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Matching genetics with oceanography: directional gene flow in a Mediterranean fish species

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Abstract

Genetic connectivity and geographic fragmentation are two opposing mechanisms determining the population structure of species. While the first homogenizes the genetic background across populations the second one allows their differentiation. Therefore, knowledge of processes affecting dispersal of marine organisms is crucial to understand their genetic distribution patterns and for the effective management of their populations. In this study, we use genetic analyses of eleven microsatellites in combination with oceanographic satellite and dispersal simulation data to determine distribution patterns for *Serranus cabrilla*, a ubiquitous demersal broadcast spawner, in the Mediterranean Sea. Pairwise population F_{ST} values ranged between −0.003 and 0.135. Two genetically distinct clusters were identified, with a clear division located between the oceanographic discontinuities at the Ibiza Channel (IC) and the Almeria-Oran Front (AOF), revealing an admixed population in between. The Balearic Front (BF) also appeared to dictate population structure. Directional gene flow on the Spanish coast was observed as *S. cabrilla* dispersed from west to east over the AOF, from north to south on the IC and from south of the IC towards the Balearic Islands. Correlations between genetic and oceanographic data were highly significant. Seasonal changes in current patterns and the relationship between ocean circulation patterns and spawning season may also play an important role in population structure around oceanographic fronts.

Keywords: connectivity, microsatellites, population structure, seascape genetics, *Serranus*, surface currents

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Introduction

Population connectivity is driven by the dispersal of individuals (Palumbi 2003), and it is generally assumed that for marine organisms pelagic stages represent the main vector of genetic exchange because of their high potential for dispersal (Galarza *et al.* 2009a). Seventy per cent of all marine life has a pelagic larval phase in their life cycle (Pinet 2009), but difficulties in studying early life stages still limit the understanding of how connectivity works within the marine environment. The high effort of measuring larval movement in the field, owing to the r-selection strategy of most marine organisms by producing hundreds and thousands of offspring in one brood or spawning event, is one of the first hurdles when analysing larval dispersal. Furthermore, larvae are extremely small in size (200 μm–20 mm, Levin 2006), which makes tagging studies problematic or highly elaborate (Thorrold *et al.* 2006). Moreover, especially for the pelagic larval phase biological features are closely connected with physical processes (Levinton 2001) such as winds and currents, which affect the passive transport of early-life stages (Leis 2007).

In marine waters, there is an apparent continuity potentially allowing larvae of marine organisms to be

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dispersed over large spatial scales. In such cases, we would expect genetic isolation only by distance (Kimura 1953; Wright 1969), meaning that genetic differentiation increases because of increasing geographic distance. However, numerous marine species do not follow this stepping stone model of isolation by distance (Bradbury & Bentzen 2007), and recent genetic studies suggest that the extent of dispersal and gene flow between populations is more complex than previously assumed (Calderon *et al.* 2007; Carreras-Carbonell *et al.* 2007; Uriz *et al.* 2008; Planes *et al.* 2009). Moreover, small-scale genetic structuring is a common phenomenon even for highly mobile species with long larval phases such as cod (*Gadus morhua*) and spiny lobster (*Palinurus elephas*) (Knutsen *et al.* 2003; Palero *et al.* 2008).

Recent studies indicate that oceanographic processes and barriers to dispersal may greatly influence or even determine the connectivity of marine populations (Johansson *et al.* 2008; Galarza *et al.* 2009a; Selkoe *et al.* 2010; White *et al.* 2010). Phylogeographic or speciation events can be measured by studying ancient breaks or past climate patterns (Palero *et al.* 2008); however, the study of current genetic exchange patterns is essential especially in the presence of ever-increasing anthropogenic pressure (Saenz-Agudelo *et al.* 2009) and requires the analysis of present-day oceanographic processes (Gilg & Hilbish 2003). Fortunately, studies coupling oceanographic and genetic data are becoming increasingly popular (Galindo *et al.* 2006; Selkoe *et al.* 2006; Banks *et al.* 2007; Galarza *et al.* 2009a). Coastal and ocean circulations, as well as eddies and current discontinuities, have been demonstrated to strongly affect population connectivity (White *et al.* 2010; Schunter *et al.* 2011). Nevertheless, there is still limited knowledge on how oceanographic conditions may pose barriers for the population structure of a species and especially why different species with similar life-history traits show distinct responses to oceanographic discontinuities (Bargelloni *et al.* 2008; Galarza *et al.* 2009a).

Plasticity of oceanographic processes presents a key challenge in understanding and predicting larval distribution patterns. Numerous fronts can be seasonal in intensity and even change direction in which the driving current flows (Astraldi *et al.* 1995). Neumann (1968) described how ocean currents may vary in terms of speed and direction, which highlights the difficulty in determining a general gene flow direction. Such current fluctuations can greatly influence recruitment and the genetic exchange between populations (Shanks & Eckert 2005; Stenseth *et al.* 2006). In particular, seasonal shifts in current direction can result in seasonal variation in larval transport. Thus, two groups of offspring from one same location may follow highly varied distribution patterns depending on the time of spawning. Hence, combining information of temporal and directional variation at different spatial scales in oceanographic patterns is of great interest and importance in the investigation of connectivity patterns.

The Mediterranean Sea is an ideal study area for a survey incorporating oceanographic features and gene flow. The circulation patterns within the Mediterranean Sea have been subject to studies for many decades and are well described (Fig. 1, e.g. Millot 1999; Fernandez *et al.* 2005; Rio *et al.* 2007). Moreover, several oceanographic discontinuities, mostly on the Spanish coast, originated by the entry of less saline Atlantic waters in the Mediterranean Sea, have been studied at the population genetic level in different marine organisms. The oceanographic processes occurring off the Gibraltar Strait has been demonstrated to act as barriers to gene flow for two fish species (e.g. Galarza *et al.* 2009b; Sala-Bozano *et al.* 2009). The most well-studied and quasi-permanent Almeria-Oran Front (AOF) has been proposed as the point of genetic break between the Atlantic

