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Genetic characteristics of Polish whitefish (*Coregonus lavaretus maraena*) broodstocks – recommendations for the conservation management

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ABSTRACT: European whitefish *Coregonus lavaretus maraena* is an important fish species in Poland. Unfortunately, most of the whitefish populations are currently threatened with extinction, generally due to water pollution and overfishing. Because the whitefish populations have decreased drastically in Poland, a protection plan has been developed, which includes the creation of whitefish broodstocks in aquaculture conditions. During its implementation, genetic analysis of these valuable fish populations should be performed. This manuscript describes a study, in which microsatellite DNA analysis was applied to investigations into the genetic structure of two whitefish broodstocks maintained in Poland (Pomorska Bay and Galadus) and two natural populations from Lebsko Lake and Mamry Lake, northern Poland. Genetic differentiation was detected between the analyzed populations through the pairwise genetic differentiation index (F_{ST}). The D_A measure of genetic distance between pairs of populations indicated that the shortest distance was between the Pomorska Bay broodstock and the Lebsko Lake population (0.062), while the longest one was between the Lebsko Lake and the Galadus populations (0.172). The present results reveal genetic characterization of important populations of whitefish in Poland and provide the first information about the genetic condition of these fish stocks.

Keywords: aquaculture; genetic monitoring; population; genetic analysis; microsatellite DNA; whitefish

INTRODUCTION

Coregonids are an important group of fish in Poland, which appear in two forms: lake-dwelling (in oligotrophic lakes) and migratory fish (inhabiting the Baltic Sea). In recent years, most populations of whitefish have become endangered because of anthropogenic activity (e.g. water pollution, intensive fishery exploitation, eutrophication). Unsuitable environmental conditions prevent the natural reproduction of whitefish. Therefore, the existence of whitefish populations in Poland is dependent on artificial reproduction conducted during the spawning season. Some populations have been included in the program of restitution, e.g. Lebsko Lake, Pomorska Bay, while some other populations have a spawning stock maintained and controlled by fish breeding specialists, e.g. Galadus Lake. In Poland, some populations of whitefish have been covered by a protection program and now have an individual broodstock. The Galadus broodstock has been created under aquaculture conditions in the Department of Sturgeon Breeding, Inland Fisheries Institute in Olsztyn, Poland. This broodstock is

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maintained for artificial breeding and the production of stocking material. The broodstock for the Pomorska Bay whitefish population is maintained in the Department of Salmonid Breeding in Rutki, Inland Fisheries Institute in Olsztyn, Poland. The whitefish population from Lebsko Lake is a wild population, now under a restitution program and annually supported by stocking with fish bred from spawners caught in the wild; finally, the Mamry population of whitefish is a small, natural population inhabiting Mamry Lake. Recently, the Mamry population has not been reproduced artificially because a good quality offspring from these fish is difficult to obtain.

While implementing the natural population protection plan, any risk of negative ecological, genetic or economic impacts of aquaculture should be controlled *via* careful survey of genetic inventories of stocked systems (Allendorf 1986; Wedekind 2002; Aho et al. 2006). Moreover, knowledge of the genetic variability of a valuable stock or population allows one to notice signals of domestication selection and to monitor negative events in a population, for example the bottleneck effect and loss of genetic diversity (Allendorf 1986; Wedekind 2002). Therefore, the whitefish protection plan based on broodstocks created in aquaculture should include genetic assays of fish that are being protected.

The main objective of this study was to apply genetic studies for the recognition of genetic diversity of two broodstocks and two natural populations of whitefish from Poland. The results provide valuable information for conservation programs on the examined populations of whitefish.

MATERIAL AND METHODS

Fin clips of the European whitefish *Coregonus lavaretus maraena* from four sites were sampled in 2009–2012. The samples were collected from 572 specimens of whitefish and preserved in 96% ethanol until extraction of DNA. The whitefish populations and broodstocks from the following areas were studied: Lebsko Lake (497 samples), Galadus Lake (29 samples), Mamry Lake (14 samples), and the Pomorska Bay (32 samples). Genomic DNA for microsatellite amplification was extracted from tissue samples using a Sherlock AX Kit (A&A Biotechnology, Gdynia, Poland).

