



SHORT COMMUNICATION

# High gene flow promotes the genetic homogeneity of the fish goby *Pomatoschistus marmoratus* (Risso, 1810) from Mar Menor coastal lagoon and adjacent marine waters (Spain)

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## Keywords

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## Abstract

The extreme environmental variability of coastal lagoons suggests that physical and ecological factors could contribute to the genetic divergence among populations occurring in lagoon and open-coast environments. In this study we analysed the genetic variability of lagoon and marine samples of the sand goby, *Pomatoschistus marmoratus* (Risso, 1810) (Pisces: Gobiidae), on the SW Spain coast. A fragment of mitochondrial DNA control region (570 bp) was sequenced for 196 individuals collected in five localities: Lo Pagan, Los Urrutias and Playa Honda (Mar Menor coastal lagoon), and Veneziaola and Mazarrón (Mediterranean Sea). The total haplotype diversity was  $h = 0.9424 \pm 0.0229$ , and the total nucleotide diversity was  $\pi = 0.0108 \pm 0.0058$ . Among-sample genetic differentiation was not significant and small-scale patterns in the distribution of haplotypes were not apparent. Gene flow and dispersal-related life history traits may account for low genetic structure at a small spatial scale. The high genetic diversity found in *P. marmoratus* increases its potential to adapt to changing conditions of the Mar Menor coastal lagoon.

## Problem

Coastal lagoons are habitats exposed to wide environmental variations, particularly as regards salinity and temperature, which may cause strong selective pressures on organisms. These characteristics suggest that environmental factors directly modify the genetic patterns of species (González-Wangüemert *et al.* 2006, 2009) and could contribute to the genetic divergence among populations, associated with physical and ecological discontinuities between coastal lagoon and open-coast environments (Bilton *et al.* 2002; Iannotta *et al.* 2008).

The sand goby *Pomatoschistus marmoratus* (Risso, 1810), a small fish found inshore over sandy bottoms, is able to enter brackish and hyper-saline waters, with a larval pelagic duration of 2 weeks (Mazzoldi & Rasotto 2001). Several species of the genus *Pomatoschistus* show little or no migration behaviour (Gysels *et al.* 2004;

Berrebi *et al.* 2005) and have a limited swimming ability (Bardin & Pont 2002). There are few papers about *P. marmoratus* population genetics. Recently, Berrebi *et al.* (2009) analysed the genetic structure of two sedentary gobiids (*Pomatoschistus microps* Krøyer, 1838 and *P. marmoratus*) in several Mediterranean lagoons using RFLPs; they found that almost no common haplotypes are shared between populations of *P. microps* inhabiting neighbouring lagoons. Therefore, a high level of isolation is deduced for *P. microps* populations. However, the most common haplotype (BBBADBB) was shared between Vaccarès and Thau lagoons for *P. marmoratus* and the authors could not establish any genetic differentiation between lagoon samples. Research on other *Pomatoschistus* species population genetics using allozymes and microsatellites indicated genetic differentiation between estuarine, coastal and marine samples of *Pomatoschistus minutus* Pallas, 1770 (Stefanni *et al.* 1996; Pampoulie

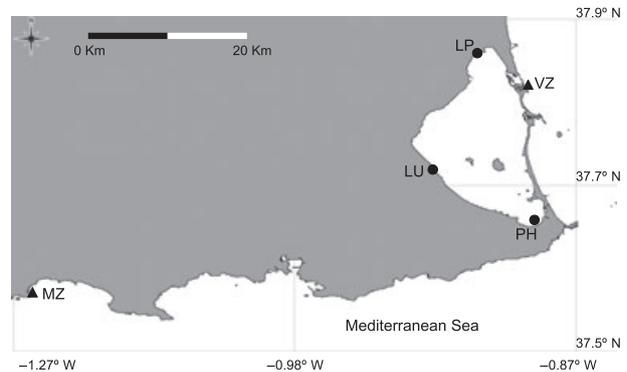
*et al.* 2004). This background suggests that *P. marmoratus* could be a good model to study lagoon micro-evolutionary processes: one could expect to find genetic differentiation between lagoon and marine populations and a higher genetic heterogeneity within the lagoon populations.

The Mar Menor is a shallow water coastal lagoon (3.5–6 m deep) with a surface about 135 km<sup>2</sup>, characterized by high salinity (38–51 psu) and extreme temperature changes (from >30 °C in summer to 10 °C in winter, Pérez-Ruzafa *et al.* 2005). It is located on the Southwestern Mediterranean coast and bordered on the seaward side by the La Manga sand bar, which is crossed by three inlets (El Estacio, Las Encañizadas and Marchamalo), which regulate water exchange with the open sea. This coastal lagoon is a physically stressed environment and a good location to look into genetic diversity and gene flow levels, and to test the influence of environmental conditions on the genetic composition of lagoon populations.

