

Population subdivision of saddled seabream *Oblada melanura* in the Aegean Sea revealed by genetic and morphometric analyses

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ABSTRACT: The population structure of marine fishes is often cryptic, due to limited knowledge about life history and ecological habitats. Understanding environmental stability is a challenge to retrieve possible structuring patterns responsible for dispersal and demographic habits. In the present study, a combination of genetic and phenotypic assessments was applied in order to investigate population structure of the saddled seabream *Oblada melanura* in the Aegean Sea, eastern Mediterranean basin. Analyses of 6 microsatellite loci and 15 morphometric characters revealed that saddled seabream individuals differ among northern, southern and central Aegean populations. These observed boundaries may be related to the differentiated seascape of the Aegean Sea, suggesting that oceanographic factors are a significant stressor for population subdivision of the saddled seabream. Individual-based landscape genetic approaches and multivariate analysis of the morphometric characters suggest the presence of habitat-related limitations of saddled seabream dispersal potential. Molecular genetics and phenotypic analyses along with life-history traits provide useful informative data for the management and conservation schemes applied for this species in the Aegean Sea.

KEY WORDS: *Oblada melanura* · Microsatellite · Morphometrics · Aegean Sea · Population structure

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INTRODUCTION

The marine environment forms a continuous water body that does not exhibit clear barriers. Although, due to a lack of barriers, homogeneity is expected in marine species that have a large geographic range and high dispersal, a fine-scale population structure is often reported for these species (Bekkevold et al. 2005, Jørgensen et al. 2005, Hoelzel et al. 2007, Hoelzel 2009, Kelly & Palumbi 2010, Pilot et al. 2010).

Significant genetic differentiation has been reported for marine fishes, the extent of which varies among

species (Arculeo et al. 2003, Pinera et al. 2007, Zulliger et al. 2009, González-Wangüemert et al. 2011). Structural patterns are not always correlated with geographic components, but are the result of complex demographic stochasticity, such as local resources, social coherence and environmental stability (Galarza et al. 2009, McCusker & Bentzen 2010, Salmenkova 2011). Despite the fact that oceanographic features, such as water temperature, salinity and oxygen level, are known to play a key role in species habitats, the mechanisms driving the dispersal and population structure of marine fishes remain unclear.

The Mediterranean Sea is a closed basin ecosystem, with a relative high temperature and different seasonal rainfall patterns and flow dynamics underlying a highly oligotrophic area (Siokou-Frangou et al. 2002). Spatial and temporal genetic population structure studies in the Mediterranean Sea report differentiation between the eastern and western part of the basin (Viñas et al. 2004, Rolland et al. 2007, Coll et al. 2010), possibly due to historical demographic processes but also due to hydrological and ecological traits (e.g. Magoulas et al. 2006). The majority of these studies have focused on fish stock exploitation through the population structure of the species in question, revealing patterns via life-history traits and biogeographic barriers. Variations in oceanographic features play a key role in the life history of such species, most obviously on larval dispersal due to food availability. Species with no pelagic larval dispersal tend to evolve as highly subdivided populations, suggesting low gene flow between them (Palumbi 1994), whereas species with longer pelagic larval duration are expected to present higher gene flow values and lower population differentiation (Bohonak 1999).

The saddled seabream *Oblada melanura* L. belongs to the family Sparidae and occurs in the Mediterranean Sea, eastern Atlantic, Biscay Bay and from the Strait of Gibraltar to Angola. It is also recorded in Madeira, Cape Verde and the Canary Islands (Bauchot & Hureau 1986, Bauchot et al. 1981). It is a gregarious species, living in coastal regions, on rocky and seaweed (*Posidonia oceanica*) beds up to 30 m deep, creating small schools near the coastline. During the day it hides in small crevices and cavities of rocky beds (Bauchot & Hureau 1990). It is an omnivorous species, preying on small invertebrates and phytoplankton (Klaoudatos et al. 1997, Lenfant & Olive 1998). In the Mediterranean Sea spawning is from April to June (Bauchot & Hureau 1986) and, according to Jardas (1996), in the Adriatic Sea in late summer. The saddled seabream is of high economic importance in the Mediterranean Sea, as it is a species of interest not only for fisheries but also for tourists (i.e. angling). It is one of the species that is targeted to be set in aquaculture enterprises (Klaoudatos et al. 1997), as are many others members of the Sparidae family.

To evaluate the effectiveness of biodiversity management programs detailed knowledge of the population ecology of the managed species is necessary. Recent information on the stock definition of sparids, in particular saddled seabream, is scarce, although they are commercially and ecologically important fish

in the Mediterranean Sea. Genetic and abundance data, in conjunction with morphometric assessments, may provide important information on saddled seabream stock composition, the evolutionary mechanisms related to specific demographic patterns in the Mediterranean Sea and the effect of selective environmental pressures.

It is hypothesized that variation in food availability and abundance affect the density and dispersal of saddled seabream in the Mediterranean, and therefore, its population structure should correlate with oceanographic characteristics that cause such variation. To test such a hypothesis, the genetic structure of saddled seabream was analyzed and correlated to seascape characteristics. The Aegean Sea (eastern Mediterranean basin) is a complex ecosystem with a highly irregular coastline and semi-isolated deep basins (Olson et al. 2007), and presents a novel challenge as a transition between the eutrophic Black Sea and the oligotrophic Mediterranean basin. Six microsatellite loci were analysed to evaluate genetic polymorphism in the Aegean Sea using a combination of recent individual-based landscape genetic approaches.

