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Biomonitoring and Genetic Analysis of Sturgeons in Serbia: A Contribution to Their Conservation

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Abstract: While the historical sturgeon catch in Serbia encompassed mainly four species (beluga sturgeon – *Huso huso*, Russian sturgeon – *Acipenser gueldenstaedtii*, stellate sturgeon – *Acipenser stellatus*, and sterlet – *A. ruthenus*) most of the research, especially since 1956, has been conducted on sterlet. More detailed studies started in 2001 and comprised analyses of contamination levels of heavy metals and other pollutants in sterlet tissues, histopathological analyses and genotoxicity tests. However, there is still a lack of data on sturgeon spawning, nursing and overwintering habitats, and the abundance of sturgeons that migrate for spawning up to the Djerdap II dam is unknown. Although a ban of sturgeon catch was proclaimed in 2006 by Romania, followed by Serbia and Bulgaria, there is still illegal sturgeon fishery in the Lower Danube River, which will require intensification of international cooperation to solve this problem efficiently.

Keywords: Historic data, commercial fishery, ecotoxicology, molecular genetics, histopathology, genotoxicity

Introduction

Sturgeons in Serbia were important already in the Mesolithic and early Neolithic age, when large whirlpools located in the vicinity of Lepenski Vir were recognized as attractive fishing spots. Both archaeozoological and isotopic data suggest that fishing played a major role in the economy of Lepenski Vir and neighboring sites. Many fish bones found in this area belong to acipenserids, notably to beluga sturgeon (*Huso huso*). The sandstone sculpture "Danubius" found in Lepenski Vir represents a human-fish hybrid (Srejović, Babović 1983) and the elements of sturgeon anatomy on this and other sculptures are hardly coincidences. It is striking that the majority of whirlpool fishing spots that were used in sturgeon fishery during the 19th century overlap with the locations of mesolithic-neolithic sites in the Djerdap Gorge. The six native sturgeon species (beluga sturgeon, European sturgeon – *Acipenser sturio*, Russian sturgeon – *A. gueldenstaedtii*, ship sturgeon – *A. nudiventris*, stellate sturgeon – *A. stellatus*, and sterlet – *A. ruthenus*) have been commercially harvested in the Serbian part of the Danube River, with the first negative anthropogenic effect manifested in the late 19th century through river regulation in Djerdap region due to navigation. The major following negative impact on sturgeon populations was the construction of Djerdap I and II dams in 1972 and 1984, respectively. Research activities conducted during 1948-1954 on the Danube section between river km 886 and 944 revealed that the catch of beluga, Russian sturgeon and stellate sturgeon had declined in comparison with the catch at the beginning of the 20th century, and only five specimens of ship sturgeon and six specimens of European sturgeon were caught during the sevenyear period of investigations. After that period, the investigations in the Serbian part of the Danube River were mainly focused on sterlet. Some studies of the ecology of sterlet were conducted in 1956 (Janković 1958) and included the analysis of growth, feeding and reproduction. There was a large gap in sturgeon investigations, which lasted up to 2001, when new research efforts were directed at sturgeons focusing mainly again on sterlet.

Sturgeon catch and protection in Serbia

Data on sturgeon species catch in Serbia were published for the first time by the Federal Statistical Office in 1953. During 1951-1957 only the sterlet catch was reported separately, while the catch of the other sturgeon species (beluga, Russian, stellate, ship, and European sturgeon) was reported as pooled data. Although only a few specimens of the European and ship sturgeon were being caught, these two species were not placed under protection until 1993 and 2009, respectively. Data on sturgeon catch from 1958-1959 are missing. For the period 1960-2004 separate data are available for the catch of sterlet, beluga, Russian sturgeon and stellate sturgeon (Fig. 1).

Russian, European and stellate sturgeon were all fully protected by the Decree on Natural Rarities Protection in 1993 (Official Gazette of the Republic of Serbia, No. 50/1993 and 93/1993). CITES rules concerning beluga sturgeon quotas were applied in Serbia from 2003 to 2006, and reported beluga catch remained below allowed annual CITES quotas (Smederevac-Lalić *et al.* 2011). In accordance with the moratorium proclaimed by Romania (2006-2016), Serbia has adopted a ban on catch of all sturgeon species except sterlet. Only in 2009 the catch of ship sturgeon and beluga was permanently banned by national regulations (*Order on measures for conservation and protection of fish fund,* Official Gazette of the Republic of Serbia, No. 104/2009). The rule about proclamation of strictly protected and protected species of plants, animals and fungi (Official Gazette of the Republic of Serbia, No 05/2010) finally banned the catch of all sturgeon species except sterlet; thus, the application of the conservation management of the endangered wild populations was ensured. Based on the data on sturgeon catch collected by the Statistical Office of the Republic of Serbia, the extinction risk for Russian sturgeon and beluga sturgeon was predicted (Lenhardt *et al.* 2006).

