SPATIAL GENETIC STRUCTURE IN THE SADDLED SEA BREAM (*Oblada melanura* [Linnaeus, 1758]) SUGGESTS MULTI-SCALED PATTERNS OF CONNECTIVITY BETWEEN PROTECTED AND UNPROTECTED AREAS IN THE WESTERN MEDITERRANEAN SEA

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ABSTRACT

Networks of marine protected areas (MPAs) are advocated worldwide for the achievement of marine conservation objectives. Although the knowledge about population connectivity is considered fundamental for the optimal design of MPA networks, the amount of information available for the Mediterranean Sea is currently scarce. We investigated the genetic structure of the saddled sea bream (*Oblada melanura*) and the level of genetic connectivity between protected and unprotected populations, using a set of 11 microsatellite loci. Spatial patterns of population differentiation were assessed locally (50-100 km) and regionally (500-1000 km), considering three MPAs of the West Mediterranean Sea. All values of population differentiation (Fst and Jost's D) were non-significant after Bonferroni correction, indicating that, at a relatively small spatial scale, protected populations were in general well connected with non-protected ones. On the other hand, at the regional scale, other analyses (discriminant analysis of principal components, AMOVA and STRUCTURE) revealed the presence of a subtle population structure that reflects the main oceanographic features (currents and barriers) of the study area. This genetic pattern (population divergence in presence of high gene flow) could be a consequence of different processes acting at different spatial and temporal scales among which species dispersal capacity, the presence of admixed populations or large population size could play a major role. These information can have important implications for the conservation biology and fisheries management of saddled sea bream like other coastal fish, as spatial variability in connectivity patterns may promote long-term stability of fish populations. From this perspective, multi-scale patterns of genetic connectivity should be taken into account when future MPA networks will be established in the West Mediterranean Sea.

KEYWORDS: genetic connectivity; marine protected areas; spatial scale; sea bream; Mediterranean Sea

1.INTRODUCTION

Marine protected areas (MPAs) are considered an effective tool for the restoration and the management of fishery resources within their borders (Claudet et al., 2008; Pérez-Ruzafa et al., 2008a; Fenberg et al., 2012). A number of recent evidences highlighted the potential capacity of MPAs to sustain fisheries outside their borders through the export of propagules (eggs and larvae) and the density-dependent spillover of juvenile and adult individuals (Goñi et al., 2010; Grüss et al., 2011a, 2011b; Harrison et al., 2012; Hackradt et al., 2014). From this perspective there is an increasing tendency to study and design MPA-based protection strategies in terms of 'networks', defined as a set of MPAs that operate synergistically in order to enhance protection benefits within and between protected areas, and sustaining the recovery of unprotected fishery resources (IUCN, 2008). This trend is corroborated by the global effort of marine management policies in promoting the establishment of effective MPA networks for the achievement of national and international conservation objectives (e.g. the Marine Strategy Framework Directive of the European Union, (European Commission, 2013)).

The effectiveness of MPA networks would depend on a series of criteria both related to the size, location and zoning of the single MPAs (Pérez-Ruzafa et al., 2008b; Almany et al., 2009) and to the relative positioning and spacing of MPAs among them (Jones et al., 2007; Pérez-Ruzafa et al., 2008b). The latter concepts refer to the spatial arrangement of MPAs within networks and strictly rely on the dispersal potential of marine organisms (Green et al., 2014). Connectivity refers to the demographic link between local sub-populations through the exchange of individuals at whatever life stage (Cowen and Sponaugle, 2009) and is inherently related to species dispersal capacity (Jones et al., 2007; Botsford et al., 2009). Connectivity is recognized to have a fundamental importance for conservation issues because it determines the range of distances over which marine fish populations interact and the geographical scales that should be considered in order to properly manage fishery resources (Leis et al., 2011). From this point of view, information on connectivity patterns are thought to be a key element for designing MPAs and networks (Halpern and Warner, 2003; Jones et al., 2007; Almany et al., 2009; Green et al., 2014).

In spite of the amount of MPAs already present and the call for the establishment of MPA networks in the next feature, the number of connectivity-based studies that have addressed the appropriate arrangement of MPA networks in the Mediterranean Sea is still scarce (Calò et al., 2013). Recent works have focused on the dispersal potential of larvae and/or the movements of juveniles from existing MPAs using, alone or combined together, genetic analysis, otolith chemical analysis and biophysical larval dispersal models (Di Franco et al., 2012a, 2012b; Pujolar et al., 2013; Andrello et al., 2013).

From this perspective, populations genetics is the most frequently adopted approach to assess the structure of fish populations and the gradient of genetic differentiation among spatially distinct units (Palumbi, 2003; Jones et al., 2009). Such information allow to investigate larval dispersal, providing an indirect measure of connectivity (Hellberg et al., 2002; Waples and Gaggiotti, 2006; Jones et al., 2009), and can be used to address specific management issues (González-Wangüemert et al., 2004; Pérez-Ruzafa et al., 2006; Waples et al., 2008). Among the molecular markers currently suitable for these kind of studies, microsatellites have proved to be a powerful tool for investigating population structure and gene flow in many fish species (Balloux and Lugon-Moulin, 2002). These markers are highly polymorphic and have fast mutation rates, thus allow to reveal genetic differences even at relatively small spatial scales (Elphie et al., 2012).

In this study we investigated the genetic structure of the saddled sea bream, *Oblada melanura* (Linnaeus, 1758) (Perciformes: Sparidae), a gregarious fish widely distributed on rocky reefs and seagrass (*Posidonia oceanica*) beds of Mediterranean coastal ecosystems (Bauchot and Hureau, 1986; García-Charton et al., 2004). It is also a species of relatively high commercial value for artisanal and recreational fisheries (Claudet et al., 2008; Lloret et al., 2008) Recruits of *O.melanura* (i.e. individuals of 3-4 months of age) were used to investigate patterns of genetic connectivity and population differentiation at two different spatial scales (50-100 and 500-1000 kilometers) and

assess the genetic flow between protected and unprotected areas, considering three MPAs of the Western Mediterranean Sea. The choices to analyze individuals of the same (0-age) class and the selection of a model species with a relatively short pelagic larval duration (less than 14 days in the west Mediterranean Sea; Calò et al. in prep.) were made in order to focus on recent genetic flows and reasonably investigate pattern of population differentiation even at the small spatial scale considered.
The outcomes of the present study will provide insights on the spatial scale and arrangement that should be considered when new MPAs are to be established in the Western Mediterranean Sea for the creation of networks of MPAs.