Additionally, 15 morphological assessments were used to determine the geographical variation of the given stocks. Multivariate statistical analysis of morphometric characters has provided useful results in the past for assessing stock structure of several marine fish species (Schaefer 1989), however, the use of morphological characteristics does have some limitations—they are polygenically inherited, have low heritability and are subject to considerable environmental plasticity (Karakousis et al. 1991). Multivariate analysis of a set of phenotypic characters is regarded as a more appropriate method than the use of a single character for determining relationships between populations of a given species (Thorpe 1987, Palma & Andrade 2004).

MATERIALS AND METHODS

Sample collection and study area

Adult saddled seabream individuals were collected as bycatch from 8 locations in the Aegean Sea. Sampling sites and sizes along with main oceanographic features are shown in Fig. 1. The total sample size was 514 individuals; sampling was carried out by local fishermen using gillnets. A small amount of muscle tissue was obtained and stored in 70% ethanol for further analysis.

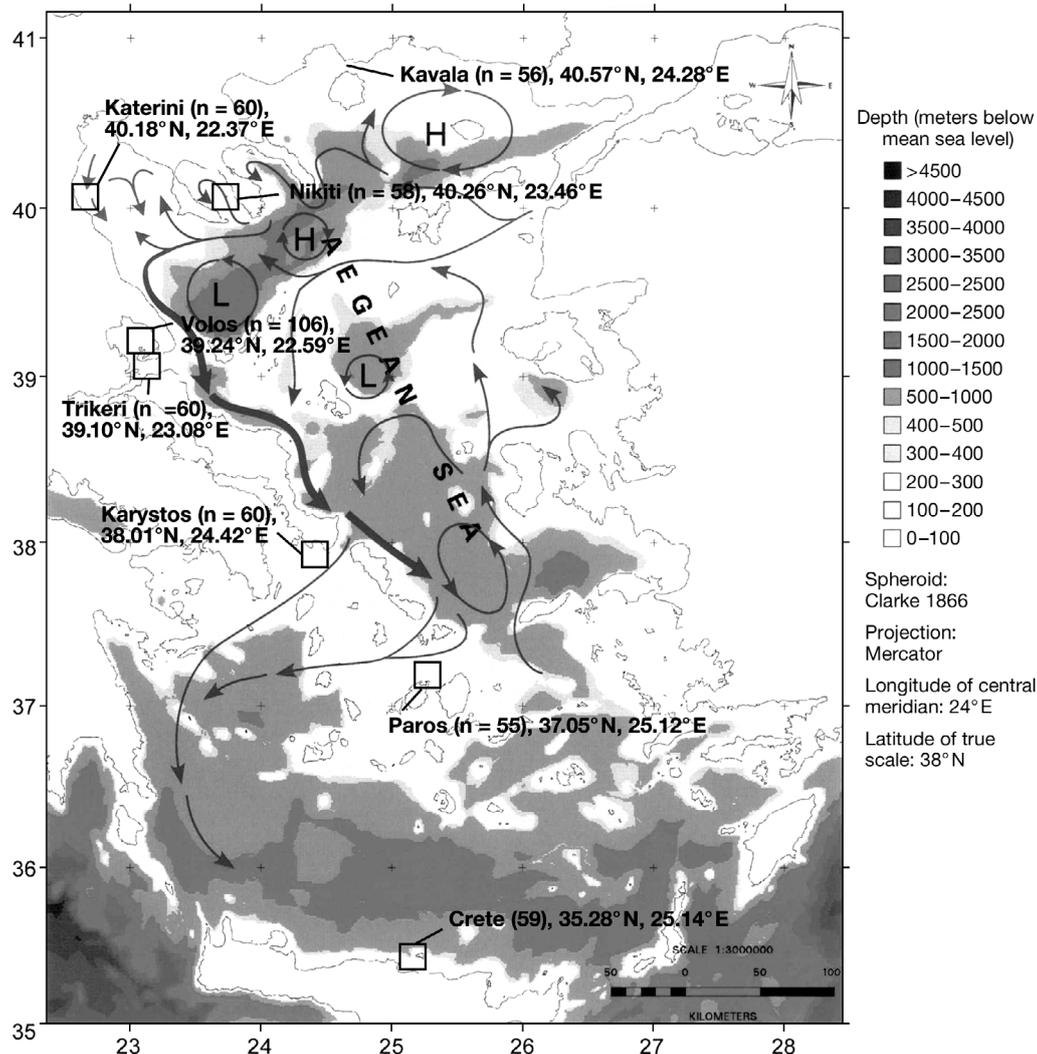


Fig. 1. *Oblada melanura*. Oceanographic features (current patterns are illustrated with arrows), sample sites and number of saddled seabream specimens (n) in the Aegean Sea (after Olson et al. 2007)

DNA extraction and PCR amplification

DNA was extracted from 0.01 g of muscle tissue following the standard phenol/chloroform protocol (Sambrook & Russel 2001). DNA was preserved in 10 mM TE and stored in -20°C . A panel of 6 microsatellites was tested and optimized for the genetic analysis: SAI10, SAI12, SAI19 (Brown et al. 2005) and Pma1, Pma2 and GA2A (Adcock et al. 2000). Roques et al. (2007) characterized an additional panel of species-specific microsatellites which ought to be under future consideration for screening. The total volume of the PCR reaction was 10 μl ; consisting of 40 ng of template DNA, 10X Buffer, 1.5 mM MgCl_2 , 0.2 mM dNTPs, 1 U Taq polymerase (all Invitrogen) and 0.5 μM of each primer (Operon-Invitrogen). The fluorescence primers used were labelled by 4 different dyes: FAM,

HEX, ROX and TAMRA (Invitrogen). The PCR conditions were as follows: 95°C for 3 min, followed by 30 cycles of 1 min at 95°C , 50 s at $T^{\circ}\text{C}$ annealing of the primer set, and 30 s at 72°C , with a final step of 15 min at 72°C . PCR products were verified by 1% agarose gel electrophoresis (Invitrogen). Fluorescently labelled PCR products were run on an ABI 3700 automated sequencer (Applied Biosystems). Each specimen's alleles were scored by STRand software v.2.0 (Toonen & Hughes 2001) and 10% of genotypes were rerun for error checking.

