www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 17: 7-14 (2017) DOI: 10.4194/1303-2712-v17_1_02

RESEARCH PAPER

Genetic Structure of Wild European Sea Bass (*Dicentrarchus labrax* L, 1758) Populations in Aegean and Levantine Sea Using Microsatellite Markers

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Received 15 February 2016
Accepted 17 July 2016

Abstract

The aim of this study is to investigate the genetic structure of *Dicentrarchus labrax* populations sampled in the North-East Mediterranean. These are the main areas where the hatcheries collect their broodstock candidates from the wild in Turkey, which is the biggest European sea bass producer in Europe. Five samples collected from the Turkish Levantine and Aegean Sea coasts were analysed in addition to the Atlantic and Ionian samples (total 305 individuals) for 12 microsatellite loci. The present results revealed that the Aegean populations from Homa and Doğanbey, where the sea bass culture is mostly conducted in Turkish Aegean Sea, were closely related (F_{ST} 0.00347, P>0.01). Another close relation was found between Yumurtalık and Doğanbey (F_{ST} 0.01148, P>0.01), which might be the result of massive fry transfers from Yumurtalık (East Levantine coast) to Doğanbey till 2000 in Turkey. Obtained results also show gene flow from Greek to Turkish Aegean population which most probably was the consequence of frequent juvenile transfers from Greek hatcheries to Turkish fish farms between 2000 and 2010.

Keywords: European sea bass, Mediterranean, population genetics, microsatellite.

Introduction

European sea bass is one of the most important commercial fish and simultaneously one of the best genetically studied marine species in Europe. Previous population genetic studies in D. labrax used a variety of molecular markers to show that there is fragmentation of breeding populations not only between the Atlantic and the Mediterranean (Benharrat *et al.*, 1983; Naciri *et al.*, 1999) but also within the Atlantic (Benharrat *et al.*, 1983; Castilho and McAndrew, 1998) and the Mediterranean (Benharrat *et al.*, 1983; Allegrucci *et al.*, 1997; Garcia de Leon *et al.*, 2009; Quéré *et al.*, 2012) ptableopulations. These studies explain well enough that D. labrax population is divided into three main metapopulations as Atlantic, Western, and Eastern Mediterranean (Benharrat *et al.*, 1983; Garcia de Leo´n *et al.*, 1997; Naciri *et al.*, 1999; Bahri-Sfar *et al.*, 2000; Lemaire *et al.*, 2005; Fritsch *et al.*, 2007; Coscia and Mariani, 2011; Quéré *et al.*, 2012) while there are fewer studies investigating differentiation of sea bass within the Eastern Mediterranean (Bahri-Sfar *et al*,. 2000; Castilho and Ciftci, 2005).

There has been a continuous effort for marker development, studies on genetic structures of population and quantitative trait loci (QTL) mapping of D. labrax (Chatziplis *et al.*, 2007; Massault *et al.*, 2010; Louro *et al.*, 2016). To this date, few hatcheries have already initiated specific breeding programs in order to develop genetic improvements of this species for sustainable aquaculture practice (Chavanne *et al.*, 2016). The determination of genetic structure in available aquaculture stocks is an important prerequisite to achieve results for selective breeding programmes but also for protecting biodiversity and reducing the effect of escapees (Karahan, 2009). Since it is fundamental to identify the genetic structure of wild populations, it is of importance to reveal the genetic structure of D. labrax in Turkish coasts where the hatcheries collect broodstocks.

Turkey is the biggest European sea bass producer in Europe with 74.653 tons in 2014 (FAO, 2015, TURKSTAT, 2015). Moreover, juvenile production has increased in the last few years in sea bass hatcheries (178 million juveniles were produced in 2014, FEAP, 2015) because Turkish laws have banned the collection of juveniles from nature since 2000 (Memiş *et al.*, 2002). Recently, there are five main lagoon systems around the Mediterranean coast of Turkey where the hatcheries collect wild broodstocks in order to renew their stocks (Emiroğlu *et al.*, 2005) in addition to renewing broodstocks with their offspring and/or offshore fishing.

Although there is relatively extensive knowledge on the genetic structure of European sea bass population in the Mediterranean, there are very limited studies in Levantine populations from Turkey. In the first study on genetic structure of European sea bass in Turkish coast, no differentiations were found in these four samples along the Mediterranean, Aegean coast, Marmara and Black Sea (Ergüden and Turan, 2005). This was probably due to the fact that most of the allozyme loci they used were monomorphic and was therefore not informative enough to see diversity between populations.

