Microsatellite analysis of red mullet *Mullus barbatus* (Perciformes, Mullidae) reveals the isolation of the Adriatic Basin in the Mediterranean Sea

Teresa Maggio, Sabrina Lo Brutto, Flavio Garoia, Fausto Tinti, and Marco Arculeo


The red mullet *Mullus barbatus* is commercially one of the most important demersal fish resources in the Mediterranean. Molecular data on its genetic population structure throughout the Mediterranean are reported. Six microsatellite loci displayed a high degree of expected heterozygosity and a high allele number per locus. The Hardy–Weinberg equilibrium test revealed an overall tendency towards heterozygote deficiency, probably caused by the admixture of various demes. Population differentiation was assessed by analysis of molecular variance (AMOVA) and Bayesian analysis. AMOVA showed that most of the variation was within the population, but the mean value of $F_{ST}$ was significant, indicating genetic differentiation among the samples analysed. This differentiation is primarily attributable to the isolation of the Adriatic samples and partly to a weaker substructuring of the populations in the Gulf of Lions, Tyrrenian Sea, Strait of Sicily, and Ionian Sea. Bayesian analysis also revealed genetic differentiation among the samples analysed, identifying two genetic clusters. The restricted gene flow from and to the Adriatic, also recorded for other fish species, most likely reflects the environmental separation of the Adriatic and suggests that management protocols for the red mullet in the Mediterranean should be revisited.

**Keywords:** microsatellite, *Mullus barbatus*, population structuring.

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**Introduction**

Studies of population genetics on marine organisms, which employ variable and discriminatory genetic markers such as microsatellites, have often refuted the genetic homogeneity in fish species, which have a pelagic life phase. Much experimental evidence has contributed to highlighting the fact that biological factors, physico-chemical factors, and characteristics relating to life history can act singly or in combination in determining the genetic structure of marine species (Waples, 1987; McMillan and Palumbi, 1995; Shulman and Bermingham, 1995; Patarnello et al., 2007).

Biological and physico-chemical factors contribute to defining patterns of genealogical concordance (*sensu* Avise, 1996) among species co-distributed in the same area, and to detecting marine barriers to gene flow. The Mediterranean Sea is an area within which evidence of breakpoints in gene flow has been demonstrated widely in the Almeria-Oran Front, the Siculo-Tunisian Strait, and the Peloponnesse region (Bahri-Sfar et al., 2000; Patarnello et al., 2007; Pérez-Losada et al., 2007). Less evidence of population isolation in the Adriatic has been detected to date, although its specific hydrological and oceanographic characteristics, such as depth, temperature, and salinity, are well known (Astraldi et al., 1999).

One of the marine species distributed throughout the Mediterranean, but not yet well studied in terms of gene flow from and to the Adriatic, is the red mullet, *Mullus barbatus*. It is a demersal fish, belonging to the family Mullidae, distributed in the eastern Atlantic from the British Isles to Senegal, as well as in the Mediterranean. It prefers sandy/muddy substrata from 10 to 300 m deep, where it feeds using its sensory appendices. It spawns in spring, producing pelagic eggs; the juveniles are also pelagic, and they feed at the surface until they attain adulthood. From pelagic juvenile to adult, red mullet move from the surface to deeper water (10–300 m), then remain benthic (Tortonese, 1975).

The red mullet is commercially one of the most important demersal fish resources in the Mediterranean, annual catches of almost 4000 t being made (Mamuris et al., 1998a). Its catch is seasonal, probably a consequence of variable effort at different times of the year, as reported for several areas of the Mediterranean (Pipitone et al., 2000, and references therein). The disappearance of red mullet from fishing grounds also has been explained in the past by the offshore migration of adults (Machias and Labropoulou, 2002).