Genetic analysis

All loci were tested for the presence of null alleles or allelic dropout using the software MICRO-

CHECKER v.2.2.3 (van Oosterhout et al. 2004). Exact tests for Hardy-Weinberg equilibrium (HWE), linkage disequilibrium (LD, using Fisher's exact tests), expected heterozygosity (H_{EXP}) and observed heterozygosity (H_{OBS}) were carried out using the software Genepop v.3.4 (Raymond & Rousset 1995). F_{ST} and F_{IS} indices (using the formulation described by Weir & Cockerham 1984) and number of alleles per locus were calculated using FSTAT v.2.9.3.2 software (Goudet 2001). This software was also used to calculate the allelic richness (rarefaction) per locus and per population. A Bonferroni correction was carried out for each pairwise analysis (Rice 1989). A recalculation was made in order to estimate null and visible allele frequencies for each locus and population; for such estimations, the ENA method was used as described in Chapuis & Estoup (2007). The ENA correction method was found to efficiently correct the positive bias induced by the presence of null alleles on F_{ST} estimation and provide a more accurate estimation of F_{ST} . To evaluate hypothesized patterns of spatial genetic structure, a hierarchical analysis of molecular variance (AMOVA) (Excoffier & Lischer 2010) was performed using Arlequin 3.5 software in order to partition variance components attributable to (1) variance between areas; (2) variance between populations within areas; and (3) variance among samples within populations. The significance of the resulting F -indices and variance components were permuted 10 000 times using the Bonferroni correction (Rice 1989).

Population structure was assessed using the software STRUCTURE v.2.3 (Pritchard et al. 2000a), where identified migrants and admixed individuals were assigned. The Correlated Allele Frequency Model (Falush et al. 2003b) records the allele frequencies in a hypothetical 'ancestral' population without specifying geographic area as a prior. To test the convergence of the priors and the appropriateness of the chosen burn-in length and simulation length, 3 independent repeats were run for each value of K , the number of clusters ($2 \leq K \leq 10$). Burn-in length and length of simulation were set at 500 000 and 1 000 000 repetitions, respectively. Isolation by distance software (IBD) was used to assess whether the association between genetic similarity ($F_{\text{ST}}/[1-F_{\text{ST}}]$; Rousset 1997) and geographic distance is statistically significant using a Mantel test based on 1000 randomizations (Bohonak 2002). The software uses partial correlation coefficients between genetic and geographical distances. We tested for evidence of recent bottleneck events using the software BOTTLENECK v.1.2.02 (Piry et al. 1999) and the Stepwise Mutation

Model (SMM). A one-tailed Wilcoxon signed rank test was used to determine numbers of loci in heterozygosity excess. The mode shift indicator tested the allele frequency distribution that discriminates bottlenecked populations from stable populations (Luikart et al. 1998).

The software Barrier v.2.2 (Manni et al. 2004) was utilized in order to identify locations and the directions of barriers using a computational geometry approach. The Monmonier (1973) maximum difference algorithm provided a more realistic representation of the barriers in a genetic landscape and a significance test was implemented by means of bootstrap matrix analysis. In order to obtain a geometric satisfactory map from a list of geographic x,y coordinates, a Voronoï tessellation (Voronoi 1908) calculator was used. Out of this tessellation a Delaunay triangulation (Brassel & Reif 1979) was obtained. Additionally, Migrate v.3.3.2 (Beerli & Palczewski 2010) was implemented in order to reveal any directionality of the migration patterns.

Morphometric analysis

Fifteen morphometric characters were measured on each specimen according to Hubbs & Lagler (1967). These morphometric characters were: standard length, maximum body depth, minimum body depth, caudal peduncle length, head length, diameter of eye, preorbital distance, postorbital distance, predorsal fin distance, dorsal fin height, dorsal fin base length, anal fin height, anal fin base length, pectoral fin length and wet weight (Fig. 2). All measurements were taken to the nearest 0.1 mm or mg.

Parameters such as sampling timing, sexual dimorphism, allometric growth, sampling more than one population in each water body, different phenotypic groups within the sample, and maturation stage of the fish could impose some limitations on a study (Roughgarden 1972, 1974). The effect of allometry and sexual dimorphism was minimized after the transformation of the original measurements. In order to overcome the fourth parameter (sampling more than one population in each water body) sampling was restricted to as small a geographic area as possible. As for maturation stage, every effort was made to choose only mature fish for the analysis. To minimize any variation resulting from allometric growth, all morphometric measurements were standardized according to:

