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ORIGINAL ARTICLE

Environmental variables, habitat discontinuity and life history shaping the genetic structure of *Pomatoschistus marmoratus*

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Abstract Coastal lagoons are semi-isolated ecosystems exposed to wide fluctuations of environmental conditions and showing habitat fragmentation. These features may play an important role in separating species into different populations, even at small spatial scales. In this study, we evaluate the concordance between mitochondrial (previous published data) and nuclear data analyzing the genetic variability of *Pomatoschistus marmoratus* in five localities, inside and outside the Mar Menor coastal lagoon (SE Spain) using eight microsatellites. High genetic diversity and similar levels of allele richness were observed across all loci and localities, although significant genic and genotypic differentiation was found between populations inside and outside the lagoon. In contrast to the F_{ST} values obtained from previous mitochondrial DNA analyses (control region), the microsatellite data exhibited significant differentiation among samples inside the Mar Menor and between lagoonal and marine samples. This pattern was corroborated using Cavalli-Sforza genetic distances. The habitat fragmentation inside the coastal lagoon and among lagoon and marine localities could be acting as a barrier to gene flow and contributing to the observed genetic structure. Our results from generalized additive models point a significant link between extreme lagoonal environmental conditions (mainly maximum salinity) and *P. marmoratus* genetic composition. Thereby, these environmental features could be also acting on genetic structure of coastal lagoon populations of *P. marmoratus* favoring their genetic divergence. The mating strategy of *P. marmoratus* could be also influencing our results obtained from mitochondrial and nuclear DNA. Therefore, a special consideration must be done in the selection of the DNA markers depending on the reproductive strategy of the species.

Keywords Connectivity · Ecological gradient · Generalized additive model (GAM) · Transitional waters · Microsatellites

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Introduction

Studies of coastal lagoon fauna are extremely important from ecological and evolutionary perspectives. Coastal lagoons are semi-isolated ecosystems separated from the sea by both physical barriers and ecophysiological boundaries. These ecosystems suffer frequent environmental disturbances, being exposed to wide fluctuations on their physical–chemical parameters (Pérez-Ruzafa et al. 2005, 2007). Such environmental variation may subject the local fauna to severe adaptive challenges that could have a direct effect on the genetic composition of some species such as *Aphanius fasciatus*, *Diplodus sargus, Elysia timida, Cerastoderma glaucum, Ostrea edulis, Ostreola stentina*

and *Bursatella leachii* (Cognetti and Maltagliati 2000; Maltagliati 2002; González-Wangüemert et al. 2004, 2006, 2009; Giménez-Casalduero et al. 2011; González-Wangüemert and Pérez-Ruzafa 2012) or separate species into different populations (Bilton et al. 2002; Trabelsi et al. 2004; González-Wangüemert et al. 2009; Richards et al. 2010; Fluker et al. 2011; Vergara-Chen et al. 2013; González-Wangüemert et al. 2014). The isolation and habitat fragmentation have been also supposed to contribute to the genetic structure of marine species with populations inhabiting coastal lagoons, enhancing the effects of microevolutionary processes (Watts and Johnson 2004).

Several physical and biological factors can reduce gene flow between populations and hence produce the appearance of spatial genetic structure in marine species. Patterns of gene flow in benthic shore fishes appear to stem in part from aspects of life history, larval dispersal, adult migration, habitat discontinuity and coastal water currents (Marques et al. 2006; Giovannotti et al. 2009; Earl et al. 2010; Riginos et al. 2011; Hirase et al. 2012; Durand et al. 2013). Moreover, differences in reproductive strategy may have an important impact on genetic diversity and population structure (González-Wangüemert and Pérez-Ruzafa 2012; Portnoy et al. 2013). In addition, there is evidence of genetic adaptation as result of natural selection capable of sustaining adaptive divergence on contemporary time scales (Conover et al. 2006; Nielsen et al. 2009; Yoboué et al. 2012; Wang et al. 2013). All these factors could have a profound effect on the arising of population differentiation.

Population differentiation in marine fishes through drift is hoped to be weak because of their populations have relatively shallow histories and are often very large (Hauser and Carvalho 2008). However, as it was indicated above, other factors as a restricted gene flow, selection events or some mating systems such as polygyny could favor the genetic structuring of fishes. In fact, evidence of genetic differentiation across large and small spatial scales has been reported in a great number of marine fishes using microsatellites (Pampoulie et al. 2004; Larsson et al. 2007; Bradbury et al. 2009; Earl et al. 2010; González-Wangüemert et al. 2010, 2012; Larmuseau et al. 2010a; Horne et al. 2011; González-Wangüemert and Pérez-Ruzafa 2012). These nuclear markers are especially useful to study the fine geographical variation of marine populations because they tend to be highly variable and can discern even subtle genetic differences (Waples 1998; Hedgecock et al. 2007), resolving population structures that are not detected by mitochondrial DNA and allozyme markers (De Innocentiis et al. 2001; Pampoulie et al. 2004; Bisol et al. 2007; Canino et al. 2010). In this sense, several studies based on microsatellite markers have suggested that estuaries and coastal lagoons may offer special opportunities (because of their environmental features) to find local genetic structuring of fish populations (Beheregaray and Sunnucks, 2001; Bisol et al. 2007; Roberts et al. 2010; McCraney et al. 2010; González-Wangüemert and Pérez-Ruzafa 2012).

The marbled goby *Pomatoschistus marmoratus* (Risso 1810; Teleostei: Gobiidae) is a small benthic fish, with lagoonal, estuarine and marine populations, inhabiting sandy, inshore, shallow waters from the eastern Atlantic, Mediterranean, Black and Azov seas and Suez Canal (Miller 1986) usually in high densities (Verdiell Cubedo et al. 2008). During the reproductive season, marbled goby males build a nest by cleaning the inside of empty bivalve shells and covering the outside with sand (Mazzoldi and Rasotto 2001). They defend the nest and take care of the eggs deposited on the upper valve by one or more females, cleaning and fanning the eggs until larvae hatch (Mazzoldi and Rasotto 2001). There is no information about the pelagic larval duration (PLD) of *P. marmoratus,* although congeneric species, such as *Pomatochistus minutus* (Pallas 1770) and *Pomatochistus lozanoi* (de Buen 1923), have a PLD of 30–39 days under laboratory conditions (Fonds 1970). Several species of the genus *Pomatoschistus* show little or no migration behavior (Gysels et al. 2004; Berrebi et al. 2005) and have a limited swimming ability because the pelvic fins have been fused into a suction disk (Miller 1986; Bardin and Pont 2002).

Therefore, considering all these biological and ecological features, *P. marmoratus* could be a good target species to study small-scale genetic structure and adaptation under highly variable environmental conditions in coastal lagoons. However, in the last years, only works considering great spatial scales have been published using different species from *Pomatochistus* genus and no study considering simultaneously mitochondrial and nuclear markers. Mejri et al. (2009) using mtDNA markers proposed that the complex genetic structure of *Pomatochistus tortonesei* was shaped by recurring shifts in sea level and sea surface temperatures of Mediterranean Sea which caused the desiccation of shallower lagoons and therefore colonization and re-colonization events of the brackish populations. Moreover, Berrebi et al. (2009) studied the genetic structure of two sedentary gobiid fishes (*Pomatoschistus microps* and *P. marmoratus*) along several French Mediterranean coastal lagoons using mitochondrial DNA RFLP markers; they found that populations of *P. microps* inhabiting neighboring lagoons showed a high level of isolation, which was not shown by *P. marmoratus*. In general, previous data published using mtDNA markers and medium/great spatial scales pointed that the *Pomatoschistus* populations inhabiting different brackish habitats could be characterized by unique mitochondrial haplotypes which are well defined in relation to the limited gene flow

and restricted dispersal abilities of the different species (Mejri et al. 2011, 2012). Therefore, as concluded by Mejri et al. (2011), the role of the Mediterranean lagoon habitat should be related to how much it amplifies the effects of historical (e.g., past sea level changes) and environmental (e.g., present-day hydrographic regime) processes in regard to the genetic structure of the inhabiting *Pomatochistus* species.

Recently, Vergara-Chen et al. (2010a) carried out a study about the genetic structure of *P. marmoratus* considering small spatial scales (<25 km) into Mar Menor coastal lagoon (SE Spain) and between adjacent localities in Mediterranean Sea (<100 km) using mitochondrial DNA sequences (control region). This study found very low population differentiation considering F_{ST} values and high gene flow rates between lagoon and nearby open sea locations. However, a higher genetic diversity and occurrence of exclusive haplotypes into the different lagoon localities were observed, suggesting a possible adaptive potential in this species.

Considering these interesting data, we analyze the same five localities using eight polymorphic microsatellite loci, which could show us the genetic gradient of *P. marmoratus* at small spatial scale with higher accuracy. We also look for factors explaining the observed genetic patterns, including environmental features and life history. Finally, we assess the concordance of genetic patterns obtained from our previous mitochondrial results (control region; Vergara-Chen et al. 2010a) and current nuclear data (eight polymorphic microsatellite loci).

