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Application of allozyme markers for screening of turbot populations along Western Black Sea coast

VENELIN NIKOLOV, PETYA IVANOVA*, NINA DZHEMBEKOVA, MARINA PANAYOTOVA, VIOLIN RAYKOV, IVAN DOBROVOLOV

Institute of Oceanology, BAS, First May Street 40, P.O.Box 152, Varna 9000, Bulgaria, *e-mail: pavl_petya@yahoo.com

Abstract: Data for electrophoretic pattern of 26 loci, resolved for the 8 protein systems (7-enzyme and 1-non-enzyme) assayed were used to investigate population structure of turbot along the Bulgarian and Romanian Black Sea coasts using genetic diversity measures. Seventeen loci were polymorphic in all populations and a total of 34 alleles were identified. Four types of tissue: muscle, retina, plasma and haemoglobin were analyzed. The percentage of polymorphic loci was high (65.38%) within populations. A low level of genetic differentiation among populations was detected, based on the Shannon's information index (0.446-0.448) and the coefficient of genetic differentiation between populations (FST =0.014). The overall mean of within-population inbreeding estimate (FIS) was (-0.209) and demonstrated low level of inbreeding. The genetic distance (DNei) between the populations was low and vary between 0.003 and 0.014. Genetic distances among turbot populations were positively correlated with geographic distances (r = 0.474), but the association was not significant according to the Mantel test (p=0.651) and showed a lack of correlation between genetic distance and the geographic location of populations. Results identified one genetic stock with sufficient gene flow between all the three sites to prevent genetic differentiation from occurring. Only 1.4% of the genetic variation was observed among populations. Results revealed that adopting a single stock model and regional shared management could probably be appropriate for sustainable long-term use of turbot along western Black Sea coast. Determination of the contemporary state of the population distribution will be the prerequisite for determination of adequate measures for exploitation and protection of the existing turbot populations along western Black Sea coast.

Key words: allozymes, turbot, populations, genetic distance, western Black Sea.

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Introduction

The turbot *Psetta maxima* is naturally distributed in European waters, from Northeast Atlantic to the Arctic Circle. It occurs in the Baltic and in the Mediterranean, as well as in the Black Sea, where a subspecies *Psetta maxima maeotica* has been described. Two generic names are available for the species – *Scophthalmus maximus* (Linnaeus 1758) and *Psetta maxima* (Linnaeus 1758). Recent studies of Bailly & Chanet (2010) strongly recommended using Scophthalmus as the valid generic name for the turbot.

The Black Sea turbot is one of the most valuable commercial species in all countries of the Black Sea basin. It is subject of intensive exploitation and endangered from extinction. Current status of the turbot population in the Black Sea characterized the stock as exploited unsustainably and at risk of collapse (STECF 2014) and "overexploited" and "in overexploitation" (GFCM 2014).

The preservation of the turbot population requires knowledge for the population genetic structure and constant monitoring of its biodiversity.

Different opinions exist regarding the availability of turbot local populations (ecotypes) in the Black Sea. Shlyakhov (2014) considered that turbot in the Black Sea is presented by several local populations, which mix in the adjacent areas. The strongest one of them - "Western" - is distributed in the waters of Ukraine, Romania and possibly in Bulgaria, where it mixes partially with the local population and the "North-Eastern" population is distributed in the waters of the Russian Federation, Ukraine and partially in Georgia (Shlyakhov 2014). Stock identification and stock boundaries are still not well defined and for the time being the turbot population in the Black Sea is assessed as a single stock (STECF 2014).

The population structure of turbot was subject of several allozyme studies (Blanquer *et al.* 1992, Bouza *et al.* 1994, 1997, 2002, Exadactylos & Thorpe 2001, Exadactylos *et al.* 2001, Imsland *et al.* 2003, Nielsen *et al.* 2004, Ivanova *et al.* 2006, Tsekov *et al.* 2008). According to Danancher & Garcia-Vazquez (2006) very little is known about population structure in wild turbot.

Recently the molecular marker technologies become an essential tool for analysis of genetic diversity applied in fish systematics, population genetics and conservation biology. Genetic methods are the most important tools for defining stock structure and evaluating levels and patterns of genetic diversity in fishes Liu & Cordes (2004).