$$e = \log Y - b (\log X - \log X_1)$$

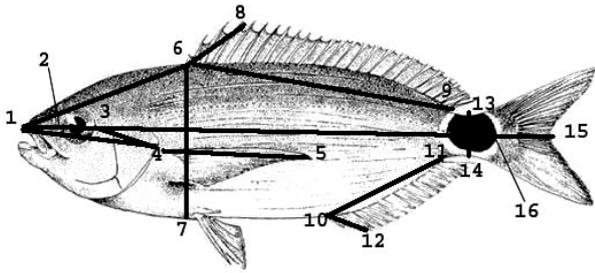


Fig. 2. *Oblada melanura*. Morphometric measurements: 1–2: Preorbital distance; 2–3: diameter of eye; 3–4: postorbital distance; 1–4: head length; 4–5: pectoral fin length; 1–6: predorsal fin distance; 6–8: dorsal fin height; 6–9: dorsal fin base length; 6–7: maximum body depth; 10–11: anal fin base length; 10–12: anal fin height; 13–14: minimum body depth; 11–16: caudal peduncle length; 1–15: standard length

where e is a standardized measurement, Y is character length, b is the slope of $\log Y$ against $\log X$ plot of each population, X is the standardized length of the specimen and X_1 is the mean standard length of the specimen examined. According to Reist (1985), this transformation best reflects shape variation among groups independently of size factor. Therefore, total length and standard lengths of each specimen were excluded from the final analysis.

Principal component analysis (PCA) was used to test for the contribution of the remaining 13 morphological characters in the configuration of variance. Forward stepwise discriminant analysis (DA) based on the generalized Mahalanobis distance was used to determine the similarity between populations and the ability of these characters to identify the specimens correctly. The above analysis was performed using SPSS v.15.0 statistical software.

RESULTS

A moderately high level of polymorphism was detected in all tested loci, ranging from 18 alleles at the Pma1 locus to 93 alleles at the SAI19 locus. However, all loci showed evidence of the presence of null alleles. These 6 loci also showed departures from HWE ($p < 0.01$). Observed heterozygosity values were quite low across all loci for all populations (mean $H_{OBS} = 0.597$) and were comparatively lower than overall expected heterozygosity values (mean $H_{EXP} = 0.898$). Values of H_{OBS} , H_{EXP} , F_{IS} and allelic richness, as well as the numbers of alleles are shown in Table 1.

F_{ST} values between the 8 sampled geographical areas of the saddled seabream ranged from 0.007

between Volos and Karystos to 0.1162 between Paros and Karystos (Table 2). Most of the values were highly significant after Bonferroni correction (Rice 1989). Pairwise F_{ST} values and their significances did not differ from the original pattern, even when recalculated using the ENA method (see Table 2). A hierarchical analysis (AMOVA) of the genetic structure showed that the proportion of total genetic variation that can be ascribed to differentiation between the southern, central and northern Aegean areas (F_{CT}) is close to zero (Table 3). Furthermore, genetic structuring appears to take place among populations within each area ($F_{SC} = 0.042$) and within populations ($F_{ST} = 0.322$). Approximately 82% of the total genetic variation was due to variation within populations and 11% was due to variation among populations within the 3 areas. A Bayesian individual assignment implemented in STRUCTURE is shown in Fig. 3, without using geographical area as a prior. The highest posterior probability was for $K = 4$ and $\ln(PD) = -7866.6$ (Fig. 4). The Wilcoxon sign-ranked test under the SMM model, implemented in BOTTLENECK, revealed no deviance from the mutation-drift equilibrium overall (data not shown). Furthermore, the implemented IBD model of geographical and genetic distances was insignificant.

The geometric map, using Barrier software, illustrated 4 barriers (Fig. 5). The first barrier separates the Crete and Paros populations from the Karystos population, the second separates the Karystos population from the Volos and Trikeri populations, the third separates the Volos and Trikeri populations from the Katerini and Nikiti populations, and the fourth determines a barrier to gene flow between the Katerini, Nikiti and Kavala populations. Analysis using Migrate software did not reveal any migration directionality. The connection type matrix pinpointed that migration rates were free to vary, without any clear direction of the migrants (data not shown).

Moreover, univariate ANOVA carried out on the transformed data (Zar 1984) indicated significant differences between the 8 populations for all morphometric characters except maximum body depth and anal fin base length ($F_{212,0.05} = 3.23$, $p \ll 0.05$). The PCA extracted 3 factors with eigenvalues > 0.75 , explaining 70.6% of the variance (Table 4).

The characters of primary importance in distinguishing between the groups were head length for the first canonical variable and dorsal fin height for the second. Using these morphometric characters, each specimen could be classified (using DA) correctly to the 8 populations with an accuracy of 66.5% (Table 5, Fig. 6).

Table 1. *Oblada melanura*. Genetic variation at each locus for each population of saddled seabream in the southern, central and northern Aegean Sea. H_{OBS} : observed heterozygosity; H_{EXP} : expected heterozygosity; F_{IS} : inbreeding index. ** $p < 0.01$

