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Recent genetic changes in enhanced populations of sea trout (*Salmo trutta* m. *trutta*) in the southern Baltic rivers revealed with SNP analysis

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Abstract – The genetic structure of a fish population is usually thought to be stable over time. In the southern Baltic, *Salmo trutta* m. *trutta* (sea trout) populations have been characterized by low degree of genetic differentiation. All studied populations had been heavily stocked with mixed material for many years prior to the sampling period, including releases of Pomeranian sea trout to the Vistula River in Poland, Southern Baltic. However, the strategy of stocking became river based a few years before the sampling began. Juveniles from artificial reproduction are released only to their parental river, which reduces the mixing of the gene pool of fish from different populations. Changes in sea trout populations in the southern Baltic over time were studied using single nucleotide polymorphisms (SNPs). Genetic composition of populations of sea trout in the Vistula and Drwęca river system were found to increasingly resemble the non-admixed hatchery population from Aquamar (Miastko, Poland), whereas the Pomeranian populations were stable. The implementation of a new stocking strategy for the restoration and protection of Vistula sea trout was noted as possible explanation. With the increase of natural breeding, artificial enhancement of sea trout populations should be reduced.

Keywords: Population genetics / management of populations / brown trout / homing / population structure / changes over time

1 Introduction

The supplementing of diminishing endangered natural populations or restitution of extinct populations with reared specimens has been a common practice applied as a countermeasure to deterioration of living resources and the environment (Araki and Schmid 2010; Laikre et al. 2010). The enhancements have often been reported to have had negative effects on natural populations of fish (Ward 2006). These enhancements have included releases of artificially reared stocks or escapes of large numbers of individuals characterized by lower fitness (Satake and Araki 2011; Baskett et al. 2013; Milot et al. 2013; Naish et al. 2013) and lower genetic diversity have been reported for some species, including Adriatic sturgeon (Boscari and Congiu 2014), Korean starry flounder (An et al. 2013) and black sea bream in Japan (Blanco Gonzalez and Umino 2009). Other effects have been introductions of nonindigenous populations, such as salmon in Poland (Poćwierz-Kotus et al. 2015a; Bernaś et al. 2016); or alien populations of the same species and their subsequent hybridization with local native populations, as with salmon and brown trout in Bulgaria (Chelenkova et al. 2012), Norway (Thaulow et al. 2012), Hungary (Horváth et al. 2013) and in Spain (Madeira et al. 2005), pikeperch in Finland (Salminen et al. 2012); unintentional escapes from hatcheries as red sea bream in Japan (Blanco Gonzalez et al. 2015), salmon in Norway (Liu et al. 2013) and Canada (Fraser et al. 2008). Accidental or deliberate introductions of exotic and invasive species to new environments, often beneficial economically, causes distortions in the functioning of local ecosystems and can threaten native populations of fish and shellfish, as in the case of brown trout in Japan (Hasegawa and Maekawa 2008), Patagonia (Vigilano et al. 2007; Young et al. 2010) and in North America (Turek et al. 2016), mussel *Mytilus* in Europe and South Africa (Kijewski et al. 2009; McQuaid et al. 2015 and oyster *Crassostrea gigas* (Carlton 1979; Meehan et al. 1989; Miller et al. 2012; Lallias et al. 2015). Stocking and introductions to marine farms can also be the means of spreading diseases, as in salmon in Chile (Murray 2013; Marshall et al. 2014) and oyster *Crassostrea gigas* in Europe (Mineur et al. 2014). The application of genetic research facilitated more effective stocking e.g. as in sea bass in the USA (Darden et al. 2013) and a reduction of

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their negative effects in white seabass populations (Gruenthal and Drawbridge 2012) and bay scallop (Bert et al. 2011).

In the last decades, a range of new molecular genetic techniques have been used to study fish and shellfish evolution, and changes in wild populations including those associated with exploitation, stocking and research related to aquaculture (e.g. Utter et al. 1987; Wenne 1992; Poteaux and Berrebi 1997; Halvorson et al. 2001; Wąs et al. 2004; Poćwierz-Kotus et al. 2007; Wenne et al. 2007; Filipowicz et al. 2008; Beaumont et al. 2010; Poćwierz-Kotus and Wenne 2010; Poćwierz-Kotus et al. 2010; Wenne et al. 2011; Kohout et al. 2013; Nakajima et al. 2014). Microsatellites are regularly used in fisheries related applications and research (Wąs and Wenne 1998; Griffiths et al. 2013; Perrier et al. 2013; Pukk et al. 2013; Bernaś et al. 2014; Koljonen et al. 2014; Thaulow et al. 2014; Valiquette et al. 2014). However, the high potential of more recently discovered and developed single nucleotide polymorphisms (SNPs) to uncover population structures and find genomic regions (islands) under selection has become increasingly recognized (Lamaze et al. 2012; Pustovrh et al. 2012; Ozerov et al. 2013; Milano et al., 2014; Poćwierz-Kotus et al. 2015b; Sušnik Bajec et al. 2015). SNPs are biallelic markers characterized by codominant inheritance, lack of homoplasy and an infinite model of mutation (Morin et al. 2004; Beacham et al. 2010; Seeb et al. 2011) Analyzing the drawbacks of use SNPs the most important are lower discrimination power in comparison with microsatellites, non-neutrality, possibility of random genetic drift and ascertainment bias. Glover et al. (2010) demonstrated that identification of highly informative SNP markers from the screening of larger pools represents a powerful technique to create molecular tools, both to study individual membership of populations and for population differentiation.

