GENETIC ANALYSIS OF STERLET (*ACIPENSER RUTHENUS*) RELEATED TO RESTOCKING PROGRAM IN HUNGARY

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Abstract. *In the last decades many of negative effects, as different hydraulic structure, hydropower plant, and dams and river regulation influenced on the Danube river system. These artificial structures alter the water dynamic of river, the morphology of the bed-load, and the river bank. As a result of the changed hydro –ecology conditions, have been degradated the important spawning sites and habitat of sturgeon. The crucially migration to the the upper-river basin of the sturgeon was restricted, after the construction of the two dams of Iron Gate. Of the five native sturgeon species, only the sterlet (A. ruthenus) has naturally breeding population in the Hungarian region of the Middle-Danube basin. However, the size of their population is extremely decreasing. The HAKI ex situ gene bank reserves nearly 200 sexually matured sterlet specimens however, no further information about the genetic structure of this stock is available. The aim of this study was to gain a preliminary knowledge on the genetic structure of brood stock in relation to the wild population. Four microsatellite loci were used this far to assess the genetic diversity of two sterlet population. 57 specimen originated from HAKI brood stock (25% of the total brood stock), and 37 individual sampled in 2016 from the Hungarian part of the Danube. Allele frequency, number of polymorphic loci, effective alleles as well as, expected and observed heterozygosity were determined by GenAlex statistical software package. Altogether 54 alleles were detected in the two observed populations. Based on four microsatellite loci data, there is no genetic differences (Fst=0,051) between the two populations this far. In the future this genotyping data can be used to improve the genetic management of the ex-situ gene bank, and it will be useful in the restocking program also. Although, for more detailed and reliable genetic results, genotypic data from further microsatellite loci are needed.*

Keywords*: Sterlet, Microsatellite, Danube River, Restocking, Hungary*

INTRODUCTION

Sturgeons had played an important role in the history of Hungarian fisheries, but due to overexploitation, followed by extensive river regulations and deterioration of water quality decrease in their populations has led most of them to the verge of extinction in the middle Danube and its tributaries (GUTI, 1998).

According to the Red list of IUCN four native sturgeon species are in the critical endangered category. Sterlet (*Acipenser ruthenus*) is in vulnerable category, and it has only naturally breeding population in the Hungarian region of the Danube River, but the sterlet catches has become decreasing, that show the catching data in the last twenty years (*Figure 1.)* Sterlet was caught for commercial and recreational purposes in the last decade, but since 2015 sterlet catches is forbidden, because it is under protection.

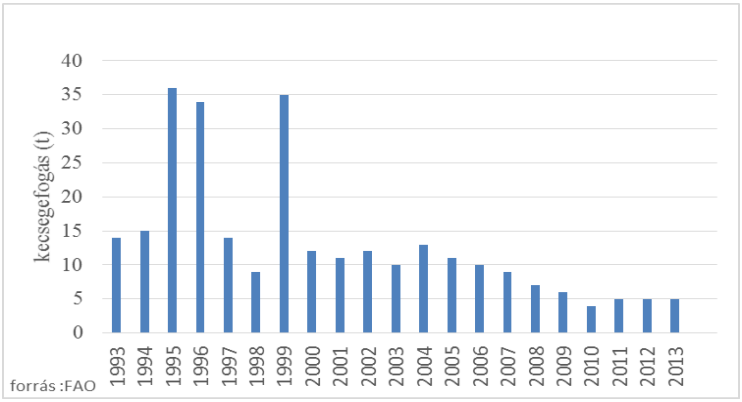

Figure 1. Sterlet catches in Hungary

Urgent action should be initiated for their restoration strangely for saving of the available gene-pool.

In the fisheries institute (HAKI) the live gene bank of sterlet was established at the end of ’80-ies and was formed from captured fish originated from Hungarian sections of the Danube and Tisza Rivers. These fish therefore is utilized as breeding stock in a restocking program to provide the stocking material. Thus, the aim of this study was to gain a preliminary knowledge on the genetic structure of the captive brood stock in relation to the wild fish, originating from recent catches in the Middle Danube.

MATERIAL AND METHODS

Tissue samples (fin-clip) were taken from two place, 57 fish originated from the brood stock of the research institute (HAKI), and 37 wild fish from a natural habitat, from the Danub river basin of Hungary (Lányfalu). All fish were tagged. The genomic DNA was extracted using the standard protocoll of the E.Z.N.A. Tissue Kit (Omega Bio-Tek, USA). The quality and quantity of the extracted DNA was controlled. DNA concentrations were determined by spectrophotometer (NanoDrop) and integrity of the DNA was controlled in 1% agarose gels.