Locus	Parameter	Volos	Trikeri	Kavala	Katerini	Karystos	Nikiti	Paros	Crete
SAI10	H_{OBS}	0.697	0.5	0.5	0.611	0.5	0.529	1	0.778
	H_{EXP}	0.963	0.938	0.936	0.96	0.868	0.927	1	0.928
	Allelic richness	7.053	6.430	6.436	6.965	5.600	6.270	8	6.279
	F_{IS}	0.277**	0.474**	0.471**	0.37**	0.498**	0.436**	0	0.17
	No. of alleles	45	13	19	10	15	8	11	15
SAI12	H_{OBS}	0.598	0.6	0.688	0.5	0.6	0.611	0.8	0.889
	H_{EXP}	0.957	0.954	0.901	0.954	0.953	0.951	0.933	0.967
	Allelic richness	6.912	6.795	6.024	6.849	6.772	6.749	6.6	7.077
	F_{IS}	0.376**	0.377**	0.243**	0.482**	0.383**	0.364**	0.158**	0.086
	No. of alleles	41	16	20	13	17	8	14	15
SAI19	H_{OBS}	0.708	0.6	0.5	0.842	0.7	0.765	0.8	0.667
	H_{EXP}	0.973	0.933	0.929	0.959	0.974	0.963	0.956	0.954
	Allelic richness	7.303	6.345	6.369	6.982	7.263	7.042	6.756	6.923
	F_{IS}	0.273**	0.363**	0.47**	0.125**	0.292**	0.211**	0.179**	0.314**
	No. of alleles	58	13	23	15	21	8	13	16
Pma1	H_{OBS}	0.467	0.556	0.438	0.222	0.4	0.412	0.2	0.125
	H_{EXP}	0.788	0.883	0.704	0.654	0.7	0.797	0.511	0.792
	Allelic richness	4.397	5.415	3.542	3.511	3.273	4.471	2.778	3.664
	F_{IS}	0.408**	0.377**	0.386**	0.667**	0.442**	0.491**	0.636**	0.851**
	No. of alleles	13	9	5	4	8	3	5	6
Pma2	H_{OBS}	0.614	0.9	0.563	0.684	0.8	0.833	0.4	0.778
	H_{EXP}	0.935	0.964	0.944	0.963	0.942	0.96	0.844	0.948
	Allelic richness	6.48	7.039	6.579	7.029	6.61	6.985	5.178	8
	F_{IS}	0.344**	0.068**	0.412**	0.295**	0.158**	0.135**	0.556**	0.188**
	No of alleles	35	18	20	13	20	6	12	15
GA2A	H_{OBS}	0.516	1	0.5	0.579	0.6	0.556	0.2	0.333
	H_{EXP}	0.917	0.921	0.911	0.861	0.921	0.886	0.867	0.791
	Allelic richness	6.089	6.069	5.927	5.182	5.986	5.608	4.733	3.505
	F_{IS}	0.438**	-0.089	0.459**	0.333**	0.361**	0.38**	0.789**	0.593**
	No of alleles	22	11	9	9	13	5	5	12
Total	H_{OBS}	0.600	0.693	0.532	0.573	0.600	0.618	0.567	0.595
	H_{EXP}	0.922	0.932	0.888	0.892	0.893	0.914	0.852	0.897
	Allelic richness	6.372	6.349	5.813	6.086	5.917	6.188	5.674	5.990
	F_{IS}	0.353**	0.262**	0.407**	0.379**	0.356**	0.336**	0.386**	0.367**

Table 2. *Oblada melanura*. F_{ST} values between the 8 sampled geographical areas of saddled seabream (ns: non-significant, ** $p < 0.01$, *** $p < 0.001$, after Bonferroni correction). Values in parentheses are the recalculated F_{ST} values after application of the ENA method

	Volos	Trikeri	Kavala	Katerini	Karystos	Nikiti	Paros	Crete
Volos	–							
Trikeri	0.02527*** (0.021***)	–						
Kavala	0.01270 ^{ns} (0.012 ^{ns})	0.04246*** (0.032***)	–					
Katerini	0.07151*** (0.06***)	0.04333*** (0.0321***)	0.09333*** (0.088***)	–				
Karystos	0.00704 ^{ns} (0.003 ^{ns})	0.04205*** (0.031***)	0.00875 ^{ns} (0.005 ^{ns})	0.09069*** (0.089***)	–			
Nikiti	0.06357*** (0.051***)	0.03426*** (0.023***)	0.08249*** (0.074***)	0.01131 ^{ns} (0.008 ^{ns})	0.08088*** (0.73***)	–		
Paros	0.09476*** (0.082***)	0.04993 ^{ns} (0.031 ^{ns})	0.11406*** (0.095***)	0.01539 ^{ns} (0.009 ^{ns})	0.11622*** (0.109***)	0.03062 ^{ns} (0.016 ^{ns})	–	
Crete	0.07369*** (0.065***)	0.05079** (0.045**)	0.08885*** (0.081***)	0.02221 ^{ns} (0.015 ^{ns})	0.08365*** (0.0712***)	0.01958 ^{ns} (0.011 ^{ns})	0.03425 ^{ns} (0.02 ^{ns})	–