Studies on D. labrax genetic structure along the Turkish coast suggested in their results that more comprehensive research must be done to understand the genetic structure of European sea bass along the Turkish coasts (Ergüden and Turan, 2005; Karahan, 2009; Bekcan *et al*., 2009) because nearly all of these few studies had limited samples and/or individuals. For this reason, the main objective of the current study was to assess the population genetic structure of five D. labrax samples from localities where the hatcheries collect broodstocks in Turkey for the future selective breeding programmes and also to gain knowledge on wild D. labrax genetic characteristics. Additionally, two more populations outside of Turkish water (from West Greece and the Atlantic) were analysed as a out group to investigate the current level of genetic differentiation between D. labrax populations from east Mediterranean and Atlantic water reservoirs.

Materials and Methods

Sampling Locations

Two hundred and nine individuals (N=209) were collected in cooperation with the fishermen from five lagoon systems along the Levantine and Aegean coasts of Turkey. In addition, ninety six European sea bass individuals (N=96) were sampled both in the Ionian Sea (Messolonghi Lagoon) and the Atlantic Ocean (Bay of Biscay). All collected samples were used for genetic analyses to investigate differences between Turkish (Levantine) stocks and other localities. The details of the populations studied are given in Table 1 and sampling locations are shown in Figure 1.

All fish were transferred to the laboratory in an ice box whereupon small clippings of dorsal muscle were collected, placed in 10 ml tubes with absolute ethanol and stored at -20°C until DNA extraction.

DNA Extraction and Multiplex PCR

DNA from Turkish samples was extracted following the Phenol:Chloroform:Isoamyl Alcohol protocol (25:24:1) and stored at -20°C (Aksakal, 2009). DNA samples from Bay of Biscay (AO) (Fritsch *et al*. 2007) and Mesolonghi Lagoon (IS) were supplied from Hellenic Centre for Marine Research (HCMR, Greece). PCR amplification and

Table 1. Details of samples, locations, code and sampling numbers (*N*), years, providers

Origin	Location	Abbreviation	N	Sampling Year	Provider
Aegean Sea	Homa, İzmir, TR	ASH	34	Jan 2011	T. Bodur
Aegean Sea	Doğanbey, Aydın, TR	ASD	43	Dec 2010	T. Bodur
Levantine Sea	Köyceğiz, Muğla, TR	LSK	45	Jan 2011	T. Bodur
Levantine Sea	Beymelek, Antalya, TR	LSB	40	Jan 2011	T. Bodur
Levantine Sea	Yumurtalık, Adana, TR	LSY	47	Dec 2010	T. Bodur
Ionian Sea	Mesolonghi, GR	IS	48	Dec. 2010	C. Tsigenopoulos
Atlantic Ocean	Bay of Biscay, FR	AO	48	Dec. 2002	B. Guinand

Figure 1. Map of sampling locations (abbreviations are given in Table 1) and Rhodes Abyssal Zone.

genotyping of microsatellite loci were conducted as described in Guinand *et al*. (2015) for the 12 microsatellite loci (Table 2). Scoring was performed with STRand computer software (http://www.vgl.ucdavis.edu/STRand) and binning was manually carried out in MS Excel to minimize the genotyping errors.

Statistics Analysis

The received genotype data was checked for null alleles, in consistent values, scoring errors and large drop-out in samples with MicroChecker software (Van Oosterhout *et al*,. 2004). The mean number of alleles, allelic richness (AR), the observed and expected heterozygosity (Nei, 1987) were computed for each locus and each population. Divergences within and among populations were measured using Wright's FIS and FST, respectively. Both parameters were estimated according to Weir and Cockerham (1984). All data analysis was performed using the FSTAT v2.9.3 (Goudet, 2002) and the GENETIX v4.02 (Belkhir *et al*., 2004) softwares. The genetic structure was also investigated using a Bayesian approach implemented in the program STRUCTURE (Pritchard *et al*., 2000) to estimate the most likely number of genetic clusters (K) in the studied populations. K was tested from 1 to 7, using the default setting with five independent runs for each K, 1.000.000 iterations and burn-in period of 250.000. The most probable K value was estimated on the bases of algorithm developed by Evanno *et al*. (2005). We acknowledged that an individual belongs to a certain group if the members' probability was higher than 75%, otherwise we assigned this individual as not belonging to any group.