Brown trout (*Salmo trutta* L.) is a palearctic salmonid species, which is naturally distributed in Europe and adjacent waters (Klemetsen et al. 2003). It was introduced to, and subsequently colonized drainages, lakes and coastal waters in North (Canada and USA) and South America (Chile and Argentina), Kerguelen, New Zealand, southern Australia and Japan. The anadromous form of brown trout, sea trout (*S. t. trutta*) usually spawn in freshwater streams and rivers with juveniles generally staying 1–3 years in the natal rivers before smoltification from the *parr* stadium and migration to the sea. A decline in natural reproduction resulted in a significant reduction in the number of smolts descending to the sea which, in turn, justified development of massive stocking in Europe and most Baltic countries since many years including Poland (Bartel 1993). On average between the years 2002–2012, 34 mln smolts were released in Baltic countries, including 9.5 mln in Poland (ICES 2013). Many more alevins and fry were released; 7 mln in Poland in 2012 alone.

In Poland there are about 25 rivers, in which sea trout occur (Pedersen et al. 2012). Building dams without appropriate passages prevented sea trout migration and spawning up the rivers. Regular stocking of the Vistula and Pomerania rivers has been conducted since the 1960s. But this was carried out without regard to the origin of the sea trout stocked. Therefore, populations were mixed before the 1990s. Mixed stocking may have started in the nineteenth century but

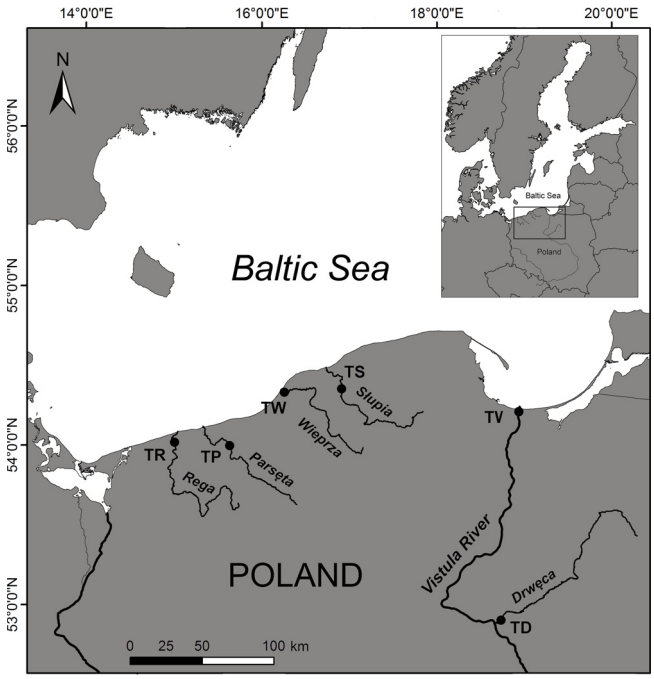


Fig. 1. Sea trout sampling locations: TV – Vistula River; TD –Drwęca River; TR – Rega River; TP – Parsęta River; TS – Słupia River; TW – Wieprza River, TM – the hatchery in Miastko.

political upheavals over the period make a precise tracking of the stocking process difficult. All populations in rivers are considered as mixed after approximately 120 years of occasional, and more than 50 years of intensive stocking. According to the HELCOM (2011) report, the status of the sea trout populations from the Vistula River was presented as 'reared' (no, or almost no, natural reproduction) with migratory hindrances. Of the populations from Pomeranian rivers: Parsęta and Słupia (Fig. 1) were qualified as mixed, with natural reproduction and with large-scale fish releases; whereas Rega and Wieprza rivers showed lower (below 10% of smolts) natural reproduction. In the case of the Drwęca River, a tributary of the Vistula, the level of wild smolts was much lower than in Pomeranian rivers (HELCOM 2011).

In the past, the Vistula sea trout had the largest catchments compared to other river populations in the Baltic Sea drainage (Bartel 1988) and consisted of two stocks: winter and summer. The main part of the population used to be the winter stock (Żarnecki 1963). Th decline in the sea trout Vistula population, compared to the 1950s and 60s, was caused by the construction of a dam in Włocławek in 1968 (Bartel 1993). The low number of eggs obtained from Vistula specimens resulted in the translocation of stocking material in the 1970s from Pomeranian sea trout, originating from the Parsęta – Słupia rivers area, to the Vistula River. This translocation caused the decay of original Vistula sea trout characteristics (Borzęcka 2010).

Several million sea trout at early stages of development (alevins, fry, and parr) and smolts have been released annually into the Vistula River and its tributaries. For smolts released to the Vistula River and its tributaries, homing was very high and ranged from 94% to 99.3% (Dębowski and Bartel 1995). However, smolts released into Pomeranian rivers estuaries had

Table 1. The location and time of sampling.

No	Sample	Name	Localization		Sampling time	Fish no.	Population
1	Vistula River	TV 96	Świbno	1996	September	40	Vistula (wild)
2	Drwęca River	TD 96	Lubicz		November	42	Vistula (wild)
3	Rega River	TR 96	Trzebiatów		November	42	Pomeranian (wild)
4	Parsęta River	TP 96	Karlino		December	40	Pomeranian (wild)
5	Słupia River	TS 96	Słupsk		October	41	Pomeranian (wild)
6	Wieprza River	TW 96	Darłowo		October	42	Pomeranian (wild)
1	Vistula River	TV 09	Świbno	2009	October	50	Vistula (wild)
2	Drwęca River	TD 09	Lubicz		September- November	49	Vistula (wild)
3	Rega River	TR 09	Trzebiatów		September- November	50	Pomeranian (wild)
4	Parsęta River	TP 09	Białogard		September- November	50	Pomeranian (wild)
5	Słupia River	TS 09	Słupsk		November	50	Pomeranian (wild)
6	Wieprza River	TW 09	Darłowo		September- November	50	Pomeranian (wild)
7	Miastko Hatchery	TM 05	Miastko hatchery	2005	November	50	Vistula (hatchery originally from Świbno)
					Total	595	

a significantly lower percentage of homing. The stocking of the Pomeranian rivers comprises 30–40% of the total of Polish sea trout releases (Pedersen et al. 2012). The 'Stocking of Polish marine areas' programme of The Ministry of Agricultural and Rural Development Board currently regulates both stocking and the duties of particular fishing region users. Juveniles from artificial reproduction are released only to their parental river, which restricts the mixing of the gene pool of fish from different populations as happened in the past, before 1990s. In addition, the passages in Poland have been improved since and this may also have contributed to the enhanced restitution of natural spawning.