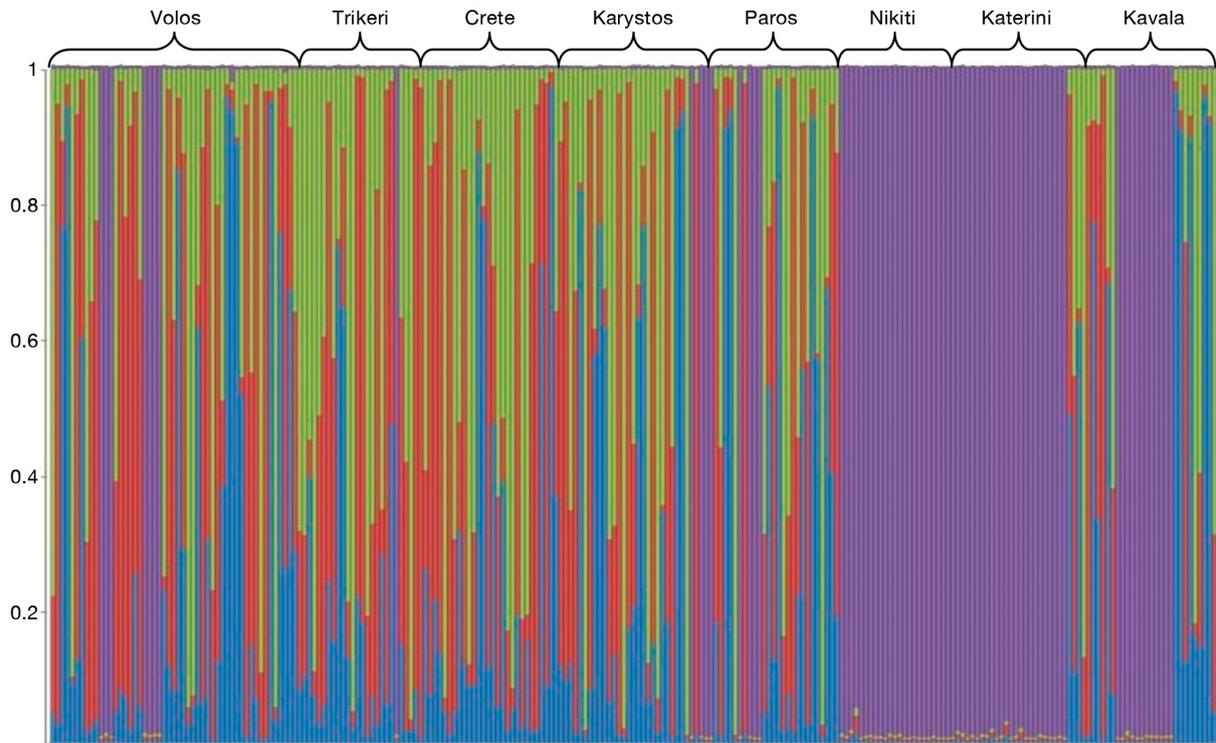


Fig. 3. *Oblada melanura*. Bayesian individual assignment implemented in STRUCTURE for $K = 4$ clusters without using geographical area as a prior. The y-axis represents the probability of assignment of an individual to each cluster and each color corresponds to the suggested cluster

Table 3. *Oblada melanura*. AMOVA of grouped into 3 areas: northern, central and southern Aegean Sea. ns: non-significant, *** $p < 0.001$

	df	Variance component	SS	Variance explained (%)	Fixation index
Between areas	2	19.054	-0.00623	11.2	$F_{CT} = -0.00233^{ns}$
Among populations within areas	5	36.388	0.11322	25.5	$F_{SC} = 0.04227^{***}$
Within populations	217	735.800	0.82539	63.3	$F_{ST} = 0.32174^{***}$

DISCUSSION

The genetic individual-based approaches show that most of the saddled seabream in the central and south Aegean Sea form a relatively continuous population, despite a complex coastline in the area. However, the significant and relatively high F_{ST} values between central Aegean populations (Volos, Trikeri, Karystos) and

south Aegean populations (Paros, Crete) revealed a subdivision of the species. The striking result of the relatively low but significant level, in some cases, of genetic differentiation between the northern Aegean group of populations and the group of central and south Aegean populations illustrates a potential small-scale population structure in the targeted area for the species in question. The Bayesian individual assignment proposes a differentiation between the northern grouping of specimens and the rest of the study area. However, the geometric map analysis revealed barriers to gene flow between the south and central Aegean and between the central and north Aegean Sea.

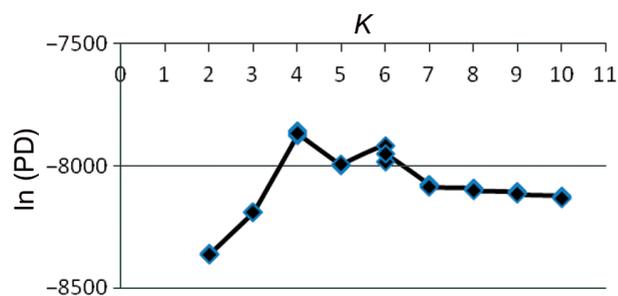


Fig. 4. *Oblada melanura*. Determination of the number of clusters (K) including all 3 repetitions for each K without geographical area as a prior. The highest peak denotes the most likely number of clusters according to the Pritchard Bays formula. PD: probability of data

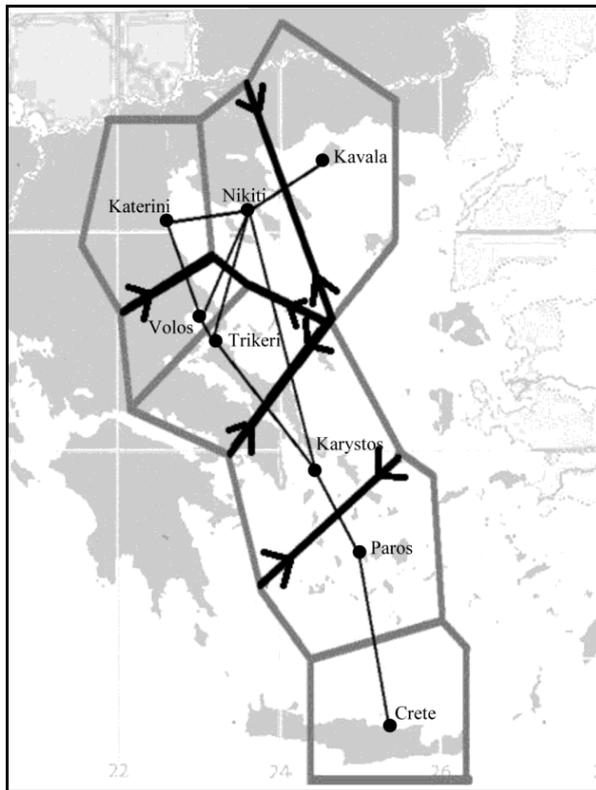


Fig. 5. *Oblada melanura*. Voronoi tessellation (in grey) of the points (populations) according to geographical locations (black circles) and the corresponding Delaunay triangulation (in dark grey). The bold black lines with arrows indicate the 4 barriers. Dotted lines represent biogeographic sub-areas of the Aegean Sea (after Voultziadou 2005b)