Six microsatellite loci were chosen for the genetic analysis: *Clav-8*, *Clav-28*, *Clav-80*, *Clav-18* (Rogers et al. 2004), *Str-73* (Estoup et al. 1993), *Sfo-292* (Perry et al. 2005). PCR reaction mixture was prepared in a total volume of 25 µl with 40 ng DNA template, 1× PCR reaction buffer (50mM KCl, pH 8.5; Triton X-100), 0.4mM of each primer, 0.25mM of each deoxynucleotide triphosphate (dNTP), 3.3mM $MgCl_2$, and 0.6 unit Go *Taq* Flexi DNA Polymerase (Promega, Madison, USA). Redistilled 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 94°C for 5 min, 30 cycles at 94°C for 30 s, the locus-specific annealing temperature for 30 s, 72°C for 30 s, and final extension at 72°C for 10 min. Amplification was conducted in a Mastercycler gradient thermocycler (Eppendorf AG, Hamburg, Germany).

In order to enable genotyping of PCR products with a 3130 Genetic Analyzer (Applied Biosystems, Foster City, USA), forward primers were 5'-labelled with different fluorescent reporter dyes (Clav-8-PET, Clav-28-6FAM, Clav-80-NED, Clav-18-VIC, Str- 73-NED, Sfo-292-6FAM). The lengths of amplified DNA fragments were determined using an Applied Biosystems 3130 Genetic Analyzer sequencer against GeneScan 600 (LIZ) size standard (Applied Biosystems). Individual microsatellite loci amplified using primers with different fluorescent dyes attached were arranged into sets and analyzed in a multiplex mode. The fragment sizes and alleles were determined using GeneMapper and the Genetic Analyzer software (Applied Biosystems) according to the manufacturer's recommendations. Genetic profiles containing lists of alleles detected within the examined loci were prepared for each specimen.

The allele frequencies, expected heterozygosity (H_e), observed heterozygosity (H_o), and the mean numbers of alleles per locus (N_a) for each population were computed using the microsatellite toolkit makro for MS Excel 97-2003 (Park 2001). The number of effective alleles (N_e), number of private alleles (N_{pa}), fixation index (F), D_A distance matrix, and analysis of molecular variance (AMOVA) were calculated using GeneAlex software (Version 6, 2006). In AMOVA analysis, the matrix of genetic distances among the sampled individuals was used. Arlequin software (Version 3.5, 2010) was used for calculation of $F_{ST.}$ Conversion of the gentyping data to Arlequin format (*.arp file) has been performed using the Genassemblage

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Table 1. Number of different alleles (N_a), number of effective alleles (N_e), observed heterozygosity (H_o), expected heterozygosity (H_e), fixation index (F), and number of private alleles (N_{pa}) in four studied populations of whitefish *Coregonus lavaretus maraena* based on microsatellite DNA analysis

Population	*n*	Mean/SE	N_a	N_e	H_o	H_e	F	N_{pa}
LE	497	mean	10.250	4.073	0.480	0.531	0.088	4.250
		SE	4.122	1.365	0.110	0.126	0.040	2.320
GA	29	mean	5.125	2.702	0.487	0.485	0.012	0.375
		SE	1.246	0.649	0.103	0.096	0.074	0.183
MA	14	mean	5.750	3.698	0.571	0.553	–0.045	0.375
		SE	1.485	0.976	0.116	0.114	0.051	0.183
PO	32	mean	5.875	3.331	0.492	0.524	0.033	0.375
		SE	1.641	0.852	0.111	0.118	0.057	0.183

SE = standard error, LE = Lebsko Lake population, GA = Galadus Lake broodstock, MA = Mamry Lake population, PO = Pomorska Bay broodstock

software (Version 1.0, 2015). FSTAT software (Version 2.9.3.2, 2001) was used to calculate the allelic richness (A_R) per locus and population of whitefish. The significance of genetic differences revealed in the mean number of alleles per locus (N_a), expected and observed heterozygosity (H_e and H_o respectively) were evaluated between the studied pairs of whitefish populations by the use the Wilcoxon test (STATISTICA, Version 10.0, 2010). The confidence interval for the testing was 0.95. A phylogenetic tree was constructed using UPGMA method based on genetic distance, with the aid of MEGA software (Version 5.1, 2011).