Two main questions are raised with the present study: (i) Is gene flow between lagoon and marine waters sufficient to prevent genetic divergence of these areas? (ii) Is the genetic diversity higher in ecologically variable lagoon populations? To find answers to these questions, the genetic variation in the population of the sand goby *Pomatoschistus marmoratus* from the Mar Menor coastal lagoon (SW Spain) and adjacent marine locations was investigated using mitochondrial DNA control region sequences, which have been demonstrated to be informative for population level studies in other *Pomatoschistus* species (Stefanni *et al.* 2002; Stefanni & Thorley 2003).

## Material and Methods

Adult specimens were collected at three Mar Menor coastal lagoon localities (Lo Pagan, Playa Honda and Los Urrutias) and two marine sites (Veneziola and Mazarrón, Mediterranean Sea) (Fig. 1). The tissue was dissolved using lysis buffer solution with proteinase K, and total genomic DNA was isolated by protein precipitation and final precipitation with ethanol (Sambrook *et al.* 1989). A fragment of the control region of mitochondrial DNA was amplified using primers UG-PROA (5'-AACTCCCRCCCCTGGYCCCAAAGCCAGCATT-3') and MIN-FB (5'-TCAAAATTGATGGTAAAGTCAGGAC-3') (Stefanni *et al.* 2002). A double-stranded DNA of D-loop was PCR synthesized in 25- $\mu$ l reactions containing 2.5  $\mu$ l amplification buffer (10 $\times$ ), 2.5  $\mu$ l each of the above primers (10  $\mu$ M), 1.0  $\mu$ l MgCl<sub>2</sub> (50 mM), 0.5  $\mu$ l of dNTP mix (25 mM) and 1 U Taq polymerase (Ecogen), 1  $\mu$ l DNA and purified water to complete the final volume. Amplification proceeded with an initial denaturation at 94 °C for 1 min, then 35 cycles of denaturation at 94 °C for 1 min, annealing at 48 °C for 1 min, and extension at 72 °C for



**Fig. 1.** Lagoon and marine localities of *Pomatoschistus marmoratus* sampled for this study. LP, Lo Pagán; LU, Los Urrutias; VZ, Veneziola; PH, Playa Honda; MZ, Mazarrón; Black circles, lagoon locations; Black triangles, marine locations.

1 min followed by a final extension at 72 °C for 3 min. A 5- $\mu$ l sample of each PCR product was run on 2% agarose gel stained with ethidium bromide before being viewed under UV light. A 2.5- $\mu$ l volume of each amplified product was purified using ExoSAP-IT (Amersham Pharmacia Biotech) and sequenced according to the protocols of the University of Murcia Molecular Biology Service (<http://www.um.es/sai/servicios/molecular.php>; Murcia, Spain), using an ABI Prism 3130 automated genetic analyser (Applied Biosystems). The sequences were aligned using the BIOEDIT software (Hall 1999).

Genetic heterogeneity tests using fixation indices ( $F_{ST}$ ) and the analysis of molecular variance (AMOVA) considering two groups, coastal lagoon (Lo Pagan, Playa Honda, Los Urrutias) and marine (Veneziola, Mazarrón), were performed using ARLEQUIN 2000 (Schneider *et al.* 2000). A statistical parsimony network of haplotypes was built with TCS software (Crandall & Templeton 1993). Neutrality tests, Tajima's D (Tajima 1989) and Fu's  $F_s$  (Fu 1997), and mismatch distribution analyses (Rogers & Harpending 1992) were carried out using ARLEQUIN 2000 (Schneider *et al.* 2000) to infer population expansion events and to test the deviations from a strictly neutral model of evolution. We carried out a maximum likelihood approach to calculate the effective population sizes ( $\Theta$ ) and asymmetrical migration rates between samples. For this analysis, we used the computer program MIGRATE 3.0.3 (Beerli & Felsenstein 2001; <http://popgen.scs.fsu.edu>).

## Results

The control region of mtDNA was amplified for 196 individuals and obtained sequences were edited to fragments of 570 bp. All sequences were deposited at GenBank

**Table 1.** Molecular diversity indices for populations of *Pomatoschistus marmoratus* using 570 bp of mtDNA control region (D-loop) based on Kimura's (1980) 2-parameter substitution model.