2. MATERIALS AND METHODS

2.1. Study area and sample collection

Sampling of *O. melanura* was carried out between September and October 2013. Three sectors (i.e. stretches of coastline of ca. 80-100 km) spaced about 400-600 km among them were selected along the European coast of the Western Mediterranean Sea (Central France, Northern Spain and Southern Spain) (Fig. 1). In each sector 3 locations were selected (see Fig. 1 for location names and abbreviations). The central location of each sector corresponded to an MPA, respectively: National Park of Porquerolles (become part of the National park of Port-Cros in 2012), Cap de Creus MPA (established in 1998) and Cabo de Palos MPA (established in 1995). The other 2 locations of each sector were unprotected and located about 40-50 km northwards and southwards of each MPA (Fig. 1). In each location, both protected and unprotected, 25 - 32 recruits, for a total of 258 individuals, were sampled during the night, by snorkeling, using a hand net and a torch. Specimens were firstly euthanized immersing them in a water solution with few drops of 95% alcohol for minimizing their suffering (Leary et al., 2013) and, after cessation of opercular movements, preserved in absolute ethanol used for genetic analysis. In the laboratory, caudal fins were dissected from each specimen and stored in absolute ethanol at -20ºC

2.2. DNA extraction and PCR amplification

Total genomic DNA was extracted from a minute section of caudal fin (~ 10-20 mg) using Sambrook et al. (1989) protocol.
DNA concentration of each individual was evaluated using NanoDrop 1000 (Thermoscientific) spectrophotometer, using 5 µl of ultra-pure water as blank measure. A dilution with polymerase chain reaction (PCR) ultra-pure water was made to standardize each sample to 50 ng/µl of DNA.
Genotypes were examined at a total of 11 polymorphic dinucleotid microsatellite loci: 7 (Omel primers) specifically developed by Roques et al. (2001) for *O. melanura* and 4 (Dvul primers) cross-validated in *O. melanura* by Roques et al (2007) from a set originally developed for *Diplodus vulgaris*. PCR products were obtained in a MG96Y PCR Thermocycler (AORI Technology Group) using 2 different multiplex mixes for the 2 sets of primers used. For Omel primers, PCRs were performed in a total volume of 10 µl containing 50 ng of DNA, 2 mM of MgCl2, 0.2 µM of each primer, 0.3 µM dNTP's, 1× reaction buffer [75 mm Tris-Hcl, 20 mm (NH4)2SO4], 1 mg/ml of BSA and 0.75 U Taq polymerase (BIOTAQ). PCR conditions were as follows: an initial denaturation step of 5 min at 95 °C, eight cycles consisting of 45 s at 92 °C, 45 s at 53 °C annealing temperature, 45 s at 72 °C followed by an additional 24 cycles consisting of 30 s at 92 °C, 30 s at 55 °C annealing temperature, and 20 min at 72 °C. For Dvul primers, PCRs conducted in a total volume of 20 µl containing 50 ng of DNA, 2 mM of MgCl2, 0.25 µM of each primer, 200 µM dNTP's, 1× reaction buffer [75 mm Tris-Hcl, 20 mm (NH4)2SO4] and 0.5 U Taq polymerase (BIOTAQ). Amplification conditions were the same as for Omel primers. PCR product was run on 1.5% agarose gel stained with safe- DNA® before being viewed under UV light and were visualized by capillary electrophoresis using ABI Prism 3730 automated genetic analyser (Applied Biosystems). Allele scoring was done using GeneMapper v.3.5 software (Applied Biosystems, Foster City, California).