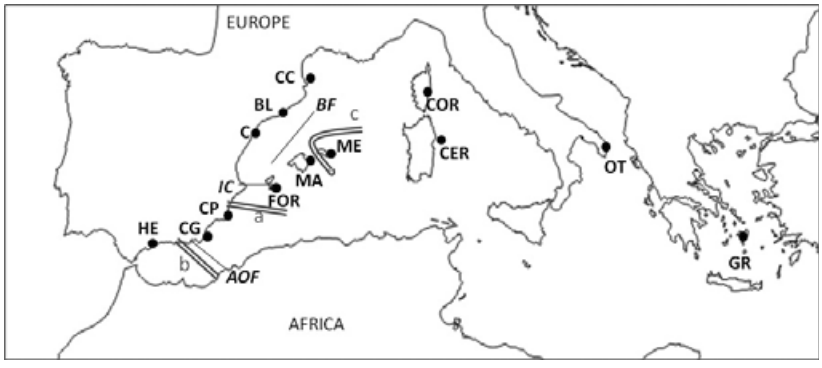

Fig. 1 Map of sample locations across the Mediterranean Sea showing putative barriers to gene flow (grey lines): Almeria-Oran Front (AOF), Balearic Front (BF), Ibiza Channel (IC). Inferred genetic barriers identified in the BARRIER analysis are plotted with a double line and are labelled a, b, c with a being the strongest. CC, Cap de Creus; BL, Blanes; C, Columbretes; ME, Menorca; MA, Mallorca; FOR, Formentera; CP, Cabo de Palos; CG, Cabo de Gata; CER, Sardinia; COR, Corsica; OT, Otranto; GR, Greece.

Ocean and the Mediterranean Sea in many species of different taxa including seaweeds, sponges, molluscs, crustaceans, fish and mammals (see the review in Patarnello *et al.* 2007). However, only few genetic studies sampled populations at either side of the front (Galarza *et al.* 2009a), and thus, the genetic break inferred can include several oceanographic discontinuities as well as genetic differentiation because of isolation by distance. The Balearic Front (BF), a temporal front, has only recently been studied, but has shown to be a strong barrier for genetic exchange in littoral fish species (Galarza *et al.* 2009a). Besides, the Ibiza Channel (IC) has rarely been considered in genetic population studies of marine species, but see the genetic study in red gorgonian by Mokhtar-Jamaï *et al.* (2011), although the oceanographic patterns and temporal processes are well known (Fernandez *et al.* 2005; Monserrat *et al.* 2008). Other oceanographic processes occurring within the Mediterranean Sea, for example along the Sicily Channel and the Sardinian Channel, can also act as barriers to gene flow such as unveiled in prawns and seaweeds (Zitari-Chatti *et al.* 2009; Serra *et al.* 2010).

Our study species *Serranus cabrilla,* also called the comber, is a common demersal fish inhabiting the Eastern Atlantic Ocean, and the Mediterranean and Black Seas. It inhabits sea-grass beds, and is found on rocky, sandy and muddy bottoms between 5 and 100 m deep. The comber is considered one of the most important predators of early fish stages and vagile invertebrates (Guidetti & Cattaneo-Vietti 2002). Combers are economically relevant and included in the Food and Agricultural Organization of the United Nations (FAO) catalogues as species of interest to fisheries in the Eastern Atlantic, the Mediterranean and the Black Sea. Much is known about its ecology and biology (Torcu-Koc *et al.* 2004); however, there is no information about its population structure and degree of connectivity between populations. Larvae of comber remain in the plankton stage for 21–28 days (Raventós & Macpherson 2001; Macpherson & Raventós 2006) and have been collected inshore and over the continental shelf at a considerable distance (50 km offshore) from the habitats of the adults (Sabatés 1990). This implies that comber larva may have a wide dispersal potential to maintain high connectivity between populations (Planes 2002).

The main aim of this study is to analyse the effects of oceanographic processes on the population structure of fish species. More specifically, we evaluate the connectivity pattern of the comber *S. cabrilla* with genetic markers and identify different genetic units as well as direction of gene flow. With the help of oceanographic satellite and dispersal simulation data, we analyse the flow of particles to assess the influence of predominant current patterns on the genetic structure of *S. cabrilla*. Finally, we discuss the seasonal and directional effects of oceanographic processes on the genetic connectivity of the species.

Materials and Methods

Sampling and DNA extraction

Thirteen locations were sampled to investigate the genetic structure of *S. cabrilla* within the Mediterranean Sea. Two locations from the East-Mediterranean basin [Greece (GR), n = 22 and Otranto (OT), n = 30] and two island localities from the central part of the Mediterranean basin [Sardinia (CER), n = 30 and Corsica (COR), n = 30] were chosen. More intensive sampling was carried out along the Spanish coast, because there is more information on oceanographic processes in this area. To examine the effects of oceanographic discontinuities on the gene-flow patterns, nine coastal and island locations were selected along the Spanish coast: Cap de Creus (CC) (n = 30), Blanes (BL) (30), Columbretes (C) (25), Mallorca (MA) (30), Menorca (ME) (30), Formentera (FOR) (30), Cabo de Palos (CP) (29), north of Cabo de Gata (CG) (31) and Herradura (HE) (35) (Fig. 1, Table 1). A total of 382 specimens were collected in the field by hook and line or spear gun, and CG samples were bought from local fishermen at the sampling location. All specimens were collected in 2003 with the

Table 1 Summary statistics of all localities

Population	N	Allelic richness	H_e	H_o	F_{IS}	Cluster 1	Cluster 2
GR	22	8.10	0.65	0.64	**0.015**	21	1
OT	30	8.43	0.69	0.62	**0.104**	30	0
CER	30	8.35	0.69	0.65	**0.047**	28	2
COR	30	8.27	0.69	0.66	0.041	30	0
CC	30	8.10	0.70	0.67	**0.045**	30	0
BL	30	7.49	0.67	0.60	**0.112**	28	2
C	25	8.03	0.69	0.64	**0.075**	24	1
ME	30	8.37	0.73	0.73	**−0.002**	27	3
MA	30	8.79	0.73	0.72	**0.016**	25	5
FOR	30	8.75	0.72	0.71	0.015	27	3
CP	29	9.03	0.77	0.74	0.039	8	21
CG	31	8.62	0.79	0.70	**0.110**	5	26
HE	35	8.04	0.76	0.73	**0.048**	0	35

CC, Cap de Creus; BL, Blanes; C, Columbretes; ME, Menorca; MA, Mallorca; FOR, Formentera; CP, Cabo de Palos; CG, Cabo de Gata; CER, Sardinia; COR, Corsica; OT, Otranto; GR, Greece, HE, Herradura; N, Number of individuals; H_o, Observed Heterozygozity; H_e, Expected Heterozygozity; F_{IS}, Fixation Index (significant values in bold after FDR ($P < 0.0157$)); Cluster 1 and Cluster 2, number of individuals assigned by STRUCTURE to each cluster with over 75%. Population abbreviations are as in Fig. 1.

exception of HE collected in 2005. The sampled fish were young adults (total length 15–20 cm), sexually active and therefore represent the young spawning population. Pectoral fin clips were removed and preserved in 100% ethanol at room temperature. Total genomic DNA was extracted from fin tissue using the Chelex 10% protocol (Estoup *et al.* 1996).

