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Natural Sturgeon Hybrids along Bulgarian Black Sea Coast and in Danube River

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Abstract: During October 2005 and June 2006 eleven individuals of sturgeon hybrids were caught in Bulgarian waters of Black Sea and Danube River. Electrophoresis (isoelectric focusing, IEF) ascertained the observed morphological differences of these hybrids on the genetic level. Three different hybrids of various offspring generations were identified: *Acipenser gueldenstaedti x Acipenser ruthenus; Acipenser ruthenus x Acipenser stellatus; Huso huso x Acipenser ruthenus.*

Key words: Acipenseridae, sturgeons, hybrids, isoelectric focusing, Black Sea, Danube River

Introduction

The family Acipenseridae includes some of the most ancient representatives of Osteichthyes fish class which is distributed only in the Northern Hemisphere, in the waters of Europe, Asia and North America (Billard and Lecointre 2001). They are freshwater, semi-migratory and migratory species, the latter living in the sea and entering large rivers for feeding and reproduction.

Presently, it is assumed that only four out of six native species of sturgeons in Danube River continue their reproduction: Great sturgeon – *Huso huso*; Russian sturgeon – *Acipenser gueldenstaedti*; Sterlet – *Acipenser ruthenus* and Stellate sturgeon – *Acipenser stellatus.* Atlantic sturgeon – *Acipenser sturio* is extinct in Danube and Black Sea. Ship sturgeon – *Acipenser nudiventris* is highly endangered to extinction (Reinartz 2002, AP 2006).

Sturgeon stocks and catches in Lower Danube River have decreased dramatically due to confusing fisheries legislation and lack of fishing regulations (AP 2006). Navodaru *et al.* (1999) provide evidence for over-exploitation of sturgeon stocks in Lower Danube River, with an estimated proportion of poaching of up to 90%. According to Cites, black caviar export during 1998-2003 declined by 70% and the quota were set to zero for 2006 (CITES 2006). Sturgeon catches still decrease continuously in Danube River.

An indicator of decreasing sturgeon stocks is - apart from changed biometry (Ceapa *et al.* 2002) - the increase of natural hybrids between different sturgeon species in Danube River and Black Sea. Sturgeons are known to easily form hybrids (Congiu *et al*. 2001, Ene and Suciu 2001, Reinartz 2002).

Spawning populations of Acipenseriformes show a complex multi-age structure. When spaw-

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ning habitats are lost (by technical impacts and/or hydromorphological alterations) an increase in natural hybridization may occur. This is caused by the strong tendency of sturgeons towards a breakdown in pre-mating isolating mechanisms, especially if animals are confined to only a few suitable spawning sites and/or one species is rare compared to another (AP 2006).

The same sturgeon species can display different life histories and migration patterns in different river systems, while similar habitats within a river system may be used for different developmental stages by different sturgeon species (AP 2006). The life history of sturgeons is highly adaptive.

The presence of hybrid sturgeons in riverine ecosystems therefore is a result of the specific biology and ecology of sturgeon species and forms, the quality and availability of spawning habitat as well as the number and composition of shoals respectively groups of individuals (e.g. species, sex ratio, age structure) involved in simultaneous spawning in the same area. If the size of wild spawning sturgeon stocks is not diminished, relatively few natural hybrids will form and not be a problem for the genetic integrity of native species and forms. However, the decreasing sturgeon stocks allow hybrids to participate in the reproduction (introgressive hybridization), and F_1, F_2 and further generations may endanger the genetic structure of wild sturgeon populations. Further, Gilpin and Soule (1986) reported negative effects due to inbreeding in hatcheries and loss of genetic variability.

This is the first time to report on the presence of natural sturgeon hybrids along Bulgarian Black Sea and Danube River, based on morphological observation and electrophoretical analysis.

Material and Methods

During October 2005 – June 2006 three expeditions on Danube River and Black Sea were performed by licensed fishermen. One individual of hybrid N1 was caught on October 20, 2005 in the area of Gorni Vadin (Danube River km 654). Five specimens of hybrid N2 were caught 200 m offshore of Cape Galata, Varna during January – February 2006. Hybrid N3 is represented by four individuals caught in the Black Sea during February – March 2006 in the area of Varna and Sozopol, and one specimen caught on April 4, 2006 in the area of Oryahovo (Danube River km 678).

Each specimen was examined in detail – weight, overall body length (*Tl*), head length (*lc*), snout length (*r*), number of dorsal (*SD*), lateral (*Sl*) and ventral scutes (*SV*). The relations lc/Tl, r/Tl and r/lc were defined (Table 1).

