Genetic protocol of Atlantic sturgeon *Acipenser oxyrinchus* (L.) fry for restocking the Vistula river, Poland

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Abstract

In Poland the conservation program of Atlantic sturgeon *Acipenser oxyrinchus* is conducted during over 10 years by Inland Fisheries Institute in Olsztyn, Poland. Material of *A. oxyrinchus* is annually transported from Canada to Poland and the broodstock of this species is created. Part of imported fish material is breed and released into local rivers e.g. Drweca River, Vistula River. All the imported material is obligatory genetically analyzed. The analysis must include the species verification and the genetic diversity of fish. Such analyses are necessary to issue a permit for restocking the local rivers. This paper presents a genetic characteristics of juvenile Atlantic sturgeon imported from Canada by Fish Farm Komorowo, Poland intended for restocking the river Vistula. Genetic analysis of the 45 juvenile Atlantic sturgeon was conducted using five microsatellite DNA markers (*Spl-106*, *Spl-101*, *ATR-113*, *Aox-23A*, *Aox-23B*) and one fragment (240 base pairs) encoding the *cytochrome b* gene (*Cytb*). In the present paper the genetic information about optimal genetic diversity and good condition of restocked materials of Atlantic sturgeon were described.

Keywords: Atlantic sturgeon, Microsatellite loci, Genetic diversity, Conservation

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Introduction

In Poland, for over 10 years, researchers from the Institute of Inland Fisheries in Olsztyn implement restitution program *A.oxyrinchus* Atlantic sturgeon. The initial restocking material of this species (fertilized eggs and larvae), is imported since 2003, every year, to Polish from Canada (Kolman *et al*., 2014). The fish eggs are derived from natural spawners (during artificial reproduction) from the population inhabiting the river St. John, Canada (Kolman *et al*., 2014). Growing to stocking material is carried out under controlled conditions, and fry released into local rivers is obligatorily subjected to genetic analysis.

The conservation and restitution of endangered fish populations is one of the most important aims in fish stocking programmes. The restocking procedure of fish is usually associated with production the stocking material by breeding individuals from the selected population and then releasing fish fry to the natural environment (Wang *et al*., 2001; Kolman *et al*., 2014). An appropriatere stitution plan of endangered fish population, based on genetic analysis, should have reliable protocol that allows optimal implementation of the recovery plan. Moreover, the recovery plan should maintain the genetic variation of the population to maximize the probability of success when the population is introduced into the wild.

Many of the contemporary methods of analyzing polymorphism of a nuclear and mitochondrial DNA have proven to be applicable to fisheries and aquaculture (Hayes *et al*., 2005; Fopp-Bayat and Ciereszko, 2012; Kaczmarczyk and Fopp-Bayat, 2013). One of the most popular markers that are usually applied in fish species conservation and restitutions of endangered species are microsatellite DNA (microsatellites). Due to high level of polymorphism the microsatellites are useful in verification of genetic diversity of studied populations. Microsatellite loci were applied in study of sturgeons by several authors, therefore there are numerous primer pairs for such analyses (McQuown *et al*., 2000; Rodzen and May, 2002; King *et al*., 2001). In species or population conservation, were the genetic monitoring of reintroduced fish is necessary, the appropriate number of markers should be used in the analysis. In sturgeons, usually from 4 to 10 micro satellite were used in conservation genetic studies. For example, analysis of genetic variation in the Chinese sturgeon (*A.siensis*) for estimating the contribution of artificially produced larvae in a wild populations was performed by four microsatellites (Zhu *et al*., 2002). King *et al*. (2001) applied six microsatellites in the study of genetic analysis of Atlantic sturgeon populations (*A.oxyrinchus*) from USA, while 10 microsatellite loci were used for genetic characteristics of green sturgeon (*A.medirostris*) populations from Nort America (Israel *et al*., 2009). Conservation of endangered fish population or restitution of fish species should be realized based on species identification and application of gene diversity indicators. All genetic analyses

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should be well documented, archived, and applied duringmonitoring of the genetic condition of fish population.

The purpose of the present study was the genetic analysis of Atlantic sturgeon fry produced for restocking the river Vistula in Poland. In this study five microsatellite DNA markers and one fragment of *cytochrome b* gene were used to examine the genetic condition of restocking material of Atlantic sturgeon.

Material and methods

Fin clips from a total of 45 specimens of *A.oxyrinchus* were sampled and fixed in 96% ethanol until DNA extraction. DNA was isolated from collected fin clips using standard Chelex 100 procedure (Walsh *et al*., 1991).