2.3. Data analysis

All loci were tested for the presence of null alleles using the software MICRO-CHECKER v.2.2.3 (Van Oosterhout et al., 2004). The software POWSIM (Ryman and Palm, 2006) was used to assess the statistical power of the markers used in the study using Chi-squared and Fisher's exact tests. A range of predefined levels of expected divergence (Fst=0.001, 0.005, 0.01, 0.05) was tested using an Ne (effective population size) of 1000 and t (time of divergence) of 10. The total number of alleles (N), the number of private alleles (PA), observed (Ho) and expected (He) heterozygosities for each locus and location were obtained with GenAlex v.6. (Peakall and Smouse, 2006). Standardized allelic richness (AR) and inbreeding coefficient (Fis) and their estimated probabilities were calculated via 10^4 random permutations using FSTAT version 2.3.9.2 (Goudet, 2002). A Student's t test was used among all pairs of locations to test for potential differences in the genetic diversity.
Deviations from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were tested using GENEPOP version 3.4 (Raymond and Rousset, 1995). Where multiple comparisons were tested, the sequential Bonferroni procedure (Rice, 1989) was used to adjust the statistical significance.
Population structure was investigated by a series of statistical approaches. Firstly population differentiation was explored with a Bayesian approach implemented in the software STRUCTURE (Pritchard et al., 2000), a model-based clustering algorithm that infers the most likely number of groups in the data. The software organizes individuals into a predefined number of clusters (K), with a given likelihood, which might represent the number of putative populations. Ten runs of 50,000 Markov Chain Monte Carlo (MCMC) and 10,000 burn-in-length were carried out using admixture model with no prior population information and $1<K<9$. The most likely K was determined with the criterion of Evanno et al. (2005) using the web tool Structure Harvester v0.6.94 (http://taylor0.biology.ucla.edu/structureHarvester/). The method is based on the rate of change of mean posterior probability between 2 different clusters (ΔK) in function of the number of cluster (K), and the optimal number of clusters is the one that corresponds to the higher value of ΔK.
Population structure was also investigated through the Discriminant Analysis of Principal Components (DAPC, (Jombart et al., 2010)) as implemented in ADEGENET version 1.3-6 (package used in R software, version 3.1.1; R Development Core Team 2011). DAPC does not rely on explicit population genetics models, and is useful when the structure is subtle (Jombart, 2008; Jombart et al., 2010; Vander Wal et al., 2013). The *dapc* function is based on 2 steps: data are firstly transformed using a PCA, then a discriminant analysis (DA) is performed. As recommended in Jombart et al. (2010), for the *dapc* we retained a number of principal components that corresponded to more than ~90 % of cumulated variance and all the linear discriminants. Two methods for DAPC are available, depending on whether the number of origin populations (K) is known or not, and were both used in study. In the first case (no info on K), before performing *dapc*, the function *find.clusters* was implemented to identify the optimal number of clusters (populations). This function runs a k-means algorithm after transforming the data through a PCA (a step done in order to reduce the number of variables and to speed up the clustering algorithm, (Jombart, 2013)). K-means is run sequentially with increasing values of K, and different clustering solutions are compared using Bayesian Information Criterion (BIC). The optimal clustering solution is the one that corresponds to the lowest BIC, indicated by an elbow in the curve of BIC values as a function of K. In our case all the PCs were retained for the analysis (Jombart et al., 2010) and the maximum number of possible clusters was set to K=9. In the second case, the function *dapc* was directly used considering the information on the putative origin populations and the outcomes were visualized through a scatterplot.
Finally genetic differences among sampling locations were assessed by hierarchical AMOVA in ARLEQUIN 3.5.1.2 (Excoffier et al., 2005), considering different location groupings. The first one was performed partitioning genetic differentiation among sectors and among locations within each sector. The other AMOVAs were run on the base of the genetic clusters depicted by DAPC (with and without information on K). ARLEQUIN was also used to test for Isolation-by-distance (IBD) using a Mantel test, correlating genetic distance versus linear coastal distance between locations and to calculate pairwise Fst between locations. Pairwise genetic differentiations were also calculated

using the more recent heterozygosity-independent Jost's *D* (Jost, 2008), which is thought to be a more appropriate statistic for highly variable markers such as microsatellites (Meirmans and Hedrick, 2011) This statistic, based on a bootstrap method (1000 bootstrap repeats) to estimate p-values, was calculated with DEMEtics (Gerlach et al., 2010) (package used in R software, version 3.1.1; R Development Core Team 2011).

3. RESULTS

All 11 loci considered were polymorphic, with the total number of alleles ranging from 2 to 22. The most of loci showed a high level of polymorphism. The lowest mean number of alleles (2.8) was recorded for locus 'Omel20', while the highest (18.8) for locus 'Dvul84'. The software MICROCHECKER detected no evidence for null alleles or genotyping errors due to stuttering or large allele dropout. Simulations using recorded allelic frequencies in POWSIM showed that the markers used in this study have enough statistical power to reveal population structure at Fst values ranging from 0.001 to 0.05 (p = 1.000).

Values of Ar, PA, Ho, He and Fis for each combination of locus x population are shown in (Tab. 1). Observed values of genetic diversity did not differ significantly between protected and unprotected locations in each sector (Student's t test, $p>0.05$). Observed heterozygosity values were relatively high across all loci, apart from locus Omel20 (for which the lowest number of alleles was recorded). Differences in mean values of Ho (range 0.724 – 0.794) were not significant among locations ($p>0.05$ for all pairwise Student's t test) similarly to what observed for mean values of He (range 0.752 – 0.788) and Ar (range 10.54 – 11.36) ($p>0.05$ for all pairwise Student's t test). Only 4 out of 99 tests departed significantly from HWE after Bonferroni correction (locus Omel02 at Les and Tor; locus Omel61 at Cav and CC MPA, see Fig. 1 for abbreviations of location names). No LD was observed between any pair of loci after Bonferroni correction.

The results from the Bayesian approach implemented in STRUCTURE, applying the Evanno's method, are shown in Tab 2. When we considered all the 9 locations together, the higher value of ΔK was recorded for K=3 (3 clusters) with a similar posterior probability that an individual belonged to one specific cluster (Tab 2). The analysis preformed on each sectors separately revealed in the three cases a higher value of ΔK for K=2. However, importantly Evanno's method needs at least 2 clusters in order to calculate a ΔK and there is no possibility for a single homogeneous population to be selected.

DAPC analysis was firstly performed without any *a priori* group information. Using the function *find.cluster*, the lowest BIC value (381.115) corresponded to K=3 (Fig. 2). For DAPC analysis, 75 PCA axes and two discriminant functions were retained. One cluster (C2 in Fig. 3) included mainly individuals from Por MPA, Les and Col, which together account for ~50.0% of the total membership probability of the cluster. In a second cluster (C1) the highest membership probability was recorded for individuals from Cav and Aig (14.9% and 13.6% respectively). In a third cluster (C3) the highest membership probability was recorded for individuals from Aig and CC MPA. Individuals from the Southern Spain sector showed similar values of individual membership probability for the three clusters. In the second *dapc* analysis, clusters were defined *a priori*, retaining 85 principal components and the maximum available number of discriminant functions. In a first *dapc* all the 9 putative populations sampled were considered separately: the first axis in the scatterplot separated southern locations (Southern Spain sector) from northern ones (Central France and North Spain sector), with Cav, CC MPA and Aig partially separated from Por MPA, Les and Col (Fig. 4a). The second axis grouped Cav and Aig, separating them from CC MPA and the remaining locations. The main groupings identified by the first axis of the scatterplot were used for running other 2 *dapc* for a better visualization of the outcomes (Fig. 4b and 4c).