The molecular markers were applied only to the closely related Mediterranean turbot. Suzuki *et al.* (2004), Prado *et al.* (2005), Bouza *et al.* (2002), Vera *et al.* (2011) used mitochondrial and nuclear DNA markers for taxonomic studies of the Mediterranean population. Atanassov *et al.* (2011) using mitochondrial control region of DNA variation to analyze the turbot populations from Bulgarian and Romanian Black Sea coasts.

The goal of the study is to evaluate genetic structure in natural turbot stocks (populations) along the Bulgarian and Romanian coast based on allozyme data.

Material and Methods

Allozyme analyses

65 turbot samples from the western Black Sea coast (Fig.1) caught between 2010-2012 were analyzed. Data for electrophoretic pattern of 26 loci were used to investigate population structure of turbot along the Bulgarian and Romanian Black Sea coasts, analyzing four types of tissue: muscle, eye, haemoglobin and plasma.

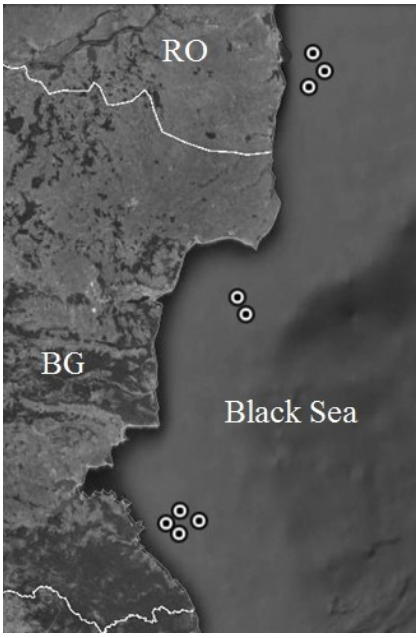

Fig.1. Sampling localities of turbot samples

For the analysis of the enzymes and non-enzyme protein systems, a homogenate of white dorsal muscle was used. Proteins were separated by horizontal starch gel electrophoresis according to Smithies (1955) methods, modified by Dobrovolov (1973).
The following 7 enzymatic and one nonenzymatic systems were studied: General muscle proteins (PROT), esterase (EC 3.1.1.1 - EST), lactate dehydrogenase (EC 1.1.1.27 - LDH), malate dehydrogenase (EC 1.1.1.37 - MDH), malic enzyme (EC 1.1.1.40 - MEP), superoxide dismutase (EC 1.15.1.1 - SOD), fumarase (FH) and phosphoglucoisomerase PGI.
The proteins were stained with Commassie Brilliant Blue R-250. Staining of different enzymes was performed according to Shaw & Prasad (1970). Buffer systems of Dobrovolov (1976) and Clayton & Gee (1969) were used for the electrophoresis.

The nomenclature of mentioned loci and alleles followed essentially the recommendation of Shaklee *et al.* (1990).

Genetic diversity analyses

Gene frequencies of the polymorphic loci were calculated using the Hardy-Weinberg equilibrium. Calculation of indices of genetic similarity and genetic distance was performed according to Nei (1972).

Genetic diversity was determined as allele frequencies, effective number of alleles (Ne), test of Hardi-Weinerg equilibrium (HWE), observed (Ho) and expected (He) heterosigiosity F-statistics and Nei's genetic distance (D) Nei (1972) using GENALEX 6 (Peakall & Smouse 2006). Percentage of polymorphic loci (PPL), number of different alleles (Na), average effective number of alleles per locus (Ne), average gene diversity (He), Shannon's information index (SI) as well as hierarchical analysis of molecular variance (AMOVA) were done using GENALEX 6 software package.

To visualize the genetic relationship among populations, a dendrogram was constructed based on Nei's genetic distance (D) Nei (1972), by an unweighted pair-group method of cluster analysis using arithmetic averages (PAUP), version 4.0 (Swofford 1998) and Treeview. To test the correlation between Nei's genetic distance (D) between populations and geographic distances (in km) among populations, a Mantel test was performed using IBDWS program.

The program BOTTLENECK (Piry *et al.* 1999) was used to test whether populations have recently passed through a bottleneck. Both the stepwise mutation model (SMM) and the infinite allele model (IAM) were run. The sing test was conducted to determine the significance of heterozygosity excess (Cornuet & Luikart 1996).