New methods in sturgeon genetics allow the identification of individuals and their caviar, as well as the characterization of populations for management purposes (Birstein *et al.* 1999, Campton *et al.* 2000, Jenneckens *et al.* 2001, Ludwig *et al.* 2002, Dobrovolov *et al*. 2005). According to Dobrovolov *et al.* (2005) genetic-biochemical methods (isoelectric focusing and starch gel electrophoresis) may be used for rapid identification of sturgeon species and hybrids as well as unknown sturgeon caviar and fillet. Electrophoretical analyses using isoelectric focusing (IEF) on blood and muscle on thin polyacrylamide Ampholine gel with pH-gradient 3.5-10.0 were carried out to identify the hybrids on genetic level.

Results and Discussion

Hybrid N1 (Fig. 1) weighed 130 g and was 37 cm long. At first, this specimen was identified by visual observation as a Ship sturgeon (or a hybrid with Ship sturgeon participation) because of the first dorsal scute being prominent and bigger and the length of the snout being like that usually found in Ship sturgeon. However, the number of lateral scutes (42) is smaller in comparison with Ship sturgeon and Sterlet, but bigger in comparison with Russian sturgeon, Stellate sturgeon, Great sturgeon and Atlantic sturgeon. The percentage correlations *(lc/Tl)*, (*r/Tl)* and *(r/lc)* was smaller than those of hybrid N2 and larger than, or in the range of, those of hybrid N3 (Table 1). These obvious differences allow the possibility that hybrid N1 is second (F_2) or third (F_3) generation offspring which might prove the reproduction capability of certain hybrid combinations. Comparative electrophoretical analyses (Fig. 2) show the partici-

Fig. 1. Sturgeon hybrid N1 (between Russian sturgeon and starlet), caught in October 2005. Photos: Dr. A. Tsekov .

pation of Russian sturgeon and Sterlet in the heredity of this generation. Since reference individuals of Ship sturgeon are lacking, we cannot determine if this species is represented by hybrid N1.

Two larger specimens of hybrid N2 (Fig. 3) were different from the three smaller ones and show star-like lamellae between the dorsal and ventral rows of bone scutes. The results of morphometric measurements of the caught individuals (Table 1) show that these hybrids have a weight between 260 and 450 grams. In comparison with hybrid N1 and N3 the number of lateral scutes is larger (51-55), and *(lc/Tl)*, (*r/Tl)* and *(r/lc)* percentage correlation is high. According to the morphological data hybrid N2 is closer to Sterlet than to Stellate sturgeon (Table 1, Fig. 4). The results from isoelectric focusing show that this form is a hybrid (samples 2 and 4) between Sterlet (*A. ruthenus*) and Stellate sturgeon (*A. stellatus*) and have the same electrophoretical spectra (Fig.5).

Hybrid N3 (Fig. 6) was the only form caught in the Sea and in the River. The hybrid N3 specimens (Table 1) weighed between 354 and 470 g, and body length varied between 45 and 52 cm. The number of the lateral scutes (43-45), as well as the percentage correlation of lc/Tl, r/Tl and r/lc are similar to those of hybrid N1, but smaller than that of hybrid N2 (Table 1). The results from IEF analyses show the presence of Great sturgeon and Sterlet in the hybrid heredity (Fig. 7).