Results

Overlap failures were observed at two loci and these two loci were discarded from all samples and the subsequent analyses (DLA0051 and DLA0075). According to the MicroCheker software no stuttering or allele drops out was found in any locus. Possibility for null alleles was detected for DLA0073 and DLA0078 loci in LSY. In addition, DLA0068 and DLA0086 loci had evidence of null alleles in IS and AO populations, respectively. A total of 184 alleles were identified in studied microsatellite DNA loci, at an average of 18.2 alleles per locus. Mean allele numbers per locus ranged from 8 in LSB to 13.9 in AO populations. Allelic richness was found highest at the DLA078 locus (AR=15.948) and lowest at the DLA0060 locus (AR=5.588). Allele numbers, Allelic richness and private allele information are given in Table 3.

Lower gene diversity in LSK (He=0.682±0.159) and higher in AO (He=0.802±0.123) were observed. DLA0068 and DLA0060 loci exhibited the lowest expected heterozygosity value in all Turkish populations and non-Turkish populations, respectively. Observed gene diversity were homogenous ranging from Ho=0.663 for LSB to Ho=0.710 for IS populations. Number of private allele found highest at AO population and no private allele found at LSK and LSB population. The observed (Ho) and expected heterozygosity (He) values and Allelic Richness (AR) for all populations in all loci are given in Table 3.

All loci exhibited significant departure from

Table 2. Locus and primers used in this research (F: forward, R: reverse, bp: base pair) (* discarded from the analysis afterwards)

Locus	Motif	Size Range (bp)	Fluorochrome		Primers 5'→3'
DLA0044	$(CT)_{19}$	101-149	HEX	R	ACCGCCCAAGGGTTGGACTG
				F	TCCGCTCCGCACCGAGTGAC
DLA0051*	(GT)17	149-181	ROX	R	AGTGACAGCAGCCTCCAGAG
				F	AGGTTCTTGGCCTGGGAATC
DLA0060	$(CA)_{12}(TA)_3AA(CA)_2$	111-141	FAM	R	TGTAGTAATAATGCGCTCTGCAA
				F	GAGAGTTCATCCTGTTCGCTC
DLA0061	$(TG)_{14}$	145-175	FAM	R	CTCCCTGTCCATCTGTCCTC
				F	AAAGGCCAGTGAAACTCATGT
DLA0068	$(CA)_7CGCACG(CA)_3$	233-269	TAMRA	R	GCATTAGCATTGATTGTCCTG
				F	CAACACCTGTTCCTCTGAACC
DLA0073	$(CT)_{36}$	148-188	TAMRA	R	AGTTCAGAGCGGCAACTGT
				F	CATGACTTCATGTGCTAATGTCC
DLA0075*	(CA)15	180-186	ROX	R	GGCAGAGATGGGAAATAGACA
				F	CACATACACAAGCTTAACCC
DLA0078	$(AG)_{29}$	191-261	HEX	R	CACAAGGAACCGAGACAAGA
				F	AAGACTGGACCTCTGGAGACC
DLA0081	$(CA)_{16}$	191-227	ROX	R	ATACCGAGCGACCATGTTG
				F	GACGAAGACTTCAGACGAGCTAT
DLA0086	$(AC)_{26}$	172-222	FAM	R	ACCTGGTGATTGGCAATTCT
				F	GCTAGAGGATTCATGTCGCTT
DLA0089	$(GT)_{15}$	107-147	TAMRA	R	GTCAAAACAGCCCACCTA
				F	ACGAGTAATGAGGACCCA
DLA0096	$(GT)_{16}$	242-270	FAM	R	TCGATGCATCTAGGACAGGA
				F	AACTTAGTGAAGTAACTTGTGGCAA

Hardy-Weinberg Equilibrium (HWE). Locus DLA0044 showed significant heterozygote excess (FIS=-0.045, P<0.001). Except ASD (FIS=0.157, P<0.001) and AO populations (FIS 0.246, P<0.001), heterozygote excess was observed at DLA0086 locus in all other populations. Furthermore, all populations revealed significant departure from HWE except LSK population (FIS=0.035, P>0.001) (Table 3).

FST values, measures of the genetic differences between populations, were found the lowest between ASD and ASH (0.00347, P>0.01), ASD and LSY (0.01148, P>0.01) populations. The highest FST value was observed between LSK and AO populations (FST=0.06177 P<0.01) (Table 4).