Studies carried out in the middle of 1990s using allozyme markers, shortly after implementation of the more restrictive stocking strategy indicated that the genetic structure of populations from the Pomeranian rivers Rega and Parsęta, as well as from the Słupia and Vistula rivers was disturbed (Woźnicki et al. 1999; Łuczyński et al. 2000; Wenne et al. 2000). A low level of differentiation between Vistula River and łupia River populationshas been demonstrated using mtDNA (Włodarczyk and Wenne 2001), and growth hormone gene 2 (GH2) marker analysis (Włodarczyk et al. 1999). A slightly higher level of differentiation was observed by the application of microsatellite DNA (Wąs and Wenne 2002; 2003). However, no molecular markers have been found suitable for application in tracing the origin of spawners entering the Vistula river. Newer studies based on SNP methodology enabled the differentiation of the Vistula and Pomeranian (Słupia) sea trout populations (Drywa et al. 2013).

The aim of this paper was to find changes over 13 years in genetic structure of very weakly spatially differentiated and intensively enhanced sea trout populations in southern Baltic, Poland.

2 Material and methods

2.1 Sample collection and DNA extraction

Two sets of sea trout samples collected from 6 rives in 1996 and 2009 were studied. Approximately 5 mm^2 fin clip samples were collected from 595 individuals. Adult spawners were caught in traps in 5 rivers and electrofished in Parsęta River in 1996 and 2009, and adult spawners from the Aquamar hatchery in Miastko, Poland in 2005 used for artificial spawning (Fig. 1, Table 1). The hatchery stock in Aquamar is thought to represent a native winter migrating group of sea trout in the Vistula River and is used for production of stocking material for releases in the Vistula only. Fin clips were stored in 95% EtOH solution at 4 °C prior to DNA extraction. Genomic DNA was extracted using Genomic Mini Kit (A&A Biotechnology). DNA quality was assessed by running sample aliquots on 1% agarose gels. Samples were quantified using the Epoch^{TM} Spectrophotometer System (BioTek) and diluted to a final concentration of 20ng/μl in TE buffer.

2.2 SNP genotyping and evaluation of genetic differences between populations

Assays were designed for 62 candidate SNPs selected from results of genotyping with the Atlantic salmon custom design Illumina iSelect SNP array containing 15 225 markers of 24 samples of sea trout from Vistula and Slupia rivers (Drywa et al. 2013; 2014; Poćwierz-Kotus et al. 2014). SNP genotyping was performed using the Sequenom MassARRAY iPLEX platform (Gabriel et al. 2009) in Centre for Integrative Genetics, Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, Ås, Norway. Polymorphic loci in the studied sea trout populations were selected from genotyping results using locus-by-locus AMOVA (Analysis of Molecular Variance) after 10 000 permutations. Population genetic differentiation was analyzed using global weighted average F-statistic over loci and pairwise F_{ST} (Weir and Cockerham 1984; Weir 1996) with 1000 permutations and significance level $P < 0.05$. The critical probability value for each test was adjusted using the Bonferroni correction (Rice 1989) using FSTAT software 2.9.3.2 (Goudet 1995). Genetic diversity was assessed by number of polymorphic SNPs (P_O) and values of observed (H_O) and expected (H_E) heterozygosity (Nei 1978; 1987). All tests, except Bonferroni correction, were carried out by Arlequin 3.5.1.2 (Excoffier and Lischer 2010).

Takezaki's POPTREE program was used to create a neighbor joining tree using D_A distances (Nei 1978) with 1000 bootstrap replications (Takezaki et al. 2010). The identification of distinct genetic populations and individuals assignment to them was performed with a Bayesian-model-based, clustering analysis using Structure 2.3.4 (Pritchard et al. 2000). Individuals were assigned to predefined K populations (from K =1–16 with 10 iterations for each K), where each K was characterized by a set of allele frequencies for each locus. An admixture model with no a priori information on the origin of the individuals with 10 000 repetitions as burn in, and 100 000 repetitions after burn in, was applied. Individuals were probabilistically assigned to one or more clusters if their genotypes indicated a mixing. The true K was estimated following the method described by Evanno et al. (2005) using STRUCTURE HARVESTER program (Earl and vonHoldt 2012). The CLUMPP 1.1.2 (Jakobsson and Rosenberg 2007) software was used to average the results from the ten replicate cluster analyses. Full Search algorithm and 10 000 random permutations were applied.

Correspondence between populations was assessed using a three-dimensional Factorial Correspondence Analysis (FCA) implemented in GENETIX 4.05.2 software (Belkhir et al. 2000; Benzécri 1992). The ability of the selected markers set to assign individuals to the most likely population, indicated by STRUCTURE 2.3.4 software, was assessed using ONCOR software (Kalinowski et al. 2007). Assignment tests to evaluate the accuracy of identification of individuals to populations by leave-one-out method were performed. Two approaches were applied. First, with a priori baseline populations and second with reporting groups based on clustering results.

3 Results

3.1 Characterization of SNPs, quality control and basic statistics

A total of 595 sea trout specimens from 6 rivers and a hatchery in Poland were genotyped. Candidate SNPs were divided into three groups: failed (lack of genotyping results), monomorphic and polymorphic. Missing data rate, set at a 5% level, reduced the number of specimens to 576 and loci to 47, of which 14 loci were monomorphic and another 4 had minor allele frequency (MAF) values lower than 0.01. The remaining 29 loci were used for further analysis. AMOVA analysis resulted in the selection of 22 polymorphic loci (P_O) with statistically significant results in all populations. Table 2 shows frequencies of alleles for the 13 studied sea trout populations.