Materials and methods

Study sites

The Mar Menor is a hypersaline coastal lagoon located in a semiarid region in the southeastern Spain (Fig. 1). It is one of the largest coastal lagoons in the Mediterranean basin, with a surface area of 135 km^2 and an average depth of 3–4 m. It is separated from the Mediterranean Sea by a 22-km-long sand bar called La Manga with three narrow inlets connecting the lagoon to the open sea: Las Encañizadas, El Estacio and Marchamalo. The lagoonal salinity ranges from 38 to 51 psu,

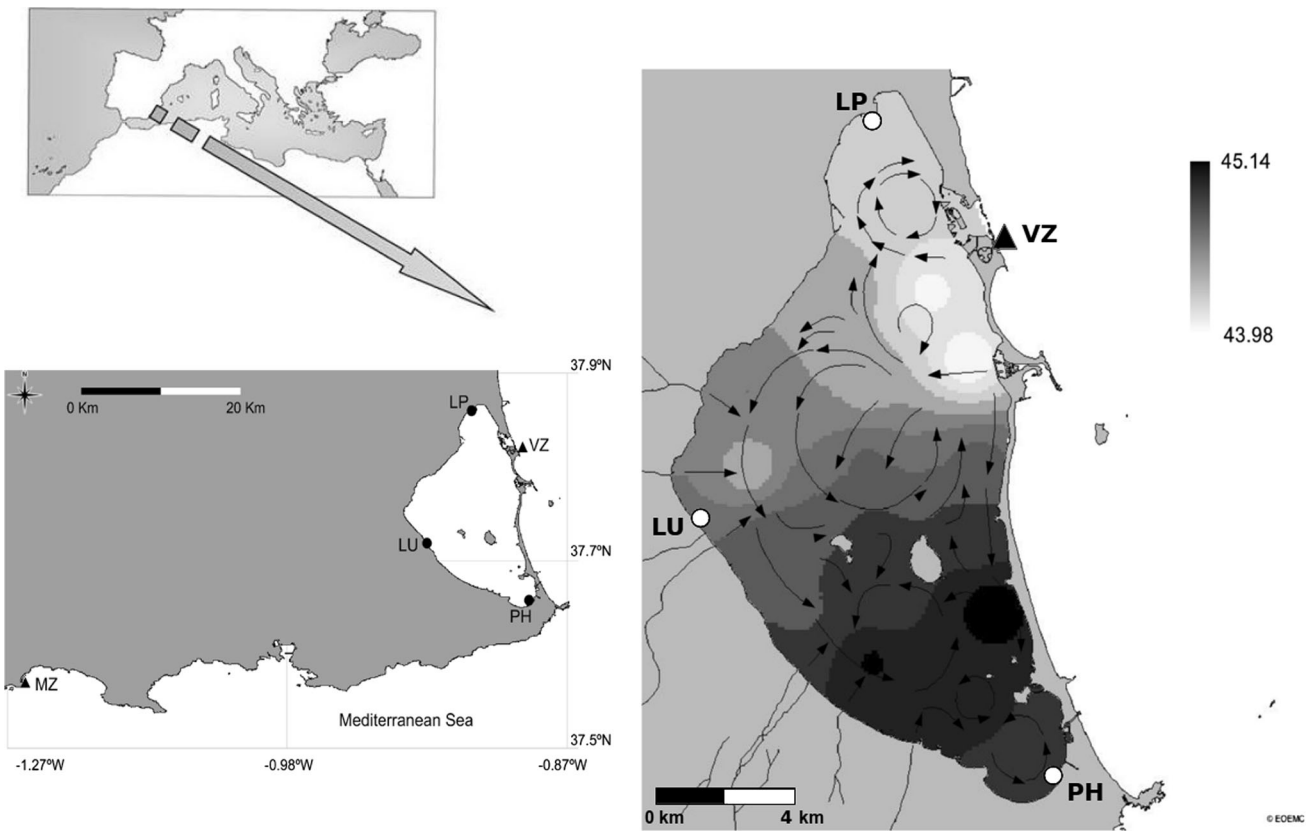

Fig. 1 Study area: the Mar Menor lagoon-SW Mediterranean transition (SE Spain) showing sampling locations for *Pomatoschistus marmoratus*. The Mar Menor map is showing the interpolated surface of mean salinity and the current pattern representing by the *arrows* (González-Wangüemert et al. 2009). *White circles* indicated lagoonal sites: Lo Pagán (LP), Los Urrutias (LU), Playa Honda (PH). *Black triangles* indicated marine locations: Veneziola (VZ), Mazarrón (MZ)

and temperature varies from 10 °C in winter to 32 °C in summer (Pérez-Ruzafa et al. 2005; González-Wangüemert et al. 2009). Its bottom is principally covered by dense meadows of the invasive macroalga *Caulerpa prolifera*, although shallowest areas are covered by scarce patches of the phanerogams *Cymodocea nodosa* and *Ruppia cirrhosa* (García-Sánchez et al. 2012; Martinez-Garrido et al. 2014). The lagoon maintains a diverse fish community due to its environmental heterogeneity: sandy without vegetation and muddy bottoms, rocky bottoms and seagrass beds composed of monospecific and multispecific meadows of *C. nodosa, R. cirrhosa* and *C. prolifera* (Pérez-Ruzafa et al. 2006). The Mar Menor coastal lagoon hydrodynamic is driven by the wind (mainly northeast), generating a circulation pattern comprising three main gyres and dominant currents from north to south along the internal coast of the lagoon (González-Wangüemert et al. 2009; de Pascalis et al. 2012). According to its hydrographical characteristics, three main basins have been identified in the lagoon (Fig. 1) (Pérez-Ruzafa et al. 2007; González-Wangüemert et al. 2009). The northern basin has the greatest influence of Mediterranean waters through the entrance channels at the north of La Manga and El Estacio with the lowest mean salinity values. The southern basin is a more confined area with the most saline water, and the central basin has intermediate salinity values due to the mixing of Mediterranean and lagoonal waters.

Otherwise, the southwestern Mediterranean Sea is characterized by lower extreme values of temperature and salinity than Mar Menor lagoon, oscillating its salinity between 36.84 and 37.41 psu and its temperature between 13.83 and 26.44 °C (available information at: www.puertos.es/oceanografia_y_meteorologia/).

Thus, the environmental heterogeneity observed along this ecological gradient may be considered as an outdoor laboratory to assess the relationships between the environmental conditions and the spatial distribution of the genetic variation in coastal transitional waters. Three sampling sites were selected inside the Mar Menor coastal lagoon: Lo Pagán, located on the northern coast of the Mar Menor; Los Urrutias, located on the west side; and Playa Honda, located on the south. Two sampling locations were selected in the Mediterranean Sea: Veneziola (seaward side of La Manga) and Mazarrón (southern Murcia) (Fig. 1). We highlight the lack of appropriate habitats and absence of *P. marmoratus* populations along the Murcia coast from Mar Menor to Mazarrón.

Field sampling procedures

Fish samples were collected using a beach seine over shallow sandy bottoms. The sample size consisted of 35–40 individuals per collecting site. The marbled gobies were identified on the basis of external morphological characters, killed by freezing and preserved in 100 % ethanol until tissue dissection. Tissue samples of muscle were removed from each specimen and preserved in 100 % ethanol.

DNA extraction and microsatellite genotyping

The tissue was dissolved using lysis buffer solution with proteinase *K*, and total genomic DNA was isolated by protein precipitation and final cleanliness with ethanol (Sambrook and Russell 2001). PCRs were carried out for eight microsatellite loci (Pmar03, Pmar05, Pmar08, Pmin04, Pmin09, Pmin29, Pmin35 and Pmin38) (Berrebi et al. 2006; Larmuseau et al. 2007) (Table 1). The loci Pmin04, Pmin09, Pmin29, Pmin35 and Pmin38 designed on *P. minutus* and tested only by cross-species amplification for *P. microps*, *P. lozanoi* and *P. pictus* (Malm 1865) (Larmuseau et al. 2007) amplified well for *P. marmoratus*. The PCR was a volume of 10 µl containing 300 ng of genomic DNA, 10× buffer, 1.75 mM MgCl2, 0.4 mM of each dNTPs, 3.75 µm of each primer [using forward primers labeled with FAM (SIGMA), HEX (SIGMA) and NED (Applied Biosystems)] and 0.3 U of Taq polymerase (Ecogen). Amplification was performed in a 2,720 thermal cycler (Applied Biosystems) programmed for an initial denaturation at 95 °C for 2 min, followed by 35 cycles composed of denaturation at 95 °C for 1 min, annealing at an appropriate temperature (Table 1) for 1 min, extension at 72 °C for 1 min and a final extension at 72 °C for 3 min. Allele sizes were scored according to the protocols from Molecular Biology Service (Center of Marine Science, Faro, Portugal) using an ABI Prism 3130 automated genetic analyzer (Applied Biosystems).

Microsatellite data analysis

The genotype data were scored using the STRand software (v. 2.4.59 http://www.vgl.ucdavis.edu/STRand). The mean numbers of alleles per locus, allele frequencies, observed (H_O) and unbiased expected heterozygosity (H_E) were calculated in GENETIX version 4.05 (Belkhir et al. 2004). Deviations from Hardy–Weinberg equilibrium (HWE) were characterized by F_{IS} and tested using exact test in the software GENEPOP version 4.0.10. Also, we registered the F_{IS} values per locality and locus, estimating the exact *P* values by the Markov chain method (GENETIX version 4.05). In instances where the observed genotype frequencies deviated significantly from HWE, the program Micro-Checker version 2.2.3 (van Oosterhout et al. 2004) was used to infer one of the most probable causes of such HWE departures. Some authors have described that the presence of null alleles led to the overestimation of both F_{ST} and genetic distance in cases of significant population differentiation (Chapuis and Estoup 2007).