Analysis of molecular variance was conducted using different groupings. The first AMOVA, performed considering the geographical groupings, suggested no genetic structuring, with no significant differences among sectors and within sectors (Table 3). A second AMOVA was run considering 2 groups: northern locations (Central France and Northern Spain sector) and southern ones (Southern Spain sector). In this case the variation between the two groups resulted to be

significant (0.23 and $p<0.05$) while the differences among locations within the groups resulted not significant. Finally, an AMOVA was run considering the 3 main groups clustered by the first axis of the scatterplot: (1) Por MPA, Les and Col; (2) Cav, CC MPA and Aig; (3) Southern Spain locations. In this case differences among groups resulted to be significant ($p<0.01$) (Table 3). On the other hand, no Fst comparison resulted significant after Bonferroni correction (only two comparisons, Cav-Por MPA and CP MPA – Por MPA, were significant before correction). Overall, values of Fst, were low with an average value, considering all the 9 locations, of 0.002. The highest value were recorded between Cav - Por MPA and CP MPA – Por MPA (0.0083 for both comparisons) (Table 4). The Jost D values showed similar results to the Fst values (Table 4). Mantel test also resulted not significant, indicating that no relation between geographical and genetic distances occurs.

4. DISCUSSION

The results of the study revealed a high level of genetic diversity in *O. melanura*, expressed as both He and AR. Similar results were recorded, for the same species, using a different set of microsatellite primers (Gkafas et al., 2013), and in other sparid fish in the Mediterranean Sea (Franchini et al., 2011). High genetic diversity is fundamental for maintaining the adaptability of natural fish populations and sustainable yields in fisheries (Kenchington et al., 2003).

At a relatively small spatial scale (within sectors, 50-100 km) protected populations of *O.melanura* were in general well connected with non-protected ones. The lowest values of Fst and Jos'st D were recorded for pairwise comparisons between locations within the same sector, suggesting a high genetic flow between MPAs and populations outside their borders, within each sector considered. Moreover, no differences were found between protected and unprotected populations in terms of genetic variability. This latter result contrasts with the work of Pérez-Ruzafa el al. (2006) that found higher values of both total and standardised AR in protected populations of *Diplodus sargus sargus* than in unprotected ones, considering two MPAs of the Western Mediterranean Sea (two within the Southern Spain sector and one within the Northern Spain sector of this study). Genetic diversity loss in commercial important species can be a consequence of fishing selection (Pérez-Ruzafa et al., 2006). However, the inference about benefits due to protection from fishing was not an aim of the present study. In fact, the choice to analyze recruits and consider recently established MPAs (e.g. Por MPA) have to be seen in the light of a study mainly focused on recent genetic connectivity patterns, among MPAs and unprotected areas, rather than MPA effects.

The low level of population differentiation, considering all the sampling locations, suggested that local populations of saddled sea bream form a unique, continuous population along the coast of the Western Mediterranean Sea.

On the other hand, at the regional scale (considering the three sectors, distant 500-1000 km apart), further analyses revealed the presence of a subtle population sub-structuring. The DAPC both performed with and without *a priori* information on the origin populations showed the presence of 3 main clusters. The analysis with *a priori* information revealed a separation between the north of the study area (Central France and Northern Spain) and the Southern Spain sector. This potential sub-structure was confirmed by a hierarchical analysis of molecular variance, in which the overall Fst, although low (0.0023), resulted statistically significant and 4 times higher then the Fst when groups corresponded to the geographic sectors considered. The genetic differentiation between the northern and the southern part of the western Mediterranean Sea was already pointed out in several recent studies focused on the genetic structure of different coastal fish. Schunter et al. (2011a) specifically addressed the role of the Ibiza channel, that in our study separated the Southern Spain sector from the other two sectors considered, by acting as an important barrier for the circulation of the Western Mediterranean Sea, thus determining genetic differentiation across this boundary (but see also, Schunter et al., 2011b). Similar results were found for other commercial species of the Sparidae family (Pérez-Ruzafa et al., 2006; Galarza et al., 2009, for an example with *O.melanura*) and for the red mullet *Mullus barbatus* (Galarza et al., 2009), along the same stretch of Mediterranean coastline.

Within the northern sectors of our study area (France and Northern Spain) a potential separation was found by the function *find.cluster* between a group formed by Cav, CC MPA and Aig and a second group composed by Por MPA, Les and Col. This further sub-structure was confirmed by the AMOVA. This pattern, although subtle, is concordant with the geographical arrangement of the sampling locations and the oceanographic circulation of the western Mediterranean Sea. In particular, Por MPA, Les and Col, could be genetically more similar due to the circulation of the Liguro-Provençal current along the gulf of Lion, separating them from populations outside the gulf. This oceanographic system is one of the most important current in the Mediterranean Sea and follows a south-western trajectory skimming the coasts of France and Spain (Mounier et al., 2005). The Liguro-Provençal could be responsible of a high dispersal of larvae directly towards the Catalan coast and a low dispersal towards locations inside the gulf of Lion. This could explain the

relatively high values of Fst between Por MPA and Cavalaire (one of the two pairwise comparison that resulted statistically significant before Bonferroni correction), and the high genetic similarity between Cav and the two southern locations of the Northern Spain sector (south of Cap de Creus peninsula). A similar conclusion was reached by Lenfant and Planes (1996), that found a genetic distinction between populations of *Diplodus sargus*, relatively close to each other, across the western edge of the gulf of Lion.

The result from the Bayesian approach, implemented in STRUCTURE, was concordant with the above clustering results, showing the presence of 3 genetic clusters. However, individuals' posterior probability to belong to one specific cluster was relatively low; this could indicate that the 3 groups identified by the analysis were likely to be made by an admixture of individuals from different locations.

Despite the overall homogeneity found among populations, our results detected a certain degree of divergence at the regional scale. Increasing amounts of data have supported the importance of population divergence in the presence of ongoing gene flow. This mode of differentiation could depend on the strength of disruptive natural selection and the factors affecting the linkage among genes under disruptive selection. This process includes population differentiation, in which gene exchange occurs since onset of divergence, and secondary contact following a period of geographic isolation, as well as all sorts of situations in which gene flow happens intermittently (Pinho and Hey, 2010). An explanation to our observations could be the self-recruitment to the sampled locations and distinct patterns of connectivity by oceanographic flow that could allow fitness selection and the maintenance of the exclusive alleles enhancing the divergence with gene flow (González-Wangüemert and Pérez-Ruzafa, 2012). On the other hand, the concurrent lack of spatial genetic partitioning (resulting from low, non-significant Fst values and high gene flow) among populations suggests the occurrence of high levels of admixture of differentiated genetic pools rather than stable populations among the locations considered. Reduced levels of population structure can be promoted in marine organisms with large effective population sizes and can also indicate different patterns of connectivity between populations over small and large geographic scales (Baeza and Fuentes, 2013; Lemer and Planes, 2014; Vergara-Chen et al., 2014).