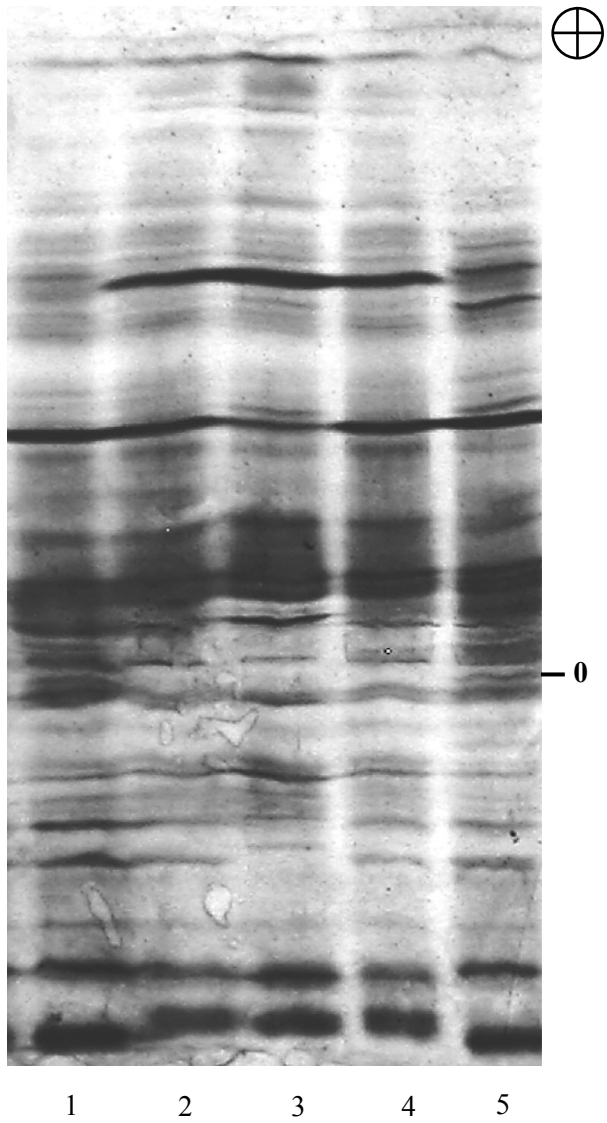

Fig. 2. Isoelectric focusing of general muscle proteins (PROT) on thin polyacrylamide Ampholine gel with pH gradient 3.5 – 10.0: Russian sturgeon (Boljarci) – 1 and 5; hybrid N1 (between Russian sturgeon and starlet - Danube River) – 2 and 4; Sterlet (Danube River) – 3, 0 - origin.

Conclusions

The existence of natural sturgeon hybrids in Bulgarian aquatic waters of Black Sea and Danube River is ascertained. Electrophoretical investigations show the participation of the sterlet (*A. ruthenus*) in the three

Table 1. Morphometric data of sturgeon hybrids caught in Danube River and Black Sea in 2005-2006.

Indicators:	**Hybrid N1** between Russian sturgeon and sterlet	**Hybrid N2** between Stellate sturgeon and sterlet	**Hybrid N3** between Gread sturgeon and sterlet
Weight – (g)	130	260-450	354-470
Length, *Tl* – (mm)	370	432-535	450-520
Length of head, *lc* – (mm)	75	112-123	83-95
Length of snout, r – (mm)	35	75-76	39-45
SD: number of dorsal scutes	12	13-15	12-13
SL: number of lateral scutes	42	51-55	43-45
SV: number of ventral scutes	11	10-13	10-12
lc/ Tl - %	20.3	22.5-25.9	18.3-19.5
r / Tl - %	9.5	14.2-17.4	8.6-8.7
r / lc - %	46,7	61.8-68.0	44.4-47.0

Fig. 3. Sturgeon hybrid N2 (F_1 between sterlet and stellate sturgeon), caught in Black Sea region of Varna (Bulgaria) and kept in the aquarium of Institute of Fisheries and Aquaculture. Photos: Dr. A. Tsekov, March 2006.

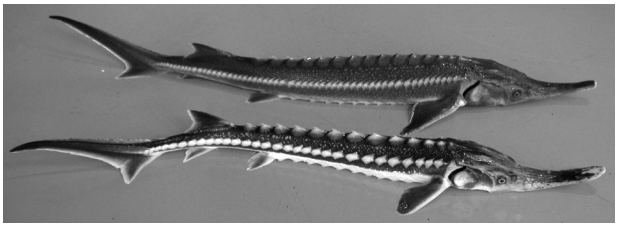

Fig. 4. Hybrid N2 between stellate sturgeon and starlet (above) and stellate sturgeon, caught in March 2006. Photo: Dr. A. Tsekov.

hybrids analyzed. Two of them (hybrid N2 and N3) are found as F_1 offspring. Morphological and electrophoretical differences identify hybrid N1 as either second or third generation (F_2 or F_3) offspring.

The increasing presence of natural sturgeon hybrids in Danube River and Black Sea, is another evidence for decreasing sturgeon stocks. Not only their reproduction is endangered, but also the genotypes of sturgeons are changed.

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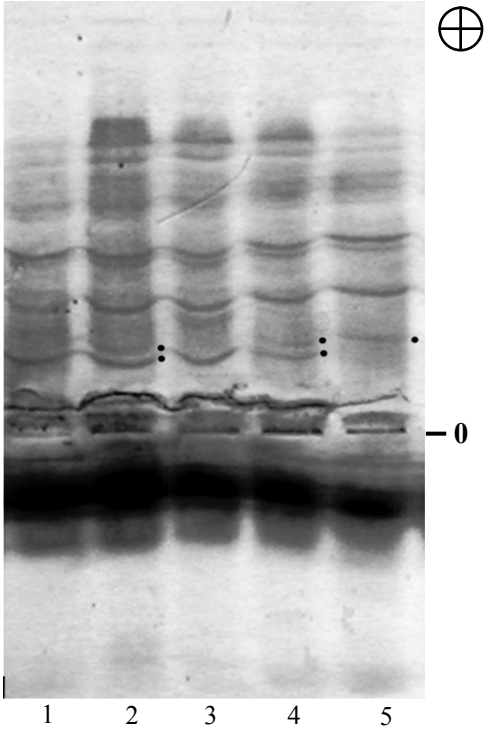