Results and Discussion

Allelic variability in turbot populations

Common electrophoretical mobility of LDH–B* (eye-retina), mMDH, sMDH-1*, sMDH-2*, GPI-1*,GPI-2* and FU* (muscle tissue), SOD-1* and SOD-2* (haemoglobin) were observed.
The allelic frequencies of polymorphic loci EST-2*, EST-3*, PROT-1* and PROT-2* (haemoglobin), PROT-1*, PROT-2* and EST-2* (plasma, retina), EST-2*, EST-3*, MEP-1* and MEP-2* (muscle and retina), LDH-A* and LDH-C* (retina) are presented on the Table 1.
General muscle proteins (PROT) - Electrophoretical spectra on general muscle proteins were different on three tissue analyzed (Fig.2 A.). Polymorphism was found on haemoglobins (PROT-1* and PROT-2*), (Fig.2 B) and plasma tissues. The data received for gene frequencies were presented on Table 1.

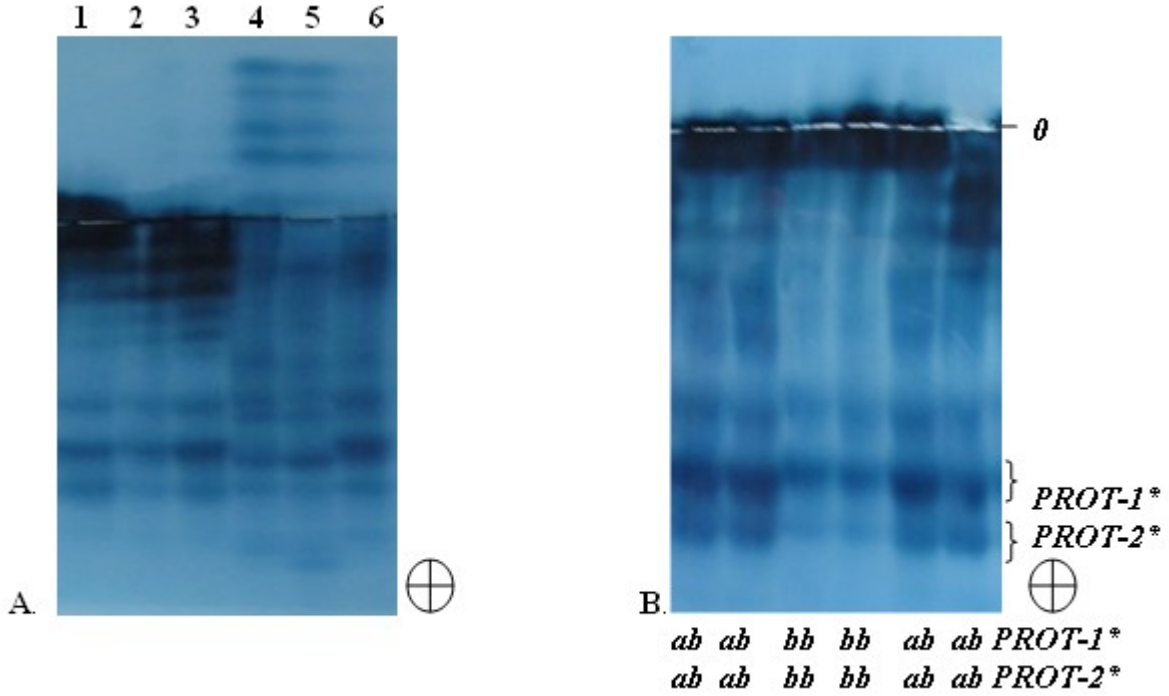

Fig. 2. A. Electrophoregrams on PROT from turbot (Bulgarian and Romanian coast), using different tissues:1-3 – haemoglobins, 4-5- eye (retina) and 6 – muscle, 0 – origin. B. Electrophoregrams on PROT from turbot Bulgarian and Rumanian coast haemoglobin tissue. PROT-1* and PROT-2* were polymorphic, 0 – origin.

The esterases are highly polymorphic. Two esterases loci (EST-2* and EST-3*) were polymorphic in all tissues analyzed and could be used as a marker enzyme system for distinguishing of turbot populations (Fig.3, Table1). The allele frequencies of EST-2* and EST-3* (haemoglobins), PROT-2* and EST-2* (plasma), EST-3*, MEP-1* and MEP-2* (muscle), LDH-C* and PROT-2* (retina) (Table 1) on north Bulgarian and Romanian populations are closely related.