Genetic analysis of the 45 juvenile Atlantic sturgeon was conducted using five microsatellite DNA markers: *Spl-106*, *Spl-101*, *ATR-113*, *Aox-23A*, Aox-*23B* (McQuown *et al*., 2000; King *et al*., 2001; Rodzen and May, 2002) and one fragment (240 base pairs) encoding the *cytochrome b* gene (Cytb, Wolf *et al*., 1999). PCR reaction mixture for microsatellite DNA amplification was prepared in a total volume of 25 µl with 40 ng DNA template, 1x PCR reaction buffer (50 m MKCl, pH 8.5; Triton X-100), 0.4 mM of each primer, 0.25 mM of each deoxynucleotide triphosphate (dNTP), 3.3 mM $MgCl_2$ and 0.6 unit Go *Taq* Flexi DNA Polymerase (Promega, Madison, WI, USA). Re-distilled water was used to bring the reaction mixture to the desired final volume. PCR reactions were conducted under the following reaction profile: one cycle at 94ºC for 5 min, 30 cycles at 94ºC for 30s, the locus-specific annealing temperature for 30s, 72ºC for 30s, and final extension at 72ºC for 10 min. Amplification was conducted in a Mastercycler gradient thermocycler (Eppendorf, Germany).

In order to enable genotyping of PCR products with an Applied Biosystem 3130 Genetic Analyser, forward primers were 5'-labeled with different fluorescent reporter dyes (Spl-106-6FAM, Spl-101-VIC, ATR-113-6FAM, Aox-23A-NED, Aox-23B - NED). The lengths of amplified DNA fragments were determined using an Applied Biosystems 3130 Genetic Analyser sequencer against Gene Scan 600 [LIZ] size standard (Applied Biosystems, California, USA).

The amplification of the fragment of *cytochrome b* (mtDNA) gene was performed using primers and conditions described by Wolf *et al*. (1999). Purification of amplified DNA templates was conducted using the GF-1 PCR Clean-up Kit (Vivantis, USA). All sequencing reactions were prepared using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems California, USA) following the manufacturer's protocol. The amplified, fluorescently labeled and terminated DNA was salt-precipitated with Big Dye Xtermitator Purification Kit (Applied Biosystems California, USA) and screened on an Applied Biosystems 3130 Genetic Analyzer.

In order to visualize the results of microsatellite DNA analysis, software provided by manufacturer Genemaper v4.1 software and Data Collection

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Software v3.0 (Applied Biosystems, California, USA) were used. The genetic profiles containing the list of alleles detected within the studied loci were prepared for each fish. For display and savethe sequences of *cyt b* gene, the manufacturer's Sequence Scanner soft warev1.0 (Applied Biosystems, California, USA) was used.

The raw data matrix of the microsatellite allele was established and filed in Microsoft Excel as well as subsequently converted into the input files for the applied genetic analysis programs using Convert software (version 1.31) (Glaubitz, 2004). Microsatellite allele frequencies, number of allele per locus, allelic range, Shannon's-Wiener's index (*I*) and the Polymorphism Information Content (*PIC* value) for each loci within investigated specimens of *A.oxyrinchus* were calculated by Gen AlEx computer software (version 6.5) (Peakall and Smouse, 2012). The allelic richness (A_r) and fixation index (*Fis*) were computed using Fstat software (version 2.9.3.2) (Goudet, 2002). The observed (*Ho*) and expected heterozygosity (*He*), Garza-Williamson index (*M*), the Exact Hardy-Weinberg equilibrium (H-WE) test as well as Linkage Disequilibrium (LD) were calculated using Arlequin software (version 3.5) (Excoffier and Lischer, 2010). Each locus and each population was tested separately. The effective population size (*Ne*) was estimated for examined specimens of *A.oxyrinchus* by NeEstimator computer program (version 2.01) (Do *et al*., 2013). The linkage disequilibrium method was used for computing *Ne*, where the lowest allele frequency used was 0.05 and 95% parametric confidence intervals (95%CI) were calculated. A test for bottleneck assessment was also conducted using the Bottleneck computer software (version 1.9) (Piry *et al*., 1999) via two implemented methods. The first method tests for departure from mutation drift equilibrium based on heterozygosity excess or deficiency. Recent broodstock bottlenecks assuming a Stepwise Mutation Model (*SMM*) and Infinite Allele Model (*IAM*) for examined specimens of *A.oxyrinchus* were tested. This method is based on the assumption that in non-bottlenecked broodstock (close to mutation drift equilibrium) the value of expected heterozygosity (*He*) is equal to *Heq* (heterozygosity expected in a mutation-drift equilibrium). The excess of *He* over *Heq* is the evidence of severe reduction in broodstock effective size that may occur because of a bottleneck event. Statistical tests were performed using the one-tailed Wilcoxon signed rank test. The second method is related with testing of the allele frequency distribution. The population that does not suffer any bottleneck is expected to show a normal L-shaped distribution. In contrast, a bottlenecked population exhibits mode shifts (Luikart and Cournet, 1998). The obtained partial sequence of *cytochrome b* gene was compared to sequences deposited in NCBI gene bank using BLAST software (NCBI-NIH).