Table 1 Characterization of the eight microsatellite loci used to genotype five populations of *Pomatoschistus marmoratus* from the Mar Menor coastal lagoon and adjacent marine sites (SE Spain)

Locus	Reference	Repeat sequence	Primer sequences (5′–3′) and the fluorescent dye to mark the 5′ end of the forward primer	Annealing temperature (°C)	Alleles	Size range (bp)
Pmar03	Berrebi et al. (2006)	$(AC)_8GC(AC)_{12}$	F: FAM-AGGTTTCCGCTGTTACTGCGAC R: GAGATAACAAGCGCAAAGTCC	57	20	314–350
Pmar05	Berrebi et al. (2006)	$(GT)_6GC(GT)_{16}$	F: FAM-TCTCCGTGGCTCTCCCCAGTGC R: CTGATCAGGGCCACAGCATCT	54	18	218–248
Pmar08	Berrebi et al. (2006)	$(ATCTA)_5$	F: NED-GTCTGGTCAATACTGAACAGCTC R: ACAGTCTCCAACGGCCGTTCAG	61	8	232–252
Pmin04	Larmuseau et al. (2007)	$(GT)_7ACA(TG)_8$	F: FAM-TCTAACAGGCAGCTGAACGA R: GGCTCAAACACACACGAAAA	57	15	209–231
Pmin09	Larmuseau et al. (2007)	$(TG)_2CG(TG)_{20}$	F: HEX-GCCGTGGATGCATTATCAGT R: GGGATGTGTGAGTGTGCAAG	57	16	196–222
Pmin29	Larmuseau et al. (2007)	$(CA)_9$	F: NED-GGGCTCCACTTTGTTAGCAG R: CGTGGGAATTCCTTGATTGT	55	5	206–210
Pmin35	Larmuseau et al. (2007)	$(CA)_3TA(CA)_{20}$	F: HEX-GTGACTGGGAGCGTTTGAGT R: GCCCTATCTGCCTGACAAAG	55	2	162–168
Pmin38	Larmuseau et al. (2007)	$(TG)_4TA(TG)_5$	F: FAM-TGAATCCGAAGCCTGGTAAC R: TCCCTTCTGCTTCCTTTTGA	54	31	194–258

Note: The loci Pmar03, Pmin04 and Pmin09 were amplified by multiplex PCR with an annealing temperature of 57 °C. The loci Pmin29 and Pmin35 were amplified by multiplex PCR with an annealing temperature of 55 °C. The loci Pmar05 and Pmin38, despite amplified using the same annealing temperature (54°), were not used as multiplex PCR

We used the software GENEPOP version 4.0.10 to estimate the genotypic (G-based) and genic differentiation for all pairs of populations (Raymond and Rousset 1995; Rousset 2008). Genetic linkage disequilibrium between locus pairs was estimated according to Weir and Cockerham (1979) and tested on contingency tables under the null hypothesis of independence ($P < 0.05$). To determine whether a population exhibits a significant number of loci with heterozygosity excess, we used the Sign and Wilcoxon tests implemented in the program BOTTLENECK version 1.2.2 (Cornuet and Luikart 1996). Computations were based only on the infinite allele model (IAM) because the two-phase mutation (TPM) model still assumes that loci mutate within repeat number, but some our loci were off repeat.

The Wright's single locus F-statistics (Wright 1969) were calculated from allele frequencies for all loci examined for each population according to Weir and Cockerham (1984) in ARLEQUIN version 3.11 (Excoffier et al. 2005). Significance of F_{ST} for all loci and pairwise population comparisons was assessed by permutation of the values 10,000 times. Standard deviations of single-locus F_{ST} values were obtained by jackknifing over all populations. The Bonferroni correction for multiple comparisons (Rice 1989) was applied to all P values from F_{ST} estimates to compensate for possible type I errors resulting from multiple pairwise comparisons. Genetic distances (D_{CE}) (Cavalli-Sforza and Edwards 1967) were computed between pairwise samples in GENETIX. We used Cavalli-Sforza distance because it is less affected by null alleles (Chapuis and Estoup 2007). Probabilities of random departure from zero for distance values, according to the null hypothesis, were read directly from the distribution of 10,000 randomized matrices computed by permutation. The Bonferroni correction was applied to all P values.

Populations were spatially clustered using correspondence analysis (CA) implemented with BiodiversityR package in *R* software (*R* Development Core Team 2007), which utilizes the genotype frequencies of populations as variables in order to visualize similarities among locations without assuming tree-like relationships. We used as genotype data, the allele frequencies obtained from the microsatellite data and the haplotype frequencies from our previous study with mitochondrial DNA control region sequences (Vergara-Chen et al. 2010a). An analysis of molecular variance (AMOVA) (Excoffier et al. 1992) was carried out in ARLEQUIN to assess the hierarchical partitioning of genetic variability within and among populations and among lagoon and marine groups.

Population structure was inferred using Structure version 2.3 software by the method of Pritchard et al. (2000) from multilocus genotype data. Each K was replicated 20 times for 100,000 iterations after a burn-in period of 5,000, without any prior information on the population of origin

of each sampled individual. The height of the modal value of distribution for the posterior probability of the data for a given K was used as an indicator of the strength of the signal detected by Structure (Evanno et al. 2005).

To investigate gene flow linking to the lagoonal localities and between lagoonal and marine sites, migration rates based on maximum likelihood were obtained with the program MIGRATE v. 3.2.7 (Beerli and Felsenstein 2001; http://popgen.scs.fsu.edu). MIGRATE uses a Markov Chain Monte Carlo-based (MCMC) approach to explore all possible gene genealogies to provide maximum likelihood estimates of the population size and migration rates compatible with the data. These estimates are computed as $\theta = N_e\mu$ as population size and $M = m/\mu$ as the mutation-scaled migration rate for migration (nomenclature according MIGRATE-n software), where N_e is the effective population size, m is the fraction of the new immigrants in the population per generation, and μ is the mutation rate of the gene. The MCMC run consisted of 10 short chains (sampling 10,000 trees) and one long chain (sampling 10,000 trees) with a burn-in period of 10,000 trees.

To assess the presence of microsatellites loci under selection, we used LOSITAN version 1.0.0 (http://popgen.eu/soft/lositan/) (Antao et al. 2008). This program evaluates the relationship between F_{ST} and expected heterozygosity (H_E) to identify outlier loci. We ran 75,000 simulations with "neutral mean F_{ST}" and "force mean $F_{ST,}$" to increase the reliability of the mean F_{ST} and the entire microsatellite dataset under the infinite allele model (IAM). The number of populations was four according to F_{ST} values and Cavalli-Sforza genetic distances. We chose the confidence intervals of 99 % to carry out a more conservative test for selection.

To test the possible relationships between environmental variables and genetic diversity, we developed generalized additive models (GAMs) for the first principal component of the CA (which usually explains the higher values of variance). The coordinates of each locality derived of the CA were included in GAMs as dependent variables. GAMs are a nonparametric extension of generalized linear models (GLM) that fit a wide variety of forms of stochastic variation in the response. GAMs represent the relationship between the response variable and the predictors by smooth functions, which can take virtually any form (Hastie and Tibshirani 1990). These models have been applied on genetic and environmental data previously (Snäll et al. 2004; Parisod and Bonvin 2008; González-Wangüemert et al. 2009; Vergara-Chen et al. 2010b). The GAMs were evaluated by examining the proportion of explained deviance and minimizing the generalized cross-validation (GCV; Wood 2000; Wood and Augustin 2002) and the Akaike information criterion (AIC; Venables and Ripley 2004) scores. As independent variables, we used maximum, minimum and mean values of temperature and salinity (data from: http://www.noaa.gov; Research Group "Ecología y Ordenación de Ecosistemas Marinos Costeros"). GAMs were performed using "ade4" (Chessel 1992) and "mgcv" (Wood 2006) packages from R statistical software (R Development Core Team 2007).

Results

Genetic diversity

A total of 177 fishes were analyzed from three lagoonal and two marine locations. Mean expected heterozygosities (H_E) were high, ranging from 0.669 (Los Urrutias) to 0.748 (Lo Pagán). A similar level of allelic richness was observed across all samples with a mean of 9.025 alleles across all loci (ranging from 8.375 to 10.0 alleles). Regarding to the fixation index (F_{IS}), the Mazarrón sample showed the highest F_{IS} value (0.161) while the lowest value (0.065) was detected in the Veneziola sample (Table 2). Significant deviations from Hardy–Weinberg equilibrium (heterozygote deficit) were detected at all five populations. Four loci, Pmar03 and Pmin38 (3 localities), Pmin04 (4 localities) and Pmin29 (5 localities), deviated significantly from Hardy–

Table 2 Estimates of genetic diversity of the five samples of *Pomatoschistus marmoratus* from the Mar Menor coastal lagoon and adjacent marine sites (SE Spain) based on eight microsatellite markers

Populations	Sample size	Alleles	Exclusive alleles	Allelic richness	H_O	H_E	F_{IS}
Lo Pagán (lagoon)	38	72	6	9.000	0.661	0.748	0.118***
Playa Honda (lagoon)	34	71	7	8.875	0.601	0.669	0.103***
Los Urrutias (lagoon)	42	71	2	8.875	0.617	0.676	0.088***
Veneziola (marine)	30	67	3	8.375	0.646	0.689	0.065*
Mazarrón (marine)	33	80	9	10.000	0.560	0.713	0.161***
Total	177	72	27	9.025	0.617	0.669	0.107***

H_O is the observed heterozygosity, H_E the expected heterozygosity and F_{IS} measure deviation from Hardy–Weinberg equilibrium

*** $P < 0.001$; ** $0.001 < P < 0.01$; * $P < 0.005$

Table 3 Pairwise fixation indices (F_{ST}, below) and Cavalli-Sforza genetic distances (above) between five sampling localities of *Pomatoschistus marmoratus* from the Mar Menor coastal lagoon and adjacent marine sites (SE Spain) based on eight microsatellite markers

Locations	LP	PH	LU	VZ	MZ
Lo Pagán (LP)	–	0.02	0.02	**0.0240***	**0.0300***
Playa Honda (PH)	0.0054	–	**0.0210***	**0.0240***	**0.0310***
Los Urrutias (LU)	0.0084	**0.0157***	–	**0.0220***	**0.0280***
Veneziola (VZ)	0.0069	0.0069	**0.0137***	–	**0.0310***
Mazarrón (MZ)	**0.0303***	**0.0167***	0.0176*	0.0178*	–

* Significant values ($P < 0.05$). The significant values after Bonferroni' correction are indicated in bold

Weinberg expectations ("Appendix 1"). Analysis of all samples indicated that some of the heterozygote deficits could be due to the presence of null alleles, but they were not attributable to genotyping errors. The recalculated frequency data using MICROCHECKER software to consider null alleles were not significant different from the detected first values, and therefore, we continued using them to all posterior analyses. Also, inclusion or exclusion of these loci did not quantitatively affect either population comparisons (F_{ST} values and genetic distances, data not shown).

Significant linkage disequilibrium was detected in four loci pairs from some localities (Pmar03/Pmin29 in Lo Pagán, Pmar03/Pmin38 and Pmar05/Pmin38 in Lo Pagán and Mazarrón, Pmar08/Pmin35 in Playa Honda). The IAM model was applied to find possible bottlenecks; however, they were not detected in any populations.