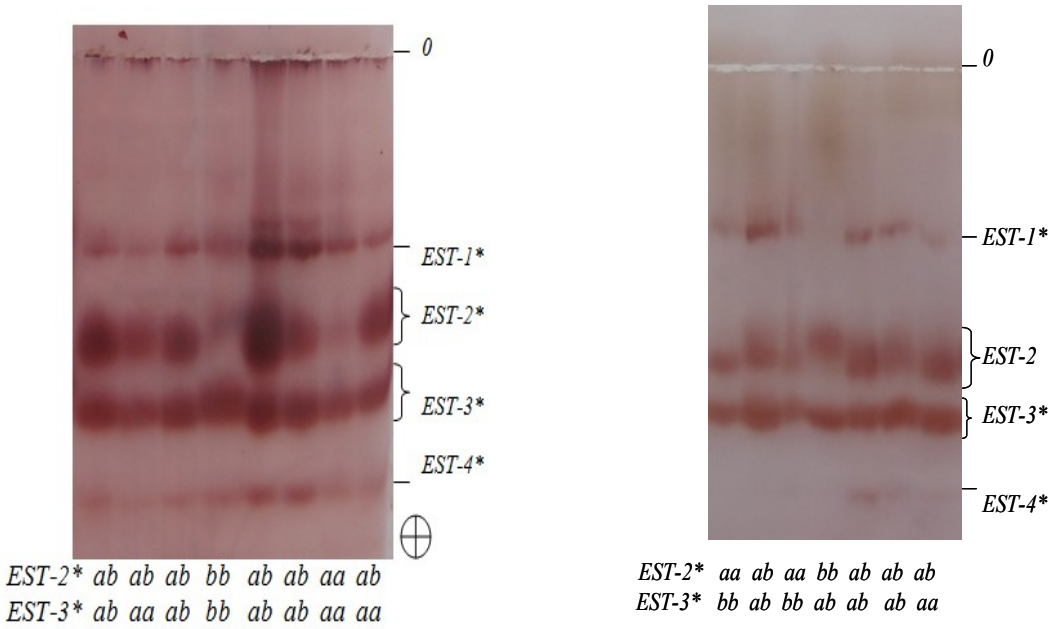

Fig. 3. Enzymograms of general unspecified esterases (EST) from muscle tissue of turbot (1) and haemoglobin (2), EST-2* and EST-3* - polymorphic loci, 0 – origin.

A new polymorphic LDH-C*, sMEP-1* and sMEP-2* loci, useful for identification of turbot stocks along Bulgarian and Romanian coast were found after analyses of retina and muscle tissue (Table 1).

Genetic diversity and genetic structure of western Black Sea populations

Summary statistics i.e. number of individuals screened (n), number of alleles found for each sample at a locus (Na), observed (Ho) and expected (He) heterozygosity for each locus and FIS are shown in Table1.

The percentage of polymorphic loci (PPL) for a single population was 65.38 %, (Table 2). The number of different alleles (Na) was 1.654. The average effective number of alleles per locus (Ne) at the population level was 1.63 ranged from 1.626 to 1.633. The average gene diversity (He) for all 26 loci was estimated 0.321. The Shannon's information index (SI) ranged from 0.446 to 0.448 at the population level (Table 2). No private alleles were found.

Table 1. Allelic frequencies, genetic variation and heterozygosity statistics of 17 polymorphic allozyme loci in turbot populations. n- number of individuals screened; a and b allele frequencies, Ho – observed heterosigosity, He-expected heterosigosity and FIS.

Tissue	Locus	Allele/ Parameter	Romania n=21	BG North n=23	BG South n=21
haemoglobin	*EST-2**	***a***	0.571	0.522	0.452
		b	0.429	0.478	0.548
		H_o	0.571	0.609	0.714
		H_e	0.490	0.499	0.495
		F_{IS}	-0.167	-0.220	-0.442
haemoglobin	*EST-3**	***a***	0.476	0.435	0.595
		b	0.524	0.565	0.405
		H_o	0.667	0.609	0.619
		H_e	0.499	0.491	0.482
		F_{IS}	-0.336	-0.238	-0.285
haemoglobin	*PROT -1**	***a***	0.476	0.565	0.571
		b	0.524	0.435	0.429
		H_o	0.571	0.609	0.667
		H_e	0.499	0.491	0.490
		F_{IS}	-0.145	-0.238	-0.361
haemoglobin	*PROT-2**	***a***	0.548	0.500	0.524
		b	0.452	0.500	0.476
		H_o	0.619	0.739	0.762
		H_e	0.495	0.500	0.499
		F_{IS}	-0.249	-0.478	-0.527
plasma	*PROT-1**	***a***	0.643	0.522	0.524
		b	0.357	0.478	0.476
		H_o	0.524	0.609	0.571
		H_e	0.459	0.499	0.499
		F_{IS}	-0.141	-0.220	-0.145
plasma	*PROT-2**	***a***	0.476	0.478	0.452
		b	0.524	0.522	0.548
		H_o	0.476	0.499	0.714
		H_e	0.499	0.510	0.500
		F_{IS}	0.045	-0.045	-0.429
plasma	*EST-2**	***a***	0.595	0.587	0.500
		b	0.405	0.413	0.500
		H_o	0.524	0.565	0.619
		H_e	0.482	0.485	0.500
		F_{IS}	-0.087	-0.166	-0.238
muscle	*EST-2**	***a***	0.524	0.543	0.524
		b	0.476	0.457	0.476
		H_o	0.667	0.739	0.476
		H_e	0.499	0.496	0.499
		F_{IS}	-0.336	-0.490	0.045