Results

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Sequencing analysis of the *cytochrome b* gene fragment verified the restocked material as species *A.oxyrinchus*. The obtained sequence was deposited in the NCBI gene bank under accession number KC987018.

All five examined microsatellite loci were polymorphic. The length of the identified alleles in the studied loci ranged between 94 and 295 base pairs (bp). A total number of 40 different alleles were found in the studied specimens of *A.oxyrinchus*. The observed number of alleles in all the analyzed loci ranged from 6 to 10 alleles with average value of 8 alleles. The highest genetic diversity was observed in the *ATR-113* and *Aox-23B* loci (10 alleles were identified) (Table 1).

The genetic diversity parameters (*Ho*, *He*, A_r, A_o, A_e, *I* and *PIC*) of the studied *Acipenseroxyrinchus* specimens are shown in table 1 and table 2. The studied group of fish was characterized by a high level of genetic diversity. The polymorphic information content (*PIC)* and the rate of Shannon-Wiener index (*I*) indicated the value ranges 0.500-0.823 and 1.070-2.004, respectively. The observed values of allelic richness (A_r) varied from 3.000 to 4.000 in studied fish. The rate of fixation index (*Fis*) ranged between 0.604 and -0.471 taking the average value at -0.031 indicating a slight excess of heterozygotes in the group of fish (Table 1).

Observed heterozygosity (Ho) in the studied loci showed values between 0.333 and 0.978 while the expected heterozygosity (He) ranged between 0.585 and 0.852. All of the examined microsatellite loci deviated from Hardy-Weinberg equilibrium (H-WE), indicating that the studied specimens of *A.oxyrinchus* were not in H-WE. Tree of the loci (*Spl-101*, *Aox-23A* and *Aox-23B*) exhibited heterozygosity excess while the rest (*Spl-101* and *ATR-113*) heterozygosity deficiency (Table 2). Significant (α=0.05) Linkage Disequlibrium (LD) was found between four out of the ten locus pairs in the tested fish. The estimated effective population size (*Ne*) value was at the level 7.1 (95% CI=3.5-12.2). Table 2 also shows expected heterozygosity in two models of a mutation-drift equilibrium (*Heq*). In tested fish, only one locus (*Spl-106*) exhibited statistically significant *He>Heq* differences, which were observed under the Stepwise Mutation (*SMM*) model. Applied the Wilcoxon sign test showed significant (p<0.05) overall heterozygosity excess exclusively under the *IAM*. Moreover, the analysis of allele frequency distribution revealed an L-shaped distribution. All investigated loci differed in terms of the Garza-Williamson index (*M*), ranging from 0.080 to 0.400. The average value of *M* in the investigated broodstock equaled 0.162 (Table 2). Comparative analysis of mitochondrial DNA sequence of the gene encoding a fragment of *cytochrome b* gene with sequences deposited in the NCBI gene bank indicated that the test group of fish is not affected by the phenomenon of interspecific hybridization, confirming their belonging to the species *A.oxyrinchus*.

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Table 1: Genetic diversity parameters of studied *A.oxyrinchus* individuals. A_r: allelic richness, A_o: observed alleles, A_e: expected alleles, *I*: Shannon's index, *PIC*: polymorphism information content, *Fis*: fixation index.