Table 4. *Oblada melanura*. Results of principal component analysis (PCA) and factor loadings for each morphometric variable on the 3 extracted PCA factors after varimax normalized rotation

Factor	Eigenvalue	% of variance	Cumulative %
1	7.237	55.671	55.671
2	1.194	9.187	64.857
3	0.746	5.738	70.595
Character	Factor 1	Factor 2	Factor 3
Maximum body depth	0.868	-0.145	0.019
Minimum body depth	0.767	-0.138	-0.260
Caudal peduncle length	0.724	-0.024	0.318
Head length	0.923	-0.146	0.003
Diameter of eye	0.725	0.192	-0.197
Preorbital distance	0.615	0.096	-0.450
Postorbital distance	0.789	0.074	-0.231
Predorsal fin distance	0.906	-0.197	0.038
Dorsal fin height	0.466	0.704	0.119
Dorsal fin base	0.763	0.089	-0.040
Ana fin height	0.468	0.604	0.292
Ana fin base length	0.845	-0.129	0.098
Pectoral fin base length	0.662	-0.396	0.412

Moderately high levels of polymorphism were displayed at all 6 microsatellite loci, which is not an unexpected result given the proposed higher rates of microsatellite mutation previously reported in marine fishes (O'Reilly et al. 2004). The reported significant departures from HWE support the presence of null alleles found in this study, which likely reveal issues regarding biased patterns of the disequilibrium. Moreover, the observed deviation from HWE and the relative high inbreeding values may be related to small sample sizes and/or individuals from the same progeny. The observed heterozygosity deficit related to the high F_{IS} values suggests that saddled seabream do not mate randomly in the study area and that gene flow may be spatially restricted. However, the relatively limited gene flow and the high F_{ST} values among samples, along with the differentiation from HWE, might indicate a subdivision of the populations, suggesting sub-structuring of the species in the Aegean Sea.

This differentiated pattern in the Aegean Sea was previously reported in benthopelagic species; these species in the Aegean Sea seem to exhibit spatial differences with respect to their benthogeographical distribution (Vafidis 2002, Kefalas et al. 2003, Voultziadou 2005a). The authors argue that this dissimilarity of the north, central and south Aegean Sea conforms with the prolonged differences in species' genetic and morphological data in relation with geographical and physicochemical features of the area. The results of the present study further support such a hypothesis. The phenotypic univariate analysis indicated significant differences between the 8 populations for all morphometric characters. DA of morphometric characters further revealed a clear pattern of population structure segregating the saddled seabream individuals between the 3 major sub-areas of the Aegean Sea. The high significance of the morphometric analysis compared with the genetic analysis suggests that oceanographic factors (i.e. currents, cyclonic circulation, bathymetry) are a significant stressor for environmental plasticity.

According to the literature, barriers in the Aegean Sea coincide with strong oceanographic fronts of different nature as a result of a near-surface thermaline circulation involving the low salinity outflow from the Black Sea in conjunction with the freshwater input of the continental shelf and the high salinity input from the south Aegean Sea (Poulos et al. 1997, Kourafalou et al. 2004). In addition, a basin-wide cyclonic circulation is taking place, resulting in sub-basin scale gyres connected to the complex topography of the area (Nittis & Perivoliotis 2002). Taking

Table 5. *Oblada melanura*. Discriminant analysis output, showing the percentage of specimen classification in each sampled group (1 to 8). Overall classification = 66.5 %

Population	1	2	3	4	5	6	7	8
Volos	75.1	4.2	0	5.7	0	15	0	0
Karystos	20	70	0	0	0	0	10	0
Kavala	0	0	31.3	31.2	0	12.5	12.5	12.5
Katerini	10	5	15	35	0	15	20	0
Crete	0	14.3	0	0	71.4	0	14.3	0
Trikeri	10	0	0	10	0	80	0	0
Nikiti	11.1	5.5	33.3	5.5	0	0	42.4	2.2
Paros	0	0	0	0	66.6	0	0	33.4
Wilks' lambda	<i>F</i>	<i>df</i>	<i>p</i>					
0.82288	5.60709	7	0.00186					

them in turn, the conditions in the north Aegean contain extended shelf areas, 2 cyclonic and 1 anticyclonic system and a deep trench of 1500 m, which present a physical geographical boundary to the central Aegean. The central Aegean forms extended deep areas, a cyclonic system and a boundary current along the continental shelf of an elongated island with Karystos at the southern end. The south Aegean contains many island complexes, a main cyclonic system and different water masses, and communicates with the western Mediterranean basin through Crete (for a review, see Olson et al. 2007). The different cyclone systems in the Aegean Sea contribute to different upwelling events and thus differentiated nutrient-enriched water masses, suggesting differences in food resources and availability for biota (Theodorou 2004).