Population genetic differentiation

Pattern of population differentiation based on F_{ST} statistics pointed to significant differences between six sample comparisons, while we detected lack of differentiation between four sample comparisons (Table 3). The highest F_{ST} value (0.0303) was observed between Lo Pagán (lagoon) and Mazarrón (marine) samples, whereas the lowest significant value (0.0137) was found between Los Urrutias (lagoon) and Veneziola (marine) samples. Pairwise F_{ST} estimates among lagoonal samples indicated that Los Urrutias and Playa Honda were genetically different ($F_{ST} = 0.0157$) while Lo Pagán–Playa Honda and Lo Pagán–Los Urrutias showed absence of genetic differentiation. The marine locality of Veneziola showed genetic similarity with two lagoonal localities, Lo Pagán and Playa Honda. All lagoonal localities and the Veneziola marine sample were genetically differentiated from the Mazarrón marine sample. These results were corroborated by Cavalli-Sforza genetic distances (Table 3).

Furthermore, genotypic differentiation (G-based) for all pairs of populations showed significant differences between all comparisons across all loci except for some lagoon localities, Lo Pagán–Playa Honda ($P = 0.3198$) and Lo Pagán–Los Urrutias ($P = 0.0863$). The genic differentiation test showed the same pattern without significant differences between Lo Pagán and Playa Honda ($P = 0.1087$) ("Appendix 2").

This structure was confirmed using a correspondence analysis (CA) based on allele frequencies. The surface plot of the two first ordination axes scores 64.58 % of the total variance in the data revealing a small-scale geographic structure (Fig. 2) and showing two groups (Fig. 2a): (1) Mazarrón sample on the positive side of Axis I; (2) a group composed of all remaining localities on negative side of Axis I. The Axis II discriminated the Veneziola sample from the lagoonal localities. This pattern indicating the differentiation of Mazarrón sample was reconfirmed with the CA plot using our previous data (frequencies of haplotypes) from mitochondrial DNA control region (Vergara-Chen et al. 2010a) pointed a score of 67.47 % of the total variance for the two first ordination axes (Fig. 2b).

The analysis of molecular variance (AMOVA) pointed to nonsignificant differences among groups: coastal lagoon (Lo Pagán, Los Urrutias and Playa Honda) and marine (Veneziola and Mazarrón). A low percentage of the variance was attributed to differences among groups (0.12 %; $P > 0.05$). However, the analysis revealed significant differences among populations within groups and within populations (1.23 and 98.65 %, respectively; $P < 0.05$).

The *P. marmoratus* populations did not show a definite structure such as was obtained by the Bayesian approach. This analysis suggests that $K = 1$ best describes the situation among samples (Fig. 3).

The MIGRATE analysis pointed the lowest value of effective population size (Θ) in Los Urrutias (2.04) and the highest value in Lo Pagán (3.06). Migration rates showed a predominant gene flow from Playa Honda to Lo Pagán (3.78) and the lowest value of genetic exchange from Lo Pagán to Veneziola (0.87) (Table 4).

LOSITAN tests did not identify any microsatellite as an outlier (Fig. 4), so no loci can be consider under positive or balancing selection.

Relationships among genetic structure and environmental variables

Our analyses, except for AMOVA and assignation tests, have detected a small but significant genetic differentiation between coastal lagoon and marine populations of *P. marmoratus*, which could be influenced (among others factors) by environmental variables.

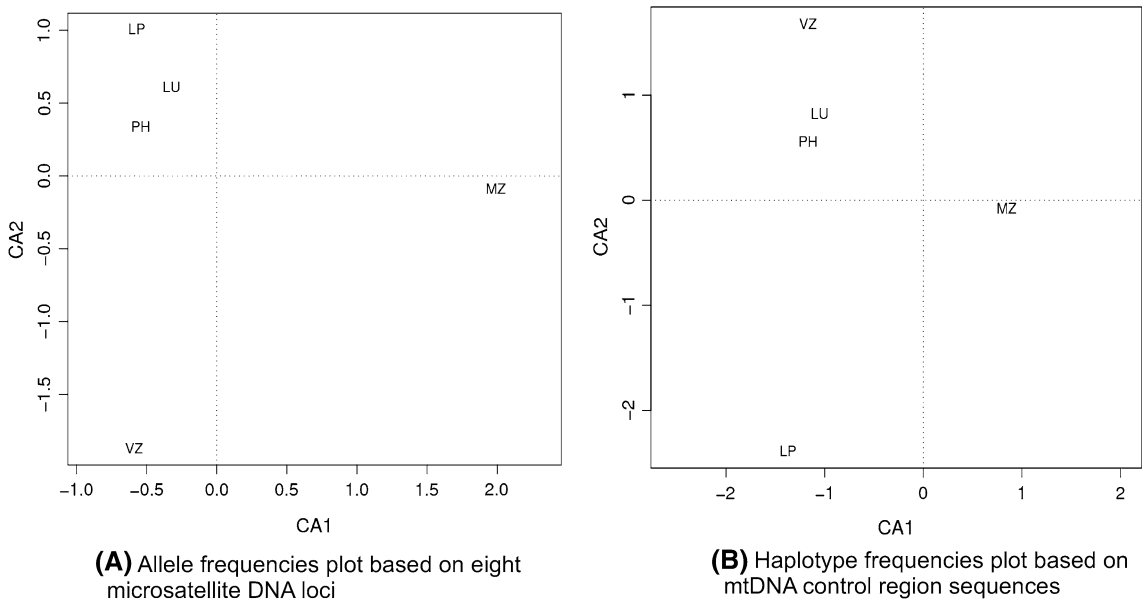

Fig. 2 Correspondence analysis of genotype frequencies of five populations of *Pomatoschistus marmoratus* along the Mar Menor lagoon-SW Mediterranean transition (SE Spain). **a** Plot based on allele frequencies from eight microsatellite loci. **b** Plot based on haplotype frequencies from mitochondrial DNA control region sequences obtained by Vergara-Chen et al. (2010a). Populations are identified as follows: Lo Pagán (LP), Los Urrutias (LU), Playa Honda (PH), Veneziola (VZ), Mazarrón (MZ)

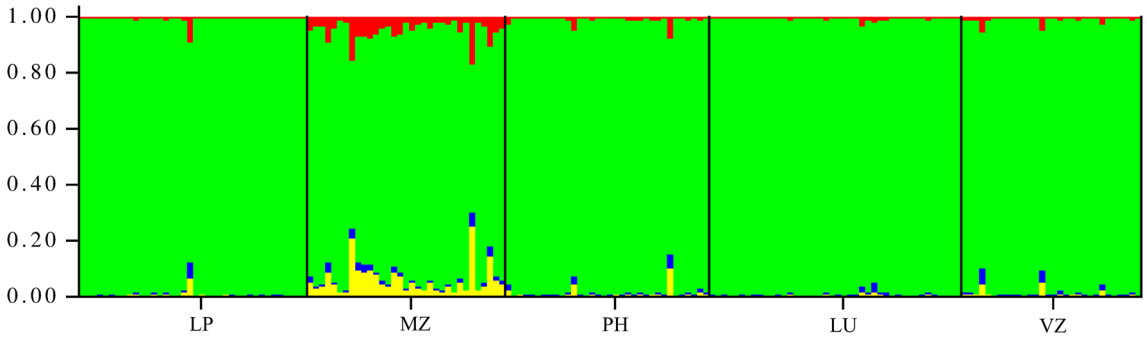

Fig. 3 Estimated population structure of *Pomatochistus marmoratus* and surface population using STRUCTURE (Pritchard et al. 2000) for $K = 1$. Each individual is represented by a *thin vertical line*, which is partitioned into K segments that represent its estimated population group membership fractions

Table 4 Effective population size ($\Theta = N_e\mu$) and migration rates (m/μ = Nem/Θ) based on eight microsatellite loci of *Pomatoschistus marmoratus* from the Mar Menor coastal lagoon and adjacent marine sites (SE Spain)

Locality	Θ	Migration rates				
		Lo Pagán to	Playa Honda to	Los Urrutias to	Veneziola to	Mazarrón to
Lo Pagán	3.06		3.78	3.08	2.55	1.99
Playa Honda	2.25	1.34		1.98	1.19	1.70
Los Urrutias	2.04	2.51	2.01		2.53	3.28
Veneziola	2.76	0.87	3.31	2.88		1.46
Mazarrón	2.98	0.98	0.93	1.83	3.04	

The application of GAMs to our previous published data from control region in *P. marmoratus* (Vergara-Chen et al. 2010a) supports this relationship among genetic structure and environmental variables, obtaining a linear response to maximum salinity. The deviance explained by the GAM was high ($Dev_{maximum\ salinity} = 98.2\ \%$, Pr (intercept) = 0.0014, $Pr_{maximum\ salinity} = 0.0010$). The GCV and AIC scores were significant ($GVC_{maximum\ salinity} = 0.0329$,

