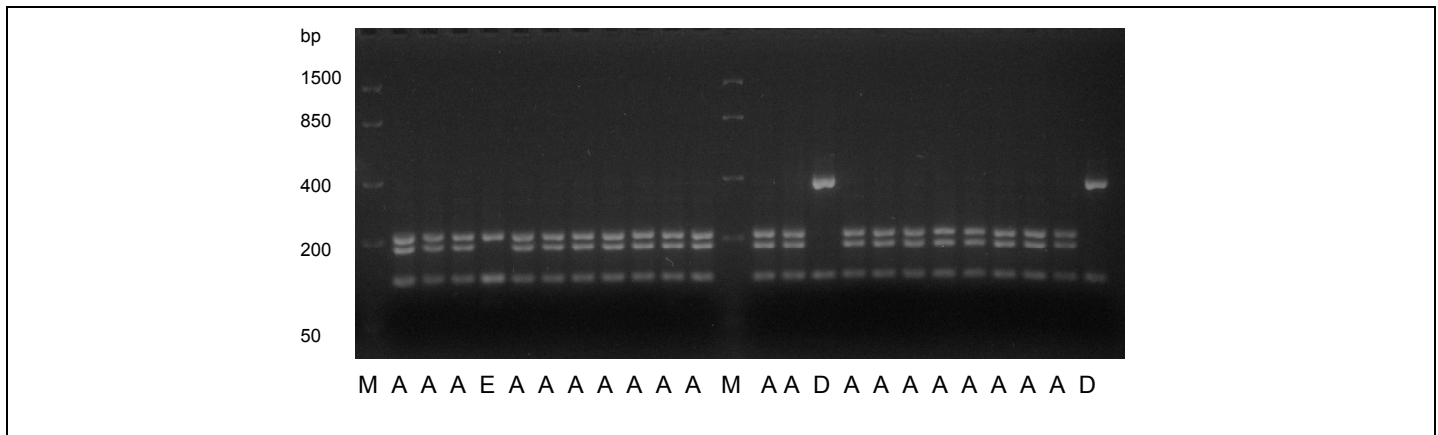


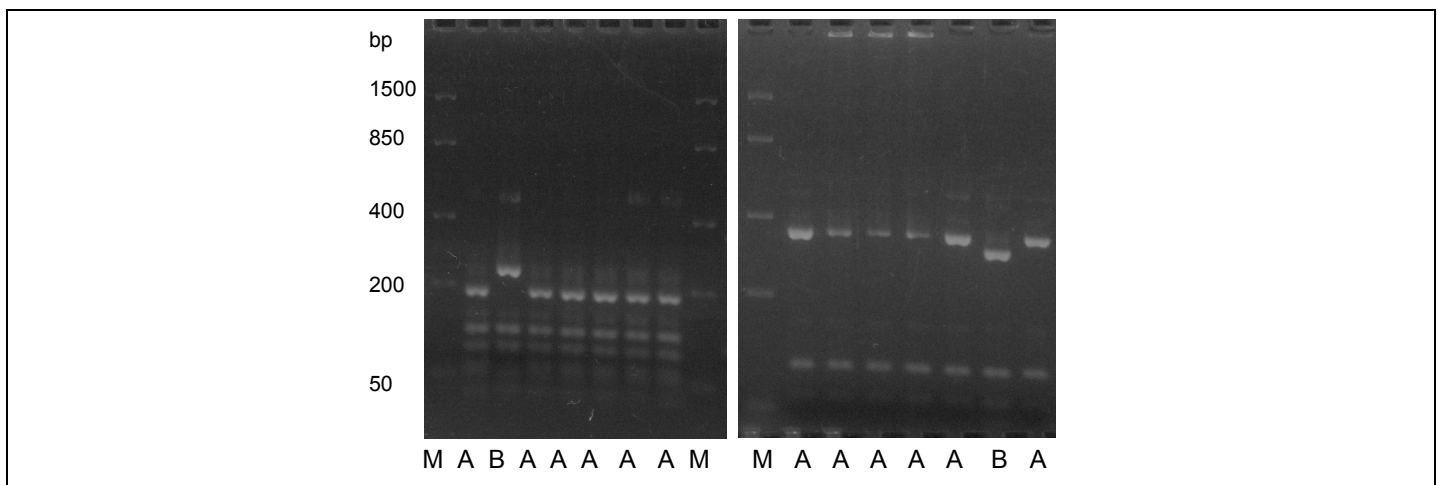
Fig. 2. Regression analysis between total length (TL) and total weight (TW) of stellate sturgeon captured in the current study.

**Stellate sturgeon**

Three restriction enzymes were used to assess the polymorphism at *D-loop region of mtDNA*: *HaeIII* (Promega R6171), *BsII* (NEB R0555) and *BsrSI* (Promega R7241) (D. Holostenco, unpublished data - [24]). Four haplotypes were identified with *HaeIII* (A, C, D, E), 2 haplotypes (A, B) with *BsII* and 2 haplotypes (A, B) with *BsrSI*. (Fig. 3 and Fig. 4).