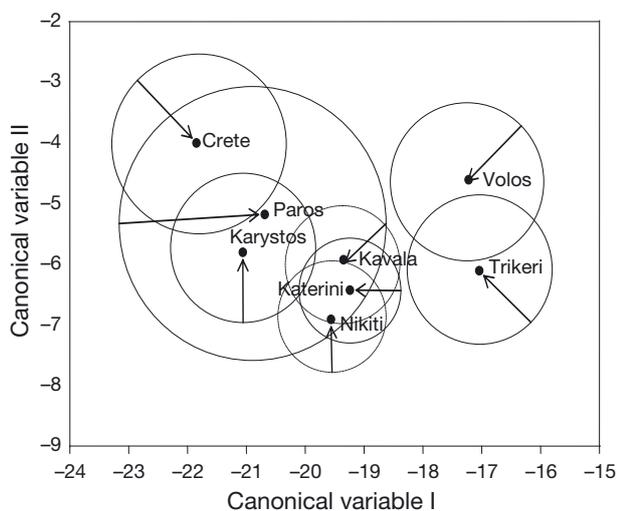


Fig. 6. *Oblada melanura*. Discriminant analysis plot of populations, where the 13 morphometric characters were used. Circles represent the percentage of the total of specimens correctly classified per population. Arrows indicate corresponding population

Biogeographically speaking, this documented cyclonic circulation is not only a food specialization habitat, and therefore boundary for saddled seabream dispersal potential, but also a transition zone with respect to ecological niches for many marine species (e.g. Voultziadou 2005b). The saddled seabream is considered to have moderate pelagic larval duration with pelagic eggs and offshore larvae, suggesting a shorter pelagic duration than other marine species and an ultimately restricted gene flow due to tidal currents (Macpherson & Raventos 2006, Galarza et al. 2009).

Despite the reported extensive phenotypic variability within and among populations, the fact is that specimens were collected on a differential seasonal basis during sampling. Therefore, it is probable that a large proportion of the intra-sample variation (i.e. various age groups) is the result of such heterogeneous phenotypic groups. To what degree these differences are the result of environmental conditions or genetic divergence remains to be determined, although the present data on microsatellite variability do confirm such differentiation. Within the limits of accuracy of the biometric experimental procedure, the characters of primary importance in distinguishing between the studied populations were those related to head and fin height dimensions; such characters explained approximately 70% of the total variance between the groups. Nonetheless, the DA classification accuracy for saddled seabream populations was somewhat lower and/or similar to values reported in the literature for other marine species (e.g. Alexander & Breden 2004, Cabral et al. 2003, Mamuris et al. 1998, Karakousis et al. 1993, Claytor & MacCrimmon 1988). However, one ought to bear in mind that such morphometric characteristics have a lower ability to discriminate populations than meristic (Karakousis et al. 1991) or even evolutionarily influenced characteristics. In any case, pairwise F_{ST} significances reflect the relative described differentiation: AMOVA was highly significant on 2 of the 3 hierarchical levels. Regarding the phenotypic analysis, results from both PCA and DA were highly significant.

Morphological variability among different geographical populations could be attributed to the variable genetic structure of populations and/or to different environmental conditions prevailing in each geographic area. Multivariate analyses can provide

an efficient tool, along with genetic data interpretation, for stock definition, and thus would be useful for a conservation management project plan for Hellenic coastal resources. However, one cannot exclude the existence of possible undetected genetic structure of such populations, which could account for any detected morphological variation. Such findings are not unique, as some phenotypic adaptations are not dependent on genetic mutations. The novelties of some phenotypic adaptations are not immediately expressed in the species gene pool. The lack of differences in a study does not preclude the existence of stock differentiation, even when including the input of genetic forces, since this is a direct consequence of environmental adaptation (Schweigert 1991).

Historical processes producing isolation, or low connectivity in combination with potential bottlenecks in one or more subpopulations (although not detected in the present data set), could also lead to morphological and genetic differentiation. Moreover, the observed absence of HWE is maybe due to genetic drift (e.g. small sample size), and inbreeding is such a crucial process and is remarkably quite common for species favoring reproduction in the sea. As inbreeding is caused by mating of genetically related individuals (common in fishes), it results in increased homozygosity. Therefore, observed heterozygosities are likely to be lower than expected. Although this is not always the case, in the present study inbreeding within populations was high enough. Furthermore, intense fishing pressure, mainly overfishing, can also lead to potential bottlenecks and population divergence (see Perez-Ruzafa et al. 2006, Walsh et al. 2006, Baibai et al. 2012, Limborg et al. 2012), and thus apparent small populations often suffer from the loss of genetic diversity due to genetic drift and inbreeding effects. However, the present study assumed a structure between the 3 areas, which affects local effective population size and thus the level of inbreeding. Therefore, the considerable population structure revealed by the morphometric characteristics has the potential to influence the impact on fitness-related traits to a greater extent than may be expected from the genetic analysis. The Aegean Sea, being a semi-enclosed marginal basin of relatively small volume compared with the open sea, shows an amplified and very rapid response to climate change (Anagnostou et al. 2005). A large number of faunal studies in the area have succeeded in reconstructing the climatic conditions and documenting the general trend from the cold, glacial climate conditions of the late Pleistocene to the warm, interglacial conditions of the Holocene (see Cramp et al. 1988, Geraga et al. 2000).

Cyclonic circulation, current flows and life history seem to be the factors that best represent the nature of barriers to gene flow encountered across the Aegean Sea in saddled seabream populations. The revealed population structure and the species' geographic dispersal might be closely linked to the oceanographic features encountered in the studied area, and could act as a useful tool for the study of other physical processes, such as the abundance of plankton and nutrients, which regulate the ecological niche of bio-society in small scaled areas. Further analysis is required to address the demographic patterns of the saddled seabream, as well as the ecological aspects of plasticity in this species.

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