RESULTS

All the selected markers were polymorphic and displayed successful amplification in all the groups of whitefish studied. Among the six studied microsatellite loci, two (*Clav-28* and *Sfo-292*) were tetrasomic (1–4 alleles in locus). Each tetrasomic locus was examined as two disomic loci (for example the locus *Clav-28* was analyzed as *Clav-28A* and *Clav-28B*) and as a result the average frequency was considered for estimation of genetic variation. Table 1 contains data on the number of alleles, number of private alleles, number of effective alleles, expected and observed heterozygosity, fixation index, and number of private alleles. The average expected and observed heterozygosity ranged from 0.485 (Galadus stock – GA) to 0.553 (Mamry population – MA) and 0.480 (Lebsko population – LE) to 0.571 (MA), respectively. The highest mean number of alleles (10.250) was observed in the LE population, but the lowest (5.125) occurred in the GA stock of whitefish (Table 1). Private alleles were observed in all the populations (Table 1). The highest number of

Table 2. Allelic richness (A_R) per locus and population of whitefish based on microsatellite DNA analysis

Locus	LE	GA	MA	PO	All
Clav-8	8.168	8.498	10.000	7.843	9.219
Clav-28	6.498	4.088	5.000	4.773	6.770
Clav-80	13.297	8.082	14.000	11.128	14.654
Clav-18	2.000	2.737	4.000	3.125	2.305
Str-73	1.126	1.737	2.000	1.688	1.233
Sfo-292	2.524	2.784	3.000	2.206	2.776

LE = Lebsko Lake population, GA = Galadus Lake broodstock, MA = Mamry Lake population, PO = Pomorska Bay broodstock

Table 3. Gene diversity (Nei 1987) per locus and population of whitefish *Coregonus lavaretus maraena*

Locus	LE	GA	MA	PO
Clav-8	0.832	0.840	0.904	0.836
Clav-28	0.751	0.575	0.703	0.725
Clav-80	0.922	0.822	0.912	0.893
Clav-18	0.410	0.538	0.673	0.508
Str-73	0.009	0.068	0.071	0.061
Sfo-292	0.289	0.264	0.309	0.256

LE = Lebsko Lake population, GA = Galadus Lake broodstock, MA = Mamry Lake population, PO = Pomorska Bay broodstock

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Table 4. F_{ST} matrix (normal) and D_A distance matrix (bold) of four studied populations of whitefish

	LE	GA	MA	PO
LE	0.000	**0.172**	**0.131**	**0.062**
GA	0.121	0.000	**0.116**	**0.167**
MA	0.079	0.072	0.000	**0.114**
PO	0.043	0.117	0.062	0.000

LE = Lebsko Lake population, GA = Galadus Lake broodstock, MA = Mamry Lake population, PO = Pomorska Bay broodstock

$P = 0.05$

Table 5. Genetic differences between the studied pairs of whitefish populations based on mean number of alleles per locus (N_a), expected and observed heterozygosity (H_e and H_o respectively). The differences were calculated using the Wilcoxon test (STATISTICA, Version 10.0, 2010)

Population	Mamry	Galdus	Lebsko
Mamry			
Galdus	(A) + (H_o) – (H_e) –		
Lebsko	(A) + (H_o) + (H_e) –	(A) – (H_o) – (H_e) –	
Pomorska Bay	(A) + (H_o) – (H_e) –	(A) – (H_o) – (H_e) +	(A) – (H_o) – (H_e) –

+ = significant values ($P = 0.05$), – = non-significant values

private alleles (4.25) was found in the LE population, while in the other three groups of fish (GA, MA, PO) the number of private alleles was lower (0.375) (Table 1).