Locations	Sample size	Haplotypes	Singletons	Polymorphic sites	Haplotype diversity (h)	Nucleotide diversity ( $\pi$ )
Lo Pagan (lagoon)	40	22	12	60	0.9462 $\pm$ 0.0190	0.0178 $\pm$ 0.0092
Playa Honda (lagoon)	37	20	6	20	0.8979 $\pm$ 0.0399	0.0069 $\pm$ 0.0039
Los Urrutias (lagoon)	43	27	13	37	0.9435 $\pm$ 0.0236	0.0108 $\pm$ 0.0058
Veneziola (marine)	41	25	11	21	0.9634 $\pm$ 0.0146	0.0079 $\pm$ 0.0044
Mazarron (marine)	35	23	15	39	0.9613 $\pm$ 0.0177	0.0108 $\pm$ 0.0058
Total	196	77	57	91	0.9424 $\pm$ 0.0229	0.0108 $\pm$ 0.0058

(accession numbers GQ265703 to GQ265779). The overall mean values of haplotype and nucleotide diversity were  $h = 0.9424 \pm 0.0229$  and  $\pi = 0.0108 \pm 0.0058$ , respectively. In general, the sequence data were characterized by low nucleotide and high haplotype diversity (Table 1). An AMOVA was performed to test the null hypothesis that haplotypes were randomly distributed among populations, considering two groups (Mar Menor coastal lagoon and adjacent Mediterranean sites). There were non-significant differences among groups or among populations within groups. The highest proportion of the total variance was attributed to differences within sampled locations (98.12%;  $P < 0.05$ ), 0.96% of the variation was attributed to differences among groups (coastal lagoon–marine) but was not significant ( $P > 0.05$ ), and 0.92% was distributed among sampled locations.  $F_{ST}$  values based on comparisons of sample pairs revealed non-significant differences ( $P > 0.05$ ) between coastal lagoon (Lo Pagan, Playa Honda, Los Urrutias) and marine (Veneziola, Mazarron) samples (Table 2).

We detected a total of 77 haplotypes for all sampling locations; 20 were shared haplotypes and 57 were singletons, of which 31 haplotypes were only present in the lagoon samples and 26 haplotypes only in marine localities. The statistical parsimony network of haplotypes showed a very complex structure with several ambiguous connections and a small number of shared haplotypes (CR-5, CR-9, CR-12) between all sampling locations (data not shown). Given the absence of significant population differentiation, we pooled all individuals (196 sequences)

to construct a mismatch distribution diagram (Fig. 2). The dataset exhibited non-significant SSD values (SSD = 0.003;  $P = 0.297$ ), significant Tajima's  $D$  ( $D = -1.827$ ;  $P = 0.004$ ) and significant Fu's  $F_s$  value ( $F_s = -24.800$ ;  $P = 0.000$ ), which corroborated a sudden population expansion.

The estimation of effective population sizes indicated the lowest value for Playa Honda and the highest values for Lo Pagan and Los Urrutias (Table 3). Migration rates showed a predominant flow from Playa Honda to the rest of localities and nearly nonexistent flow from Mazarron to Veneziola ( $3.52e-11$ ) and from Lo Pagan to Playa Honda ( $9.36e-14$ ).

## Discussion

The study of control region (mtDNA) from lagoon and marine *Pomatoschistus marmoratus* samples detected high genetic diversity, high gene flow and no population differentiation between lagoon and marine locations. These characteristics could be explained by the dispersion during 2 weeks of the pelagic larvae, which could allow displacements of about 200 km with the current speed at this zone (0.2–0.6 knots). Our results agree with previous work carried out using the control region in *Pomatoschistus minutus* (Stefanni & Thorley 2003) and a fragment of mtDNA (including the D-loop and cytb regions) in *P. marmoratus* (Berrebi *et al.* 2009) where significant genetic differentiation between populations is not detected. However, other authors have described genetic

**Table 2.** Pairwise fixation indices ( $F_{ST}$ ) between five *Pomatoschistus marmoratus* samples based on mtDNA control region (D-loop) sequences ( $F_{ST}$  values, below diagonal) and geographic distances (km) between locations (above diagonal).  $P$  is the probability that any random value obtained after 1000 permutations is greater than the observed value.

	Lo Pagan	Playa Honda	Los Urrutias	Veneziola	Mazarron
Lo Pagan (lagoon)		20.725	14.682	4.946	86.561
Playa Honda (lagoon)	0.0053		10.495	16.682	59.548
Los Urrutias (lagoon)	0.0005	-0.0034		12.810	76.251
Veneziola (marine)	0.0108	0.0133	0.00003		71.035
Mazarron (marine)	0.0013	0.0149	0.0039	0.0121	

**Table 3.** Effective population size ( $\Theta = Ne\mu$ ) and migration rates ( $m/\mu = Nm/\Theta$ ) calculated for mitochondrial DNA control region (D-loop) of *Pomatoschistus marmoratus*.