Continued ▼

muscle	*EST-3**	***a***	0.524	0.522	0.476
		b	0.476	0.478	0.524
		H_o	0.571	0.696	0.762
		H_e	0.499	0.499	0.499
		F_{IS}	-0.145	-0.394	-0.527
muscle	*MEP-1**	***a***	0.571	0.630	0.452
		b	0.429	0.370	0.548
		H_o	0.667	0.565	0.524
		H_e	0.490	0.466	0.495
		F_{IS}	-0.361	-0.213	-0.057
muscle	*MEP-2**	***a***	0.619	0.587	0.476
		b	0.381	0.413	0.524
		H_o	0.571	0.652	0.571
		H_e	0.472	0.485	0.499
		F_{IS}	-0.212	-0.345	-0.145
retina (eye tissue)	*LDH-A**	***a***	0.476	0.565	0.571
		b	0.524	0.435	0.429
		H_o	0.571	0.609	0.571
		H_e	0.499	0.491	0.490
		F_{IS}	-0.145	-0.238	-0.167
retina (eye tissue)	*LDH-C**	***a***	0.405	0.391	0.667
		b	0.595	0.609	0.333
		H_o	0.429	0.435	0.571
		H_e	0.482	0.476	0.444
		F_{IS}	0.111	0.087	-0.286
retina (eye tissue)	*EST-2**	***a***	0.476	0.587	0.595
		b	0.524	0.413	0.405
		H_o	0.667	0.565	0.619
		H_e	0.499	0.485	0.482
		F_{IS}	-0.336	-0.166	-0.285
retina (eye tissue)	*EST-3**	***a***	0.524	0.500	0.500
		b	0.476	0.500	0.500
		H_o	0.571	0.565	0.524
		H_e	0.499	0.500	0.500
		F_{IS}	-0.145	-0.130	-0.048
retina (eye tissue)	*PROT-1**	***a***	0.500	0.457	0.429
		b	0.500	0.543	0.571
		H_o	0.524	0.565	0.667
		H_e	0.500	0.496	0.490
		F_{IS}	-0.048	-0.139	-0.361
retina (eye tissue)	*PROT-2**	***a***	0.548	0.587	0.333
		b	0.452	0.478	0.667
		H_o	0.524	0.485	0.476
		Mean H_o	0.571± 0,017	0.596± 0,020	0.613± 0,022
		Mean H_e	0,492± 0,003	0,491± 0,002	0,489± 0,004
		Mean F_{IS}	-0.162±0.033	-0.213±0.038	-0.255±0.042
	Total	Mean H_o	0,594± 0,011		
		Mean H_e	0,490± 0,002		
		Mean F_{IS}	-0.210 ± 0.022		

Table 2. Genetic variability within populations of turbot detected by allozyme analysis. PPL - percentage of polymorphic loci; No-number of different allels; Ne-effective number of alleles per locus; He - expected heterosigosity for 26 loci; SI -Shannon's information index.

Population	PPL (%)	No	Ne	He	SI
Pop R	65.38	1,654±0.095	1,633 ±0,092	0,321±0,047	0,448 ±0,065
Pop N	65.38	1,654±0.095	1,631±0,092	0,321±0,047	0,447±0,065
Pop S	65.38	1,654±0.095	1,626±0,092	0,320±0,047	0,446±0,065

Mean expected heterozygosity (He) per population was relatively high (0.321; Table 2) as compared to genetic diversity values found for turbot wild samples in Liverpool, UK, (0.027 in Exadactylos *et al.* 2001; 0.0295 in Bouza *et al.* 1997, 0.02 - Blanquer *et al.* 1992 and 0.02 - Bouza *et al.* 2002). Our data for the mean heterozygosity He values were higher than previous results, and more close to the data of Florin & Höglund (2007) (He =0. 580), based on microsatellites. The group of flatfish has even shown higher heterozygosity than the average values in marine fish (Smith & Fujio 1982, Brulé 1989). Populations of widespread fish species often show significantly higher heterozygosity estimates than for population of species with more restricted distribution (Gopalakrishnan *et al.* 2009).