Impact of pollution on sterlet

Pollution is considered to be one of the main threats to sturgeon species, in particular, through accumulation of persistent environmental contaminants in their tissues (Williot *et al.* 2002, Agusa *et al.* 2004, Pikitch *et al.* 2005, Poleksić *et al.* 2010). These species are highly susceptible to pollutant bioaccumulation due to their high lipid contents and their specific life history, such as a delayed maturity, high longevity, and benthivorous diet (Kruse, Scarnecchia 2002, Stanić *et al.* 2006, Webb *et al.* 2006, Jarić *et al.* 2011). As a bottom feeding species, sterlet is exposed to contaminants both from sediments and water, as well as through food chains.

To overcome the lack of knowledge on the actual impact of water and sediment pollution on the Danube sterlet populations, some extensive studies focused on the analysis of pollutant accumulation in sterlet tissues and organs, the extent of histopathological alterations and the level of genotoxicity (Poleksić *et al.* 2010, Janković *et al.* 2011, Jarić *et al.* 2011, Lenhardt *et al.* 2011).

A high degree of differential heavy metal and trace element accumulation in Danube sterlet tissues was observed. Concentrations were the highest in the sterlet liver, which is their main storage tissue, while they were the lowest in the muscle (Jarić *et al.* 2011). A comparison of the observed heavy metal levels with the maximum allowed concentrations (MAC) in fish meat prescribed by both EU (European Commission Regulation 2006) and national regulation (Official Gazette of RS 2011) indicated that most of the heavy metals accumulated in the muscle tissue were below the MAC (Jarić *et al.* 2011). However, the concentrations in 10% of the assessed individuals slightly exceeded European MAC for Cd and in 3% exceeded national MAC for Fe. Consequently, sterlet can be considered as safe for utilisation in human diet, but a constant monitoring of heavy metal concentrations in sterlet meat will be necessary.

Heavy metal concentrations in sterlet were site specific, and they could be attributed to pollutants in the environment. Concentrations of Zn, Fe and Cu were the highest in livers of sterlet caught in the Danube River downstream of Djerdap II dam, probably due to the pollution from the copper and gold mines in the area (Poleksić *et al.* 2010). However, it is important to note that, while the concentrations

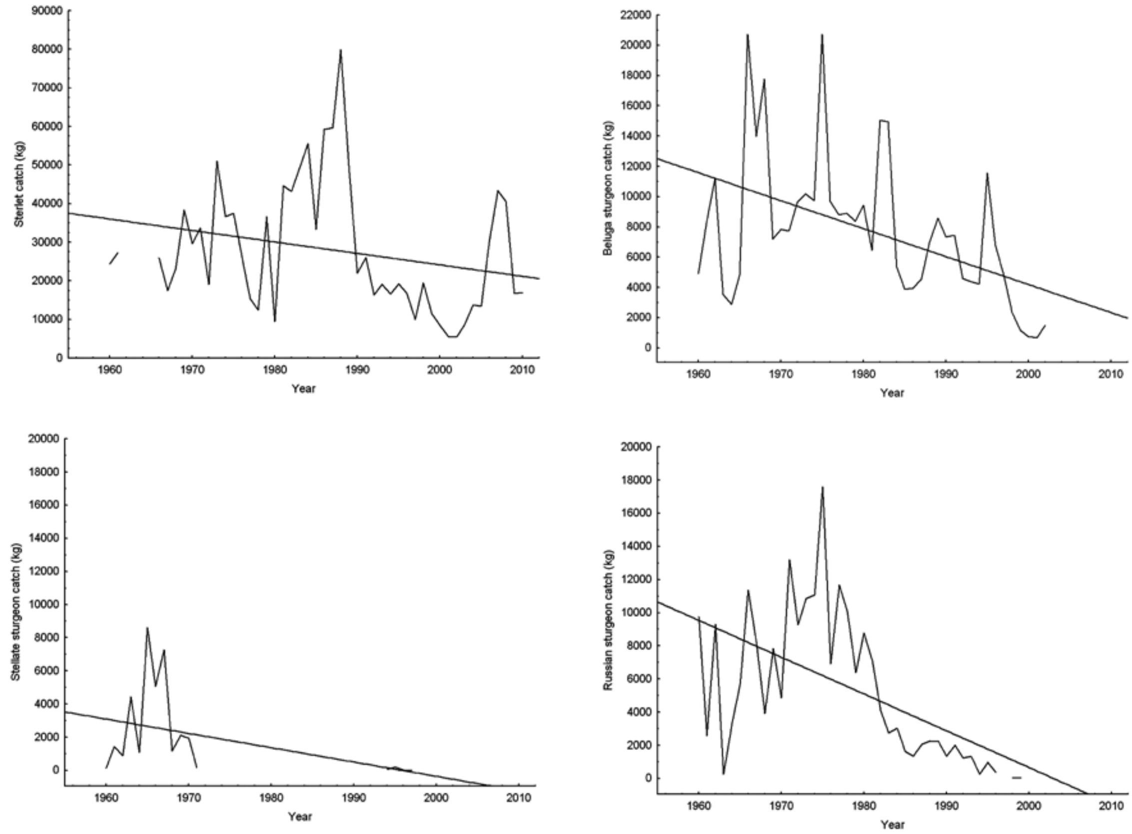

Fig 1. Data on catch and trends of sturgeons in Serbia from 1960 till present time collected by the Statistical Office of the Republic of Serbia. All 4 sturgeon species show negative trend during this period

of some heavy metals in sterlet were correlated with water and sediment contamination, sterlet is a highly migrating species, and as such represents an indicator of the river pollution on a wider scale (POLEKSIĆ *et al*. 2010).