**Fig. 3.** *D-loop* mtDNA *HaeIII*, RFLP patterns for stellate sturgeons under study (The haplotype designation is depicted below the picture); M - FastRuler Low Range DNA Ladder, ready-to-use (Fermentas - SM1103)



**Fig. 4.** *D-loop* mtDNA *BsII* (left) and *BsrSI* (right) RFLP patterns for stellate sturgeons under study The composite haplotypes were assigned by letters indicating the restriction fragment patterns obtained with *HaeIII*, *BsII* and *BsrSI*; M - FastRuler Low Range DNA Ladder, ready-to-use (Fermentas - SM1103).

Screening the genetic diversity of stellate sturgeon adults captured and sampled in the LDR during 13 years (1998 - 2011), 10 composite haplotypes *D-loop* (*HaeIII*, *BsII*, *BsrSI*) were identified (haplo-1 - haplo-10) [25]. Additionally, a novel haplotype designated as haplo-11 was identified in the current study. Restriction fragment sizes of amplified PCR products obtained by RFLP [bp] are summarized in **Table 2**.

**Table 2.**

**Stellate sturgeon restriction fragments of 7 composite haplotypes obtained by RFLP of amplified PCR products identified among individuals captured in the Black sea Coast.**

Composite haplotypes	Restriction enzymes		
	<i>HaeIII</i>	<i>BsII</i>	<i>BsrSI</i>
haplo-1 (AAA)	209,183,114	188,107,79,57,38,30,9	357,95,60
haplo-2 (ABA)	209,183,114	245,107,79,38,30,9	357,95,60
haplo-4 (CAA)	281,114, 113	188,107,79,57,38,30,9	357,95,60
haplo-5 (AAB)	209,183,114	188,107,79,57,38,30,9	315,95,60,41
haplo-7 (DAA)	394,114	188,107,79,57,38,30,9	357,95,60
haplo-10 (DBA)	394,114	245,107,79,38,30,9	357,95,60
haplo-11 (EAA)	209, 114, 114, 68,	188,107,79,57,38,30,9	357,95,60

Six out of ten composite haplotypes identified so far among stellate sturgeon sampled in the LDR and a new composite haplotype, haplo-11 were identified in the coastal water of the Romanian Black Sea in year 2012 (haplo-1, haplo-4, haplo-5, haplo-7, haplo-10 and haplo-11). Their frequencies are presented in Fig. 5 (compared with composite haplotype frequencies in the LDR) and separately, only for the Black Sea sector in Fig. 6.

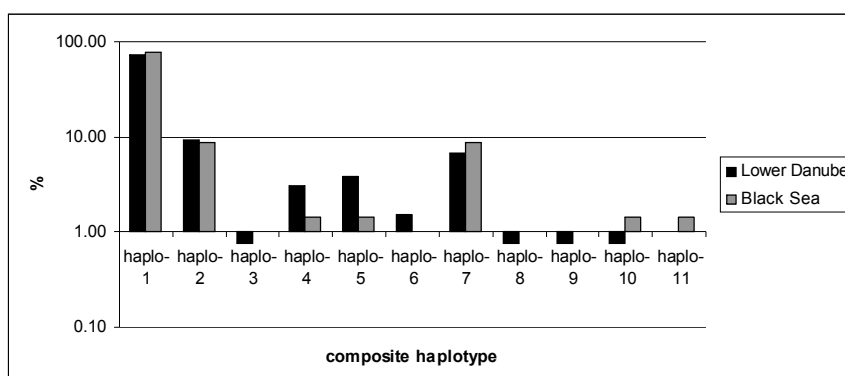


Fig. 5. Frequency (log scale) of composite haplotype D-loop (*HaeIII*, *BstI*, *BsrSI*) frequency of the stellate sturgeons captured in the LDR vs Black Sea (N=213)

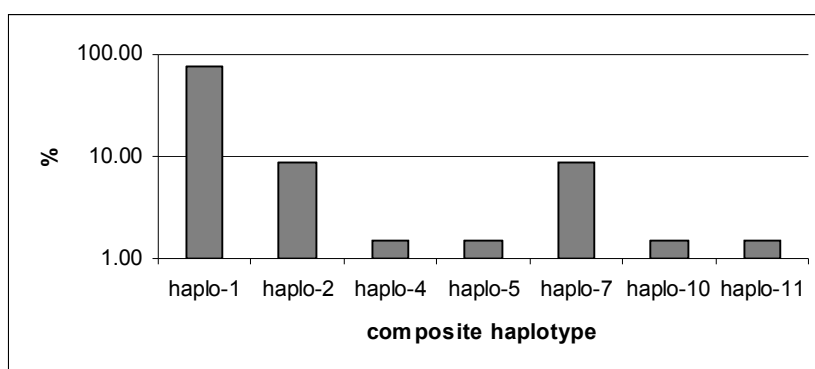


Fig. 6. Frequency of composite haplotype D-loop (*HaeIII*, *BstI*, *BsrSI*) of stellate sturgeon juveniles captured in the coastal area of the Romanian sector of the Black Sea in year 2012(N=69).