Wright's fixation index (FIT) was estimated as for each locus in each sample and represent the deviations from expected heterozigosity in overall and within populations. Negative mean value of FIT (-0.194±0.028) were found at almost all loci across all three populations of turbot, except only rPROT-2 and rLDH-C* loci (Table 3), indicating an excess of heterozygotes. A possible explanation for this pattern is that natural selection might favour heterozygotes that can cope with environment changes in highly fragmented populations.

A negative value of FIS was found for all seventeen loci, with a mean value of -0.209 (Table 3). FIS values indicating that there is heterozygote excess compared with HWE expectations and no inbreeding. Deviation from Hardy-Weinberg proportions indicates selection, population mixing or nonrandom malting. This is mentioned from other authors for natural turbot populations (Exadactylos *et al.* 2001, Florin & Höglund 2007). Our work revealed a high level of heterozygosity in all populations, however, in most cases F was not statistically significant.

Britten (1996) suggested that isolated populations might exhibit high heterozygosity due to strong selection pressures. Size selective fishing gear, destruction of habitat, alteration of prey availability, pollution stress and other such activities can impose new selection pressures on a stock or may alter the existing selection forces (Çiftici & Okomuş 2002)

According to Kang *et al.* (2005) two factors are usually involved in driving selection for heterozygotes, environmental stress and inbreeding depression.

The value of FST, a measure of the degree of differentiation among turbot populations analyzed was 0.014 (Table 3), revealing that about 98.60% of the total genetic diversity resides within populations. This indicated that 1.4% of the variation was because of allozyme differences among the three populations and showed low genetic differentiation. AMOVA analyses showed 100% of molecular variance within individuals of each populations and absence of percentage variance between individuals and populations.

Table 3. F-statistics based on 17 polymorphic loci for three turbot populations.

Locus	No of alleles	F_{IS}	F_{ST}	F_{IT}
Hb EST-2*	2	-0.276	0.010	-0.264
Hb EST-3*	2	-0.287	0.019	-0.263
Hb PROT-1*	2	-0.248	0.008	-0.238
Hb PROT-2*	2	-0.419	0.002	-0.417
Plm PROT-1*	2	-0.169	0.013	-0.154
Plm PROT-2*	2	-0.143	0.000	-0.143
mEST-2*	2	-0.260	0.000	-0.259
mEST-3*	2	-0.356	0.002	-0.353
Plm EST-2*	2	-0.165	0.008	- 0.156
mMEP-2*	2	-0.233	0.015	- 0.215
mMEP-1*	2	-0.210	0.022	- 0.183
rLDH-A*	2	-0.183	0.008	-0.174
rLDH-C*	2	-0.023	0.064	0.043
rEST-2	2	-0.263	0.012	- 0.248
rEST-3	2	-0.108	0.001	-0.107
rPROT-2	2	-0.038	0.050	0.014
rPROT-1	2	-0.181	0.003	-0.177
Mean		**-0.209**	**0,014**	**-0.194**
SE		**0.025**	**0.004**	**0.028**

Low mean level of genetic differentiation between populations within species (FST=0.029) was found for seven flatfish using allozymes (Exadactylos & Thorpe 2001). Similar results have been reported by Blanquer *et al.* (1992) and Bouza *et al.* (1997).

Turbot along western Black Sea coast had relatively high diversity He=0.321 within all of three populations in the present study. In all populations lack of rare alleles was presented. This may be explained as a result of random genetic drift caused by the recent reduction in population size. According to Kang *et al.* (2005) habitat fragmentation in natural populations could result in an immediate loss of rare alleles and a reduction of allele richness rather than a reduction of overall genetic heterozygosity.

Geographic pattern of genetic diversity

The low genetic divergence (DNei=0,003÷0,014) among western Black Sea turbot populations (Table 4) was found and confirmed with topology of PAUP dendrogram (Fig.4).

The dendrogram constructed showed one cluster, which pointed that in the investigated areas the populations are not good differentiated.