Locus	Allele size range (bp)	A_r	A_o	A_e	*I*	*PIC*	*Fis*
Spl-101	254-268	6.000	6.0	2.852	1.206	0.588	-0.463
Spl-106	188-232	6.000	6.0	2.373	1.070	0.500	0.013
ATR-113	171-295	10.000	10.0	6.338	2.004	0.823	0.611
Aox-23A	94-176	8.000	8.0	4.103	1.565	0.715	-0.283
Aox-23B	94-192	10.000	10.0	5.525	1.903	0.796	-0.183
Mean	-	8.000	8.0	4.238	1.549	0.684	-0.037

Table 2: Comparison of observed (*Ho*) and expected (*He*) heterozygosity, expected heterozygosity (*Heq*) in a Infinite Allele Model (*IAM*) and Stepwise Mutation Model (*SMM*) as well as Garza-Williamson index (*M*) in examined specimens of *A.oxyrinchus*: P-level of significance. Deviations statistically significant at $p<0.05$.

Locus	*Ho*	*He*	*P*	*IAM*		*SMM*		*M*
				Heq	*P*	*Heq*	*P*	
Spl-101	0.956	0.657	0.0000	0.582	0.371	0.726	0.140	0.400
Spl-106	0.578	0.585	0.0056	0.576	0.439	0.733	0.031	0.133
ATR-113	0.333	0.852	0.0000	0.745	0.059	0.845	0.515	0.080
Aox-23A	0.978	0.765	0.0002	0.673	0.217	0.803	0.163	0.096
Aox-23B	0.978	0.828	0.0000	0.740	0.146	0.846	0.217	0.101
Mean	0.765	0.737	-	0.663	-	0.791	-	0.162

Discussion

Genetic protocol for monitoring of gene diversity in stocked material during restitution program should be easy to implement, reliable, feasible, and resulting in obtaining important genetic indicators. Some authors, for obtain sufficient statistical power in genetic studies of fish populations, applied many markers (Kaczmarczyk and Fopp-Bayat, 2013; Panagiotopoulou *et al*., 2014; Kucinski *et al*., 2015 in press).

Application of 4-6 microsatellite markers in conservation program of sturgeons is sufficient, if the fish material is genetically variable. If the genetic variability is low (in studied stock or population), the analysis should be carried out on a larger number of markers, and such group of fish should be also checked by inbreeding and bottleneck indicators.

Comparison the analysis described in the present paper to analysis published by Panagiotopoulou *et al*. (2014) based on 17 microsatellite markers in *A. oxyrinchus* showed similar rates of genetic parameters Ho, He, PIC. Genetic analysis described by Panagiotopoulou *et al*. (2014) conducted on 603 individuals of *A. oxyrinchus* revealed Ho = 0.60, He = 0.65 and PI = 0.62 that were similar to parameters identified in the present study (Table 1). Therefore the genetic analysis based on 4-5 markers seems to be optimal for monitoring the stocking material (N=45) of genetically variable sturgeon.

In the present study the *Fis* and *I* were calculated and the information

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about the genetic condition of the examined group fish was obtained. The presented analysis based on selected genetic indicators is sufficient in genetic monitoring of fish material during restitution program. Based on this analysis we obtain the genetic information about optimal genetic diversity and good condition of restocked materials of *A.oxyrinchus*.

The protocol of genetic analysis during restitution program should include several various indicators to properly characterize genetic diversity and population differentiation. First of all, it should be determined the basic parameters of genetic diversity such as: number of alleles, allele frequencies, exact test for Hardy-Weinberg equilibrium (H-WE), and linkage disequilibrium (LD) for all loci within each population examined. Another step, in determination of genetic diversity of the examined population is estimation of the inbreeding coefficient of an individual relative to its subpopulation (*Fis*), observed (*Ho*), and expected (*He*) heterozygosities as well as effective population sizes (*Ne*). Two other indicators of genetic diversity: the Polymorphism Information Content (*PIC*) and Shannon's-Weiner's index (*I*) are also helpful in such studies. Studies on fish populations characterized by genetic diversity parameters (*PIC* and *I*) indicate that this values being close to 0.5 and 1.0, respectively are specific for populations characterized by moderate genetic diversity (You-Yi *et al*., 2009; Weiss *et al*., 2011). Lower values of this parameters (*PIC* and *I* lower than 0.4 and 0.9, respectively) are specific for populations with low genetic diversity (Ayllon *et al*., 2004; Liang *et al*., 2004). Higher values of this parameters (*PIC* and *I* higher than 0.6 and 1.3, respectively) indicate high genetic diversity (Froufe *et al*., 2004; Fopp-Bayat, 2010). The very important point in genetic protocol analysis of studied fish populations and broodstocks is estimation of occurrence of bottleneck events in the past. A bottleneck assessment test can be performed by two independent methods. One of it is a test for departure from mutation drift equilibrium based on heterozygosity excess or deficiency. In this method recent broodstock bottlenecks assuming a Stepwise Mutation Model (*SMM*) and Infinite Allele Model (*IAM*) for examined specimens. The second method is related with testing of the allele frequency distribution. The population that does not suffer any bottleneck is expected to show a normal L-shaped distribution. For detection of bottleneck events, it could also be used Garza-Wiliamson index (*M*). Values of the Garza-Williamson index being 0.8 or higher is specific for populations that were not subjected to reduction of its size. Values about 0.7 or less may suggest that a population has undergone through a recent reduction in size. Low *M* values ranging from 0.29 to 0.43 across studied populations are specific for reduced populations and may indicate essential reduction of the population size in the past (Tzika *et al*., 2008). In the genetic studies of multiple populations and broodstocks, the