Distribution of the composite haplotype frequency was plotted per each fishing areas in the Black Sea coast under study as is illustrated in Fig. 9.

## DISCUSSIONS

10 areas of the Romanian Black Sea coast were selected for evaluation of sturgeon populations feeding in the costal areas. 8 out of the 10 assessed areas were confirmed as feeding habitats for sturgeon by capturing juveniles and / or sub-adults and adults (Table 1).

Out of the 76 sturgeon specimens were captured in the coastal zone of the Romanian Black Sea sector, 70 specimens were stellate sturgeon. A good correlation ( $R^2 = 0.9624$ ) between length and weight was found in stellate sturgeon.

None of the stellate sturgeon captured during the study in the Black Sea coastal waters were born in the river in year 2012. Monitoring the natural spawning and annual recruitment at Danube River Km 123 since year 2000, all stellate sturgeon YOY were having total weigh between 1g and 39.5 g. The current study confirms previous literature data ([2]; [7]) which states that stellate sturgeon specimens are feeding at the river mouth until they reach 3 - 4 years old.

Recruitment from natural spawning of the four sturgeon species in the LDR monitored by DDNI Tulcea during 2000 – 2011 (Fig. 7) shows high/normal values in year 2000, 2005 and 2010 of natural spawning success in sterlet and beluga sturgeon [39]. Smaller CPUE values were determined in case of stellate sturgeon and Russian sturgeon. However, data concerning the species ecology of stellate sturgeon and Russian sturgeon in the Danube River is still limited. Unlike the beluga sturgeon YOY that migrate downstream in crowds [34], the stellate sturgeon larvae lift in the water column and drift downstream. Therefore, the probability to capture stellate sturgeon YOY in the Danube River is lower than in case of beluga sturgeon.

Although the number of stellate sturgeon YOY captured during the annual monitoring of natural spawning success is relatively low (e.g. 0.2 CPUE in 2011), capturing of 21 stellate sturgeon juveniles feeding in the river mouth is a positive indicator with respect to spawning success of stellate sturgeon in the LDR.

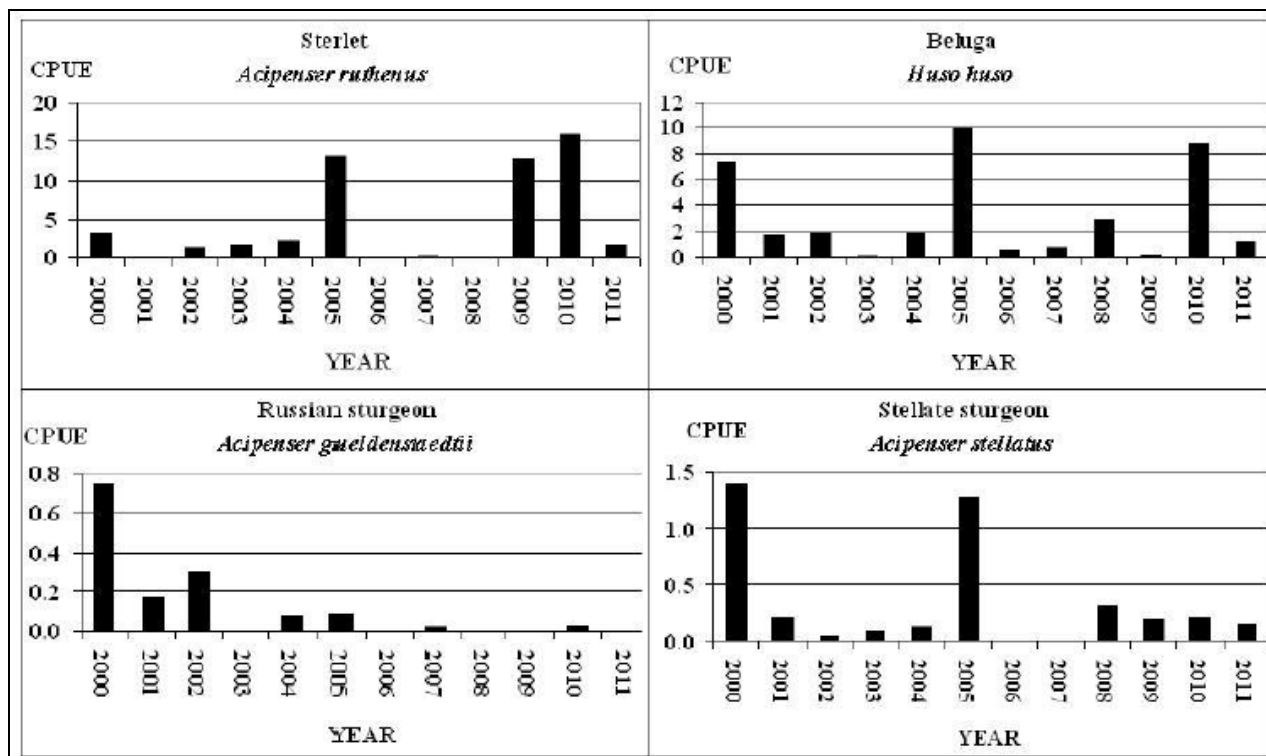


Fig 7. Recruitment from natural spawning of the sturgeon species in the LDR during 2000 – 2011.