Table 4. Genetic identity (I Nei) above diagonal and Genetic distance (D Nei) between turbot populations analyzed, calculated on 17 polymorphic loci loci.

Population	RO	BG N	BG S
RO	-	0.990	0.090
BG N	0.003	-	0.080
BG S	0.0014	0.013	-

Maximum genetic differentiation among different populations pairs was observed between Romanian and southern Black Sea region, as these areas are distantly located and have negligible gene exchange between them. Romanian population is more close to the northern Bulgarian population, which may result from gene flow during the pelagic phase. (Table 4, Fig. 4).

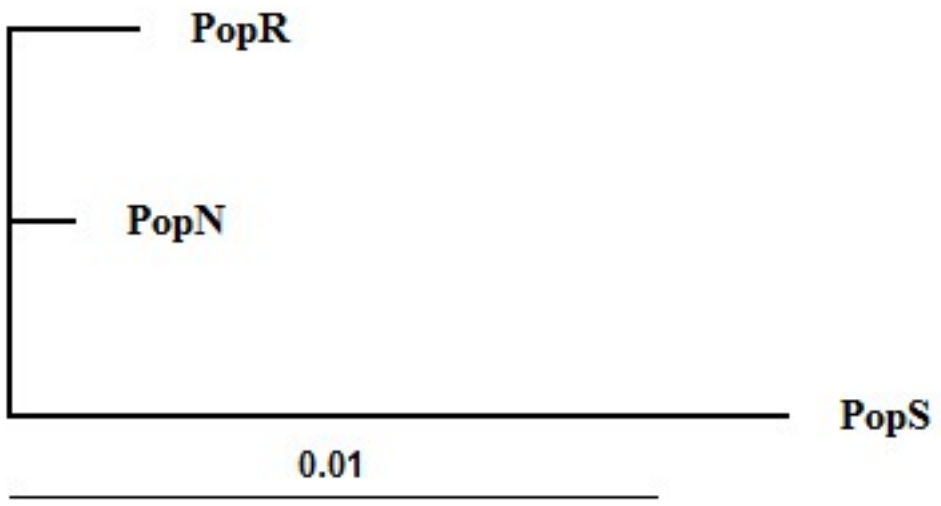

Fig. 4. Dendrogram of three turbot populations using PAUP cluster analysis of allozyme data.

The observed lack of private or locality specific allele at any of allozyme loci argues in favour of effective ongoing gene flow. Therefore, common ancestry in the past and possible continuous exchange of individuals among different areas may explain the observed low levels of genetic differentiation among turbot populations.

The results form Mantel test (Fig. 5), show no statistically significant relationship between genetic distances and geographical distances (km) of individual populations ($Z = 4.593$, $r = 0.474$, $P = 0.651$). No significant correlation between geographic and genetic distance for turbot from Baltic Sea was described also from Florin & Höglung (2007).

The Mantel test indicates that genetic differentiation among turbot populations does not seem to be correlated with geographic distance among populations, which provides further evidence of genetic drift (Shah *et al.* 2008).

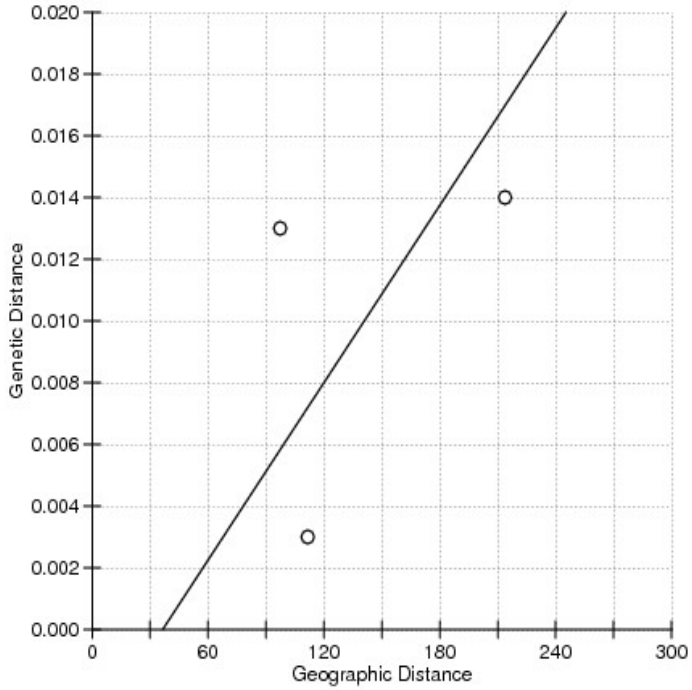

Fig. 5. The Mantel test compares a genetic distance (Y matrix) with a geographical distance (X matrix) in kilometers to test correlation between genetics and geographical location.