The most common polymorphism was A/G appearing in 12 loci (Table 3). The global F_{ST} was 0.03. The highest F_{ST} value for individual loci obtained in AMOVA was 0.095 for locus no. 14 and the lowest was 0.007 for locus No. 21. The highest heterozygosity values were observed for 6 loci with values around 0.5. And the lowest heterozygosity values were present in 3 loci with values less than 0.1 (Table 3). Most of the loci were in Hardy-Weinberg equilibrium (HWE). The highest numbers of loci in H-W disequilibrium were found in the hatchery population TM 05 (Table 4). This may have been an unfavorable effect occurring in breeding: founder effect, nonrandom mating and limited gene pool, or it may have been a high number of closely related individuals in the sample analyzed (Wąs and Bernaś 2016). SNPs were polymorphic (P_O) in different numbers for particular populations, from 18 for TR 96 population to 22 for 4 populations; TV 96, TD 96, TW 96 and TS 09. The percentage of polymorphic SNPs ranged from 82% to 100% in populations. Average gene diversity over loci in populations ranged from 0.170 for TM 05 to 0.270 for TW 09 (Table 4). A significant excess of homozygotes was noticed in 3 populations, mainly in those collected in 1996 (TV 96, TR 96) and in the population TD 09 (with the largest excess).

3.2 Genetic differences between populations

Genetic differences between pairs of populations ranged from 0 to 0.16 (Table 5). Forty seven (47.44%) of the F_{ST} values greater than 0 were statistically significant ($P < 0.05$). The samples collected from rivers in 2009 showed greater differentiation than those from 1996. Vistula samples from 2009 (TV 09 and TD 09), when compared to samples from the Pomeranian rivers from 2009 showed greater differentiation than shown by a similar comparison of the same sampling sites from 1996. After Bonferroni correction 8 (10%) of the F_{ST} values were statistically significant ($P < 0.00064$). Among the other pairs of populations statistically significant differentiation was found for the Vistula and Wieprza populations from 2009. The hatchery population (TM 05) was the most divergent and differentiated from all other samples, but less divergent from Vistula (TV 09) and Drwęca (TD 09) samples. Neighbor-joining (NJ) trees constructed on the basis of the D_A distances from 22 loci showed genetic relationships between the analyzed populations. In samples from the 1996 differentiation between Vistula River and Pomeranian rivers was not observed. Even more, the TV 96 group was clustered with TS 96 and TP 96 (Fig. 2a). 13 years later Vistula sea trout populations (including Drwęca River) were significantly different from Pomeranian populations. The analysis of 7 populations demonstrated similarity between Vistula (TV 09) and its tributary Drwęca (TD 09) and a hatchery sample TM 05 (Fig. 2b).

STRUCTURE analysis was carried out for 22 selected loci. The highest value of delta K was estimated for $K = 3$ and reached 1900.56 without other significant peaks (Figs. 3a and 3b). A Factorial Correspondence Analysis (FCA) was carried out to investigate genetic relationships among the 12 samples from rivers (Fig. 4). The first two axes accounted for 69% of the total variation. The first axis (42.42% variance) showed separation between all Pomeranian samples from 1996 and 2009 and Vistula River samples from 1996, and the Vistula trout samples from 2009: TD 09 and TV 09. In addition, all samples from 1996 formed a very homogenous group while Pomeranian samples from 2009 were more spread. The leave-one-out test, in ONCOR, found 17.7% of fish correctly assigned back to the river from which they were sampled. After the formation of two main reporting groups, by structure analysis results, the proportion of correctly self-assigned individuals increased to 82%. In the first group consisting of all samples apart from hatchery TM 05, self-assignment accounted for 85%. In the second, hatchery TM 05, self-assignment was 78%.

Table 2. Allele frequencies for 22 loci.

# locus	Locus name	Allele	1996 (wild)						2009 (wild)						2005 (hatchery)
			TV 96	TD 96	TP 96	TR 96	TS 96	TW 96	TV 09	TD 09	TP 09	TR 09	TS 09	TW 09	TM 05
1	BASS15 B7 G03 752	A	0.11	0.04	0.06	0.05	0.04	0.16	0.11	0.10	0.09	0.14	0.04	0.07	0.01
2	ESTNV 30598 384	A	0.64	0.76	0.69	0.65	0.58	0.69	0.62	0.61	0.70	0.49	0.62	0.62	0.27
3	ESTNV 32645 328	A	0.30	0.31	0.40	0.27	0.31	0.26	0.44	0.42	0.33	0.33	0.35	0.27	0.70
4	ESTNV 36818 1287	A	0.03	0.13	0.08	0.09	0.08	0.16	0.07	0.11	0.18	0.11	0.13	0.20	0.06
5	ESTV 15513 116	A	0.56	0.63	0.60	0.65	0.61	0.65	0.52	0.50	0.56	0.74	0.55	0.64	0.38
6	ESTV 21029 995	A	0.44	0.32	0.42	0.35	0.50	0.45	0.38	0.48	0.40	0.43	0.40	0.51	0.18
7	GCR cBin12977 Ctg1 237	A	0.20	0.16	0.21	0.17	0.15	0.11	0.16	0.15	0.17	0.15	0.11	0.06	0.01
8	GCR cBin2032 Ctg1 107	C	1,00	0.96	0.99	0.91	0.99	0.95	0.99	0.97	0.99	1,00	0.96	0.97	1,00
9	GCR cBin31289 Ctg1 125	A	0.99	0.99	0.95	0.89	0.97	0.97	0.98	0.98	0.95	0.92	0.87	0.99	1,00
10	GCR cBin8345 Ctg1 295	A	0.92	0.86	0.83	0.88	0.82	0.88	0.94	0.90	0.86	0.82	0.93	0.79	0.99
11	GCR cBin8670 Ctg1 184	A	0.04	0.06	0.09	0.11	0.06	0.09	0.10	0.14	0.11	0.28	0.21	0.27	0.19
12	ESTNV 36533 796	A	0.19	0.15	0.30	0.25	0.26	0.28	0.20	0.14	0.23	0.17	0.33	0.19	0.07
13	ESTNV 36651 1364	A	0.01	0.02	0.04	0.01	0.02	0.05	0.09	0.02	0.03	0.02	0.02	0.01	0.01
14	ESTV 13981 575	A	0.91	0.96	0.85	0.97	0.89	0.91	0.80	0.86	0.91	0.93	0.92	0.81	0.55
15	ESTV 15243 314	A	0.30	0.31	0.39	0.29	0.33	0.25	0.45	0.43	0.36	0.32	0.35	0.28	0.72
16	ESTV 21509 1244	A	0.23	0.27	0.24	0.26	0.27	0.16	0.19	0.13	0.29	0.28	0.21	0.21	0.03
17	GCR cBin8345 Ctg1 275	A	0.08	0.15	0.17	0.13	0.15	0.15	0.06	0.10	0.18	0.18	0.11	0.21	0.02
18	GCR hBin58067 Ctg1 173	A	0.92	0.96	0.89	0.91	0.95	0.91	0.91	0.89	0.97	0.90	0.83	0.84	0.98
19	GCR hBin7522 Ctg1 160	A	0.91	0.88	0.91	0.93	0.91	0.93	0.70	0.88	0.88	0.88	0.96	0.95	0.67
20	ESTNV 34724 1228	A	0.95	0.95	0.95	0.91	0.91	0.91	0.83	0.94	0.90	0.97	0.97	0.94	0.83
21	ESTNV 37153 794	A	0.40	0.42	0.49	0.50	0.36	0.34	0.43	0.55	0.41	0.39	0.53	0.52	0.49
22	GCR hBin20991 Ctg1 222	A	0.10	0.16	0.14	0.10	0.10	0.10	0.22	0.15	0.11	0.07	0.24	0.09	0.18