Note. CPUE (Catch Per Unit of Effort) [39].

The low number of beluga sturgeons captured in this study indicates that the species do not use any of the ten areas for feeding.

Previous studies on screening genetic diversity of beluga sturgeon migrating for spawning in the LDR (2004-2010) identified 4 composite haplotypes at *D-loop* region of mtDNA, haplo-1 being the most frequent one (76.92%) (D. Onăra, unpublished data - [32]).

Despite the very low number of beluga (N=3) captured in this study, two haplotypes were identified, haplo-1 the most frequent one and haplo-3 which is a rare haplotype detected in the LDR.

Six out of ten composite haplotypes identified in the LDR and a new composite haplotype were identified by screening genetic diversity at mtDNA *D-loop* region using PCR-RFLP methods in the Black Sea areas within the study. The most frequent haplotype, haplo-1 (over 70%) found in the LDR is the most frequent one in the batch of sample from the coastal area of the Black Sea and is present in all 8 areas where stellate sturgeon juveniles were captured except one, Vadu where only haplo-4 was detected.

Comparing the haplotype diversity of stellate sturgeon in the LDR (11 haplotypes) and in the Black Sea in 2012 (seven haplotypes) we can assume that adults having the same haplotypes identified in juveniles captured in the Black Sea successfully spawned in year 2012 or only those adults were migrating for spawning in the LDR in 2012.

60 stellate sturgeon were captured in two locations in the Black Sea: Sahalin (area 8), where the greatest genetic diversity was detected (Fig. 6) six out of seven haplotypes and Zatoane (three haplotypes) (area 7). The great number of stellate sturgeon juveniles in the two areas suggests that the two locations are the main feeding areas used by stellate sturgeon.

Estuarine area (freshwater / saltwater boundary) are used by other sturgeon species in early life stages such as: Atlantic sturgeon (*Acipenser oxyrinchus*) and shortnose sturgeon (*Acipenser brevirostrum*) juveniles use the area as feeding grounds ([13]; [17]; [20]; [28]), gulf sturgeon (*A. oxyrinchus desotoi*) juveniles use the estuarine area as wintering site [35]. European sturgeon (*A. sturio*) juveniles spend their first years in the brackish waters (5‰ to 25‰) of the estuary zone before moving out to sea ([38]; [41]). Earlier study investigating the stomach content of juvenile sturgeon [15] revealed that stellate and Russian sturgeon juveniles used to forage for little crustaceans and worms (polychaetes) in the river mouth area of the Black Sea. Therefore the river mouth represent a key location as feeding, nursery, wintering habitats for juveniles and need special protection measures.

## CONCLUSIONS

This is the first attempt aiming to investigate the distribution and genetic diversity of sturgeons in the Black Sea coast.

Although three fishing expeditions during one year of study is not much in terms of assessing the real distribution and genetic structure of sturgeon population in the Danube River basin, this study revealed some noteworthy conclusion.

Investigating the distribution of juvenile sturgeons at the Black Sea Romanian coast in study areas, 2 areas of the Black Sea coast were identified as feeding grounds especially for stellate sturgeon, Sahalin, Zatoane, Perisor, that need to be protected while fishing for other fish species in the Black Sea coastal waters.

Screening the genetic diversity of sturgeon specimen at the Black Sea 2 haplotypes were identified in Beluga sturgeon despite the low number of samples (N=3). Seven haplotypes were found in stellate sturgeon and their diversity is higher in Sahalin and Zatoane areas of the Black Sea coast.

MtDNA markers proved to be valuable tools to assessing the genetic diversity of one population which is imperative for monitoring the status of population and implementation conservation and recovery plans.

Even though the period of the study was short it shed light for the first time on the genetic diversity and distribution of sturgeon juveniles along the coast of the Black Sea.

Assessing the efficiency of the two fishing methods for capturing sturgeon specimens in the coast zone of the Black Sea, it turned out that passive fishing was more efficient (71 specimens captured) than active fishing (5 specimens).

Monitoring studies for a longer period of time (couple of years) may give valuable information on the dynamics of sturgeon juveniles born in the river as a prerequisite for better management and sustainable use of these valuable resources of the Black Sea basin.

**ACKNOWLEDGEMENTS.** This work was performed within the subcontract for "Evaluation of sturgeon and Pontic shad populations feeding in the marine area of the Danube Delta Biosphere Reserve ROSCI0066" conducted in year 2012. Special thanks go to Marian Iani who collected samples for DNA analysis and established the data base, and to Elena Taflan who extracted the DNA and helped in the work within the Molecular Biology Laboratory.