PCR amplification and screening

Eleven microsatellite loci previously isolated (Carreras-Carbonell *et al.* 2006a) were used in the analysis of all 13 populations (Supporting Information). Polymerase chain reactions were carried out under conditions described in Carreras-Carbonell *et al.* (2006a). Amplified products were scored using an ABI 3700 automatic sequencer from the Scientific and Technical Services of the University of Barcelona. Alleles were sized by GENEMAPPER™ software, with an internal size marker CST Rox 70-500 (BioVentures Inc.).

Statistical analyses

Allele frequencies, expected (H_E) and observed (H_O) heterozygosity per locus and population, were calculated using GeneAlEx 6.1 (Peakall & Smouse 2006), and standardized allelic richness was determined by FSTAT (Goudet 2002) (see Table S1, Supporting Information). Departures from the Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium were tested for each locus-population combination using GENEPOP version 4 (Rousset 2008) which employs a Markov chain method, with 5000 iterations, following the algorithm of Guo & Thompson (1992). The program MICRO-CHECKER (Van Oosterhout *et al.* 2004) was used to infer the most probable cause of HWE departures.

Genetic divergence between populations was estimated by computing the classical F_{ST} approach and the log likelihood ratio (G) tests for population differentiation was assessed by Markov chain algorithms as implemented in GENEPOP (Wright 1969; Weir & Cockerham 1984; Goudet *et al.* 1996). Also, the more recent heterozygosity-independent Jost *D* (Jost 2008), which has been shown to reliably reflect genetic differentiation, was calculated with DEMEtics (Gerlach *et al.* 2010), which uses a bootstrap method (1000 bootstrap repeats) after Manly (1997) to estimate *P*-values. To correct for possible type I errors when performing multiple tests, we employed a false discovery rate (FDR) approach described in Benjamini & Yekutieli (2001). With this approach, the cut-off of the significance value is fixed by the level at which to control FDR. The FDR can be viewed as the fraction of false positives among all tests that are declared significant. This approach has been stated to provide the critical value most relevant for biological questions (Narum 2006).

The correlation between pairwise multilocus distances ($F_{ST}/(1 - F_{ST})$) and geographical distance (Ln distance) of all locations and of only the Spanish localities was assessed using the Mantel permutation test (10 000 permutations; Mantel 1967) implemented in GENEPOP. The geographical distance in kilometres was computed as the coastline distance between continental sample locations and as the straight geographical distance for island populations. Putative barriers to gene flow were detected with BARRIER by using F_{ST} (Manni *et al.* 2004). Three barriers were chosen as there are three well-known oceanographic barriers in the sampling area on the Spanish coast. Separate tests were performed with each loci and all loci together to assure that the patterns were not driven by only one or few loci.

The program STRUCTURE 2.3.3. (Pritchard *et al.* 2000) was used to detect the number of genetically differentiated populations (*K*). The population structure was considered without prior information of the number of locations at which the individuals were sampled and to which location each individual belongs. Following recommendations from Evanno *et al.* (2005), we calculated an *ad hoc* statistic ΔK based on the rate of change in the log probability of data between successive *K* values. Twenty runs and 200 000 Markov Chain Monte Carlo (MCMC) were carried out in order to quantify the standard deviation (SD) of the likelihood of each *K* with a range of K_s between 1 and 14. Furthermore, we plotted the log probability of the data (Ln*P*(*D*)) as a function of *K* across the 20 runs and looked for the value that captured the major structure in the data (Pritchard *et al.* 2000). Structure was run with different data subsets to identify a hierarchical structure: all populations, all populations except HE, only CP, CG and HE and finally all populations except CP, CG and HE. Also, a box plot of *Q* (estimated membership of each individual to a cluster) was produced with STRUCTURE (Pritchard *et al.* 2000) indicating the possible origin for each individual. Single individuals showing a *Q* of more than 0.75 to a different cluster were identified as putative migrants.

Furthermore, a pairwise relatedness coefficient r_{xy} was computed with all the samples from all localities to receive a mean relatedness value per location. This was carried out by using the Queller & Goodnight (1989) approach which is implemented in the GenAlex software (Peakall & Smouse 2006). Population means and 95% Confidence Intervals were determined by 10 000 bootstrap replicates. The 95% CI of the null hypothesis of 'no difference' across populations was established by 9999 permutations in GenAlex. To identify population relationship in a two-dimensional space, Principle Coordinate Analyses (PCoA) of the localities were computed

and graphed with GenAlEx (Peakall & Smouse 2006). PCoA was performed using the F-statistics and the Jost *D* values.

Recent migration patterns along the Spanish coast were established by assignment tests run in GENECLASS 2.0 (Piry *et al.* 2004) and migration rate estimates in BAYESASS (Wilson & Rannala 2003). In GENECLASS 2.0, the probability of an individual belonging to a certain locality is calculated using a Bayesian method (Rannala & Mountain 1997). All assignment probabilities above 0.8 were accepted as correctly assigned and used in the migration analysis. BAYESASS uses a non-equilibrium Bayesian method to estimate recent migration rates. Default settings were used in the program and the average across three runs was used. Migration rate analyses were carried out for all Spanish localities, but joining CC and BL together as the North Spanish Coast (NSC) because locations are very close and particle simulations could only be carried out for the area including both sites. Furthermore, these two locations were not genetically differentiated (see below in Results section) as reported in other fish species (Carreras-Carbonell *et al.* 2006b).

Oceanographic General Circulation Data

Numerical simulations of particle dispersion were carried out using the Mediterranean Forecasting System (MFS) model (Tonani *et al.* 2008), characterized by the highest horizontal and vertical resolution presently available for the Mediterranean Sea: $1/16° \times 1/16°$ in the horizontal (~6.5 km) and 72 vertical levels. The model can be therefore defined as a mesoscale-resolving model for the Mediterranean Sea, as the first internal Rossby radius is ~15 km (Robinson *et al.* 2001). In order to improve the simulations, the MFS system assimilates temperature and salinity vertical profiles from eXpandableBathyThermograph (XBT) and Argo, and Sea Level Anomalies from satellite altimetry (Tonani *et al.* 2009). Daily forecast of a wide range of physical variables such as temperature, salinity, density and currents are provided (http://gnoo.bo.ingv.it/mfs/). In this study, independent satellite altimeter gridded fields (Pascual *et al.* 2007) were also used for the analysis of circulation patterns in the Mediterranean Sea. However, considering that the barriers to gene flow were only detected along the Spanish coast (see below in Results section), the simulations were only carried out along this area. Simulated surface dispersion fields are presented using numerical particles (proxy of larvae) released at specific key points at the beginning of the *S. cabrilla* spawning season (April) until the end of the larval period (3 months later) at eight different sites on the Spanish coast. However, because particles arriving at the land boundary of the model are eliminated, the release area was not placed too close to the coast. This can result in some near-shore currents not being represented in the dispersion model.