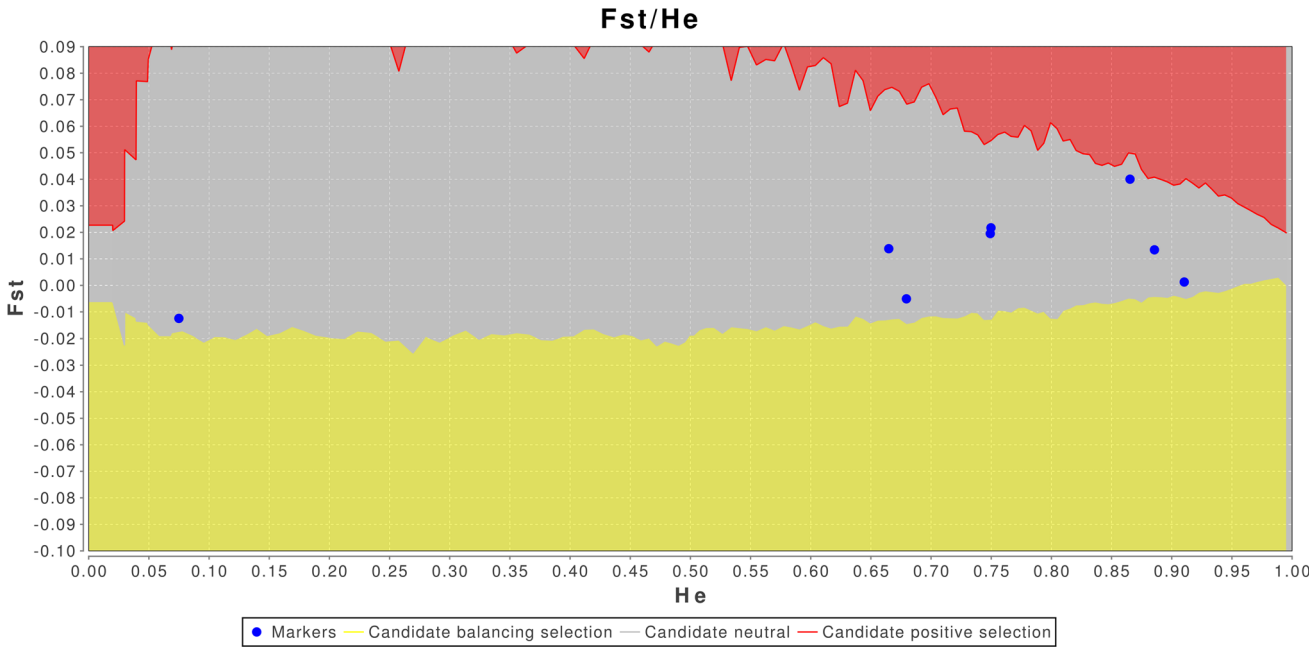

Fig. 4 Comparison of F_{ST} and H_e in polymorphic loci to identify outliers and potential candidates for selection using LOSITAN software. Graphical output shows the simulated confidence area for neutral loci (*pale gray shading*)

$AIC_{maximum\ salinity} = -1.9807$). Similar results are obtained when we apply the GAMS to our data from microsatellites ($Dev_{maximum\ salinity} = 98.5\ \%$, Pr (intercept) = 0.0017, $Pr_{maximum\ salinity} = 0.0016$; $GVC_{maximum\ salinity} = 0.0685$; $AIC_{maximum\ salinity} = 1.6816$).

Comparison between mitochondrial and microsatellite data

In contrast to the F_{ST} values from mitochondrial DNA (Vergara-Chen et al. 2010a), the microsatellite data exhibited significant evidence for population genetic structure between coastal lagoon and marine samples. This was also reflected in overall F_{ST} values that were higher for nuclear microsatellites (global $F_{ST} = 0.014$) than for mitochondrial DNA (global $F_{ST} = 0.006$). These differences were more important between Mazarrón marine sample and all remaining localities.

This pattern was confirmed when we compared the correspondence analyses from allele and haplotype frequencies (microsatellites and mtDNA, respectively); the results pointed the same grouping with Mazarrón isolated from the remaining sampling sites. Otherwise, both types of molecular markers indicated variable rates of genetic exchange among lagoonal samples and between coastal lagoon and marine localities although the overall of the pattern was very similar. The parameter Θ (a function of H_e) based on mitochondrial DNA data suggested values between 0.01 (Playa Honda) and 0.05 (Los Urrutias), while the migration rates showed significant gene flow from Playa Honda to all remaining localities, and restricted flow from Mazarrón to Veneziola (3.52e−11) and from Lo Pagán to Playa Honda (9.36e−14) (Vergara-Chen et al. 2010a). Microsatellite data pointed values of effective population size higher than those obtained with the mitochondrial marker, with the lowest (Θ) value for Los Urrutias and the highest value for Lo Pagán. Migration rates showed a predominant gene flow from Playa Honda to the Lo Pagán (Table 4). The AMOVA for both mitochondrial DNA and microsatellite loci revealed no significant evidence for genetic subdivision among groups (coastal lagoon vs. marine).

Discussion

Microsatellite loci showed high levels of polymorphism in *P. marmoratus* which is consistent with the mean number of alleles observed in other *Pomatoschistus* species (Berrebi et al. 2006; Larmuseau et al. 2007; Marques et al. 2012). The studied populations showed high genetic diversity, with values of expected heterozygosity ranged from 0.67 to 0.79. These levels of genetic variability were also similar to those found in a congeneric species, *P. minutus,* analyzed with microsatellites markers: 0.74–0.86 (Pampoulie et al. 2004), 0.74–0.78 (Larmuseau et al. 2010a) and 0.58–0.63 (Boissin et al. 2011).

Departures from Hardy–Weinberg equilibrium (HWE) were found in all *Pomatochistus* samples, such as were found in *P. minutus* and *P. microps* populations by other

authors (Pampoulie et al. 2004; Larmuseau et al. 2010a; Marques et al. 2012). The most probable causes of HWE departures may be attributed to Wahlund effect due to the recruitment of genetically variable cohorts of larvae (González-Wangüemert et al. 2007), inbreeding, null alleles (Horne et al. 2011; González-Wangüemert and Pérez-Ruzafa 2012; Milana et al. 2012), cryptic species, groupings of relatives, scoring errors or selection against heterozygotes (Pampoulie et al. 2004). The Wahlund effect, the most common explanation of heterozygote deficiency, should result in significant F_{IS} values at more than one locus, as drift causing population structuring should affect all polymorphic loci similarly (Pogson et al. 1995), but we did not find this pattern in our results ("Appendix 1"). We think that the high F_{IS} values found in the studied localities are mainly due to structuring and mixing of sand goby populations. Inbreeding seems an unlikely explanation in fish with large populations such as gobies that are not subject to drastic reduction in their effective population size by fishery. However, we cannot exclude the possibility of inbreeding derived by the polygyny in *P. marmoratus* species. We do not favor the hypothesis of null alleles because all F_{IS} estimates were positive and significant; it seems highly improbable that all loci exhibit null alleles with such a constant frequency. The heterozygotes deficiency detected in all populations can be boosted by the use of locus primers designed for other species (cross-amplification) (Rungis et al. 2004; Pashley et al. 2006; Keever et al. 2008). Finally, the selection against heterozygotes cannot be demonstrated from our results, although we have detected a significant link among genetic structure and maximum salinity (implying that selection could be acting on *P. marmoratus*). LOSITAN did not detect any locus under balancing or positive selection. Further research will be necessary to test this hypothesis.

In spite of the lack of a geographic structure at total scale showed by the STRUCTURE results, signs of a genetic substructure were found. The correspondence analyses, Cavalli-Sforza genetic distances and the F_{ST} values showed significant genetic differentiation between some localities after Bonferroni correction; however, AMOVA did not provide evidence of differences between sample groups (coastal lagoon vs. marine), being Veneziola included in the marine group. Our genetic data seem to demonstrate that this locality could be considered more lagoonal than marine; also, the Veneziola' habitat is more similar to lagoonal localities than Mazarrón, but it is not correct to carry out an AMOVA considering four samples inside "lagoon group" and one sample as "marine group." On the other hand, the lack of significance in the AMOVA comparisons between groups could be also due to low power associate with a low number of populations per group such as was described by Fitzpatrick (2009). Therefore, according to our results from F_{ST} values, Cavalli-Sforza genetic distances, correspondence analysis and significant genic and genotypic differentiation, we suggested that the highest genetic divergence observed between samples (Mazarrón and the rest of localities) could be consequence of the isolation of lagoonal populations from open sea which has been already reported in other coastal fishes such as *Pomatoschistus minutus* (Pampoulie et al. 2004; Berrebi et al. 2009), *Dicentrarchus labrax* (Lemaire et al. 2000), *Atherina lagunae* (Trabelsi et al. 2004), *Atherina boyeri* (Kraitsek et al. 2008; Milana et al. 2012), *Diplodus sargus* (González-Wangüemert and Pérez-Ruzafa 2012) and several invertebrate species (Camilli et al. 2001; González-Wangüemert et al. 2006; Tarnowska et al. 2010; Vergara-Chen et al. 2010b).

The genetic differentiation among our *P. marmoratus* samples from lagoonal and marine sites could be also influenced by environmental discontinuities along the coastal lagoon–marine transition and even inside coastal lagoon. The habitat discontinuities could cause the spatial isolation of the populations mainly on benthic marine species (Riginos and Nachman 2001; Johansson et al. 2008; Riginos et al. 2011). Inside Mar Menor lagoon, there are important habitat discontinuities related to spatial distribution of substrate type and submerged marine vegetation (González-Wangüemert et al. 2009; Quintino et al. 2010) which can be influencing the distribution and density of *P. marmoratus* populations. In fact, some studies about the distribution of fish communities in Mar Menor showed significant differences among densities and standing stocks of *P. marmoratus* depending on bottom granulometry and seagrass and algae cover (Verdiell Cubedo et al. 2008). This discontinuity of available habitats for *P. marmoratus* is also found in the Mediterranean coast from Veneziola to Mazarrón, explaining the genetic isolation and the important genetic break detected between coastal lagoon localities (including Veneziola) and Mazarrón.

On the other hand, the reproductive strategy of *P. marmoratus* plus a highly variable environmental conditions create the potential for sweepstakes reproductive success (Hedgecock 1994; Hedgecock et al. 2007) which could be also explaining the genetic differentiation detected on our target species such as was registered on other fish species previously (Planes and Lenfant 2002; González-Wangüemert et al. 2007; Christie et al. 2010; Hogan et al. 2010).

The presence of exclusive alleles and differences on the allele frequencies existing between localities could also suggest that some environmental variables are influencing the genetic differentiation among populations; this hypothesis is supported by GAM results. As *P. marmoratus* is associated with brackish and hyper-saline habitats, which

are systems suffering frequent environmental fluctuations and disturbances, this species could have been exposed to extreme conditions more frequently than other marine species inhabiting open sea habitats. Our results point a significant link between extreme lagoonal environmental conditions (mainly maximum salinity) and genetic composition, such as it was already demonstrated on cockles and sea cucumbers populations from the Mar Menor lagoon (González-Wangüemert et al. 2009; Vergara-Chen et al. 2010b). This find is very interesting because previously several authors had defined different ecological requirements for the various *Pomatochistus* species; for example, *P. marmoratus* survives to higher salinities than *P. microps*, especially in habitats whose temperature and salinity change through the year, but do not vary abruptly (Berrebi et al. 2005). Thereby, extreme environmental conditions could be acting on genetic structure of coastal lagoon populations of *P. marmoratus* causing genetic divergence (Conover et al. 2006; Marshall et al. 2010; Sanford and Morgan 2011).