## REFERENCES

- [1]. ANDERS (P. J.), DRAUCH-SCHREIER (A.), RODZEN (J.), POWELL (M. S.), NARUM (S.), CROSSMAN (J. A.), 2011 - A review of genetic evaluation tools for conservation and management of North American sturgeons: roles, benefits, and limitations. IN: *Journal of Applied Ichthyology*, vol. 27, Supplement S2, pp. 3 – 11.
- [2]. ANTIPA (G.), 1909 – Fauna ihtiologică a României. 361 p. Publisher: Institutul De Arte Grafice "Carol Göbl". Bucuresti, România. [in Romanian]
- [3]. BACALBAȘA-DOBROVICI (N.), 1997 – Endangered migratory sturgeons of the lower Danube River and its delta. IN: *Environmental Biology of Fishes*, vol. 48, No. 1, pp. 201 – 207.
- [4]. BACALBASA-DOBROVICI (N.), 2002 – Endangered migratory sturgeons of the lower Danube River and its delta. IN: *Sturgeon Biodiversity and Conservation* (eds. Birstein V., Waldman J. and Bernis W.), pp. 201 – 207. Publisher: Springer. The Netherlands.
- [5]. BALON (E. K.), 1963 – Urgeschichte de Donau – Ichthyofauna (vor dem Einfluß seitens des Menschen). IN: *Archiv für Hydrobiologie*, Suppl. XXXIV (Donauforschung III), No. 3, pp. 204 - 227.
- [6]. BALON (E. K.), 1968 – Von dem Schicksal des Hausens, der anderen Störe und weiterer Donaufische. IN: *Einfluß des Fischfanges auf die Fischgemeinschaften der Donau* (ed. BALON E. K.). IN: *Archiv für Hydrobiologie*, Suppl. XXXIV (Donauforschung III), vol. 3, pp. 228 – 249.
- [7]. BĂNĂRESCU (P.), 1964 - Pisces-Osteichthyes. IN: *Fauna Republicii Populare România*, vol. XIII. 962 p. Publisher: Editura Academiei Republicii Populare Române. [in Romanian]
- [8]. BILLARD (R.), LECOINTRE (G.), 2001 - Biology and conservation of sturgeon and paddlefish. IN: *Reviews in Fish Biology and Fisheries*, vol. 10, No. 4, pp. 355 – 392.
- [9]. BLOESCH (J.), JONES (T.), REINARTY (R.), STRIEBEL (B.), HOLCIK (J.), KYNARD (B.), SUCIU (R.), WILLIOT (P.), 2006 – Action Plan for the conservation of Sturgeons (Acipenseridae) in the Danube River Basin. IN: *Nature and environment*, no. 144, 121 p. Council of Europe.
- [10]. CAREY (R. A.), 2005 – The Philosopher Fish. Sturgeon, Caviar and the Geography of Desire. pp. 104 – 105. Counterpoint, A member of the Perseus Books Group, New York.
- [11]. CARVALHO (G. R.), HAUSER (L.), 1994 – Molecular genetics and the stock concept in fisheries. IN: *Reviews in Fish Biology and Fisheries*, vol. 4, No. 3, pp. 326 – 350.
- [12]. CHOUDHURY (A.), DICK (T. A.), 1998 – The historical biogeography of sturgeons (Osteichthyes: Acipenseridae): a synthesis of phylogenetics, palaeontology and palaeogeography. IN: *Journal of Biogeography*, vol. 25, No. 4, pp. 623 – 640.
- [13]. COLLINS (M. R.), SMITH (T. I. J.), 1997 - Management Briefs: Distributions of Shortnose and Atlantic Sturgeons in South Carolina. IN: *North American Journal of Fisheries Management*, vol. 17, No. 4, pp. 995 - 1000.