Table 3. Genetic variability of 22 SNPs in 13 sea trout populations. MAF = Minor Allele Frequency; rs = reference SNP ID from dbSNP; H_O – observed heterozygosity; H_E – expected heterozygosity. Loci marked with an asterisk have a highly significant deviations ($P < 0.01$) from Hardy-Weinberg equilibrium.

Locus#	Locus name	Region	Allele	F_{ST}	Mean MAF	dbSNP access	Averange Ho	Averange He
1	BASS15 B7 G03 752	Coding	A/C	0.014	0.078	rs119097369	0.128	0.143
2	ESTNV 30598 384	Coding	A/G	0.052	0.391	rs159406625	0.460	0.453
3	ESTNV 32645 328	Coding	A/G	0.041	0.358	rs159404820	0.350	0.442*
4	ESTNV 36818 1287	Coding	A/G	0.012	0.110		0.190	0.191*
5	ESTV 15513 116	Coding	A/G	0.022	0.420		0.538	0.477
6	ESTV 21029 995	Coding	A/G	0.022	0.406		0.501	0.474*
7	GCR cBin12977 Ctg1 237	Coding	A/T	0.014	0.136	rs159401279	0.237	0.236
8	GCR cBin2032 Ctg1 107	Coding	C/G	0.014	0.024	rs159401555	0.066	0.049
9	GCR cBin31289 Ctg1 125	Coding	A/G	0.027	0.042		0.092	0.079
10	GCR cBin8345 Ctg1 295	Coding	A/T	0.020	0.123		0.198	0.214
11	GCR cBin8670 Ctg1 184	Coding	A/C	0.041	0.144	rs159403953	0.209	0.226
12	ESTNV 36533 796	Coding	A/G	0.023	0.209		0.325	0.329
13	ESTNV 36651 1364	Coding	A/G	0.008	0.029	rs159405523	0.057	0.055
14	ESTV 13981 575	Coding	A/C	0.095	0.140	rs119097008	0.208	0.211
15	ESTV 15243 314	Coding	A/G	0.041	0.362	rs119097514	0.370	0.444
16	ESTV 21509 1244	Coding	A/G	0.022	0.209		0.425	0.329*
17	GCR cBin8345 Ctg1 275	Coding	A/T	0.016	0.129		0.216	0.224
18	GCR hBin58067 Ctg1 173	Coding	A/G	0.017	0.088		0.164	0.157
19	GCR hBin7522 Ctg1 160	Coding	A/C	0.062	0.129	rs159402846	0.216	0.208
20	ESTNV 34724 1228	Coding	A/T	0.018	0.082	rs159405162	0.161	0.146
21	ESTNV 37153 794	Coding	A/G	0.007	0.453	rs159407311	0.550	0.492
22	GCR hBin20991 Ctg1 222	Coding	A/C	0.011	0.138	rs159403997	0.246	0.233

Table 4. Genetic variability of 13 sea trout populations among 22 SNPs. H_O – observed heterozygosity; H_E – expected heterozygosity; P_O – polymorphic loci; Loci no. with highly significant HWE departure ($P < 0.01$) in populations are listed in parentheses. F_{IS} – the inbreeding coefficient (values with $P < 0.05$ are marked in bold).