The genetic changes detected on *P. marmoratus* are not explicitly linked to functionality, but really the signal recovered might be a signature of adaptive selection. This intraspecific pattern might be the base underlying evolution at the interspecific level. Some studies focused on several *Pomatochistus* species using a well-defined gene under selection (rhodopsin gene) have demonstrated that even the local light availability acts as selection pressure, sand gobies seem to be genetically adapted on the rhodopsin gene (RH1) to the differences in light between seas, lagoons and rivers (Larmuseau et al. 2009, 2010b).

Finally, the detected differences using two molecular markers (mitochondrial and nuclear) can be explained by their intrinsic characteristics and mutation rates. Nevertheless, the reproductive strategy of *P. marmoratus* could be also influencing our results. Mitochondrial DNA is a good marker for studying matrilineal movements because of its haploid nature, maternal inheritance and lack of recombination (Avise 2000). Therefore, the mitochondrial markers are influenced by mode of reproduction of species (Consuegra and de Leaniz 2007; Cano et al. 2008). The absence of significant genetic structure and high gene flow detected in our previous genetic study using control region (mitochondrial DNA) (Vergara-Chen et al. 2010a) could be considered indicative of extensive female dispersal related with *P. marmoratus* breeding behavior. This reproductive behavior shows a female spawning in different nests of different males; each male defends its nest and takes care of the eggs until larvae hatch (Mazzoldi and Rasotto 2001). Hence, the females are expected to be the most mobile sex during the breeding season (Lindström et al. 2006) favoring genetic population homogeneity detected specially using mitochondrial DNA markers. However, nuclear DNA markers (microsatellites) are diploids, with biparental inheritance. This leads to the expectation that more genetic variability and population differentiation would be detected with nuclear markers (Ruzzante et al. 1998). In fact, our microsatellite results supported this expectation, showing higher values of effective population size and lower rates of gene flow in contrast to our previous mitochondrial data (Vergara-Chen et al. 2010a). Therefore, these results corroborate the conclusions of Daemen et al. (2001) affirming that cytoplasmic markers provide a record of the historic and recent gene flow in females, while the nuclear markers estimate recent gene flow in both sexes.

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Appendix 1

See Table 5.

Table 5 F_{IS} values per locality and locus

Locus	Locality	F_{IS}	*P* value
Pmar03	LP	**0.0769**	**0.0360**
	MZ	**0.1856**	**0.0000**
	PH	0.0916	0.0925
	UR	0.1129	0.0508
	VZ	**0.1676**	**0.0034**
Pmar05	LP	−0.0049	0.5081
	MZ	−0.0977	0.7984
	PH	−0.1963	0.9955
	UR	−0.1443	0.9847
	VZ	−0.2225	0.9304
Pmar08	LP	−0.0374	0.6278
	MZ	0.0119	0.4082
	PH	−0.1035	0.7809
	UR	−0.0206	0.0747
	VZ	−0.1191	0.8719
Pmin04	LP	**0.2355**	**0.0211**
	MZ	**0.1072**	**0.0000**

Table 5 continued

Locus	Locality	F_{IS}	*P* value
	PH	**0.1423**	**0.0009**
	UR	**0.0882**	**0.0063**
	VZ	−0.3392	1.0000
Pmin09	LP	0.0120	0.0893
	MZ	**0.2426**	**0.0011**
	PH	0.0965	0.1975
	UR	−0.0647	0.8800
	VZ	0.1188	0.1623
Pmin29	LP	**0.4596**	**0.0000**
	MZ	**0.6893**	**0.0000**
	PH	**0.5114**	**0.0000**
	UR	**0.7310**	**0.0000**
	VZ	**0.6585**	**0.0000**
Pmin35	LP	–	–
	MZ	−0.0323	1.0000
	PH	−0.0154	1.0000
	UR	−0.0380	1.0000
	VZ	−0.0323	1.0000
Pmin38	LP	**0.1190**	**0.0200**
	MZ	0.0734	0.1739
	PH	**0.1884**	**0.0003**
	UR	0.0229	0.0493
	VZ	**0.1668**	**0.0002**

Estimation of exact *P* values by the Markov chain method in bold significant values

Appendix 2

See Table 6.

Table 6 Total genic and genotypic differentiation between populations

Population pair	Genic		Genotypic	
	X^2	*P* value	X^2	*P* value
LP&MZ	55.6083	**0.0000**	43.9759	**0.0002**
LP&PH	23.1914	0.1087	18.0697	0.3198
MZ&PH	52.5412	**0.0000**	∞	**0.0000**
LP&UR	30.2510	**0.0167**	24.1474	0.0863
MZ&UR	53.4901	**0.0000**	45.7717	**0.0001**
PH&UR	36.0812	**0.0028**	29.4083	**0.0213**
LP&VZ	41.3020	**0.0005**	34.5169	**0.0046**
MZ&VZ	∞	**0.0000**	43.4369	**0.0002**
PH&VZ	37.1174	**0.0020**	31.3167	**0.0122**
UR&VZ	47.3778	**0.0000**	50.0470	**0.0000**

P value for each population pair across all loci (Fisher's method). Significant values in bold

References

Antao T, Lopes A, Lopes RJ, Beja-Pereira R, Luikart G (2008) LOSITAN: a workbench to detect molecular adaptation based on a FST-outlier method. BMC Bioinformatics 9:323

Avise JC (2000) Phylogeography: the history and formation of species. Harvard University Press, Cambridge

Bardin O, Pont D (2002) Environmental factors controlling the spring immigration of two estuarine fishes *Atherina boyeri* and *Pomatoschistus* spp. into a Mediterranean lagoon. J Fish Biol 61:560–578

Beerli P, Felsenstein J (2001) Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. Proc Nat Acad Sci USA 98:4563–4568

Beheregaray LB, Sunnucks P (2001) Fine-scale genetic structure, estuarine colonization and incipient speciation in the marine silverside fish *Odontesthes argentinensis*. Mol Ecol 10:2849–2866

Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F (2004) GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, Populations, Interactions, CNRS UMR 5171, Université de Montpellier II, Montpellier (France)

Berrebi P, Rodriguez P, Tomasini JA, Cattaneo-Berrebi G, Crivelli A (2005) Differential distribution of the two cryptic species, *Pomatoschistus microps* and *P. marmoratus*, in the lagoons of southern France, with an emphasis on the genetic organisation of *P. microps*. Estuar Coast Shelf Sci 65:708–716

Berrebi P, Laserre B, Barbisan F, Zane L (2006) Isolation of microsatellite loci and cross-species amplifications in three gobiid fish of the genus *Pomatoschistus*. Mol Ecol Notes 6:724–727

Berrebi P, Rodriguez P, Rooney C, ́dAloy S, Cattaneo-Berrebi G (2009) Haplotypic confinement in two cryptic and closely-related species of sedentary gobies, *Pomatoschistus microps* and *P. marmoratus* in French Mediterranean lagoons. Folia Zool 58:123–131

Bilton DT, Paula J, Bishop JDD (2002) Dispersal, genetic differentiation and speciation in estuarine organisms. Estuar Coast Shelf Sci 55:937–952

Bisol PM, Gallini A, Prevedllo S, Rianna E, Bernardinelli E, Franco A, Zane L (2007) Low variation at allozyme loci and differences between age classes at microsatellites in grass goby (*Zosterisessor ophiocephalus*) populations. Hydrobiol 577:151–159

Boissin E, Hoareau TB, Berrebi P (2011) Effects of current and historic habitat fragmentation on the genetic structure of the sand goby *Pomatoschistus minutus* (Osteichthys, Gobiidae). Biol J Linn Soc 102:175–198

Bradbury IR, Snelgrove PVR, Bentzen P, de Young B, Gregory RS, Morris CJ (2009) Structural and functional connectivity of marine fishes within a semi-enclosed Newfoundland fjord. J Fish Biol 75:1393–1409

Camilli L, Castelli A, Lardicci C, Maltagliati F (2001) Evidence for high levels of genetic divergence between populations of the bivalve *Mytilaster minimus* from a brackish environment and two adjacent marine sites. J Molluscan Stud 67:506–510

Canino MF, Spies LB, Lowe SA (2010) Highly discordant nuclear and mitochondrial DNA diversities in Atka mackerel. Mar Coast Fish 2:375–387

Cano JM, Mäkinen HS, Merilä J (2008) Genetic evidence for male-biased dispersal in the three-spined stickleback (*Gasterosteus aculeatus*). Mol Ecol 17:3234–3242

Cavalli-Sforza LL, Edwards AWF (1967) Phylogenetic analysis: models and estimation procedures. Am J Hum Genet 19:233–257

Chapuis MP, Estoup A (2007) Microsatellite null alleles and estimation of population differentiation. Mol Biol Evol 24:621–631

Chessel D (1992) The ade4 package-I: one-table methods. R News 4:5–10

Christie MR, Johnson DW, Stallings CD, Hixon MA (2010) Self-recruitment and sweepstakes reproduction amid extensive gene flow in a coral-reef fish. Mol Ecol 19:1042–1057

Cognetti G, Maltagliati F (2000) Biodiversity and adaptive mechanisms in brackish water fauna. Mar Pollut Bull 40:7–14

Conover DO, Clarke LM, Munch SB, Wagner GN (2006) Spatial and temporal scales of adaptive divergence in marine fishes and the implications for conservation. J Fish Biol 69:21–47

Consuegra S, de Leaniz CG (2007) Fluctuating sex ratios, but no sex-biased dispersal in a promiscuous fish. Evol Ecol 21:229–245