Contamination levels of non-dioxin-like (ndl) polychlorinated biphenyls (PCBs) detected in sterlet meat indicated increased concentrations of these pollutants in the environment (JANKOVIĆ *et al*. 2011). The results also indicated a temporal increase in ndl PCB levels, as well as a recent contamination event.

Histopathological changes in sterlet were the most pronounced in liver, indicating a chronic degradation of environmental conditions (POLEKSIĆ *et al*. 2010). Gills were not heavily changed, and the most frequently encountered alterations were mild and reparable, indicating an acceptable water quality. Skin changes were restricted only to the epidermis, with picnoses observed in the epidermal matrix, which is indicating toxic conditions with no possibility of repair (ROBERTS 1989). Histopathological changes were most evident in sterlet from the Tisza River, and the lowest in specimens from the Lower Danube River (POLEKSIĆ *et al*. 2010). Results showed that the increased levels of heavy metals in the environment likely affected vital organs of sterlet and produced a range of sub-lethal changes in their tissues.

Sterlet is a good indicator of environmental pollution (LENHARDT *et al*. 2004, 2009, 2011). Future monitoring of the state of sterlet populations, as well as its use as an environmental indicator, should focus on the integrated application of different methods. Beside direct assessments of pollutant accumulation in different tissues, monitoring should also comprise histopathological analyses, monitoring of parasites, and simple genotoxicity tests, such as Comet assay and micronuleus test. While the Comet assay can be used for the monitoring of genotoxic effects, the histopathological analyses can reveal sub-lethal changes in vital organs as a result of pollution (LENHARDT *et al*. 2011).

A number of techniques for detecting DNA damage, as opposed to the biological effects (*e.g.* micronuclei, mutations, structural chromosomal aberrations) that result from DNA damage, have been used to identify substances with genotoxic activity.

The most useful approach for assessing DNA damage is the single-cell gel electrophoresis (SCGE) or Comet assay. The Comet assay was employed to acquire information through the analysis of DNA damage *in vivo* in erythrocytes of sterlet from the Danube River in Serbia. Images of randomly selected cells/fish were analyzed with fluorescence microscope Leica and image analysis software (Comet Assay IV Image Analysis system, PI, UK). Fifty nuclei were analyzed per experimental point, and the tail moment was scored as a reflection of DNA damage. The investigation showed that erythrocytes could be used as a biomarker only for acute contaminations, because of the regular cycles of their change in the bloodstream. These results confirmed that sterlet represents a good model system for genotoxicity testing within water pollution monitoring; also, the evaluated genotoxicity biomarkers are sensitive and suitable for this type of research.

Molecular genetic analysis of the Danube sturgeon populations

Over the years, a lot of research has been performed on sturgeon genetics in general (Ludwig 2006, Kriger *et al.* 2008), due to their endangered status and a high economic value. However, since sterlet caviar lacks the quality and value of other sturgeon species (*e.g.* beluga, Russian and stellate sturgeon), molecular research of sterlet was only rarely conducted worldwide, and until recently no such research has been performed in Serbia. The aim of investigations in Serbia was to conduct population genetic research with different molecular markers (D-loop mtDNA, microsatellite) on Serbian natural sterlet populations. Analyses were initiated with the optimisation and standardisation of primers for sterlet microsatellite loci (Cvijanović *et al.* 2009), as well as through incorporating genetic research within sterlet monitoring and management activities (Cvijanović *et al.* 2012). Recent findings, which included populations from the Serbian part of the Danube and Tisza Rivers, indicated that there is a gene flow between these populations, as well as the existence of a genetic drift. They also indicated a higher intra- than inter-population diversity, which is in concordance with the findings of Reinartz *et al.* (2011). Since sterlet migrate about 200-300 km around their respective resident river stretches, and human induced habitat fragmentation is relatively recent (about 100 years ago, with the dam construction about 40-50 years ago), it will be necessary to acquire samples from wild populations from both Danube and Tisza River in Hungary. However, given that there are restocking programs in Hungary, it will be also necessary to obtain samples from hatcheries that produce specimens for restocking purposes, in order to understand gene flow among wild populations.

Critical gaps that should be resolved to achieve successful sturgeon conservation

Although substantial amount of investigations related to sturgeons in the Serbian part of the Danube River were performed, there is still a lack of data on sturgeon spawning, nursing and overwintering habitats, which obstructs the development of adequate protection measures. Moreover, there are neither estimates of the abundance of sturgeons migrating upstream for spawning up to the Djerdap II dam nor investigations of their spawning success. Efficient protection and management of sturgeon species is hindered by all the above described impacts, as well as by a substantial illegal sturgeon fishery in this area, which will require intensified international cooperation, in addition to the activities on a national level.

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