The DNA samples were generally not degraded and did not contain secondary fragments. Based on the results of previous studies we selected and tested 8 microsatellites (Aox45, Aox23, KING et al. 2001; Afu19, Afu34, Afu68, MAY et al.1997; Spl101,Spl105, Spl173, McQUOWN et al.,2000). In case of four out of eigth (Aox45, Afu34, Spl101, Spl105), we detected polymorphic band pattern. Amplification reactions consisted of 100 ng genomic DNA, 1X Dream Taq PCR buffer, 2 mM MgCl2, 0.25 mM dNTPs, 0.5 µM forward and reverse primer, and 1.0 U DreamTaq DNA polymerase (Thermo Fisher Scientific) in a total volume of 20 µl. The forward primer was 5’ modified with NED, FAM, VIC or HEX (Applied Biosystems) fluorescent labels. Amplifications were carried out on a SuperCycler Triyiti (Kyratec) under the following conditions: initial denaturing at 94 ◦C for 2 min; 35 cycles of 94 ◦C denaturing for 30 sec, 53 ◦C annealing for 30 sec, 72 ◦C extension for 1 min; and a final extension at 72 ◦C for 5 min. Amplified, labelled products were subjected to capillary electrophoresis on an ABI PrismTM 310 Genetic Analyzer (Applied Biosystems). Genmaper Analysis software (Applied Biosystems) were used to determine fragment size and allele. The Fstat sofver was used to calculate the genetic distance (Fst), genetic variance within population (Fis) the allelic richness per locus and population (Ar) of sterlet. Number of alleles (Na). Number of effective alleles (Ne). Observed heterozygosity (Ho) Expected heterozygosity

(He), and Fixation Index (F) were calculated using GenAlEx 6.5 (PECALL and SMOUSE, 2012).

RESULTS AND DISCUSSIONS

Among eight microsatellite marker just four were select for the analysis. We detected polymorphic band pattern, by all the four selected markers. The number of alleles, number of effective alleles, observed heterozygosity and expected heterozygosity, and the fixation index were calculated in the GenAlex program. A high degree of genetic diversity was detected both of two group. Altogether 54 alleles, 39 in the Danube group, 42 in the Gene bank group were observed across the four loci. The allele number was ranging from 8 alleles at Afu34 to 15 at Aox45 (Table 1) The mean number of alleles per locus ranged from 9.75 (Danube) to 10.5 (gene bank). The average observed heterozygosity was 48% in fish from the gene bank and 50% in sterlet from the Danube River.

Table 1.

The genetic variability and alleles pattern

Population	Loci	Na	Ne	Ho	He	P	Sign.	F
Danube	Aox 45	15	10,61	0,88	0,91	0.39	ns	0,03
	Afu 34	5	1,98	0,33	0,50	0.00	***	0,33
	Spl101	11	3,13	0,40	0,68	0.00	***	0,41
	Spl 105	8	6,38	0,62	0,84	0.00	***	0,26
Gene bank	Aox 45	15	10,11	0,87	0,90	0.90	ns	0,04
	Afu 34	8	1,91	0,48	0,48	0.29	ns	0,00
	Spl101	9	3,63	0,68	0,72	0.00	***	0,06
	Spl 105	10	5,86	0,44	0,83	0.00	***	0,47

Number of alleles (Na). Number of effective alleles (Ne). Observed heterozygosity (Ho) Expected heterozygosity (He), ChiSq Chi-Squerd Test, Probability (P) and Fixation Index (F) ns=not significant. * P<0.05. ** P<0.01. *** P<0.001. The mean expected heterozygosity was highest 74% in the gene bank group and 68% in the Danube group. However in both two groups the observed heterozygosity (Ho) were below expected values (He), and a significant deviation from the Hardy-Weinberg equilibrium was found in case of 3 locus in the Danube group, and 2 locus in the gene bank group. The values of the fixation index (F) ranged between from 0.00 (gene bank, Afu 34) to 0.47 (gene bank, Spl105), but the mean F value was found 0.32 in the Danube, and 0.33 in the gene bank group. From this value we can conclude moderate heterozygote deficit. Presumably the small sample, and locus number are the major reasons for these lower value of Ho and for moderate high value of F index. The pairwise Fst value between the two groups was low level (0.051) as indicating no significant genetic differentiation between them.

Table 2

Fis value per population

Locus	Danube	Gene bank
Aox45	0.050	0.047
Afu34	0.540	0.086
Spl101	0.470	0.759
Spl105	0.276	0.480
Mean	0.280	0.370

Based on heterozygote value was calculated Fis variance in the population between the individual by the Fstat software. The Fis values show heterozygote deficit in both two examined group. Based on mean Fis value in the genebank group we detected higher Fis value, then in the Danube group.

Table 3

Allelic Richness per locus and population

Locus	Danube	Gene bank
Aox 45	14.48	12.96
Afu 34	4.78	6.38
Spl101	12.00	10.77
Spl105	7.93	9.12

The Fstat softwer was used to calculated allelic richness value (Table 3). The Ar values ranged between 4.78 (Afu34) and 14.48 (Aox45).

CONCLUSIONS

We have documented at the first time microsatellite based genetic analysis in the Hungarian wild and gene bank sterlet population. Although other researchers involved fish from the Hungarian Danube population in their examinations (REINARTZ et al. 2011.), but with lowest sample size. We received a current approach from the present state of the genetic content of the brood stock of the ex situ gene bank, furthermore we have got unique genotype data per specimen, since the fish were tagged. In case of the conservation of the native stock in the natural habitat, breeding methods have to take account the reservation of the genetic variability. (LEHOCZKY et al. 2017)

Three of the four microsatellites showed high levels of genetic diversity (10–15 alleles/locus) and mean heterozygosity was > 48% in the obtained population.

The Fst value (0.051) suggests a low degree of genetic distance across the wild and brood stock populations. The results could be used for proper brood stock management to prevent in- and/or outbreeding and the rare alleles/genotypes loss to preserve maintain genetic diversity of sterlet population in gene bank stock and the Danube River, as well. Moreover, to make this tool capable of determining those breeding combination that transmit the highest possible genetic variability to the next generation is need to enhancing the number of microsatellite further on.

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