Bayesian analysis indicated that individuals analysed in all populations might be clustered in two groups (ΔK=2) corresponding to Atlantic and Turkish populations. Moreover, obtained results revealed that LSK was the most isolated group among Turkish populations (Figure 2).

Discussion

In previous studies, it was reported that European sea bass subdivisions in the Mediterranean are based on the main hydrological features of the basin (Naciri *et al*. 1999, Bahri-Sfar *et al*. 2000) and two groups were recognised as Eastern and Western Mediterranean populations (Benharrat *et al*., 1983; Garcia de Leo´n *et al*., 1997; Naciri *et al*., 1999;

Table 3. Allelic richness (AR), observed heterozygosity (Ho), expected heterozygosity (He) F_{IS} values and number of private alele (Npa) of all loci and populations * P<0.05, **P<0.01, *** P<0.001

Locus	Parameters	ASH	ASD	LSK	LSB	LSY	IS	AO	Avarage
DLA0044	AR	10.000	15.469	10.263	10.697	12.923	14.405	17.238	14.440
	Ho	0.912	0.93	0.933	0.85	0.851	0.958	0.938	0.852
	He	0.858	0.894	0.833	0.813	0.835	0.878	0.918	0.906
	F_{IS}	-0.048	-0.029	-0.11	-0.033	-0.008	-0.081	-0.011	-0.045
DLA0060	AR	2.000	6.664	2.000	2.979	4.777	3.685	7.996	5.588
	Ho	0.382	0.419	0.489	0.425	0.532	0.583	0.458	0.533
	He	0.496	0.614	0.48	0.519	0.549	0.54	0.657	0.472
	F_{IS}	0.243	0.329	-0.007	0.193	0.042	-0.07	0.311	0.153
DLA0061	AR	6.000	7.539	5.928	6.958	7.436	8.309	12.213	8.923
	Ho	0.677	0.884	0.667	0.675	0.702	0.563	0.646	0.704
	He	0.654	0.754	0.645	0.759	0.692	0.722	0.773	0.694
	F_{IS}	-0.02	-0.161	-0.022	0.123	-0.003	0.231	0.175	0.054
DLA0068	AR	2.000	2.791	2.000	3.829	3.447	6.125	7.810	6.527
	Ho	0.353	0.488	0.356	0.475	0.468	0.396	0.375	0.479
	He	0.415	0.469	0.346	0.508	0.47	0.667	0.659	0.423
	F_{IS}	0.165	-0.029	-0.017	0.077	0.015	0.415	0.439	0.196
DLA0073	AR	12.000	12.978	11.224	9.784	12.327	13.274	15.540	14.344
	Ho	0.735	0.814	0.644	0.675	0.553	0.896	0.792	0.853
	He	0.884	0.902	0.804	0.768	0.875	0.886	0.908	0.712
	F_{IS}	0.183	0.109	0.21	0.133	0.377	-0.001	0.139	0.163
DLA0078	AR	18.000	12.854	9.902	9.547	12.016	15.774	20.877	15.948
	Ho	0.882	0.837	0.778	0.725	0.489	0.563	0.854	0.855
	He	0.895	0.849	0.83	0.816	0.863	0.879	0.927	0.712
	F_{IS}	0.029	0.026	0.075	0.124	0.441	0.369	0.089	0.175
DLA0081	AR	7.000	8.496	5.755	8.766	9.426	11.068	11.387	11.124
	Ho	0.588	0.605	0.711	0.55	0.766	0.667	0.833	0.695
	He	0.652	0.605	0.697	0.706	0.838	0.67	0.883	0.648
	F_{IS}	0.113	0.012	-0.009	0.233	0.096	0.016	0.066	0.072
DLA0086	AR	13.000	12.488	9.248	11.671	14.072	12.560	15.554	14.107
	Ho	0.941	0.744	0.889	0.875	0.915	0.896	0.688	0.846
	He	0.859	0.871	0.791	0.829	0.865	0.864	0.9	0.877
	F_{IS}	-0.08	0.157	-0.113	-0.043	-0.047	-0.027	0.246	0.022
DLA0089	AR	8.000	11.896	6.685	8.547	9.219	10.631	10.912	10.823
	Ho	0.618	0.721	0.6	0.75	0.723	0.854	0.625	0.739
	He	0.629	0.822	0.677	0.74	0.764	0.803	0.79	0.711
	F_{IS}	0.032	0.135	0.124	-0.001	0.063	-0.054	0.219	0.078
DLA0096	AR	7.000	7.372	5.939	4.979	7.298	7.392	8.017	8.175
	Ho	0.706	0.651	0.578	0.625	0.723	0.729	0.489	0.730
	He	0.721	0.767	0.713	0.679	0.742	0.759	0.61	0.669
	F_{IS}	0.035	0.163	0.2	0.092	0.036	0.05	0.208	0.112
Multilocus	Npa	1	1	0	0	1	1	4,0	-
	Ho	0.679	0.709	0.664	0.663	0.672	0.710	0.670	0.729
	He	0.706	0.755	0.682	0.714	0.749	0.767	0.802	0.683
	F_{IS}	0.053*	0.072**	0.036	0.084**	0.113***	0.084***	0.176***	0.093