Pop	Mean Ho	Mean He	No. of Po	Average gene diversity over loci	Number of loci with HWE departure ($P < 0.05$)	Specimens no.	F_{IS}
TV 96	0.222	0.252	22	0.211	3 (no. 3)	38	**0.082**
TD 96	0.237	0.244	22	0.213	2	41	0.037
TP 96	0.272	0.281	21	0.251	0	39	0.022
TR 96	0.243	0.262	18	0.225	0	34	**0.055**
TS 96	0.267	0.259	21	0.218	1	41	–0.030
TW 96	0.271	0.262	22	0.226	0	40	–0.027
TV 09	0.288	0.29	19	0.227	0	49	0.001
TD 09	0.251	0.268	20	0.225	1	49	**0.038**
TP 09	0.323	0.276	19	0.217	4 (no. 16)	49	–0.117
TR 09	0.307	0.288	19	0.247	3	48	–0.041
TS 09	0.264	0.279	22	0.257	1	48	0.035
TW 09	0.332	0.279	20	0.270	2 (no. 6)	50	–0.124
TM 05	0.246	0.242	19	0.170	5 (no.4)	50	0.016

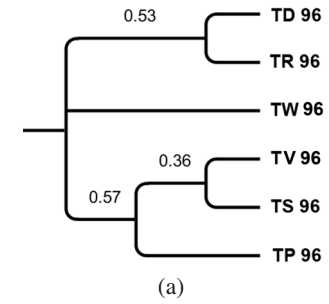
4 Discussion

The negative effect of dams on populations of freshwater and anadromous fish as manifested by blocking spawning and foraging migrations, population fragmentation or changed water regimes have been reported often (e.g. Jager et al. 2001; Morita and Yamamoto 2002). All of them have genetic consequences, for example reduction in effective population size, shift in migration-selection-drift equilibrium and in local adaptations (Meldgaard et al. 2003; Faulks et al. 2011; Paris et al. 2015). A divergence of brown trout populations isolated by dams established as early as middle ages has been found (Hansen et al. 2014).

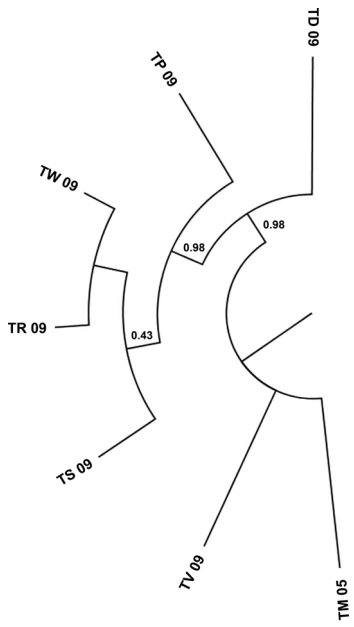
Considerable temporal stability of genetic composition of brown trout populations have previously been demonstrated (Hansen et al. 2002; Jensen et al. 2005). Studies, based on microsatellite DNA, in some cases resulted in the observation of temporal changes in the genetic composition, which may be explained by drift in small persisting populations of brown trout (Ostergaard et al. 2003). However, in larger populations of salmon, temporal variation in allele frequencies was due to the effect of hatchery practices (Koljonen et al. 2005). Hansen et al. (2009) have shown admixture of brown trout populations in Denmark with hatchery trout. The admixture may have caused reduced local adaptation. However, in contrast to Danish populations, Polish populations have been very

Table 5. F_{ST} pairwise differences for 22 SNPs. Bolded values are statistically significant. Values with an asterisk are statistically significant after Bonferroni correction.

	TV 96	TD 96	TP 96	TR 96	TS 96	TW 96	TV 09	TD 09	TP 09	TR 09	TS 09	TW 09
TD 96	0.003											
TP 96	0.000	0.003										
TR 96	0.005	–0.009	–0.009									
TS 96	–0.005	0.008	–0.005	–0.07								
TW 96	–0.002	0.007	0.004	–0.006	–0.001							
TV 09	**0.014**	**0.026**	**0.008**	**0.017**	**0.019**	**0.030**						
TD 09	0.000	**0.015**	0.000	0.006	0.006	**0.016**	0.002					
TP 09	–0.002	–0.005	–0.011	–0.010	–0.008	–0.006	**0.009**	**0.006**				
TR 09	0.009	**0.014**	**0.007**	0.004	–0.003	0.000	**0.017**	**0.016**	**0.010**			
TS 09	0.010	**0.011**	0.000	–0.008	0.004	0.009	**0.011**	**0.007**	**0.007**	**0.017**		
TW 09	**0.014**	**0.019**	0.004	0.004	0.003	0.004	**0.029***	**0.012**	0.004	0.002	**0.013**	
TM 05	**0.133**	**0.150***	**0.118***	**0.148**	**0.134***	**0.155***	**0.065**	**0.081**	**0.130***	**0.121**	**0.108**	**0.128***



(a)



(b)

Fig. 2. Bootstraped neighbor-joining tree based on the D_A distances from 22 loci using POPTREE2 software (Takezaki et al. 2010) for sea trout populations sampled in 1996 (a), and 2009 and a hatchery population in 2005 (b).

weakly differentiated due to low homing in Pomeranian populations and long term artificial supplementation (Dębowski and Bartel 1995). The Vistula sea trout populations underwent a bottleneck effect in early 70ties due to the construction of a dam in Włocławek in 1968 (Wąs and Bernaś 2016). Part of genetic unique genotype composition of the native populations in Vistula was lost before the intensive stocking with the Pomeranian sea trout was carried out and cannot be restituted in living papulations. Temporal changes in genetic composition in the studied populations of sea trout in Poland, southern Baltic as identified using SNPs, though low, were observed and concerned mainly the Vistula populations. Values of pairwise F_{ST} for populations sampled in 2009 were higher suggesting that they are more diverse than those from 1996. There were no significant values in pairwise F_{ST} in the Pomeranian and Vistula river populations (TV 96 and TD 96). This may be explained by intensive enhancement and the practice of mixing the stocking material carried out in the past. Samples from the Vistula River and Drwęca River collected in 2009 were not significantly differentiated from each other, but were different from each Pomeranian river.

The pairwise comparison between individuals from the same rivers collected at different times (1996 and 2009) revealed that over these 13 years the biggest change was observed for the Vistula River (TV 96 and TV 09) and its tributary the Drwęca River (TD 96 and TD 09) while Pomeranian populations were more stable over time. Pairwise differences of F_{ST} values between the hatchery population and all the wild populations were statistically significant. A Factorial Correspondence Analysis (FCA) and NJ tree revealed a strong separation of the Aquamar (Miastko) hatchery population. The nearest to it was the Vistula sample collected in 2009. The heterozygosity values inferred from SNPs in this study were at a similar level, around 0.3, in populations sampled in 1996 and 2009.