- [14]. ELIADES (N. - G.), ELIADES (D. G.), 2009 - "Haplotype Analysis: Software for Analysis of Haplotype Data". Distributed by the authors. Forest Genetics and Forest Tree Breeding, Georg-August University Goettingen, Germany. <http://www.uni-goettingen.de/en/134935.html>
- [15]. ENE (C.), SUCIU (R.), ENE (F.), DUMITRACHE (C.), 1996 – Cercetari asupra sturionilor marini migratori in Dunare (I). Date recente privind hrana sturionilor in zona gurilor Dunarii. IN: *Analele Stiintifice ale Institutului Delta Dunării (1995)*, vol. IV, No. 1, pp. 91 – 94. Tulcea, Romania.
- [16]. ERICKSON (D. L.), NORTH (J. A.), HIGHTOWER (J. E.), WEBER (J.), LAUCK (L.), 2002 - Movement and habitat use of green sturgeon *Acipenser medirostris* in the Rogue River, Oregon, USA. IN: *Journal of Applied Ichthyology*, vol. 18, No. 4 – 6, pp. 565 – 569.
- [17]. FERNANDES (S. J.), ZYDLEWSKI (G.), ZYDLEWSKI (J. D.), WIPPELHAUSER (G. S.), KINISON (M. T.), 2010 - Seasonal Distribution and Movements of Shortnose Sturgeon and Atlantic Sturgeon in the Penobscot River Estuary, Maine. IN: *Transactions of the American Fisheries Society*, 139.5: pp. 1436 - 1449.
- [18]. GARRIDO-RAMOS (M. A.), ROBLES (F.), DE LA HERRÁN (R.), MARTÍNEZ-ESPÍN (E.), LORENTE (J. A.), RUIZ-REJÓN (C.), RUIZ-REJÓN (M.), 2009 - Analysis of Mitochondrial and Nuclear DNA Markers in Old Museum Sturgeons Yield Insights About the Species Existing in Western Europe: *A. sturio*, *A. naccarii* and *A. oxyrinchus*. IN: *Biology, Conservation and Sustainable Development of Sturgeons - Fish and Fisheries Series*, vol 29, pp. 25 – 49. Publisher: Springer. The Netherlands.
- [19]. GUTI (G.), 2006 – Past and present status of sturgeons in Hungary. IN: *Proceedings Volume of the 36th Conference of the IAD*, pp. 143 - 147.
- [20]. HALL (J. W.), SMITH (T. I. J.), LAMPRECHT (S. D.), 1991 - Movements and habitats of shortnose sturgeon, *Acipenser brevirostrum*, in the Savannah River. IN: *Copeia*, pp. 695 - 702.
- [21]. HALLERMAN (E.), (ed.), 2003 - Population genetics: Principles and Applications for Fisheries Scientists. 458 p. Publisher: American Fisheries Society. Bethesda, Maryland.
- [22]. HENSEL (K.), HOLČIK (J.), 1997 – Past and current status of sturgeons in the upper and middle Danube River. IN: *Environmental Biology of Fishes*, vol. 48, No. 1, pp. 184 – 200.
- [23]. HERNANDO (J. A.), DOMEZAIN (A.), ZABALA (C.), CABRERA (R.), DOMEZAIN (J.), SORIGUER (M. C.), 2009 – The Regression of Sturgeons in Southern Europe. IN: *Biology, Conservation and Sustainable Development of Sturgeons - Fish and Fisheries Series*, vol 29, pp. 3 – 23. Publisher: Springer. The Netherlands.
- [24]. HOLOSTENCO (D.), 2011 – Conservation of genetic diversity in populations of stellate sturgeon (*Acipenser stellatus*) of the NW Black Sea and Lower Danube River. 64 p. MS thesis. Norwegian University of Science and Technology, Department of Biology. Trondheim, Norway.