The very low genetic variability (FST 1.4%) found by Black Sea turbot populations could be explained in terms of historical bottlenecks of different evolutionary rates as this is mention from Blanquer *et al.* (1992).

It is crucial to identify populations that have undergone ancient or recent bottlenecks, because they may have been affected by the small population size through demographic stochasticity, inbreeding or fixation of deleterious alleles, possibly leading to a reduced evolutionary potential and increased probability of extinction (So *et al.* 2006).

According to Gopalakrishnan *et al.* (2009), allozyme and microsatellite markers were useful in identifying recent genetic bottlenecks in many marine fishes.

When a population is reduced in size, the allelic diversity is reduced faster than heterozygosity leading to heterozygosity excess (Nei *et al.* 1975). All 17 loci, investigated in Black Sea turbot populations along western coast showed under Sign test a significant heterosigote excess (Table 5) and would be considered as having experienced a recent genetic bottleneck (Cornuet & Luikart 1996). This supports the existing hypothesis (Atanassov *et al.* 2011) suggested Black Sea fish population bottleneck during the height of the last glacial period.

Table 5. Results of bottleneck Sign test in the three turbot populations sampled. Ho/He, observed and expected number of loci with heterozygosity excess under the infinite allele model (IAM) and the stepwise mutation model (SMM); P - probability.

Model	IAM		SMM	
Population	He/Ho	P	He/Ho	P
Pop R	7.04/17	0.000	8.15/17	0.000
Pop N	7.45/17	0.000	8.25/17	0.000
Pop S	7.23/17	0.000	8.22/17	0.000

The genetic differentiation of many marine fish species is low. They are less differentiated into populations (Ward 1994).

Low levels of genetic differentiation was found in wild turbot based on previous allozyme studies (Exadactylos *et al.* 2001, Blanquer *et al.* 1992, Bouza *et al.* 1997). Low genetic differentiation of turbot samples from the south and north Bulgarian and north Romanian regions of the west Black Sea coast based on mitochondrial control region /CR/ analyses (Atanassov *et al.* 2011) are comparable with the data results of this allozyme study.

The low differentiation observed, could be the result of persistent gene flow during the turbot pelagic phase or from post-glacial colonization from a single refuge, without enough time having elapsed for differentiation (Exadactylos *et al.* 2001, Florin & Hoglung 2007). This could be the reason for low level of inbreeding (FIS) obtained after our data analyses.

It is concluded that there was no genetic differentiation among western Black Sea populations and that these three stocks could be considered as a single stock.

Reduction in the genetic resources of natural fish populations has become an important fisheries management problem. Much of the reduction is due to various human activities. Not only has the genetic diversity of many fish populations been altered, but many thousands of populations and species have been extirpated by pollution, overfishing exploitation, destruction of habitat, blockage of migration routes and other human developments (Ferguson 1995).

Allozyme markers were used to assess the genetic diversity and population structure in three turbot populations, a critically endangered commercial species in view of overexploatation. Ever-increasing pressures on fisheries resources intensify the need to identify stock structure on turbot populations. Understanding fish stock structure is an important component of successful and sustainable long-term management of turbot along western Black Sea coast.

Conclusions

Genetic diversity and population divergence were estimated using 26 allozyme loci and samples from 3 natural turbot populations along western Black Sea coast to analyzed population structure. The distribution of genetic variation evidenced from allozyme data clearly indicate low genetic differentiation among turbot populations along western Black Sea coast and showed no evidence for population subdivision. This lead to the conclusion that along western Black Sea coast one population (stock) existed.

For management of wild turbot stocks, an important challenge will be to maintain high levels of genetic variation over time. Over exploitation of this species will be crucial to maintain necessary large effective breeding population size.

The high level of genetic diversity, low genetic differentiation and the population structure imply that the fragmented habitats of turbot along western Black Sea coast may be due to recent over-exploitation.

Development of additional allozyme markers as well as of highly polymorphic microsatellites could be used for genetic identification of turbot stocks and will have substantial impact for further monitoring of turbot populations and is of primary importance for developing an optimal strategy for their effective management and rational exploitation.

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