The hatchery population Aquamar was bred from the winter Vistula sea trout and it did not seem to have the admixture of the Pomeranian sea trout genotype. It accounts for 15–60% of yearly releases to Vistula in 2000s. The stocking material used for releases in Drwęca River originated from spawners caught in lower river course and sometimes also from specimens electrofished below Włocławek Dam in the Vistula River. Vistula and Drwęca populations seem to become more similar to the hatchery sample from Aquamar over time, which may indicate influence of releases of hatchery fish. Increased divergence between Vistula sea trout and Pomeranian populations can be considered as step towards partial restoration of native population in the Vistula basin.

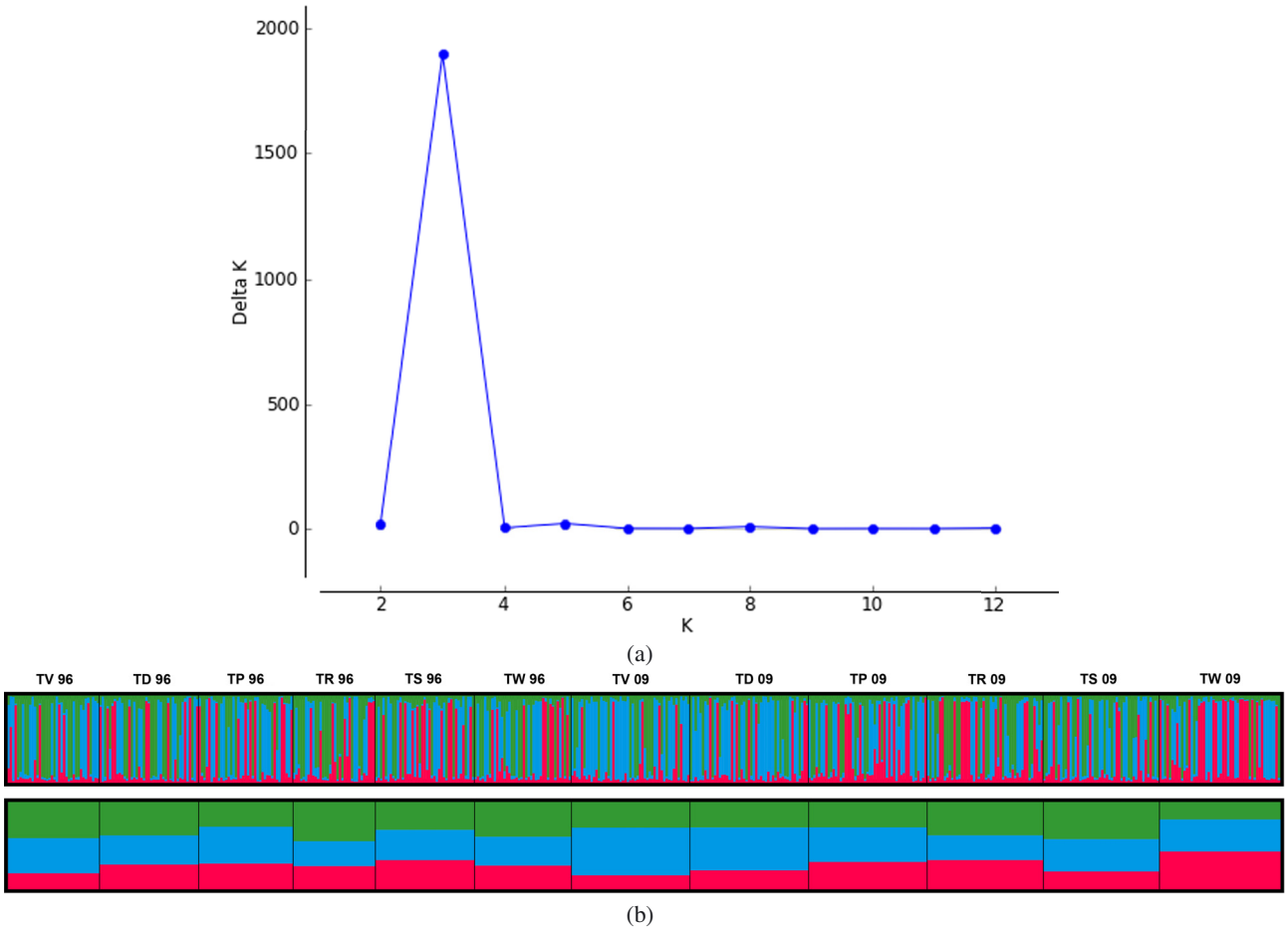


Fig. 3. (a) ΔK calculated as $\Delta K = m|L''(K)|/s[L(K)]$ against K values for sea trout populations to identify the optimum number of genetic units. The data generated with STRUCTURE HARVESTER program (Earl and vonHoldt 2012). (b) Proportion of membership of 526 individuals from 12 sea trout populations for $K = 3$ calculated by STRUCTURE v.2.3.4 (Pritchard et al. 2000) software and averaged by CLUMPP v.1.1.1 (Jakobsson and Rosenberg 2007) software. Plots were generated by DISTRUCT v.1.1 (Rosenberg 2004) software.