- [25]. HOLOSTENCO (D.), ONARA (D.), TAFLAN (E.), SUCIU (R.), 2012 - Genetic diversity of adult stellate sturgeons captured in the Lower Danube River during 1998 - 2011. IN: *Book of Abstracts The 39th IAD Conference Living Danube*. page 44. Szentendre, Hungary.
- [26]. HONTZ (Șt.), IANI (M.), PARASCHIV (M.), CRISTEA (Al.), TĂNASE (B.), BĂDILIȚĂ (A. M.), DEÁK (Gy.), SUCIU (R.), 2012 - Acoustic telemetry study of movements of adult sturgeon in the Lower Danube River (Km 175 - 375) during 2011. IN: *Book of Abstracts. The 39th IAD Conference Living Danube*. 2012: Szentendre, Hungary, page 45.
- [27]. KECSE-NAGY (K.), 2011 - Trade in Sturgeon Caviar in Bulgaria and Romania - overview of reported trade in caviar, 1998-2008. 17 p. A TRAFFIC report for WWF Austria, Budapest, Hungary.
- [28]. MOSER (M. L.), ROSS (S. W.), 1995 - Habitat Use and Movements of Shortnose and Atlantic Sturgeons in the Lower Cape Fear River, North Carolina. IN: *Transactions of the American Fisheries Society*, vol. 124, No. 2, pp. 225 - 234.
- [29]. NELSON (T. C.), DOUKAKIS (P.), LINDLEY (S. T.), DRAUCH SCHREIER (A.), HIGHTOWER (J. E.), HILDEBRAND (L. R.), WHITLOCK (R. E.), WEBB (M. A. H.), 2010 - Modern technologies for an ancient fish: tools to inform management of migratory sturgeon stocks. A report for the Pacific Ocean Shelf Tracking (POST) Project. (<http://www.postprogram.org> - accessed on February 5<sup>th</sup>, 2013)
- [30]. ONĂRĂ (D.), 2009 - Screening genetic variability of beluga sturgeon (*Huso huso*) using PCR RFLP of mtDNA control region. Second intermediary report. BestCombat project (KnRin/ - 2008/112231)
- [31]. ONĂRĂ (D.), TAFLAN (E.), SUCIU (R.), PARASCHIV (M.), HOLOSTENCO (D.), 2011 - Contributions to understanding the spawning ecology of beluga sturgeons in the Lower Danube River. IN: DANUBEPARKS International Sturgeon Conference, March 31st - April 1st. Tulcea, Romania.
- [32]. ONĂRĂ (D.), 2011 - Cercetări de biologie moleculară pentru fundamentarea conservării genofondului populațiilor de sturioni din Dunăre. Interim Reports III - V of the contract PN 09-26 05 05. Tulcea, Romania. [in Romanian]
- [33]. OȚEL (V.), 2007 - Atlasul Peștilor din Rezervația Biosferei Delta Dunării. 481 p. Publisher: Editura Centrul de Informare Tehnologică Delta Dunării. Tulcea, Romania. [in Romanian]
- [34]. PARASCHIV (M.), SUCIU (R.), SUCIU (M.), 2007 - Present status, conservation and sustainable use of sturgeon populations of the Lower Danube River, Romania. IN: Proceedings of the 36<sup>th</sup> International Conference of IAD. Austrian Committee for Danube Research, Vienna, pp. 152 - 158.
- [35]. RANDALL (M.), NOREM (A. D.), LUKE (K. E.), EDWARDS (R. E.), SULAK (K. J.), 2006 - Winter habitat use by juvenile Gulf sturgeon in the Suwannee Estuary as revealed by automated acoustic telemetry. Poster presented at the FISC Strategic Review. St. Petersburg, Florida, May 9-12, 2006.
- [36]. REINARTZ (R.), 2002 - Sturgeons in the Danube River. Biology, Status, Conservation. Literature study. International Association for Danube Research (IAD). Bezirk Oberpfalz, Landesfischereiverband Bayern e.V., pp. 118 - 131.
- [37]. 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. IN: Proceedings of 39th IAD Conference. Living Danube. 21-24 August. 2012. Szentendre, Hungary, pp. 101 - 107.
- [38]. ROCHARD (E.), LEPAGE (M.), DUMONT (P.), TREMBLAY (S.), GAZEAU (G.), 2001 - Downstream migration of juvenile European sturgeon *Acipenser sturio* L. in the Gironde estuary. IN: *Estuaries*, vol. 24, pp. 108 - 115.
- [39]. SUCIU (R.), GUTI (G.), 2012 - Have sturgeons a future in the Danube River ? IN: Proceedings of 39th IAD Conference Living Danube. 2012. Szentendre, Hungary, pp. 19 - 30.
- [40]. TAGGART (J. B.), HYNES (R. A.), PRODÖUHL (P. A.), FERGUSON (A.), 1992 - A simplified protocol for routine total DNA isolation from salmonid fishes. IN: *Journal of Fish Biology*, vol. 40, No. 6, pp. 963 - 965.
- [41]. TAVERNY (C.), LEPAGE (M.), PIEFORT (S.), DUMONT (P.), ROCHARD (E.), 2002 - Habitat selection by juvenile European sturgeon *Acipenser sturio* in the Gironde estuary (France). IN: *Journal of Applied Ichthyology*, vol. 18, pp. 536 - 541.
- [42]. \*\*\* (Anonymous), 2003 - Regional Strategy for the Conservation and Sustainable Management of Sturgeon Populations of the N-W Black Sea and Lower Danube River in accordance with CITES. IN Regional CITES Meeting - Tulcea, 26<sup>th</sup> November, 2003, 7 p. Tulcea, Romania.
- [43]. \*\*\* , 2013 - IUCN Red list [www.iucnredlist.org/](http://www.iucnredlist.org/) (accessed on 15th January, 2013)