As a direct consequence of its economic importance, the species has been studied for many years, but mainly in terms of its
reproduction, ethology, and fisheries (Jukić, 1972; Demestre et al., 1997). Some studies analysed the genetic variation in *M. barbatus* and *Mullus surmuletus*, identifying diagnostic loci between the two species using allozymes (Basaglia and Callegarini, 1988; Cammarata et al., 1991; Mamuris et al., 1998a, 2001). In terms of its genetic population structure, Arculeo et al. (1999) used an allozyme analysis of red mullet throughout the Mediterranean to demonstrate a lack of heterogeneity. Mamuris et al. (1998a, 1998b, 2001) focused on comparing allozymes, mtDNA, RFLP, and RAPD analysis of several samples collected in the eastern Mediterranean (Ionian Sea and Aegean Sea) with samples from Gulf of Lions, but their results of genetic population structure were discordant for the different molecular markers.

Garoia et al. (2004) developed six microsatellite loci to advance a hypothesis of panmixia of red mullet in the Adriatic, revealing a subtle but significant genetic differentiation. However, more data were needed to assess the population structure and the extent of isolation on a larger geographical scale throughout the Mediterranean.

Here, data on the genetic population structure of *M. barbatus* are reported from the Gulf of Lions, the Tyrrhenian Sea, the Strait of Sicily, and the Ionian Sea (FAO areas N37.1 and 37.2), and compared with four Adriatic samples (FAO area N37.2) previously analysed by Garoia et al. (2004). Data on genetic population structure can assist in detecting a potential gene-flow barrier within the Mediterranean, and they can act as a tool for formulating management programmes for stock assessment and possibly modify fishery regulations within subareas of the Mediterranean, as outlined by the FAO.

**Material and methods**

In all, 400 fish were collected from November 2002 to August 2003 from the following Mediterranean sub-basins: Gulf of Lions, Tyrrhenian Sea, Strait of Sicily, and Ionian Sea (see Figure 1 and Table 1 for details). A further 186 fish originating in the northern and southern Adriatic Sea (AD1–AD4), previously analysed by Garoia et al. (2004), were also included in the analysis (Figure 1 and Table 1). Individual PCR amplicons of newly collected red mullet from the Gulf of Lions, the Tyrrhenian Sea, the Strait of Sicily, and the Ionian Sea were analysed in the same laboratory and under the same conditions used by Garoia et al. (2004) for the Adriatic samples. Muscle or fin-clip samples were preserved in 70% ethanol until DNA extraction. Genomic DNA was extracted from 25 to 50 mg of tissue, using a saline procedure or a DNeasy tissue kit (Qiagen). Six microsatellite loci (*Mb6*, *Mb7*, *Mb15*, *Mb26*, *Mb31*, and *Mb39*) were amplified, using primers developed for the same species by Garoia et al. (2004). The PCR reactions were performed on a Perkin Elmer Cetus Thermal cycler in a 10 μl solution, containing 20 ng of genomic DNA, 0.2 μM of dNTPs, 0.5 μM of primer, 10 mM of buffer 10/2C, 1.5–2.5 mM of MgCl2, and 1 unit of Perkin Elmer Taq polymerase. The reaction profile was as follows: initial denaturation at 94°C for 2 min, 30 cycles of denaturation at 94°C for 1 min, annealing at 52°C for 45 s, extension at 72°C for 2 min, and a final extension at 72°C for 10 min. The fish were genotyped by assessing the allele size on an ABI310 automated genetic analyser (Applied Biosystems), using forward primers labelled with 6-FAM, HEX, TAMRA (MWG Biotech), and ROX 400 (Applied Biosystems) as internal standards. Allele sizing was performed using GeneScan Analysis software 2.02 (Applied Biosystems).

**Population genetic analysis**

Statistical analyses were performed using the GENETIX 4.01 (Belkhir et al., 2000), GENEPOP 3.2 (Raymond and Rousset, 1995), and Arlequin 3.1 (Excoffier et al., 2005) packages. Genetic polymorphism was measured as the number of alleles per locus, and as observed and expected heterozygosity for each locus, individually and as a multilocus estimate at each of the 14 locations. Population differentiation was analysed using Wright’s *FST*...
Table 1. Site location and corresponding FAO geographical subarea of Mullus barbatus samples.