The outcomes of this study showed that the analysis of population structure over multiple spatial scales can reveal the presence of different genetic patterns that should be considered for the management of marine resources. From this perspective, our study can have important implications for the conservation biology and fisheries management of saddled sea bream as for other coastal fish species. Spatial variability in genetic connectivity may act to promote long-term stability of populations, so it is fundamental to take into account such heterogeneity for the design of future MPA networks in the western Mediterranean Sea. The differences in local and regional patterns of population differentiations emphasize the need to consider ecological units, rather than political ones, for the management of marine resources.

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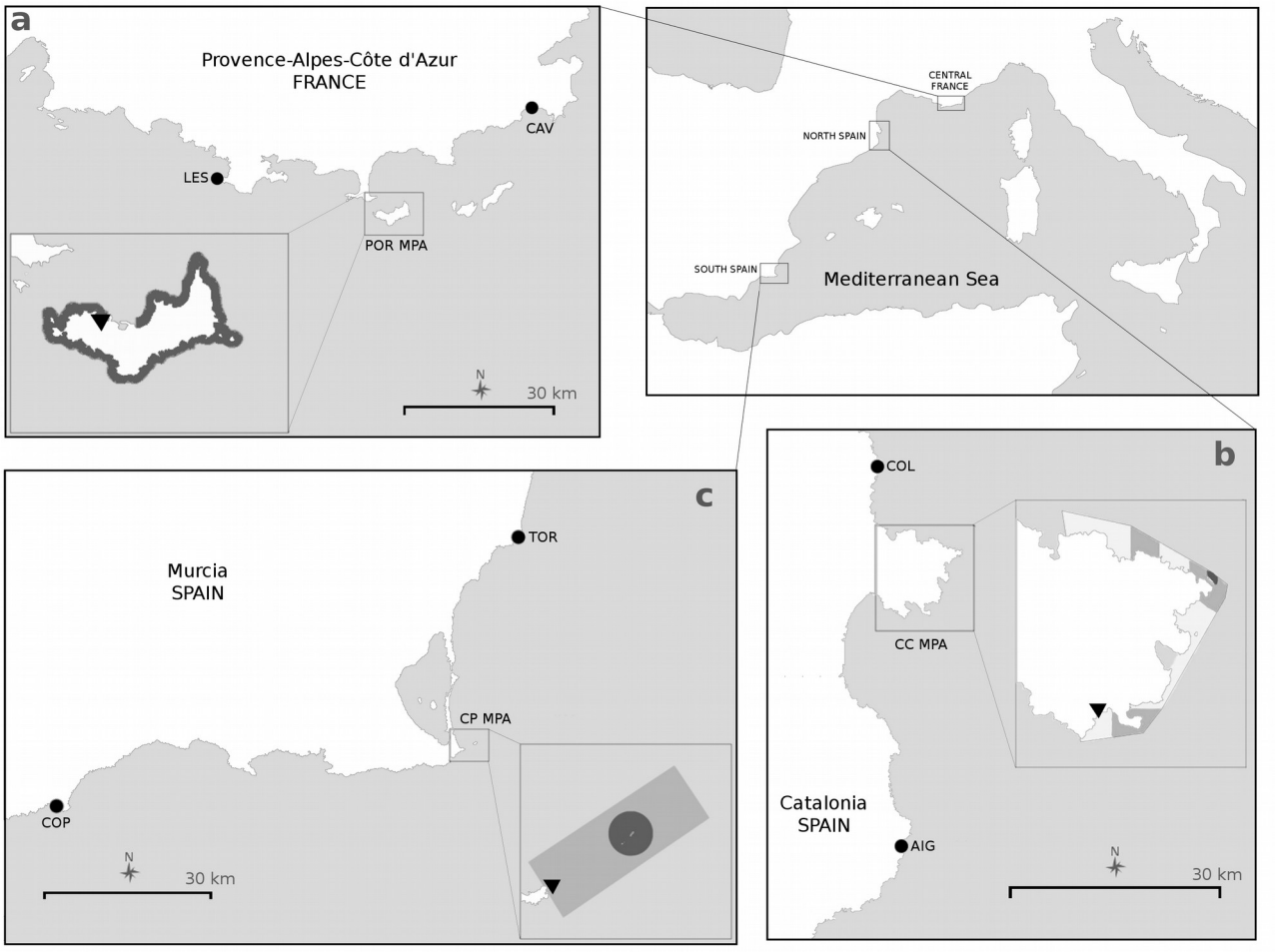

Figure 1. Study area. **a**. Central France (Cav= Cavalaire, Por MPA = Porquerolles MPA, Les = Les Embiez); **b** Northern Spain (Col = Colera, CC MPA = Cap Creus MPA, Aig = Aiguablava); **c**. Southern Spain (Tor = Torrevieja, CP MPA = Cabo de Palos MPA, Cop = Cabo Cope). In each sector (a, b and c): black dots represent unprotected locations; the MPA is highlighted and the black triangle indicates the protected sampling location. Grey scale (light, medium and dark), where present, in each MPA represents the different levels of protection (low, medium and high, respectively).