The simulations were performed for April, May and June 2001 and 2004, as most of the collected comber samples would have been larvae in those years. The particles were released in 50 km × 50 km squares at 5 km separation on the 1st day of each month and allowed to drift during 30 days with the daily currents provided by the simulation. Square matrices for each month were computed quantifying the number of particles recorded at any of the eight studied localities during the 30 days drift. Thus, one particle can be recorded at several sites. Finally, one matrix for 2001 and another for 2004 were generated by adding the matrices of April, May and June of each year. We also ran the simulations changing the initial distance separation of particles between 1 km (7803 particles) and 5 km (363 particles). Matrices obtained were then standardized by particle number and could thus be compared. The standard deviation of the different simulations computed over all the elements of the matrices were lower than 1% both for 2001 (0.22%) and 2004 (0.88%). This shows low sensitivity of the model to small changes in parameters, which in turn demonstrates that the method is robust and nondependant of the number of particles. Here, we present the data obtained from the simulation with 5 km distance, as the graphs can be more clearly interpreted and the parameter of 5 km distance adjusts better to the original resolution of the model used.

These data were then correlated with the genetic migration data calculated with GENECLASS (Piry *et al.* 2004) and BAYESASS (Wilson & Rannala 2003) with a nonparametric Spearman's rho test in SPSS. To assure that our results were not only encountered due to chance, the correlation was repeated after the randomization of the data.

Results

Genetic variability

High genetic variability was found in *S. cabrilla* in terms of extensive polymorphism per population and locus (mean allelic richness 8.3 ± 0.4), as well as high expected (0.712 ± 0.04) and observed (0.676 ± 0.04) heterozygosities (Table 1, Table S1, Supporting Information). No linkage disequilibrium between loci was observed in any of the populations; thus, the eleven loci were considered statistically independent. Private alleles were present in all populations, with a mean percentage per population of 2.88 ± 0.58%.

Significant departures from HWE were observed in most localities, with the exception of COR, C, FOR and CP (Table 1), which could be a result of selection, Wahlund effects or null alleles. When all loci where analysed separately, departures from HWE was caused mainly by locus Sc05. For this locus, null alleles were detected with MICRO-CHECKER, as well as sporadically for some loci in different localities (Table S1, Supporting Information). Null alleles appear when one allele is unamplified because of mutations in the sequence where one of the primers was designed, or when technical problems associated with amplification and scoring arise (Hoarau *et al.* 2002). Technical issues could be ruled out because all failed amplifications for loci Sc05, Sc06, Sc07, Sc08 and Sc14 were reamplified twice, lowering the annealing temperature to 50°C, verifying that the non-amplified individuals were homozygotes for null alleles. All analyses were carried out including the Sc05 locus as well as without the locus and results were almost identical. Therefore, results presented in this study include the Sc05 locus, as assignment tests have a better performance with larger number of loci (Carreras-Carbonell *et al.* 2006b).

Population differentiation

The global multilocus F_{ST} revealed significant genetic differentiation ($F_{ST\ global} = 0.032$, $P < 0.001$). Pairwise F_{ST} values ranged between −0.004 and 0.135 (Table 2). Different degrees of structuring were found between all 13 populations of *S. cabrilla*. HE was the most genetically differentiated from all other localities. Other two locations with more proximity to Atlantic waters, CP and CG, were also significantly differentiated to all other locations, but revealing a close relationship between them. The rest of the populations with greater influence from the Mediterranean Sea were only weakly genetically differentiated. Some population pairs show relatively high F_{ST} values (for instance GR-MA F_{ST}: 0.013; C-MA F_{ST}: 0.011, see Table 2) that are not significant, which could suggest a limitation of the power because of lower sample sizes in GR and C. The Jost *D* values showed similar results to the F_{ST} values and ranged between −0.018 and 0.291 however seem to be less sensitive to population sizes (Table 2).

No significant association between genetic differentiation (F_{ST} or *D*) and geographic distance was revealed by a Mantel test ($P = 0.116$ and $P = 0.147$ respectively) when all locations were included, however, the Mantel test was significant when only Spanish locations were considered (F_{ST}: $P < 0.01$, *D*: $P < 0.01$) (Fig. S1, Supporting Information).

Putative barriers to gene flow were computed with BARRIER and all loci and are represented in Fig. 1. The strongest barrier (a) with a distance value produced by BARRIER of 0.03 was placed between CP and FOR and C, which is situated at the IC. The second barrier (b) would be situated at the AOF (distance value of 0.013), and the third barrier (c) separates ME from the other localities (distance value of 0.01). All barriers were supported abundantly by the different loci: Barrier a was present in 10 of the 11 loci; Barrier b was represented by eight loci, and Barrier c was shown by seven loci.

Two genetically differentiated clusters were detected when STRUCTURE was computed with all samples, as the peak in ΔK was for $K = 2$. The height of ΔK was used as an indicator of the strength of the signal ($\Delta K = 129.1$

Table 2 Multilocus JOST *D* distances between population pairs below the diagonal and F_{ST} values above the diagonal

	GR	OT	CER	COR	CC	BL	C	ME	MA	FOR	CP	CG	HE
GR	–	0.002	−0.003	0.003	**0.011**	0.006	0.003	**0.018**	0.013	0.005	**0.058**	**0.069**	**0.135**
OT	0.023	–	0.000	0.000	**0.013**	**0.012**	**0.011**	**0.012**	**0.004**	0.000	**0.050**	**0.056**	**0.122**
CER	−0.010	0.008	–	−0.004	**0.005**	0.000	0.001	**0.009**	0.002	−0.003	**0.040**	**0.049**	**0.110**
COR	0.013	0.005	0.001	–	**0.006**	0.002	0.009	0.007	0.001	−0.001	**0.042**	**0.049**	**0.111**
CC	0.024	**0.049**	0.006	0.040	–	−0.002	**0.003**	**0.006**	**0.009**	**0.008**	**0.042**	**0.055**	**0.100**
BL	0.023	**0.048**	0.003	0.016	−0.013	–	**0.003**	**0.009**	0.008	**0.007**	**0.040**	**0.053**	**0.105**
C	0.011	**0.051**	0.008	0.038	0.020	0.015	–	**0.015**	0.011	**0.009**	**0.044**	**0.053**	**0.108**
ME	0.040	0.030	0.017	0.029	0.011	0.037	**0.057**	–	0.008	0.008	**0.027**	**0.038**	**0.085**
MA	**0.049**	0.030	0.010	0.021	0.031	**0.042**	**0.044**	0.027	–	−0.004	**0.031**	**0.032**	**0.088**
FOR	0.005	−0.005	−0.018	0.004	0.019	0.025	0.027	0.029	−0.012	–	**0.028**	**0.031**	**0.088**
CP	**0.109**	**0.122**	**0.083**	**0.097**	**0.105**	**0.095**	**0.098**	**0.078**	**0.084**	**0.071**	–	−0.001	**0.022**
CG	**0.145**	**0.136**	**0.103**	**0.131**	**0.152**	**0.133**	**0.108**	**0.132**	**0.079**	**0.072**	0.001	–	**0.013**
HE	**0.276**	**0.291**	**0.237**	**0.277**	**0.240**	**0.243**	**0.237**	**0.244**	**0.211**	**0.210**	**0.083**	**0.049**	–