The changes in the genetic structure of sea trout populations from Vistula and Drwęca rivers over the period 1996–2009, observed in this study, may be linked to the new strategy of the 'Stocking of Polish marine areas' programme of The Ministry of Agricultural and Rural Development Board. The reduction of the putative Pomeranian genotypes among Vistula populations may also be a result of this stocking strategy, and may lead to a partial recovery of the native Vistula sea trout population. Despite the release of hatchery offspring of individuals chosen for artificial spawning solely into the parental river, specimen selection is not currently based on genetic tests. This leaves a real possibility that spawning individuals can be erroneously selected from 'contaminated' stock, with a pool of ancestors from the Pomeranian populations. Therefore a further genetic monitoring of the source spawners for production of hatchery offspring needs to be developed and performed. However, implementation of genetic monitoring can reduce but not entirely eliminate, negative effects of releases of fish reared in hatcheries (Kallio-Nyberg et al. 2015). Supportive breeding programmes, in which the gametes used for carrying out breeding are collected from spawner fish obtained from the same river area to which the resulting juvenile fish are subsequently released may help to overcome the problem of potential lack of local adaptation but not necessarily a partial loss of allelic richness (Selly et al. 2014). A further process of re-adaptation of the restituted population in Vistula River will be needed over some generations.

5 Conclusion

Salmo trutta m. *trutta* populations in the southern Baltic have been characterized by low degree of genetic differentiation mainly due to admixture prior to the 1990s. The results of the present study prove the occurrence of temporal changes in populations of sea trout in Poland in the period 1996–2009. These changes can be considered to reflect the historic pattern of differentiation between 2 main groups of populations (Vistula and Pomeranian) and, in the studied period, may result from the implementation of a new stocking strategy for the restoration and protection of Vistula sea trout. Clearance of existing and building of new passes for migration of fishes around dams, restitution of river niches appropriate for natural spawning of sea trout could also have influenced the observed changes. In the last 15 years dozens of new fish passes have been constructed on Pomeranian rivers. Also an old fish pass

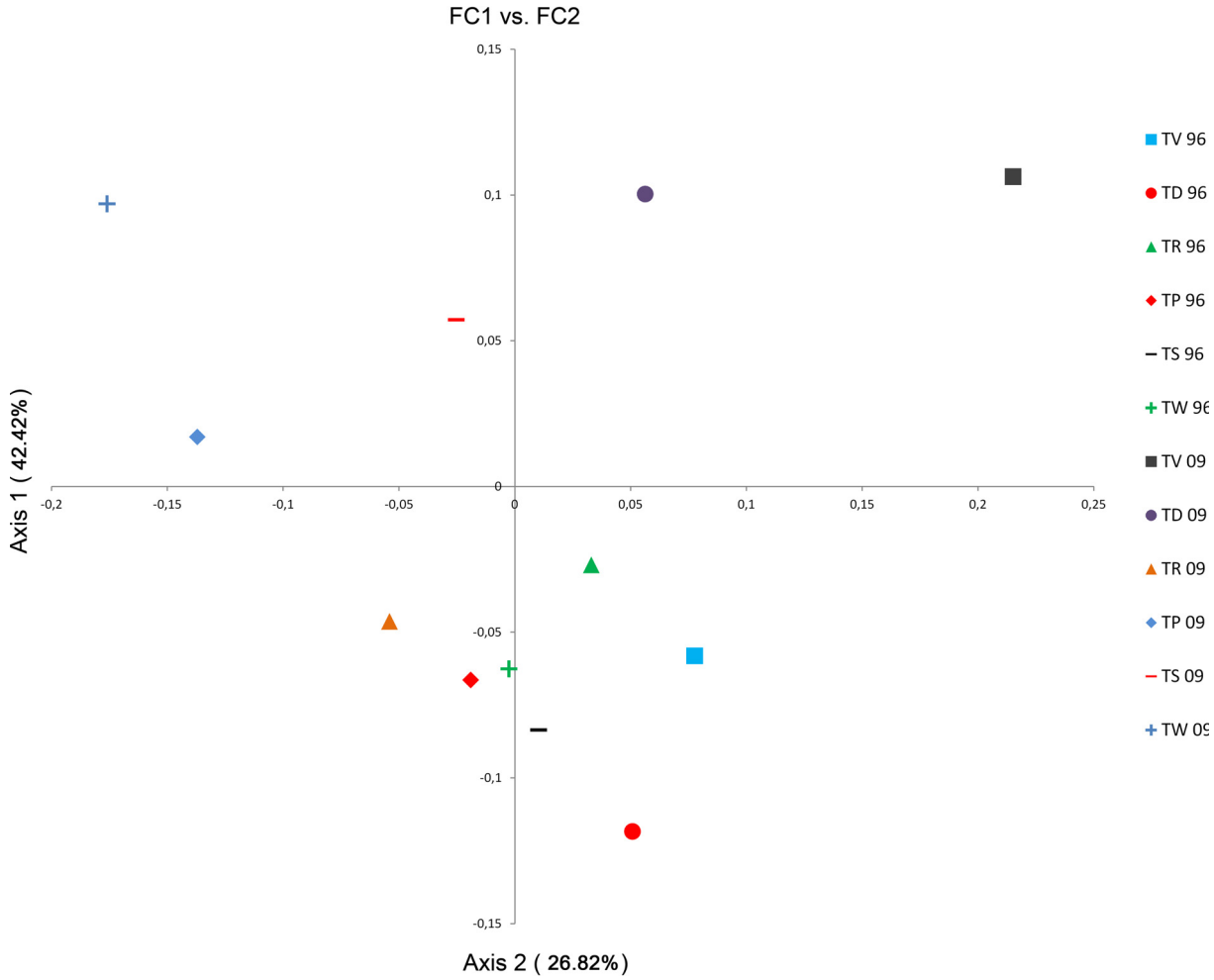


Fig. 4. First two axes of the Factorial Correspondence Analysis (FCA) determined from SNP data for studied trout populations in Poland. Each point represents one sample location. The analysis was performed with Genetix software (Belkhir et al. 2000; Benzécri 1992).

on the Włocławek dam was rebuilt in 2015. All these activities have greatly increased the possibility of sea trout migration. With an increase of natural breeding, artificial enhancement of sea trout populations should be reduced.

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