Locus	Cav	Por MPA	Les	Col	CC MPA	Aig	Tor	CP MPA	Cop
Dvul2									
Ar	8.68	8.66	9.45	10.80	9.00	9.71	10.46	10.00	11.00
PA	0	0	0	0	0	0	0	0	0
Ho	0.929	0.800	0.600	0.719	0.760	0.759	0.767	0.600	0.880
He	0.816	0.767	0.746	0.766	0.847	0.849	0.810	0.816	0.859
Fis	-0.120	-0.008	0.212	0.078	0.128	0.128	0.071	0.284	-0.004
Dvul33									
Ar	4.69	2.95	3.83	2.79	2.00	2.84	2.97	3.00	2.00
PA	2	0	0	0	0	0	0	0	0
Ho	0.643	0.750	0.633	0.656	0.654	0.533	0.700	0.680	0.560
He	0.551	0.531	0.561	0.513	0.499	0.516	0.531	0.519	0.499
Fis	-0.227	-0.402	-0.111	-0.267	-0.292	-0.016	-0.303	-0.291	-0.102
Dvul4									
Ar	6.00	6.66	6.83	6.79	6.85	7.64	6.97	6.95	5.00
PA	0	0	0	0	0	2	0	0	0
Ho	0.741	0.719	0.828	0.800	0.731	0.724	0.793	0.840	0.792
He	0.818	0.706	0.789	0.780	0.741	0.763	0.814	0.806	0.794
Fis	0.120	-0.001	-0.023	0.007	-0.018	0.077	0.050	-0.021	0.034
Dvul84									
Ar	19.41	18.94	19.54	18.20	17.00	19.39	17.16	17.00	19.00
PA	0	1	0	0	0	0	0	0	0
Ho	0.857	0.867	0.963	0.867	0.840	0.857	0.800	0.920	0.960
He	0.926	0.908	0.932	0.922	0.917	0.935	0.927	0.916	0.933
Fis	0.092	0.068	-0.009	0.081	0.067	0.104	0.154	0.016	-0.009
Omel02									
Ar	11.85	12.66	13.29	13.21	13.84	11.84	13.26	12.00	14.00
PA	0	0	0	0	0	0	0	0	0
Ho	0.893	0.875	0.900	0.935	0.846	0.828	0.897	0.720	0.880
He	0.879	0.901	0.886	0.898	0.890	0.892	0.881	0.886	0.906
Fis	0.002	0.044	0.001	-0.022	0.069	0.092	0.003	0.133	0.049
Omel20									
Ar	2.00	3.54	2.00	2.79	2.96	2.98	2.85	3.00	3.00
PA	0	1	0	1	0	0	0	0	0
Ho	0.179	0.250	0.300	0.312	0.269	0.267	0.200	0.240	0.120
He	0.162	0.225	0.255	0.268	0.292	0.309	0.183	0.215	0.311
Fis	-0.080	-0.095	-0.160	-0.150	0.098	0.155	-0.077	-0.095	0.627
Omel27									
Ar	14.94	15.16	20.49	19.04	17.81	14.44	18.30	14.00	18.00
PA	3	0	1	0	0	0	0	0	0
Ho	0.885	0.774	0.733	0.938	0.846	0.828	0.833	0.880	0.720
He	0.910	0.912	0.937	0.917	0.918	0.899	0.927	0.912	0.931
Fis	0.053	0.170	0.176	-0.006	0.098	0.100	0.117	0.055	0.246
Omel38									
Ar	9.76	8.70	8.76	7.52	6.00	8.65	7.62	7.00	7.00
PA	1	0	0	0	0	0	0	0	0
Ho	0.821	0.938	0.700	0.594	0.731	0.667	0.767	0.760	0.840
He	0.813	0.805	0.789	0.798	0.757	0.815	0.776	0.804	0.781
Fis	0.009	-0.149	0.129	0.242	0.054	0.164	0.029	0.075	-0.055
Omel54									
Ar	17.42	17.15	16.21	15.86	17.00	16.38	16.58	18.00	16.00
PA	0	0	0	0	0	0	0	0	0
Ho	0.964	0.906	0.933	0.812	0.880	0.800	0.900	0.920	0.800
He	0.925	0.905	0.911	0.913	0.904	0.926	0.928	0.931	0.916
Fis	-0.025	0.015	-0.007	0.125	0.007	0.121	0.047	0.032	0.147
Omel58									
Ar	7.80	6.60	6.68	6.60	7.96	7.64	6.97	8.00	8.00
PA	0	2	0	0	0	0	0	0	0
Ho	0.889	0.656	0.700	0.844	0.885	0.900	0.733	0.760	0.880
He	0.795	0.704	0.696	0.745	0.791	0.785	0.723	0.805	0.814
Fis	-0.090	0.083	0.011	-0.117	-0.098	-0.131	0.002	0.076	-0.060
Omel61									
Ar	18.22	16.40	17.88	16.96	19.69	15.39	17.11	17.00	16.00
PA	0	1	0	0	0	0	0	0	0
Ho	0.929	0.844	0.867	0.906	0.885	0.800	0.900	0.960	0.840
He	0.923	0.911	0.913	0.924	0.926	0.909	0.925	0.918	0.923
Fis	0.012	0.058	0.067	0.035	0.064	0.136	0.008	-0.026	0.110
All									
Ar	10.979	10.675	11.360	10.960	10.919	10.627	10.932	10.541	10.818
Ho	0.794	0.762	0.742	0.762	0.757	0.724	0.754	0.753	0.752
He	0.774	0.752	0.765	0.768	0.771	0.782	0.766	0.775	0.788

Table 1. Summary statistics of 11 microsatellite loci and overall mean among the 9 locations considered. Ar = allelic richness, PA = number private of alleles, Ho = observed heterozygosity, He = expected heterozygosity, Fis = inbreeding coefficient.

K	Mean LnP(K)	Stdev LnP(K)	Delta K	Mean posterior probability for cluster: 1	2	3
1	-11597.033	0.695	—			
2	-12145.853	233.203	0.145			
3	**-12660.827**	**318.004**	**0.374**	0.334	0.333	0.333
4	-13056.960	380.514	0.120			
5	-13407.553	544.955	0.030			
6	-13774.440	798.008	0.162			
7	-14011.960	614.006	0.029			
8	-14267.427	1109.107	0.221			
9	-14277.693	819.051	—			

Table 2. Bayesian clustering results of STRUCTURE analysis. K represents the number of genetic clusters.