Bold and shaded values are significant after False Discovery Rate application ($P < 0.01012$). Population abbreviations are as in Fig. 1. CC, Cap de Creus; BL, Blanes; C, Columbretes; ME, Menorca; MA, Mallorca; FOR, Formentera; CP, Cabo de Palos; CG, Cabo de Gata; CER, Sardinia; COR, Corsica; OT, Otranto; GR, Greece, HE, Herradura.

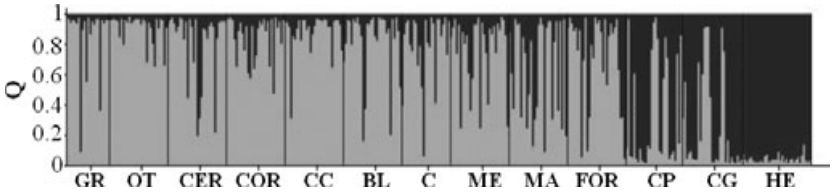

Fig. 2 Bar plot of the estimated membership fraction (*Q*) in each of the two genetically differentiated (*K* = 2) clusters identified by STRUCTURE. Each individual in the sampling locations on the *x*-axis is proportionally assigned to the clusters.

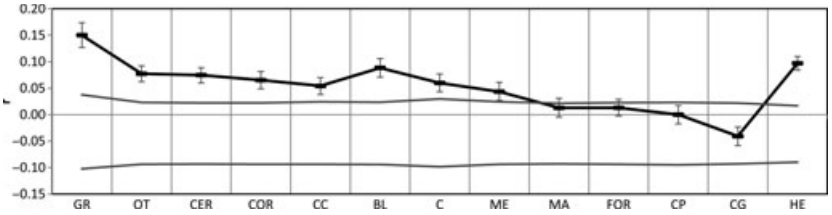

Fig. 3 Pairwise relatedness coefficient (Queller & Goodnight 1989) within each locality. Shaded area represent the 95% confidence interval of the null hypothesis of no differentiation across populations. Error bars are determined by permutations.

for *K* = 2) detected by STRUCTURE (Evanno *et al*. 2005). Furthermore, the highest likelihood was for *K* = 2 (Fig. S2, Supporting Information). The two populations (Fig. 2) could be identified by a Mediterranean unit and a more Atlantic-influenced group. This could be established by a hierarchical analysis with STRUCTURE, as running the program with different data subsets resulted in the separation of Mediterranean localities from CP, CG and HE (Fig. S2, Supporting Information). However, there was a slight substructure detected within the Mediterranean localities because L(*K*) was the same for *K* = 1 and *K* = 2. Furthermore, within each cluster, single individuals were genetically more related to the other cluster and could be considered as migrants. Seventeen individuals from the Mediterranean unit showed a *Q* value of 0.75 or more towards the other cluster with 11 of them found in the Balearic Islands (Table 1). All individuals of HE were assigned to the Atlantic group. The populations of CG and CP with mostly individuals of the Atlantic group had 21.7% of the individuals ascribed to the Mediterranean cluster (Table 1).

The mean pairwise relatedness values within localities revealed the individuals in GR to be most related with each other (*r* = 0.150) followed by BL (*r* = 0.090) and HE (*r* = 0.089) (Fig. 3). Low relatedness values close to zero could be seen for the Balearic Islands ME, MA and FOR. Negative relatedness values were found for the localities of CP and CG with the latter showing the lowest relatedness of all localities (*r* = −0.0275). Such negative values indicate that the relatedness value of the individuals tested was smaller than expected between random individuals (Queller & Goodnight 1989).

Focusing only on the western Mediterranean area to detect putative barriers on a smaller scale, PCoA were computed. The PCoAs with F_{ST} and Jost *D* values revealed similar structuring of localities, but the F_{ST} PCoA showed a higher resolution with 91.55% variation explained by the two coordinates in comparison with 82.6% variation if Jost *D* was applied (data not shown). The first axis of the PCoA clearly separated CG, CP and HE from the rest of the studied locations on the Spanish coast (Fig. 4). However, within those two groups, another division can be observed; a stronger separation of HE from CG and CP and a milder separation of the Balearic Islands (MA, ME, FOR) from the localities on the Spanish continental coast (C, BL, CC). The three separations correspond geographically with the three previously described barriers: AOF, IC and the BF (Figs 1 and 3).

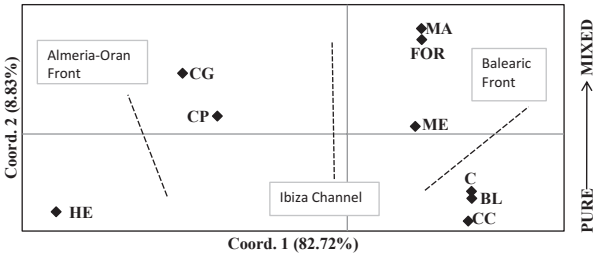

Fig. 4 Principal coordinates analysis calculated by F_{ST} values of nine Spanish locations. The positions of the oceanographic discontinuities in the area are plotted for comparison.

Assignment tests with GENECLASS resulted in 123 individuals being correctly assigned with over 80% of probability. Assignment values ranged from 0 to 0.33 for comparisons between different sampling sites (Table S2, Supporting Information), meaning that 33% of successfully assigned individuals collected in CG are from HE. When looking at self-recruitment, the values ranged between 0.21 and 0.68, with the highest two self-assignment scores found on the NSC and HE. Values from the BAYESASS analysis for pairwise comparisons were much lower between 0 and 0.04 and self-recruitment values larger, ranging between 0.78 and 0.83 (Table S2, Supporting Information). The results obtained with BAYESASS are significantly correlated with those of GENECLASS (Spearman's $\rho = 0.341$, $P < 0.01$).