Table 4. Matrices of pairwise *F*st values according to Weir and Cockerham (1984). *F*st above diagonal and Gen flow (*N*m) below diagonal (** Significant pairwise *F*st value after the sequential Borferroni correction P<0.01)

	ASH	ASD	LSK	LSB	LSY	IS	AO
ASH	-	0.00347	0.01828**	0.02502**	0.01704**	0.01466**	0.05129**
ASD	71.74	-	0.01685**	0.01431**	0.01148	0.01125**	0.0476**
LSK	13.43	14.59	-	0.03335**	0.02383**	0.03421**	0.06177**
LSB	9.74	17.22	7.25	-	0.01617**	0.0258**	0.05717**
LSY	14.42	21.53	10.24	15.21	-	0.02242**	0.05399**
IS	16.81	21.97	7.06	9.44	10.90	-	0.04273**
AO	4.62	5.00	3.80	4.12	4.38	5.60	-

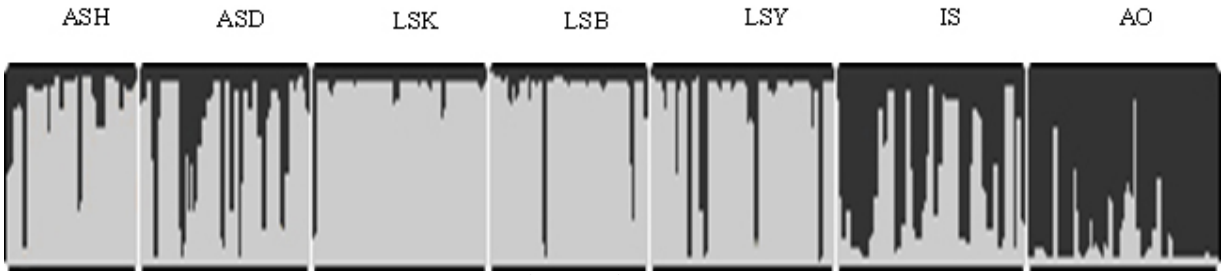

Figure 2. Bayesian clustering analysis using STRUCTURE for (*K*=2) for the European sea bass (*D. labrax*) without using geographical area as a prior. The two different shadings relate to the respective clusters suggested and the length of the bar in the y-axis represents the probability of assignment of an individual to each cluster (Abbreviation is given in Table 1).

Bahri-Sfar *et al*., 2000; Lemaire *et al*., 2005; Fritsch *et al*., 2007; Coscia and Mariani, 2011; Quére *et al*., 2012). It was stated that there are many forces in the marine environment which can contribute to the structure of inter- and intraspecific biodiversity, such as depth, light, temperature and nutrient gradients (Bahri-Sfar *et al*., 2000). Castilho and Ciftci (2005) proposed that although the present sea current patterns do not hinder intermixing, they somehow prevent the intermixing of the two populations in the Eastern Mediterranean. Güven (2011) reported that abyssal gradients have also an effect on gene flow of Sepia officinalis in the North-West Levantine region. There are subdivisions found between saddled seabream Oblada melanura populations in North Aegean Sea which might be the reason of a deep trench of 1500 m, presenting a physical geographical boundary in the Central Aegean (Gkafas *et al*., 2013). In the present study, the samples from LSK population were collected from a lagoon which has a more than 4000 meters deep trench (Rhodes Abyssal Plain) just starting from the shore. FIS analysis showed that except all other populations, LSK population is in HW Equilibrium (0.035, P>0.001). Therefore, LSK populations might be rather characterized by panmixia, limited selection and low influence of migration or also might indicate population isolation which is consistent with Structure results (Figure 2). However, Rhodes Abyssal Plain (Figure 1), which is located between Rhodes and Fethiye (LSK) has made the researchers think that besides this water current affects sea bass population divisions, the abyssal zone in the area might be another effect on subdivision. Although Bekcan *et al*. (2009) reported not-significant differences between wild populations of sea bass in Levantine and Aegean Sea a bias might be that this work was based on small sampling size (3 to 4 individuals were studied per sample); considering the present results of this study performed with codominant markers, minor differentiation between populations in Levantine was observed.