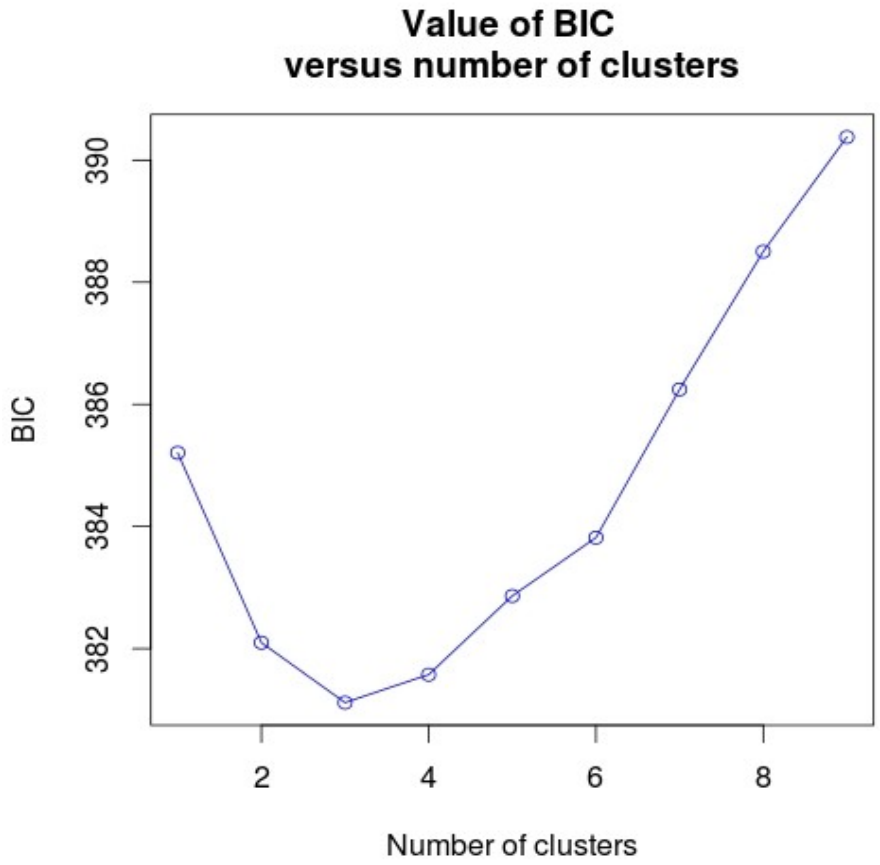

Figure 2. Bayesian Information Criterion (BIC) in relation to the number of clusters identified by the *find.cluster.*

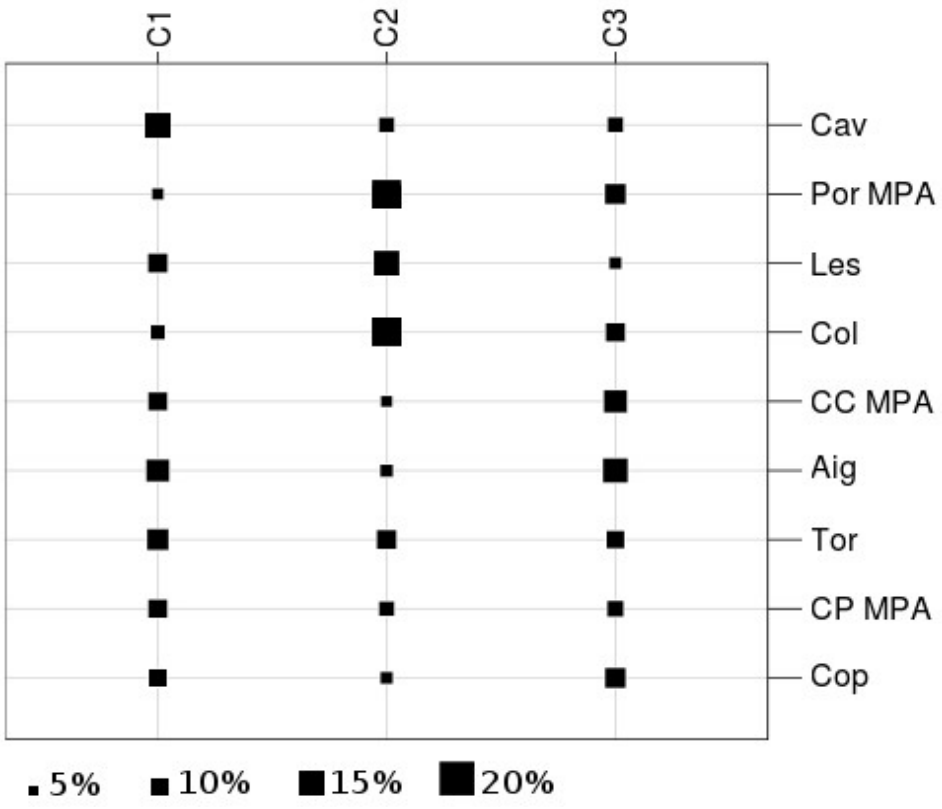

Figure 3. Repartition of the individual mean posterior probability of the 9 locations considered in the 3 clusters identified by the DAPC without *a priori* information on the origin populations. The size of the squares is proportional to the values of probability. The meaning of abbreviations to name each location is specified in Fig. 1.