Oceanography

Suspended particle circulation along the coast in the region between HE, CG and CP was almost exclusively south and eastwards (Fig. 5 and Table S2, Supporting Information), as observed by numerical particles (proxy of larvae) released at HE (green) and CP (blue) monthly from April to June at a depth of 20 m. The particles circulated through the Alboran Sea with the so-called Alboran Gyres (Tintoré *et al.* 1991) towards the coast of Morocco to then spiral back to the Spanish coast around the Almería-Oran Front (Allen *et al.* 2001). The particles released eastward of CG did not flow westward as they were stopped by the Almería-Oran Front. Although presenting monthly variation, some particles from CP crossed the IC. The particles released just south of the IC (red) partially moved slightly southwards; however, the majority flew north and once past the channel encountering the North Current which causes most of the water to be circulated towards the Balearic Current (Bouffard *et al.* 2010). Therefore, water coming up through the IC will rarely reach the east coast of the Spanish mainland. North of the IC particle movement (turquoise and yellow) was predominantly determined by the North Current, as it flows down the Spanish coast and circulates towards the Balearic Islands at the

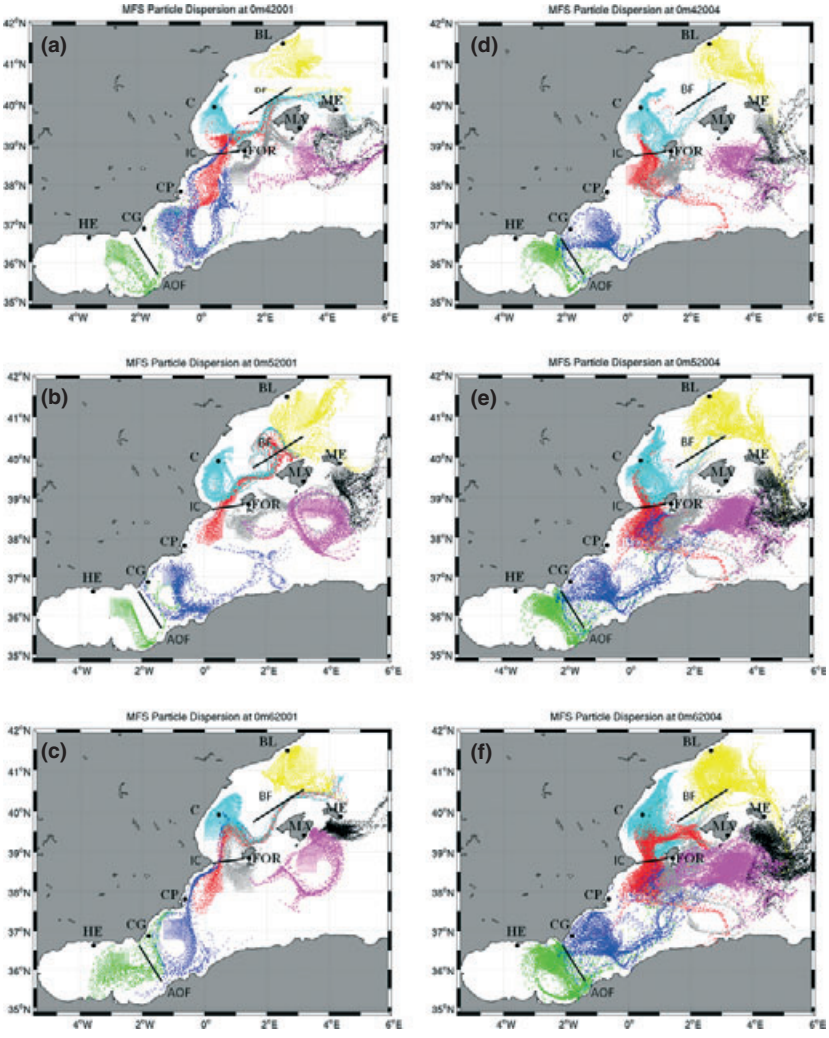

Fig. 5 Numerical particles (proxy of larvae) released at Herradura (green), Cabo de Gata (blue), South of the Ibiza Channel (red), Columbretes area (turquoise) and Cap de Creus (yellow) in (a) April 2001, (b) May 2001 and (c) June 2001 and (d) April 2004, (e) May 2004 and (f) June 2004 and allowed to drift for 30 days using the currents provided by the Mediterranean Forecasting System numerical model.

height of the channel. Simulation results for the months of April, May and June, the reproduction period of *S. cabrilla*, showed the same general pattern of dispersal. This can be also observed for different years, as simulations of 2001 and 2004 showed that these dispersal patterns are similar (Fig. 5).

The area south of Ibiza/FOR is characterized by a well-known spatial and temporal variability of the surface circulation (Pinot *et al.* 2002), a feature confirmed in our simulations that show a north-eastward flow in April 2001 crossing the MA channel, while flowing eastwards in May and northwards in June crossing the IC. In 2004, the situation was also highly variable, although with less well-defined trajectories. South of MA is characterized by weak surface circulation modulated by the presence of large-scale intermittent anticyclonic eddies detached from the Algerian Current (Puillat *et al.* 2002). In April 2001, a large and well-defined anticyclonic eddy was present in the area trapping the particle trajectories around the eddy. In 2004, the circulation was less well defined, particles being dispersed in the area (Fig. 5). In other words, in this area, the absence of a clear dominant current inhibits north/south or east/west exchanges. South of ME is also a highly variable zone as shown in 2001 simulations where an eastward/south-eastward and northeast/southward flow were obtained in April and May, while a very weak flow with low dispersion was obtained in June. The 2004 patterns from April to June were also showing predominant northeast and southward flows as in May 2001.

Correlations between genetic and oceanographic data were highly significant. The correlation between GENECLASS assignment tests and 2001 oceanographic data resulted in a Spearman's ρ of 0.691, and for the 2004 data, it was 0.685 (both $P < 0.001$) (Table S2, Supporting Information). If self-recruitment values were excluded, Spearman's ρ was also significant with both years (2001: $\rho = 0.338$, $P = 0.011$; 2004: $\rho = 0.444$, $P = 0.003$). When the data were randomized, the correlations were not significant (2001: $\rho = 0.171$, $P = 0.271$; 2004: $\rho = 0.126$, $P = 0.403$), showing that the significant results found with the original data are not a result of just chance. Correlation with the data obtained with BAYESASS was also highly significant (2001: $\rho = 0.456$, $P < 0.001$ and 2004: $\rho = 0.520$, $P < 0.001$), but revealing a slightly higher correlation in 2004 than in 2001.