Location	$\Theta$	Migration rates				
		Mazarron to	Lo Pagan to	Los Urrutias to	Veneziola to	Playa Honda to
Mazarron (marine)	0.0344		250.33	58.121	434.39	415.80
Lo Pagan (lagoon)	0.0500	237.45		562.12	84.226	908.78
Los Urrutias (lagoon)	0.0531	209.91	227.32		30.506	498.91
Veneziola (marine)	0.0167	3.52e-11	256.80	223.76		487.66
Playa Honda (lagoon)	0.0115	27.505	9.36e-14	40.799	132.88	

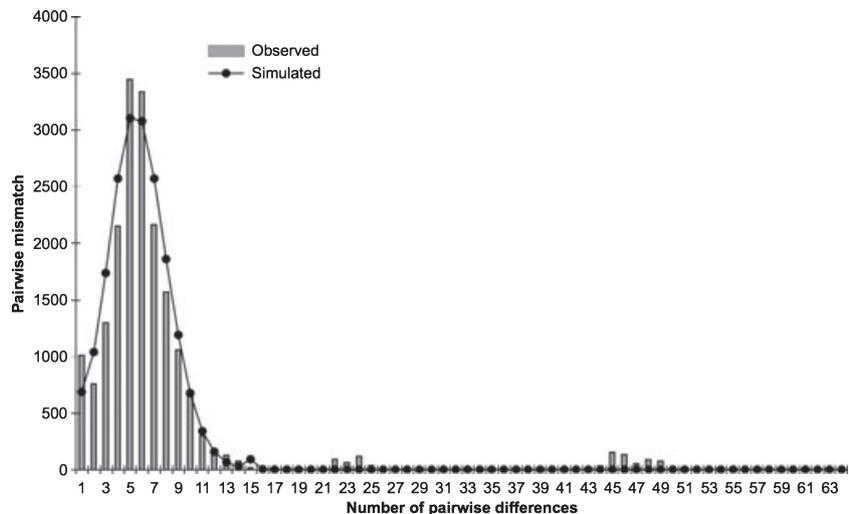
divergences between brackish and marine populations of *P. minutus* (Stefanni *et al.* 1996; Pampoulie *et al.* 2004) using allozymes and microsatellite markers. These different results in *P. minutus* could be explained by the use of non-neutral markers (allozymes) and a different geographic scale.

*Pomatoschistus marmoratus* from Mar Menor shows high values of genetic diversity, despite the fact that the lagoon environment is very unstable and susceptible to demographic fluctuations. This result is consistent with high gene flow rates, which can reduce inbreeding depression and damp the genetic drift promoted by extreme environmental variability of the lagoon (Garant *et al.* 2007). Therefore, it is acceptable to assume that gene flow between lagoon and open sea might promote the maintenance of a variable lagoon population even if population reductions/expansions occur.

Effective population sizes calculated for the lagoon and marine locations are consistent with a population bottleneck on lagoon and marine sides. We found lower  $\Theta$  values in marine locations than in lagoon locations except for the Playa Honda sample. The effect of a bottleneck appears to have been much more severe in Playa Honda

(where haplotype diversity and  $\Theta$  values are low) than in the other lagoon locations (where  $\Theta$  values were relatively high compared to the marine locations). We found evidence of greater gene flow from the lagoon (mainly Playa Honda) to marine samples. One scenario that could corroborate this pattern is based on the shared haplotypes: three haplotypes (CR-5, CR-9, CR-12) were shared between all locations but occurred with greater frequency in lagoon locations than in marine samples.

We found a high frequency of exclusive haplotypes in both lagoon and marine samples. Some authors (Avice *et al.* 1984; Ramos-Onsins & Rozas 2002) consider that singletons and recent mutations could be caused by a population growth event such as verified for our data using SSD, Tajima's D and Fu's F. As mentioned above, gene flow between marine and lagoon populations ensures high genetic diversity inside lagoon with an elevated environmental complexity that increases the adaptive potential of the species (Iannotta *et al.* 2008). In this context, the occurrence of exclusive haplotypes coupled with the existence of high gene flow between marine and lagoon samples could be an ecological survival strategy in a harsh environment such as the Mar Menor coastal lagoon.



**Fig. 2.** Pairwise mismatch distributions of mtDNA control region haplotypes for *Pomatoschistus marmoratus* sampled locations. All marine and coastal lagoon samples were pooled.

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