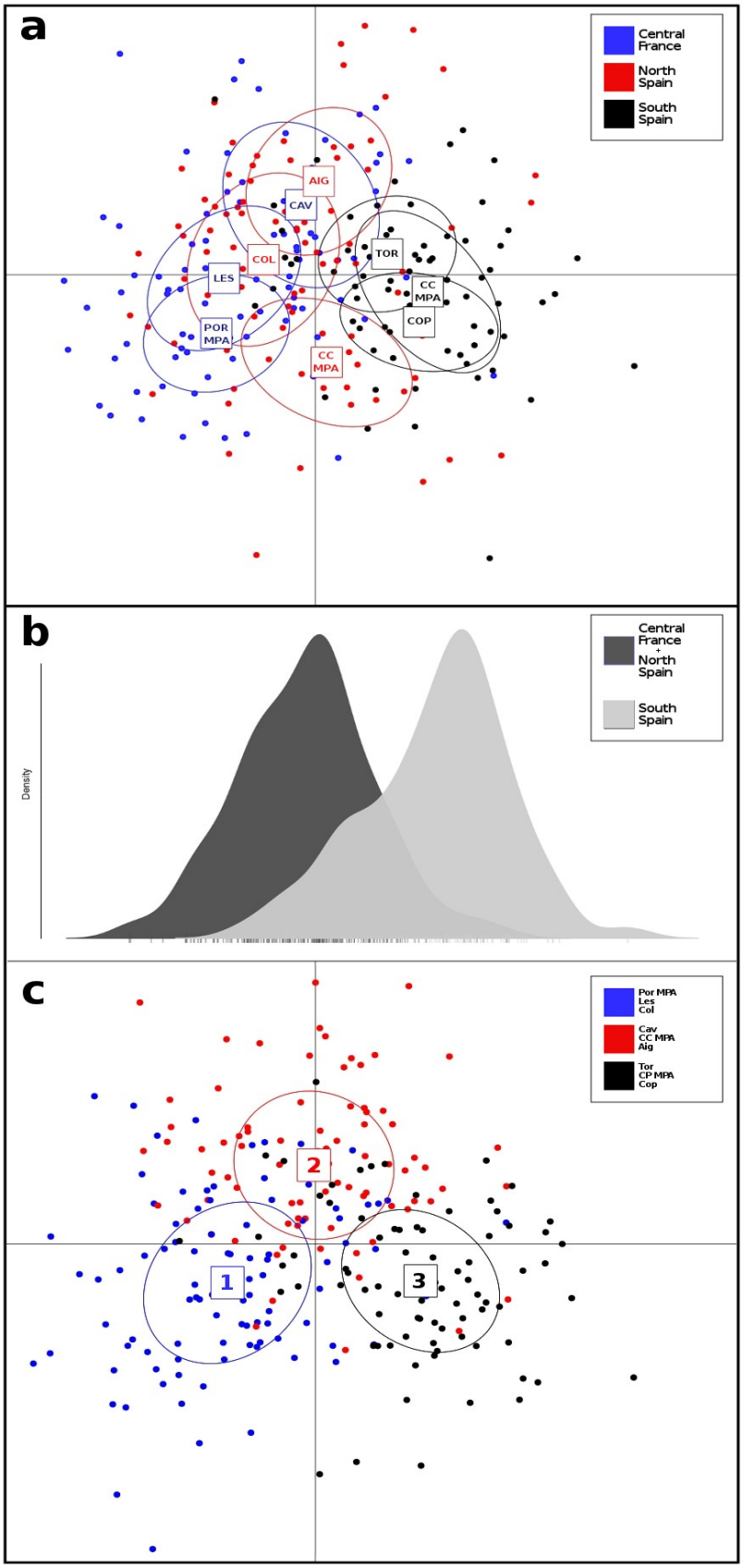

Figure 4. Scatter plot of the DAPC with *a priori* information on the origin populations: a) populations corresponding to the 9 locations sampled (the 2 axes represent the 2 major discriminant analysis eigenvalues); b) two populations (1 discriminant analysis eigenvalue); c) three populations (the 2 axes represent the 2 major discriminant analysis eigenvalues). See Fig. 1 for location names.

Grouping	Source of variation	d.f.	SS	Variance components	Percentage of variation	P-value
Sector clustering 1.France 2.North Spain 3.South Spain	Among groups	2	9.183	0.002	0.05	0.303
	Among populations within groups	6	25.281	0.000	0.00	0.493
	Within populations	507	2139.98	4.221	99.95	0.364
	Total	515	2174.44	4.223		
DAPC clustering 1.France – North Spain 2.South Spain	Among groups	1	6.206	0.0098	0.23	<0.05 *
	Among populations within groups	7	28.258	-0.0032	-0.08	0.717
	Within populations	507	2139.98	4.22087	99.84	0.364
	Total	515	2174.44	4.227		
DAPC clustering 1.Cav – CC MPA - Aig 2.Por MPA – Les – Col 3.Tor – CP MPA – Cop	Among groups	2	12.758	0.0161	0.38	<0.01 **
	Among populations within groups	6	21.706	-0.0105	-0.25	0.953
	Within populations	507	2139.98	4.22087	99.87	0.36
	Total	515	2174.44	4.227		

Table 3. Hierarchical analysis of molecular variance (AMOVA). d.f. (degrees of freedom), SS (sum of square). See Fig. 1 for location names.

	Cav	Por MPA	Les	Col	CC MPA	Aig	Tor	CP MPA	Cop
Cav		**0.0337**	-0.0007	-0.0187	-0.0044	-0.0515	-0.0080	0.0027	-0.0195
Por MPA	**0.0083**		0.0119	-0.0192	0.0004	0.0117	0.0154	**0.0450**	0.0327
Les	0.0018	-0.0006		-0.0143	0.0231	-0.0157	0.0065	0.0386	-0.0059
Col	0.0008	-0.0036	-0.0035		0.0074	-0.0141	-0.0176	0.0065	-0.0048
CC MPA	0.0014	0.0004	0.0041	0.0011		-0.0062	-0.0025	-0.0018	-0.0212
Aig	-0.0046	0.0015	-0.0005	-0.0004	-0.0007		-0.0245	0.0079	-0.0195
Tor	0.0019	0.0017	-0.003	-0.003	0.0012	-0.0018		-0.0370	-0.0416
CP MPA	0.0026	**0.0083**	0.0036	0.0009	0.0024	0.0022	-0.0043		-0.0493
Cop	0.0020	0.0056	0.0012	-0.0002	-0.0004	-0.0018	-0.0026	-0.0040	

Table 4. Pairwise comparison between populations. Fst below diagonal, Jost D above diagonal. In bold significant values before Bonferroni correction. See Fig. 1 for location names.