Discussion

Genetic population network

Within the Mediterranean Sea, two genetically distinct clusters were revealed by the data set. Only two genetically differentiated clusters of the comber *S. cabrilla* were distinguished despite Jost *D* and F_{ST} differences between pairs of samples within the clusters. Surprisingly, samples from the eastern part of the Mediterranean, like GR, were grouped together with the central Mediterranean localities, the Balearic Islands and the NSC. The break between the two clusters is positioned geographically on the Spanish coast between the IC and the AOF with the intermediate locations representing an admixed population. The IC has rarely been considered in biological or genetic population analyses, although it has been a subject of oceanographic studies for many years (Pascual *et al.* 2002; Pinot *et al.* 2002; Fernandez *et al.* 2005). Within the western Mediterranean, most research on genetic structure of marine species focused on the effects of the Strait of Gibraltar and the AOF (Naciri *et al.* 1999; Patarnello *et al.* 2007; Bargelloni *et al.* 2008; Galarza *et al.* 2009a), and only few studies have looked at other barriers such as the BF (Galarza *et al.* 2009b; Schunter *et al.* 2011). The dusky grouper (*Epinephelus marginatus)* is the only fish species for which an influence of the IC on the genetic structure was directly tested, but no significant effect was found (Schunter *et al.* 2011). Nonetheless, the species composition of epibenthic crustaceans was found to differ between the south and the north coast, with the dividing location being around the IC (Abelló *et al.* 2002) and for the crab *Liocarcinus depurator* it has a profound effect on population differentiation (García-Mechán VH, personal communication). Furthermore, for the red gorgonian *Paramuricea clavata,* the IC and BF combined are revealed as the most relevant barrier (Mokhtar-Jamaï *et al.* 2011). Besides, it has been stated that the oceanographic processes around the IC are of special interest, as it is the location where the Mediterranean surface and intermediate waters meet with the less saline Atlantic waters (Fernandez *et al.* 2005). For several species, it has been observed that genetic structuring is defined by the influence of Atlantic waters vs. more Mediterranean water conditions of higher salinity and water temperature (Patarnello *et al.* 2007; Palero *et al.* 2008). Seemingly, the separation of the populations of *S. cabrilla* is influenced by the two different water masses.

Fine-scale subdivision of clusters could not be detected with programs like STRUCTURE, which often fail to identify differences when genetic differentiation is low (Latch *et al.* 2006; Hedgecock *et al.* 2007). However, pairwise F_{ST} and Jost *D* values in combination with measures such as PCoA can provide a more detailed insight into the structure within clusters. For the nine Spanish coastal and Island localities, *S. cabrilla* was genetically divided into four units: the Spanish north coast, the Balearic Islands, the Spanish south coast and HE. Apart from the clear discontinuities caused by the

IC and the AOF, the comber apparently is also affected by the BF. The Balearic Islands appear to be a genetically admixed group with influence from the Spanish continental coast reaching the islands because of the deviation of the North Current at the IC and individuals arriving from the South coast over the IC. However, as can be seen in the particle simulations as well as the hierarchical and putative barrier genetic analysis, ME appears to be more isolated than the other Balearic Islands.

The correlation between population structure and oceanographic processes suggests that genetic exchange is not driven by adult migration but rather by larval dispersal. For several other closely related species, behavioural site fidelity of adults has been identified (*Epinephelus coioides*, Antoro *et al.* 2006; *E. marginatus*, Hereu *et al.* 2006). Based on a study with acoustic telemetry, which demonstrated that the comber spends 95% of its time in an area of 0.77 km^2 (Alos *et al.* 2011), and by the present results, we can conclude that *S. cabrilla* is a territorial species with elevated adult site fidelity. Owing to the clear effect of oceanographic discontinuities on the genetic distribution of the species, larvae of the comber most likely are transported passively by the predominant currents.

Unidirectional and seasonal barriers

Despite the clear genetic structuring and effects of oceanographic discontinuities, values of pairwise Jost *D* and F_{ST} within the Mediterranean cluster were low, suggesting elevated levels of genetic exchange between the different units. Now, the challenge is to determine the origin and destination of larval migrants for a full understanding of the dispersal patterns. Possible migrants were revealed by assignment tests and relatedness values from which several trends in directional movement can be inferred. Individuals collected at the southern localities of CP and CG belonged to an admixed population with 21.6% having mixed genotypes. This population was comprised of individuals that were genetically related to and influenced by the Mediterranean cluster, while the majority were from the Atlantic-influenced cluster and mainly arrived over the AOF from HE. This suggests that the AOF, which has been described as a relatively permanent and strong barrier to gene flow (Patarnello *et al.* 2007), allows for genetic exchange from the west side of the barrier to the east side. However, counter directional movement could not be detected, because no individuals of Mediterranean influence were detected in HE and relatedness of individuals within this locality was high showing a comparatively high self-assignment value. There can be other possible explanations for isolation between two localities besides geographic isolation because of oceanographic discontinuities. Local adaptation to certain factors, such as temperature (e.g. for Atlantic salmon, *Salmo salar*, Dionne *et al.* 2007), environmental conditions (for European flounder, *Platichthys flesus,* Hemmer-Hansen *et al.* 2007) or possibly even anthropogenic impacts as an effect of differences in fishing pressure could decrease the dispersal success from other locations to HE. Furthermore, historical colonization is reflected on present-day genetic structuring; however, little is known about *S. cabrilla* in this regard. Nonetheless, the findings of gene-flow patterns were supported by oceanographic simulations of particle suspension, suggesting that the oceanographic front plays an important role in the determination of the observed genetic flow. There was retention of particles on the west side of the AOF and a small fraction moved eastwards crossing the AOF, while the westward movement of particles across the AOF was not observed. This predominant circulation pattern would encourage *S. cabrilla* larvae from HE to flow eastwards, but *S. cabrilla* larvae found east of CG would encounter a strong counter current when moving westwards. The majority of analyses validating the AOF as the cause of genetic breaks for species were based solely on significant genetic differentiation between different locations on either side of the AOF. Hereby, numerous species were divided into genetically distinct populations by the front, whereas other species with similar life-history traits but possibly different reproduction seasons were not affected (Patarnello *et al.* 2007; Bargelloni *et al.* 2008; Galarza *et al.* 2009a). However, no direct measures of gene flow across the barrier were taken nor was the direction of (the sometimes limited) gene flow considered. In this study, F_{ST} and Jost *D* between the two locations on either side of the AOF were high and an effect of the barrier was established; moreover, we detected a unidirectional genetic movement across the AOF from the west to the east along the coast. Even though oceanographic simulations reveal consistency in the flow pattern around the AOF over the years, further analysis in multiple years between genetic flow and current patterns should be undertaken to validate the long-term relationship.