The allelic richness (A_R) observed at each locus ranged from 1.126 in the LE population at *Str-73* locus to 14.000 in the MA stock at locus *Clav-80* (Table 2). The genetic variability at microsatellite loci was quantified by calculating the gene diversity (Nei 1987) per population and locus (Table 3). The LE population of whitefish showed the lowest gene diversity (0.009) at locus *Str-73*, while the same population presented the highest gene diversity (0.922) at locus *Clav-80* (Table 3). F_{ST} values for each pair of the populations were significant ($P = 0.05$) and varied from 0.043 (the LE-PO) to 0.121 (the GA-LE) (Table 4). The D_A distance of each population pair was shown in Table 4. Genetic differences between the studied pairs of whitefish populations based on mean number of alleles per locus (N_a), expected and observed heterozygosity (H_e and H_o respectively) were presented in Table 5. The AMOVA revealed the variability of 8.0% among populations, reaching 92% within populations (Table 6). The UPGMA tree constructed according to the D_A genetic distance between each pair of populations demonstrated genetic differences (based on microsatellite DNA analysis) among the four populations of whitefish (Figure 1). The closest distance was observed between the LE population and PO stock, while the longest one was observed between the LE population and GA stock (Figure 1).

DISCUSSION

Genetic characteristics of a fish stock or population are important not only for the protection of natural populations (Hansen et al. 1999; Ostbye et al. 2005), but also for breeding valuable fish species under aquaculture conditions (Fopp-Bayat and Ciereszko 2012; Kaczmarczyk and Fopp-Bayat 2013). Data on genetic variability within and among populations are also useful for fisheries researchers and managers because such results can provide information on stock or population subdivision and relatedness in fish (Patton et al. 1997) or on hybridization events among closely related species. Genetic studies on European whitefish have been conducted by several authors, who applied various types of genetic analyses among protected natu-

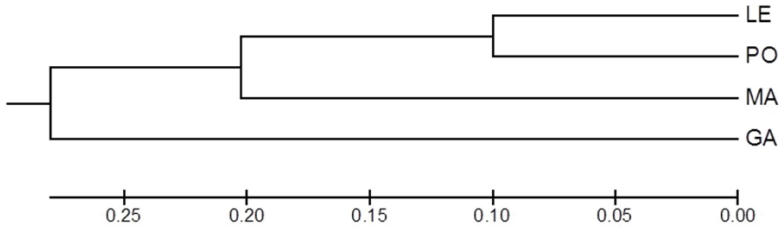

Figure 1. Evolutionary relationships of four populations of whitefish *Coregonus lavaretus maraena*

LE = Lebsko Lake population, PO = Pomorska Bay broodstock, MA = Mamry Lake population, GA = Galadus Lake broodstock

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Table 6. AMOVA analysis of four whitefish *Coregonus lavaretus maraena* populations based on microsatellite DNA variation. Input as Codominant Allelic Distance Matrix for calculation of F_{ST}

Population	LE	GA	MA	PO	
n	844	54	2864	64	
SSWP	1793.623	112.586	61.893	134.078	
Source of variation	degree of freedom	sum of square differences	mean square differences	variance component	percentage of variation
Among populations	3	57.441	19.147	0.190	8%
Within populations	990	2102.180	2.123	2.123	92%
Total	993	2159.622		2.313	100%
Statistics	value	*P* (rand ≥ data)			
F_{ST}	0.082	0.001			
PhiPT max	0.470				
PhiPT	0.174				

LE = Lebsko Lake population, GA = Galadus Lake broodstock, MA = Mamry Lake population, PO = Pomorska Bay broodstock, SSWP = sum of squares within populations, PhiPT = pairwise population values calculated from a matrix of squared Euclidean distances using AMOVA

probability, *P*(rand >= data), for F_{ST} is based on permutation across the full data set

F_{ST} = AP/(WP + AP) = AP/TOT, where AP = estimated variation among populations, WP = estimated variation within populations

ral populations of whitefish (Douglas et al. 1999; Hansen et al.1999; Ostbye 2005). Molecular genetics has generated many important and powerful methods useful for fish breeding in aquaculture, especially when a valuable fish broodstock must be obtained. Molecular genetic data may be used for quantifying genetic differences or similarities, but can also help planning the formation of a stock of spawners with distinctive genetic profiles.