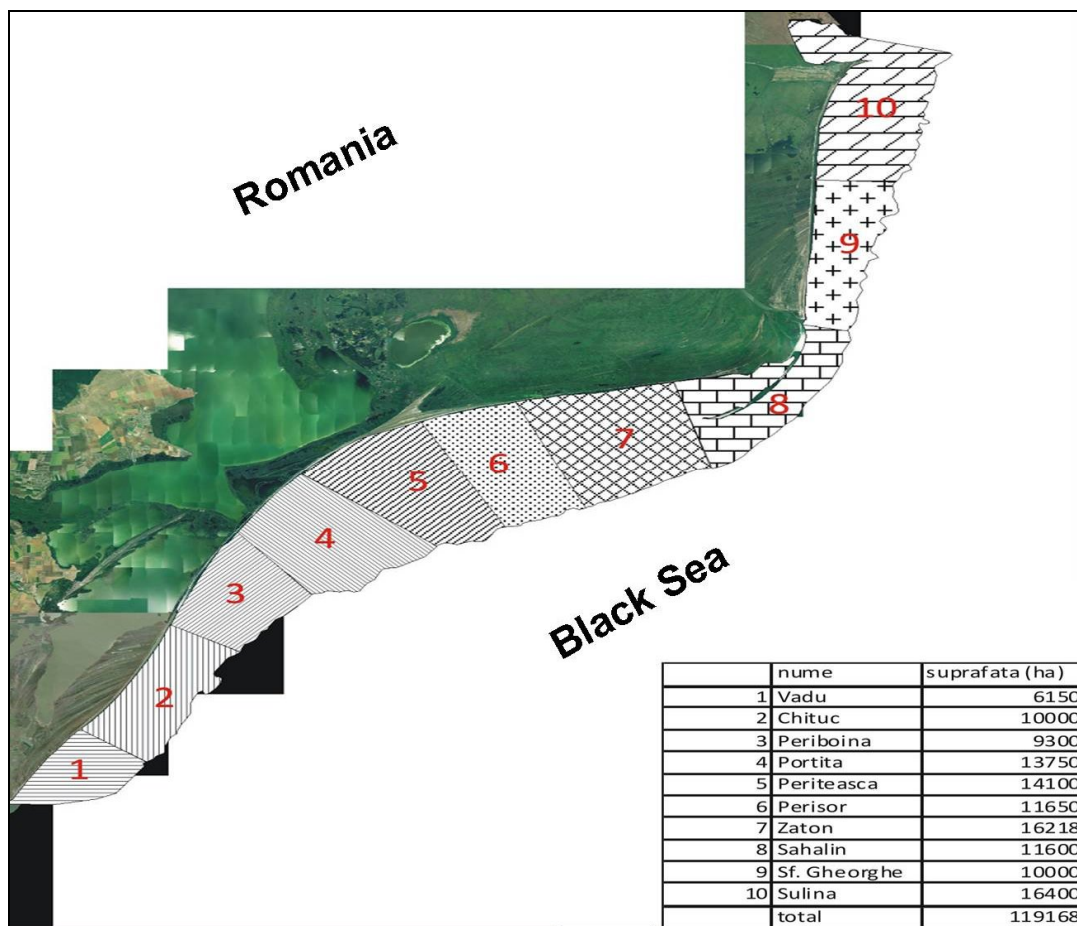


Fig. 8. Sampling map showing the 10 areas of the Black Sea coastal waters where sturgeon specimens were captured.

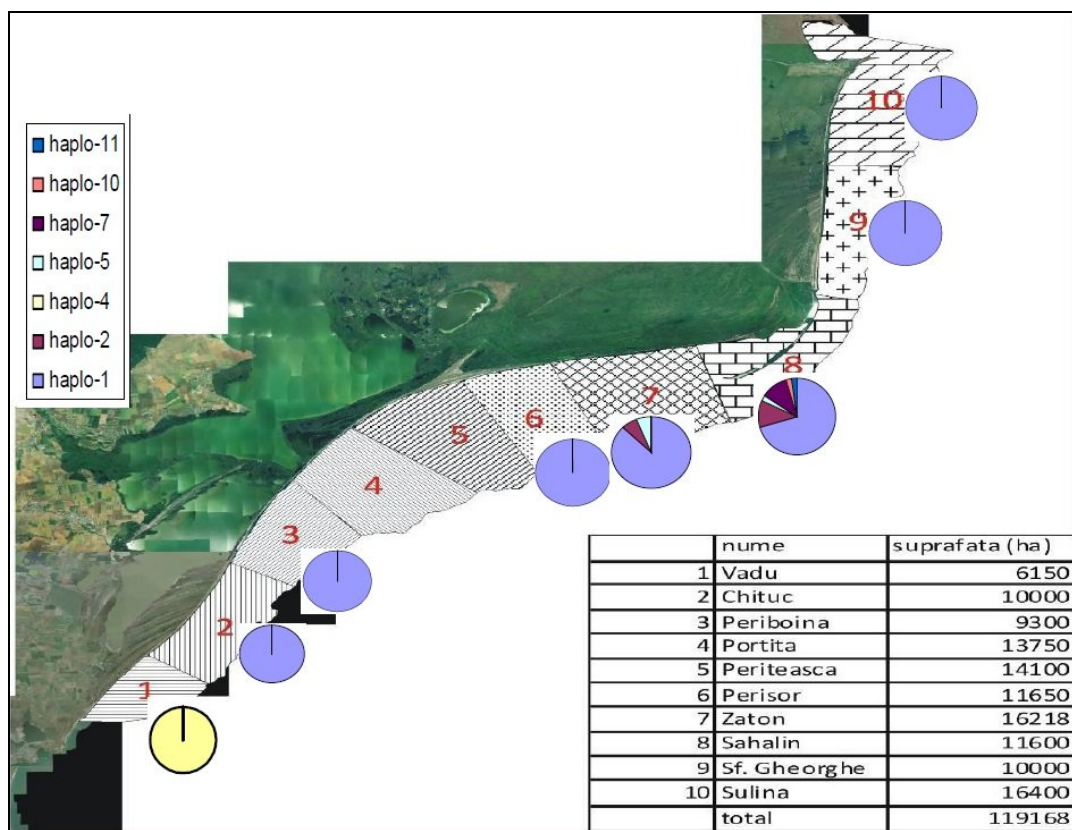


Fig. 9. Distribution of composite haplotypes frequency in 10 areas in the Black sea coast where the juveniles of stellate sturgeons were captured