Fig. 5. Isoelectric focusing of haemoglobins on thin polyacrylamide Ampholine gel with pH 3.5 – 10: Sterlet (Danube River) – 1 and 3; Sterlet (Varna) 2 and 4; Stellate sturgeon (Varna) – 5; 0 – origin.

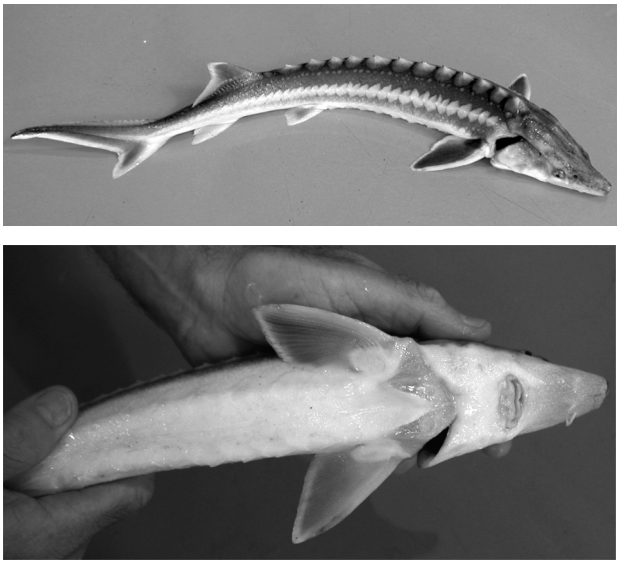

Fig. 6. Sturgeon hybrid N3 between Great sturgeon and sterlet, caught March 2006. Photos: Dr. A.Tsekov.

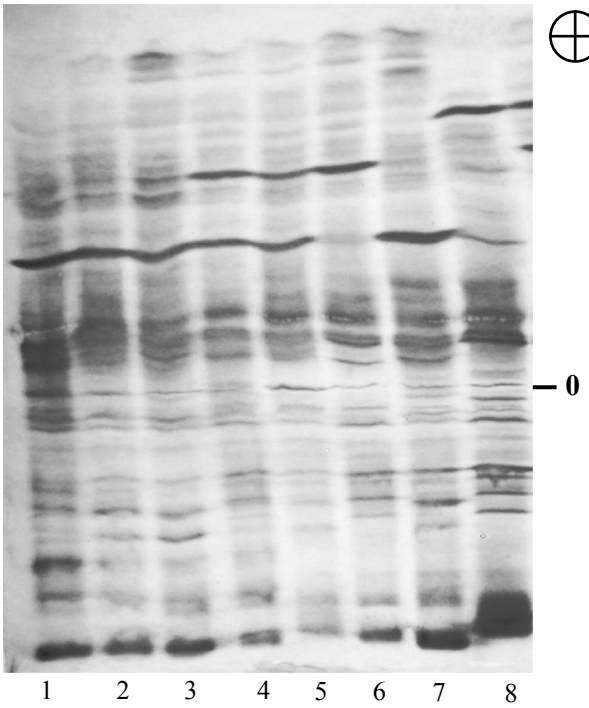

Fig. 7. Isoelectric focusing of general muscle proteins (PROT) on thin polyacrylamide Ampholine gel with pH gradient 3.5 – 10.0: Russian sturgeon 1-3; hybrid N1 – 4 and 5; Sterlet – 6; hybrid N3 -7 and Great sturgeon – 8, 0 - origin.

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Естествени хибриди при есетровите риби по българското Черноморско крайбрежие и р. Дунав

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(Резюме)

През периода октомври 2005 – юни 2006 единадесет екземпляра от есетрови хибриди бяха уловени в Българските води на Черно море и р. Дунав. Електрофорезата (изоелектричното фокусиране, ИЕФ) установи наблюдаваните морфологични различия при тези хибриди и на генетично ниво. Три различни хибрида от различни потомства бяха идентифицирани: *Acipenser gueldenstaedti x Acipenser ruthenus*; *Acipenser ruthenus x Acipenser stellatus*; *Huso huso x Acipenser ruthenus.*