The molecular analysis presented in this paper is the first attempt to compare genetic diversity (based on microsatellite DNA analysis) of two Polish natural populations of whitefish and two aquaculture broodstocks of this fish species. In this study, the mean number of alleles (5.1–10.2), allele richness (1.13–14.00), and the observed heterozygosity (0.48–0.57) parameters in the analyzed groups of whitefish were comparable to reports from studies on other whitefish populations (Douglas et al. 1999; Lu and Bernatchez 1999; Stott et al. 2004). For example, the average heterozygosity in the six populations of whitefish studied by Lu and Bernatchez (1999) ranged from 0.42 to 0.63. In the present study, the Lebsko Lake population of whitefish was characterized by a higher number of different alleles than the other examined groups of whitefish. The population from Lebsko Lake also had the highest number of private alleles. Having such a large number of private alleles in a population reflects its autochthonous character. Therefore, constant genetic monitoring of the analyzed groups of fish in the future is recommended, based on the indicators reported in this study. Our investigations did not demonstrate high genetic diversity within the four groups of fish. Therefore, it is very important to continue genetic studies and acquire more information about the genetic condition of the analyzed populations, and construct an appropriate plan as well the strategy for conservation of whitefish resources in Polish waters.

The results of the phylogenetic analysis of the four groups of whitefish showed a rather small distance between the groups, and indicated that the Lebsko Lake and Pomorska Bay populations were the same clade. The smallest genetic distance and F_{ST} values observed between LE and PO (0.062, 0.043 respectively; Table 4) suggested that there is some gene flow between the two fish populations. Because they both inhabit the Baltic Sea, some gene flow is possible. A similar F_{ST} range in a population analysis of whitefish was reported by Stott et al. (2004), while a small F_{ST} value (0.031) was observed between whitefish populations of Lake Superior and Lake Huron. In the light of our results, should a need arise in the future to supplement the genetic pool of whitefish from Lebsko Lake, then the Pomorska Bay broodstock seems

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to be the most suitable material to maintain the genetic diversity of this population (and *vice versa*). The Galadus and Mamry populations each have a more distinct character and the conservation plan of those populations should be conducted based on the fish material from each individual population. It is unacceptable to release whitefish from other populations to Galadus Lake or Mamry Lake.

In aquaculture-maintained broodstocks of whitefish, it is particularly important to sustain the genetic diversity of this fish by regular monitoring the genetic diversity of the broodstock and progeny. In such a broodstock, all spawners ought to be tagged and genetically characterized. Knowledge of genotypes of tagged spawners enables controlled breeding based on genetic profiles of spawners, so as to keep the genetic variability on an optimal level, thus preventing the inbreeding of the stock (Kaczmarczyk and Fopp-Bayat 2013). Cultured broodstocks are sometimes characterized by reduced diversity, as a result of inadequate breeding methods, e.g. using only a few individuals for reproduction (Wedekind 2002). Individuals with reduced diversity from hatcheries, released into the wild, may be deleterious to wild fish (depletion of gene diversity, increased competition, contamination with pathogens or parasites) (Aho 2006). Such studies, based on genetic analyses, allow researchers to identify suitable fish material for the creation of broodstock. Knowledge about the genetic diversity of a newly created broodstock allows genetic monitoring during a restitution program and tracing genetic changes in natural populations and in aquaculture.

The potential for evolution and adaptation to new environments is limited by the genetic diversity of a population. Adaptation is a response to selection that requires genetically based phenotypic diversity, without which no evolutionary response is possible (Ghalambor et al. 2007). Natural selection favours individuals that are better adapted to live in the natural environment than in captivity, thus research programs aimed at retaining the evolutionary genetic adaptive potential of European whitefish stocks are very important. Therefore, an optimum degree of genetic differentiation and high genetic diversity should be controlled constantly. Moreover, European whitefish habitats continue to be threatened by anthropogenic factors, and particularly unfavourable conditions may adversely impact populations characterized by a low genetic variability level. The shortage of information on the genetic structure of European whitefish broodstocks in Poland essentially limits a possible scope of sustainable conservation of this species. Therefore, baseline genetic data are crucial to guide future population specific conservation programs and research efforts on European whitefish in Poland.

CONCLUSION

The above results showed genetic characteristics of two broodstocks and two natural populations of whitefish, providing particularly useful information for the effective conservation of whitefish populations in Poland. The genetic biodiversity of those populations, identified through the present research, can serve as output indicators of the gene pool in the newly formed reproductive broodstock of Galadus Lake.

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