Cornuet JM, Luikart G (1996) Description and power analysis of two tests for inferring recent population bottlenecks from allele frequency data. Genet 144:2001–2014

Daemen E, Cross T, Ollevier F, Volckaert F (2001) Analysis of the genetic structure of European eel (*Anguilla anguilla*) using microsatellite DNA and mtDNA markers. Mar Biol 139:755–764

De Innocentiis S, Sola L, Cataudella S, Bentzen P (2001) Allozyme and microsatellite loci provide discordant estimates of population differentiation in the endangered dusky grouper (*Epinephelus marginatus*) within the Mediterranean Sea. Mol Ecol 10:2163–2175

De Pascalis F, Pérez-Ruzafa A, Gilabert J, Marcos C, Umgiesser U (2012) Climate change response of the Mar Menor coastal lagoon (Spain) using a hydrodynamic finite element model. Estuar Coast Shelf Sci 114:118–129

Durand JD, Guinand B, Dodson JJ, Lecomte F (2013) Pelagic life and depth: coastal physical features in West Africa shape the genetic structure of the bonga shad Ethmalosa fimbriata. PLoS ONE 8:e77483

Earl DA, Louie KD, Bardeleben C, Swift CC, Jacobs DK (2010) Rangewide microsatellite phylogeography of the endangered tidewater goby, *Eucyclogobius newberryi* (Teleostei: Gobiidae), a genetically subdivided coastal fish with limited marine dispersal. Conserv Genet 11:103–114

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software structure: a simulation study. Mol Ecol 14:2611–2620

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genet 131:479–491

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evol Bioinf Online 1:47–50

Fitzpatrick BM (2009) Power and sample size for nested analysis of molecular variance. Mol Ecol 18:3961–3966

Fluker BL, Pezold F, Minton RL (2011) Molecular and morphological divergence in the inland silverside (*Menidia beryllina*) along a freshwater-estuarine interface. Environ Biol Fish 91:311–325

Fonds M (1970) Remarks on the rearing of gobies *(Pomatoschistus minutus* and *P. lozanoi)* for experimental purposes. Helgol Mar Res 20:620–628

García-Sánchez M, Korbee N, Pérez-Ruzafa IM, Marcos C, Domínguez B, Figueroa FL, Pérez-Ruzafa A (2012) Physiological response and photoacclimation capacity of *Caulerpa prolifera* (Forsskål) J.V. Lamouroux and *Cymodocea nodosa* (Ucria) Ascherson meadows in the Mar Menor lagoon (SE Spain). Mar Environ Res 79:37–47

Giménez-Casalduero F, Muniain C, González-Wangüemert M, Garrote-Moreno A (2011) *Elysia timida* (Risso, 1818) three decades of research. Anim Biodivers Conserv 34:217–227

Giovannotti M, La Mesa M, Caputo V (2009) Life style and genetic variation in teleosts: the case of pelagic (*Aphia minuta*) and benthic (*Gobius niger*) gobies (Perciformes: Gobiidae). Mar Biol 156:239–252

González-Wangüemert G, Pérez-Ruzafa Á (2012) In two waters: contemporary evolution of lagoonal and marine white seabream (*Diplodus sargus*) populations. Mar Ecol 33:337–349

González-Wangüemert M, Pérez-Ruzafa A, Rosique MJ, Ortiz A (2004) Genetic differentiation in two cryptic species of Ostreidae, *Ostrea edulis* (Linné, 1758) and *Ostreola stentina* (Payraudeau, 1826), in the Mar Menor lagoon (SW Mediterranean). Nautilus 118:103–111

González-Wangüemert M, Giménez-Casalduero F, Pérez-Ruzafa Á (2006) Genetic differentiation of *Elysia timida* (Risso, 1818) populations in Southwest Mediterranean and Mar Menor coastal lagoon. Biochem Syst Ecol 34:514–527

González-Wangüemert M, Pérez-Ruzafa Á, Cánovas García F, García Charton J, Marcos C (2007) Temporal genetic variation in populations of *Diplodus sargus* from the SW Mediterranean Sea. Mar Ecol Prog Ser 334:237–244

González-Wangüemert M, Cánovas F, Marcos C, Pérez-Ruzafa Á (2009) Phosphoglucose isomerase variability of *Cerastoderma glaucum* as a model for testing the influence of environmental conditions and dispersal patterns through quantitative ecology approaches. Biochem Syst Ecol 37:325–333

González-Wangüemert G, Cánovas F, Pérez-Ruzafa A, Marcos C, Alexandrino P (2010) Connectivity patterns inferred from the genetic structure of white seabream (*Diplodus sargus* L.). J Exp Mar Biol Ecol 383:23–31

González-Wangüemert M, Vega-Fernández T, Pérez-Ruzafa A, Giacalone VM, Badalamenti F (2012) Genetic impact of a restocking experiment of white seabream in Sicily (Northwestern Mediterranean Sea). J Sea Res 68:41–48

González-Wangüemert M, Domínguez-Godino J, Giménez-Casalduero F, Serrão E (2014) Genetic signature of a recent invasion: *Bursatella leachii* (Blainville, 1817) coming to Mar Menor coastal lagoon. Biochem Syst Ecol 54:123–129

Gysels ES, Hellemans B, Pampoulie C, Volckaert FAM (2004) Phylogeography of the common goby, *Pomatoschistus microps*, with particular emphasis on the colonization of the Mediterranean and the North Sea. Mol Ecol 13:403–417

Hastie T, Tibshirani R (1990) Generalized additive models. Chapman and Hall, London

Hauser L, Carvalho G (2008) Paradigm shifts in marine fisheries genetics: ugly hypotheses slain by beautiful facts. Fish Fish 9:333–362

Hedgecock D (1994) Does variance in reproductive success limit effective population sizes of marine organisms? In: Beaumont A (ed) Genetics and evolution of aquatic organisms. Chapman and Hall, London, pp 122–134

Hedgecock D, Barber PH, Edmands S (2007) Genetics approaches to measure connectivity. Oceanogr 20:70–79

Hirase S, Kanno M, Ikeda M, Kijima A (2012) Evidence of the restricted gene flow within a small spatial scale in the Japanese common intertidal goby *Chaenogobius annularis*. Mar Ecol 33(4):481–489

Hogan JD, Thiessen RJ, Heath DD (2010) Variability in connectivity indicated by chaotic genetic patchiness within and among populations of a marine fish. Mar Ecol Prog Ser 417:263–275

Horne JB, Momigliano P, Welch DJ, Newman SJ, Van Herwerden L (2011) Limited ecological population connectivity suggests low demands on self-recruitment in a tropical inshore marine fish (*Eleutheronema tetradactylum*: Polynemidae). Mol Ecol 20:2291–2306

Johansson ML, Banks MA, Glunt KD, Hassel-Finnegan HM, Buonaccorsi VP (2008) Influence of habitat discontinuity,

geographical distance, and oceanography on fine-scale population genetic structure of copper rockfish (*Sebastes caurinus*). Mol Ecol 17:3051–3061

Keever CC, Sunday J, Wood C, Byrne M, Hart MW (2008) Discovery and cross-amplification of microsatellite polymorphisms in asterinid Sea stars. Biol Bull 215:164–172

Kraitsek S, Klossa-Kilia E, Papasotiropoulos V, Alahiotis SN, Killias G (2008) Genetic divergence among marine and lagoon *Atherina boyeri* populations in Greece using mtDNA analysis. Biochem Genet 46:781–798

Larmuseau MHD, Hellemans B, Van Houdt JKJ, Volckaert FAM (2007) Development and characterization of nine polymorphic microsatellite markers in the sand goby *Pomatoschistus minutus* (Gobiidae). Mol Ecol Notes 7:147–149

Larmuseau MHD, Raeymaekers JAM, Ruddick KG, Van Houdt JKJ, Volckaert FAM (2009) To see in different seas: spatial variation in the rhodopsin gene of the sand goby (*Pomatoschistus minutus*). Mol Ecol 18:4227–4239

Larmuseau MHD, Raeymaekers JAM, Hellemans B, Van Houdt JKJ, Volckaert FAM (2010a) Mitonuclear discordance in the degree of population differentiation in a marine goby. Heredity 105:532–542

Larmuseau MHD, Huyse T, Van Campenhout K, Van Houdt JKJ, Volckaert FAM (2010b) High molecular diversity in the rhodopsin gene in closely related goby fishes: a role for visual pigments in adaptive speciation? Mol Phylogenet Evol 55:689–698

Larsson LC, Laikre L, Palm S, Andre C, Carvalho GR, Ryman N (2007) Concordance of allozyme and microsatellite differentiation in a marine fish, but evidence of selection at a microsatellite locus. Mol Ecol 16:1135–1147

Lemaire C, Allegrucci G, Naciri M, Bahri-Sfar L, Kara H, Bonhomme F (2000) Do discrepancies between microsatellite and allozyme variation reveal differential selection between sea and lagoon sea bass (*Dicentrarchus labrax*)? Mol Ecol 9:457–467

Lindström K, St Mary C, Pampoulie C (2006) Sexual selection for male parental care in the sand goby, *Pomatoschistus minutus*. Behav Ecol Sociobiol 60:46–51

Maltagliati F (2002) Genetic monitoring of brackish-water populations: the Mediterranean toothcarp *Aphanius fasciatus* (Cyprinodontidae) as a model. Mar Ecol Prog Ser 235:257–262

Marques JF, Rego AL, Lino Costa J, Costa MJ, Cabral HN (2006) Genetic and morphologic differentiation of the Lusitanian toadfish (Halobatrachus didactylus) between estuarine and coastal areas in Portugal. Sci Mar 70:749–758

Marques J, Fonseca V, Shao Z, Cabral H, Tougard C, Berrebi P (2012) Genetic diversity of *Pomatochistus microps* (Perciformes: Gobiidae) in ecologically differentiated estuarine systems. Folia Zool 61:106–117

Marshall DJ, Monro K, Bode M, Keough MJ, Swearer S (2010) Phenotype-environment mismatches reduce connectivity in the sea. Ecol Lett 13:128–140