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distance allele sharing (DAS) (Bowcock *et al*., 1994), genetic differentiation (*Fst*) and Nei's genetic distance (Nei, 1978) analysis methods should be used for accurate determination its genetic structure. Such analysis should also take into account determination the number of genetic clusters (*K*) and probabilistically assign individuals to these clusters (Pritchard *et al*., 2000).

The presented analysis of restocking material of endangered Atlantic sturgeon allow us to expect that the application of proposed protocol based on microsatellite DNA markers will help to sustain the original level of genetic variation of recovered population during elaborated program of restitution.

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References

Ayllon, F., Davine, P., Beall, E., Martinez, J.L. and Garcia-Vasquez, E., 2004. Bottlenecks and genetic changes in Atlantic salmon (*Salmosalar* L.) stocks introduced in the Subantarctic Kerguelen Islands. *Aquaculture*, 237, 103-116.

Bowcock, A.M., Ruiz-Linares, A., Tomfohrde, J., Minch, E., Kidd, J.R. and Cavalli-Sforza, L.L., 1994. High resolution of human evolutionary trees with polymorphic microsatellites. *Nature,* 368, 455-457.

Do, C., Waples, R.S., Peel, D., Macbeth, G.M., Tillet, B.J. and Ovenden, J.R., 2013. NeEstimator V2: re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. *Molecular Ecology Resources*, 14(**1**), 209-214.

Excoffier, L. and Lischer, L., 2010. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, 10, 564-567.

Fopp-Bayat, D., 2010. Microsatellite DNA variation in the Siberian sturgeon, *Acipenserbaeri* (Actinopterygii, Acipenseriformes, Acipenseridae), cultured in a polish fish farm. *ActaIchthyologicaet Piscatoria*, 40(**1**), 21-25.

Fopp-Bayat, D. and Ciereszko, A., 2012. Microsatellite genotyping of cryopreserved spermatozoa for the improvement of whitefish semen cryobanking. *Cryobiology*, 65, 196-201.

Froufe, E., Sefc, K. M., Alexandrino, P. and Weiss, S., 2004. Isolation and characterization of *Brachymystaxlenok* microsatellite loci and cross-species amplification in *Hucho* spp. and *Parahuchoperryi*. *Molecular Ecology Notes*, 4, 150-152.

Glaubitz, J.C., 2005. CONVERT: a user friendly program to reformat diploid genotypic data for commonly used population genetic software packages. *Molecular Ecology Notes*, 4, 309-310.

Goudet, J., 2002. Fstat, a program to estimate and test gene diversities and fixation indices (version 2.9.3.2). Updated from Goudet (1995). Available from http://www2.unil.ch/popgen/softwar

[DOI: 10.18331/SFS2015.2.1.1]

es/fstat.htm Accessed 17 January 2015.

Hayes, B., Sonesson, A.K. and Gjerde, B., 2005. Evaluation of three strategies using DNA markers for traceability in aquaculture species. *Aquaculture*, 250(**1-2**), 70-81.

Israel, J.A, Bando, K.J, Anderson, E.C. and May, B., 2009. Polyploid microsatellite data reveal stock complexity among estuarine North American green sturgeon (*Acipenser medirostris*). *Canadian Journal of Fisheries and Aquatic Sciences*, 66(**9**), 1491-1504.

Kaczmarczyk, D. and Fopp-Bayat, D., 2013. Assemblage of spawning pairs based on their individual genetic profiles – as tool for maintaining genetic variation within sturgeon populations. *Aquaculture Research*, 44, 677-682.

King, T.L., Lubinski, B.A. and Spidle, A.P., 2001. Microsatellite DNA variation in Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*) and cross-species amplification in the Acipenseridae. *Conservation Genetics*, 2(**2**), 103-119.