<table>
<thead>
<tr>
<th>Mediterranean sub-basin</th>
<th>Location</th>
<th>Figure 1 reference</th>
<th>FAO geographical subarea</th>
<th>Coordinates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gulf of Lions</td>
<td>Sete</td>
<td>GL1</td>
<td>Gulf of Lions 37.1</td>
<td>43°14'N 04°10'E</td>
</tr>
<tr>
<td>Tyrrenian Sea</td>
<td>Genova</td>
<td>TIR1</td>
<td>Ligurian and North Tyrrhenian 37.1</td>
<td>44°05'N 08°47'E</td>
</tr>
<tr>
<td></td>
<td>Alghero</td>
<td>TIR2</td>
<td>Sardinia 37.1</td>
<td>40°28'N 08°08'E</td>
</tr>
<tr>
<td></td>
<td>S. Agata di Milirello</td>
<td>TIR3</td>
<td>South and central Tyrrhenian 37.1</td>
<td>38°05'N 14°37'E</td>
</tr>
<tr>
<td></td>
<td>Porticello</td>
<td>TIR4</td>
<td>South and central Tyrrhenian 37.1</td>
<td>38°03'N 13°35'E</td>
</tr>
<tr>
<td></td>
<td>Castellammare del Golfo</td>
<td>TIR5</td>
<td>South and central Tyrrhenian 37.1</td>
<td>38°09'N 12°55'E</td>
</tr>
<tr>
<td>Strait of Sicily</td>
<td>Tunisi</td>
<td>SS1</td>
<td>Northern Tunisia 37.1</td>
<td>37°30'N 08°40'E</td>
</tr>
<tr>
<td></td>
<td>Licata</td>
<td>SS2</td>
<td>South Sicily 37.1</td>
<td>36°49'N 13°59'E</td>
</tr>
<tr>
<td></td>
<td>Siracusa</td>
<td>SS3</td>
<td>South Sicily 37.1</td>
<td>36°28'N 15°18'E</td>
</tr>
<tr>
<td>Ionian Sea</td>
<td>Catania</td>
<td>IO1</td>
<td>Western Ionian Sea 37.2</td>
<td>37°24'N 15°29'E</td>
</tr>
<tr>
<td>Adriatic Sea</td>
<td>Rimini</td>
<td>AD1</td>
<td>Northern Adriatic 37.2</td>
<td>44°10'N 12°47'E</td>
</tr>
<tr>
<td></td>
<td>Fano</td>
<td>AD2</td>
<td>Northern Adriatic 37.2</td>
<td>43°54'N 13°09'E</td>
</tr>
<tr>
<td></td>
<td>Bari</td>
<td>AD3</td>
<td>Southern Adriatic 37.2</td>
<td>41°20'N 17°20'E</td>
</tr>
<tr>
<td></td>
<td>Albania</td>
<td>AD4</td>
<td>Southern Adriatic 37.2</td>
<td>41°49'N 18°45'E</td>
</tr>
</tbody>
</table>

Results

The genetic variation at six microsatellite loci was high (Table 2). The unbiased mean expected heterozygosity ranged from 0.528 (locus Mb6, TIR2) to 0.968 (locus Mb39, TIR3), and observed heterozygosity ranged from 0.342 (locus Mb15, TIR3) to 0.957 (locus Mb31, AD2). The total number of alleles per locus ranged from 16 (locus Mb7) to 69 (locus Mb39); the mean number of alleles per locus was 12.48, with a range of 6.8–14.8. Pairwise comparisons among six loci for all 14 locations revealed no significant linkage disequilibrium.

In all, 38 of 84 single-locus tests of genotype revealed a significant departure from the Hardy–Weinberg equilibrium. After applying a Bonferroni correction, 18 single-locus tests proved to be significant (p < 0.0005), all as a consequence of heterozygote deficiencies (Table 2). Heterozygote deficiency can also support the FIS value, the global value of which was estimated as 0.204 (p < 0.001). Across the loci, estimator FIS varied from 0.090 to 0.258 and was highly significant for all loci (data not shown).