Martinez-Garrido J, González-Wangüemert M, Serrão E (2014) New highly polymorphic microsatellite markers for the aquatic angiosperm *Ruppia cirrhosa* (Petagna) Grande reveal population diversity and differentiation. Genome 57:57–59

Mazzoldi C, Rasotto MB (2001) Extended breeding season in the marbled goby, *Pomatoschistus marmoratus* (Teleostei: Gobiidae), in the Venetian Lagoon. Environ Biol Fish 61:175–183

McCraney WT, Goldsmith G, Jacobs DK, Kinziger AP (2010) Rampant drift in artificially fragmented populations of the endangered tidewater goby (*Eucyclogobius newberryi*). Mol Ecol 19:3315–3327

Mejri R, Lo Brutto S, Ben Hassine OK, Arculeo M (2009) A study on *Pomatoschistus tortonesei* Miller 1968 (Perciformes, Gobiidae) reveals the Siculo-Tunisian Strait (STS) as a breakpoint to gene flow in the Mediterranean basin. Mol Phylogenet Evol 53:596–601

Mejri R, Arculeo M, Ben Hassine OK, Lo Brutto S (2011) Genetic architecture of the marbled goby *Pomatoschistus marmoratus* (Perciformes, Gobiidae) in the Mediterranean Sea. Mol Phylogenet Evol 58:395–403

Mejri R, Lo Brutto S, Hassine N, Arculeo M, Ben Hassine OK (2012) Overlapping patterns of morphometric and genetic differentiation in the Mediterranean goby *Pomatoschistus tortonesei* Miller, 1968 (Perciformes, Gobiidae) in Tunisian lagoons. Zool 115:239–244

Milana V, Franchini P, Sola L, Angiulli E, Rossi AR (2012) Genetic structure in lagoons: the effects of habitat discontinuity and low dispersal ability on populations of *Atherina boyeri*. Mar Biol 159:399–411

Miller PJ (1986) Gobiidae. In: Whitehead PJP, Bauchot Hureau M-L, Nielsen J, Tortonese E (eds) Fishes of the North-eastern Atlantic and the Mediterranean, vol 3. UNESCO, Paris, pp 1019–1085

Nielsen EE, Hemmer-Hansen J, Larsen PF, Bekkevold D (2009) Population genomics of marine fishes: identifying adaptive variation in space and time. Mol Ecol 18:3128–3150

Paetkau D, Slade R, Burden M, Estoup A (2004) Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. Mol Ecol 13:55–65

Pampoulie C, Gysels ES, Maes GE, Hellemans B, Leentjes V, Jones AG, Volckaert FAM (2004) Evidence for fine-scale genetic structure and estuarine colonisation in a potential high gene flow marine goby (*Pomatoschistus minutus*). Heredity 92:434–445

Parisod C, Bonvin G (2008) Fine-scale genetic structure and marginal processes in an expanding population of *Biscutella laevigata* L. (Brassicaceae). Heredity 101:536–542

Pashley CH, Ellis JR, McCauley DE, Burke JM (2006) EST databases as a source for molecular markers: lessons from *Helianthus*. J Heredity 97:381–388

Pérez-Ruzafa Á, Fernández AI, Marcos C, Gilabert J, Quispe JI, García-Charton JA (2005) Spatial and temporal variations of hydrological conditions, nutrients and chlorophyll a in a Mediterranean coastal lagoon (Mar Menor, Spain). Hydrobiol 550:11–27

Pérez-Ruzafa A, García-Charton JA, Barcala E, Marcos C (2006) Changes in benthic fish assemblages as a consequence of coastal works in a coastal lagoon: the Mar Menor (Spain, Western Mediterranean). Mar Poll Bull 53:107–120

Pérez-Ruzafa A, Marcos C, Pérez-Ruzafa IM, Barcala E, Hegazi MI, Quispe J (2007) Detecting changes resulting from human pressure in a naturally quick-changing and heterogeneous environment: spatial and temporal scales of variability in coastal lagoons. Estuar Coast Shelf Sci 75:175–188

Planes S, Lenfant P (2002) Temporal change in the genetic structure between and within cohorts of a marine fish, *Diplodus sargus*, induced by a large variance in individual reproductive success. Mol Ecol 11:1515–1524

Pogson GH, Mesa KA, Boutilier RG (1995) Genetic population structure and gene flow in the Atlantic cod *Gadus morhua*: a comparison of allozyme and nuclear RFLP Loci. Genetics 139:375–385

Portnoy DS, Hollenbeck CM, Renshaw MA, Cummings NJ, Gold JR (2013) Does mating behaviour affect connectivity in marine fishes? Comparative population genetics of two protogynous groupers (Family Serranidae). Mol Ecol 22:301–313

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure from multilocus genotype data. Genetics 155:945–959

QuintinoV Freitas R, Mamede R, Ricardo F, Rodrigues AM, Mota J, Pérez-Ruzafa Á, Marcos C (2010) Remote sensing of underwater

vegetation using single-beam acoustics. ICES J Mar Sci 67:594–605
R Development Core Team (2007) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, http://cran.es.r-project.org/. Accessed 15 July 2011
Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J Heredity 86:248–249
Rice WR (1989) Analyzing tables of statistical tests. Evolution 43:223–225
Richards CL, Wares JP, Mackie JA (2010) Evaluating adaptive processes for conservation and management of estuarine and coastal resources. Estuar Coast 33:805–810
Riginos C, Nachman MW (2001) Population subdivision in marine environments: the contributions of biogeography, geographical distance and discontinuous habitat to genetic differentiation in a blennioid fish, *Axoclinus nigricaudus*. Mol Ecol 10:1439–1453
Riginos C, Douglas KE, Shanahan DF, Jin Y, Treml EA (2011) Effects of geography and life history traits on genetic differentiation in benthic marine fishes. Ecography 34:566–575
Roberts DG, Gray CA, West RJ, Ayre DJ (2010) Marine genetic swamping: hybrids replace an obligately estuarine fish. Mol Ecol 19:508–520
Rousset F (2008) Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. Mol Ecol Resour 8:103–106
Rungis D, Bérubé Y, Zhang J et al (2004) Robust simple sequence-repeat markers for spruce (*Picea* spp.) from expressed sequence tags. Theor Appl Genet 109:1283–1294
Ruzzante DE, Taggart CT, Cook D (1998) A nuclear DNA basis for shelf- and bank-scale population structure in northwest Atlantic cod (*Gadus morhua*): Labrador to Georges Bank. Mol Ecol 7:1663–1680
Sambrook J, Russell DW (2001) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Press, New York
Sanford E, Morgan WK (2011) Local adaptation in marine invertebrates. Ann Rev Mar Sci 3:509–553
Snäll T, Fogelqvist J, Ribeiro PJ, Lascoux M (2004) Spatial genetic structure in two congeneric epiphytes with different dispersal strategies analyzed by three different methods. Mol Ecol 13:2109–2119
Tarnowska K, Chenuil A, Nikula R, Féral JP, Wolowicz M (2010) Complex genetic population structure of the bivalve *Cerastoderma glaucum* in a highly fragmented lagoon habitat. Mar Ecol Prog Ser 406:173–184
Trabelsi M, Maamouri F, Quignard J-P, Boussaid M, Faure E (2004) Morphometric or morpho-anatomal and genetic investigations highlight allopatric speciation in Western Mediterranean lagoons within the *Atherina lagunae* species (Teleostei, Atherinidae). Estuar Coast Shelf Sci 61:713–723
van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MicroChecker: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538
Venables WN, Ripley BD (2004) Modern applied statistics with S-plus. Springer, New York
Verdiell Cubedo D, Oliva Paterna FJ, Egea Serrano A, Torralva Forero M (2008) Population biology and habitat associations of benthic fish species in the shallow areas of a Mediterranean coastal lagoon. Sci Mar 72:319–328
Vergara-Chen C, González-Wangüemert M, Marcos C, Pérez-Ruzafa A (2010a) High gene flow promotes the genetic homogeneity of *Pomatoschistus marmoratus* (Risso 1810) from Mar Menor coastal lagoon and adjacent marine waters. Mar Ecol 31:270–275
Vergara-Chen C, González-Wangüemert M, Marcos C, Pérez-Ruzafa A (2010b) Genetic diversity and connectivity remain high in *Holothuria polii* (Delle Chiaje 1823) across a coastal lagoon-open sea environmental gradient. Genetica 138:895–906
Vergara-Chen C, González-Wangüemert M, Marcos C, Pérez-Ruzafa A (2013) Small-scalegenetic structure of *Cerastoderma glaucum* in a lagoonal environment: potential significance of habitat discontinuity and unstable population dynamics. J Molluscan Stud 79:230–240
Wang L, Liu S, Zhuang Z, Guo L, Meng Z, Lin H (2013) Population genetic studies revealed local adaptation in a high gene-flow marine fish, the small yellow croaker (*Larimichthys polyactis*). PLoS ONE 8:e83493
Waples RS (1998) Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. J Heredity 89:438–450
Watts RJ, Johnson MS (2004) Estuaries, lagoons and enclosed embayments: habitats that enhance population subdivision of inshore fishes. Mar Freshw Res 55:641–651
Weir BS, Cockerham CC (1979) Estimation of linkage disequilibrium in randomly mating populations. Heredity 42:105–111
Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. Evolution 38:1358–1370
Wood S (2000) Modelling and smoothing parameter estimation with multiple quadratic penalties. J R Statist Soc Series B 62:413–428
Wood S (2006) Generalized additive models: an introduction with R. Chapman and Hall, London
Wood SN, Augustin NH (2002) GAMs with integrated model selection using penalized regression splines and applications to environmental modelling. Ecol Model 157:157–177
Wright S (1969) Evolution and the genetics of populations. The theory of gene frequencies, vol 2. University of Chicago Press, Chicago
Yoboué AN, Adepo-Gourene BA, Seka D, Durand JD, Panfili J, Lae R (2012) Genetic diversity and adaptability of *Sarotherodon melanotheron* (Cichlidae) in coastal ecosystem. Ethol Ecol Evol 24:230–243