The IC, as previously mentioned, is one of the main dividing barriers for *S. cabrilla*; however, a directional movement of individuals across this oceanographic discontinuity was also discovered. Several individuals collected at the admixed South Spanish coast (CP and CG) were genetically related to the Mediterranean cluster, illustrating the southwards movement of migration. Meanwhile, few individual collected on the NSC (BL, CC and C) originated from the Atlantic-influenced cluster and more individuals from the Balearic Islands

were assigned to the Atlantic cluster (Table 1). Hence, the main south–north genetic flow detected was from the southern locations towards the Balearic Islands (e.g. FOR) following the current deflection towards the Balearic Islands as simulated in the particle suspension data (see also Fernandez *et al.* 2005). It is expected that these conditions would facilitate the movement of *S. cabrilla* larvae from the southern localities across the IC towards the Balearic Islands, but rarely towards the Spanish continental coast. The encountered gene flow from the NSC to the South Spanish coast across the IC could be a result of the seasonality of this oceanographic discontinuity. Monserrat *et al.* (2008) demonstrate that the deviation of North Current towards the Balearic Islands occurs in spring and early summer and is caused by the formation of an anticyclonic gyre at the IC which hinders the southward flow. This gyre, however, is not formed after relatively mild winters with temperatures above 13 degrees (Lopez-Jurado *et al.* 2008), in the absence of which the North current can flow southward through the channel. Hence, the southward movement detected by the genetic analyses in this study could be caused by periodic mild winters. Nevertheless, it is possible that there is southward larval transport just along the coastline, and more refined modelling allowing the inclusion of more coastal regions could resolve this issue.

Epinephelus marginatus, the dusky grouper, has a similar average pelagic larval duration (24. 6 days) as *S. cabrilla* (24. 3 days; Macpherson & Raventós 2006), and it would be expected for both species to have a similar dispersal potential, especially because both show high adult site fidelity. Regardless, *E. marginatus* did not reveal any effect of the oceanographic processes around the IC on its genetic structure (Schunter *et al.* 2011). It has long been discussed why similar species show different genetic patterns, and there are many plausible reasons, one of them being the evolutionary history of each species. In this case, another explanation could be the differences in reproductive periods. The dusky grouper has been shown to spawn in August on the Spanish coast (Zabala *et al.* 1997) with the larvae dispersing in the plankton from late summer to fall. On the contrary, *S. cabrilla* reproduces in spring and the larvae are suspended in the water column from April to June (Sabatés 1990) and therefore susceptible to the discontinuity occurring at the IC in spring. These results could indicate that gene exchange through the IC is possible for species with reproduction periods in late summer or fall, whereas species that reproduce in winter (as *L. depurator*, García-Merchán, personal communication) or spring (as in the present work) are affected by the IC and genetic exchange might only occur in selected years after warm winters or in waters entrained along the coast.

The influence of oceanography on the genetic structure of marine species is now widely recognized (Galindo *et al.* 2003; Selkoe *et al.* 2006; Banks *et al.* 2007; Galarza *et al.* 2009a; Serra *et al.* 2010; White *et al.* 2010), whereas seasonal changes in current flow or temporal variability in oceanographic processes are rarely considered (Astraldi *et al.* 1995; Stenseth *et al.* 2006). On the west coast of the United States, the California and Alaska Current are influenced by a warm and a cold phase, and these alterations in current direction appear to lead to alterations in the biology of a wide range of fish and crustacean species in the Eastern North Pacific (Brodeur *et al.* 1996; Shanks & Eckert 2005). Carson *et al.* (2010) indicate that predominant gene-flow patterns for two congeneric mussel species (*Mytilus californianus* and *M. galloprovincianus*) are nearly opposite owing to the changing oceanographic patterns in different reproductive seasons. Shanks & Eckert (2005) even suggest that different life-history traits of a variety of species might have evolved in response to the seasonal changes in the California Current. On the Spanish coast, such an adaptation to oceanographic events is unlikely because *E. marginatus* and *S. cabrilla* have very similar life-history traits but reveal shifted reproduction times which could be one reason for the different genetic population structure of the two species. This emphasizes the importance of seasonality of oceanographic processes in combination with the species' reproductive period on its genetic structure and dispersal patterns. Furthermore, temporal sampling would be of interest as inter-annual variation in marine current patterns could introduce temporal genetic variation which can also be traced with more direct methods, such as mark-recapture or parentage analyses (Lowe & Allendorf 2010, Saenz-Agudelo *et al.* 2011).

Conclusions

The population structure and gene-flow pattern of a species can be influenced by predominant current patterns and oceanographic processes. Four units were identified, separated by three oceanographic barriers to gene flow: the IC, the AOF and the BF. The direction of the currents also plays a determining role in the population connectivity of *S. cabrilla.* The species genetic structuring is closely related to the present currents regimes across the oceanographic barriers. At the AOF, the comber larvae only dispersed from west to east of the barrier, indicating unidirectional movement similar to simulations of passive particles. Around the IC, connectivity was found between southern locations and the Balearic Islands as well as northern coastal localities

with the southern population. The latter can only be accomplished owing to a weakening of the gyre formed at the IC after mild winters or near coastal southward flow, allowing the current to flow south through the Channel. This phenomenon is a seasonal effect and coincides with the reproduction period of *S. cabrilla*, which might explain why other species with different reproduction times are not genetically divided by the IC. It is essential to include oceanographic data into population genetic studies, to not only understand the division of the connectivity but also to study directional dispersal patterns.

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Data accessibility

Microsatellite data: DRYAD entry (Data identifier: doi:10.5061/dryad.2rr59).

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Summary statistics for the 11 microsatellites (Sc03–Sc15) and 13 populations of *Serranus cabrilla*.

Table S2 Migration rates and particle dispersal from source (rows) to sink (columns).

Fig. S1 Dispersion plot of the relationship of distance (ln Km) and $F_{ST}/(1 - F_{ST})$. Comparisons including only Spanish locations are represented in black while comparisons including other Mediterranean locations (GRE, LE, CER, COR) are represented in grey. Mantel test is non significant when comparing all populations ($R^2 = 0.1873$, $P = 0.1148$) and significant when using Spanish localities only ($R^2 = 0.4664$, $P < 0.001$).

Fig. S2 Graph representing the Structure output of the most likely number of populations (K) including the standard deviation between runs for three different data sets: All populations, all populations except HE, all populations expect CP, CG and HE and only CG, CP and HE.

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