In the current work, FST values show differences between Levantine populations (LSK, LSB, LSY) and Homa population from Aegean Sea (ASH). However, the researchers also found genetic similarities between ASD and LSY population (FST=0.01148) even if these two populations have the biggest geographic distance among Turkish populations. Those similarities might be the result of massive wild larvae and juvenile transfer from 1985 till 2000 from East Levantine (Yumurtalık Lagoon) to Aegean Sea (İzmir and Güllük Bay) where the marine aquaculture industry started in Turkey in the early 1980s. During the period of intense marine aquaculture activities in Turkey, hundred thousands of sea bass larvae and juvenile were transferred every year from wild populations to cage farms (Memiş *et. al*., 2002) and probably a lot of escapees might influence the gene pool of the wild sea bass in the Aegean Sea. Close relation between ASH and ASD (FST=0.00347) is expected as these two populations are in very close distance to each other.

Studies have recently presented the effect of aquaculture escapees on the genetic diversity of wild populations in the Eastern Mediterranean (Greece) (Souche *et al*., 2015) and Cyprus (Brown *et al*. 2015). In this study, the results of Bayesian structure analyses showed that if all Levantine populations are

regarded as a single stock, both Greece and Turkey may be exposed to escapees of Atlantic origin (Kourkouni *et al*., 2015 in preparetion). It is known that many Turkish companies have transferred sea bass and sea bream juveniles directly from Greek hatcheries to rear in Turkey since 2000s (Şereflisan and Şereflisan, 2000.). If those Greek stocks were already mixed (Atlantic and Mediterranean), we had a stepping-stone intermixing from Greece to Turkey.

FST values shows that LSK population is more similar to ASD and ASH (0.016 and 0.018, respectively) than other Levantine populations which are LSB and LSY (0.033 and 0.023, respectively). According to STRUCTURE results, LSK and LSB populations showed the highest homogeneity and the best assignment to Turkish genetic cluster, for which only few individuals were assigned to second cluster. Assuming the dark colour represents 'Atlantic type', the current results show that escapees have taken place mostly in LSH, LSD and LSY and marginally in LSK and LSB. Similar interaction was seen between Greek and Turkish Aegean populations (Nm 21.97) which might be the result of mixing of Greek and Turkish origin due to the escapees and/or possible passive drift of eggs and larvae by sea currents from Greece to Turkey in Aegean Sea.

The main aim of this research is investigating genetic structure and understanding the heterozygosity of Turkish population of European sea bass to give a perspective to hatchery managers. Interesting results found on bathymetric effects on possible subdivision in Levantine populations. But in order to understand this bathymetric boundary effect on wild sea bass populations (or on other species), more comprehensive studies are needed in this region. Genetic diversity of wild population is an important parameter for aquaculture as most of the Turkish hatcheries collect their broodstocks candidates from the natures. Generally, small effective population size and unmonitored selective breeding programs are the major causes for loss of genetic diversity in aquaculture (Hansen *et al*., 2001). Therefore, it is important to understand the distribution of the genetic diversity within a broodstock to protect capable management of the stock for selective breeding (Zhu *et al*., 2006). Relatively low heterozygosity in LSB (Ho=0.663) and LSK (Ho=0.664) populations can be considered by hatcheries to avoid collecting their broodstock candidates from these regions to prevent the possible inbreeding. This study provides some basic information about the genetic structure of wild European sea bass populations, which can be used in future studies especially on the escapees' effects of aquaculture activities in the region.

Acknowledgements

We gratefully thank to Stelios Darivianakis and Vasso Terzoglou (HCMR) for their valuable assistance in the laboratory techniques and we also thank to Gözde Yurtsever Bodur for her contribution to proofreading. This work is part of Turker Bodur's PhD thesis and was supported by The Scientific Research Projects Coordination Unit of Akdeniz University, Project Number: 2010.03.0121.015 and COST Action LarvaNet-STSM FA0801-9340.

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