King, T.L., Lubinski, B.A. and Spidle, A.P., 2001. Microsatellite DNA variation in Atlantic sturgeon (*Acipenser oxyrinchusoxyrinchus*) and cross-species amplification in the Acipenseridae. *Conservation Genetics*, 2, 103–119.

Kolman, R., Kapusta, A., Szczepkowski, M., Duda, A., Wiszniewsk, i.G. and Zdanowski, B., 2014. The current status of the work on the restitution of Baltic sturgeon *Acipenser oxyrinchus* Mitchill in Poland. In: Current status and conservation of natural populations of sturgeon Acipenseridae. (Ed. R. Kolman), Wydawnictwo IRS Olsztyn pp. 61-70 (in Polish).

Kucinski, M., Fopp-Bayat, D., Liszewski, T., Svinger, V., Lebeda, I. and Kolman, R. 2014. Genetic analysis of four European huchen (*Hucho hucho* Linnaeus, 1758) broodstocks from Poland, Germany, Slovakia and Ukraine: implication for conservation. *Journal of Applied Genetics* – in press.

Liang, L.Q., Chang, Y.M., Dong, C.Z. and Sum, X.W., 2004. Genetic analysis for *Hucho taimen* in Wusuli River with microsatellites. *Journal of Fisheries of China*, 28(**3**), 241-244.

Liukart, G. and Cournet, J.M., 1998. Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. *Conservation Biology*, 12, 228-237.

McQuown, E.C., Sloss, B.L., Sheeban, R.J., Rodzen, J., Tranah, G.J. and May, B., 2000. Microsatellite Analysis of Genetic Variation in Sturgeon: New Primer Sequences for *Scaphirhynchus* and *Acipenser*. *Transactions of the American Fisheries Society*, 129(**6**), 1380-1388.

Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, 89(**3**), 583-590.

Panagiotopoulou, H., Popovic, D., Zalewska, K., Weglenski, P. and Stankovic, A., 2014. Microsatellite multiplex assay for the analysis of Atlantic sturgeon populations. *Journal of Applied Genetics*, 55, 505-510.

[DOI: 10.18331/SFS2015.2.1.1]

Peakall, R. and Smouse, P.E., 2012. Gen AlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics*, 28, 2537-2539.

Piry, S., Luikard, G. and Cornuet, J.M., 1999. Bottleneck: A computer program for detecting recent reductions in effective population size from allele frequency data. *Journal of Heredity*, 4, 502-503.

Pritchard, J. K., Stephens, M. and Donnelly, P., 2000. Inference of population structure using multilocus genotype data. *Genetics*, 155, 945-959.

Rodzen, J.A. and May, B., 2002. Inheritance of microsatellite loci in the white sturgeon (*Acipenser transmontanus*). *Genome*, 45, 1064-1076.

Tzika, A.C., Rosa, S.F.P., Fabiani, A., Snell, H.L., Snell, H.M., Marquez, C., Tapia, W., Rassmann, K., Gentile, G. and Milinkovitch, M.C., 2008. Population genetics of Galápagos land iguana (genus Conolophus) remnant populations. *Molecular Ecology*, 17, 4943-4952.

Walsh, P.S., Metzger, D.A. and Higuchi R., 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques*,10, 506-513.

Wang, S., Hard, J.J. and Utter, F., 2001. Salmonid inbreeding: a review. *Reviews in Fish Biology and Fisheries*,11(**4**), 301-319.

Weiss, S., Marić, S. and Snoj, A., 2011. Regional structure despite limited mtDNA sequence diversity found in the endangered huchen, *Huchohucho* (Linnaeus, 1758). *Hydrobiologia*, 658, 103-110.

Wolf, C., Hübner, P. and Lüthy, J., 1999. Differentiation of sturgeon species by PCR-RFLP. *Food Research International*, 32, 699-705.

You-Yi, K., Guang-Xiang, T., Wei, X, Jia-Sheng, Y. and Xiao-Wen, S., 2009. Analysis of genetic diversity in the endangered *Huchotaimen* from China. *Acta Ecologica Sinica*, 29, 29-97.

Zhu, B., Zhou, F., Cao, H., Shao, Z., Zhao, N., May, B. and Chang, J., 2002. Analysis of genetic in the Chinese sturgeon, *Acipenser sinensis*: estimating the contribution of artificially produced larvae in a wild population. *Journal of Applied Ichthyology*, 18, 301-306.

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