An estimate of the null allele presence, using the estimators of Chakraborty et al. (1992) and Brookfield (1996), and to check scoring errors (Wattier et al., 1998).

The population structure among samples was analysed using a Bayesian approach implemented in the STRUCTURE software (Pritchard et al., 2000). That software delineates clusters of individuals (K) based on their genotypes at multiple loci, using a Bayesian approach. The true number of clusters, K, is identified as that with the highest posterior probability [P(X/K)]. As an increase in the probability value can lead to overestimation of the number of genetic clusters, we used the method suggested by Evanno et al. (2005) to obtain the true value of the cluster, searching the modal values in the ΔK graph as a function of K. ΔK measures the rate of change in the logarithm (log) probability between different K values, and the modal value of the distribution coincides with that of the real K (Evanno et al., 2005).

The genetic structure of samples was also assessed by a hierarchical analysis of molecular variance (AMOVA; Excoffier et al., 1992), performed by imposing five different clusters. Groups were defined by pooling samples in the geographical subareas defined by the FAO, and pooling samples in the genetic clusters identified by Bayesian analysis. F-statistics were calculated from genotypic frequencies, partitioning the molecular variance among groups (FCT), among populations within groups (FSC), and within populations (FST).

GENECALL (Piry et al., 2004) software was used to detect the first generation of migrants. We used a frequency-based method (Paetkau et al., 2004), and the probability was computed by Monte Carlo resampling. We also used STRUCTURE software to test for migration. In the latter case, migrants were identified by Bayesian-based methodology, using population information in the input file, and migrants were detected as individuals with a posterior probability value higher at a sampling site than at their known origin.

(Wright, 1951); global FST and pairwise FST were estimated concurrently with a Fisher exact test for homogeneity of allelic frequencies. Principal component analysis (PCA) was used to produce a visual representation of differentiation, using the genetic distance matrix generated for co-dominant data by GenAlEx 6.1 (Peakall and Smouse, 2006). The software MICROCHECKER (van Oosterhout et al., 2004) was used to test the null allele frequency in our dataset, using the estimators of Chakraborty et al. (1992) and Brookfield (1996), and to check scoring errors (Wattier et al., 1998).
Table 2. Summary of genetic variation at six microsatellite loci at 14 locations for *M. barbatus*.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Parameter</th>
<th>GL1</th>
<th>TIR1</th>
<th>TIR2</th>
<th>TIR3</th>
<th>TIR4</th>
<th>TIR5</th>
<th>SS1</th>
<th>SS2</th>
<th>SS3</th>
<th>IO1</th>
<th>AD1</th>
<th>AD2</th>
<th>AD3</th>
<th>AD4</th>
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<tbody>
<tr>
<td>Mb6</td>
<td>n</td>
<td>24</td>
<td>20</td>
<td>20</td>
<td>41</td>
<td>45</td>
<td>59</td>
<td>22</td>
<td>70</td>
<td>43</td>
<td>51</td>
<td>58</td>
<td>47</td>
<td>48</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>H*</td>
<td>0.762</td>
<td>0.600</td>
<td>0.500</td>
<td>0.676</td>
<td>0.454</td>
<td>0.500</td>
<td>0.772</td>
<td>0.657</td>
<td>0.476</td>
<td>0.568</td>
<td>0.724</td>
<td>0.617</td>
<td>0.687</td>
<td>0.727</td>
</tr>
<tr>
<td>Mb7</td>
<td>n</td>
<td>23</td>
<td>20</td>
<td>19</td>
<td>42</td>
<td>44</td>
<td>60</td>
<td>23</td>
<td>68</td>
<td>43</td>
<td>51</td>
<td>58</td>
<td>47</td>
<td>48</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>H*</td>
<td>0.565*</td>
<td>0.800</td>
<td>0.833</td>
<td>0.707</td>
<td>0.778</td>
<td>0.667</td>
<td>0.772</td>
<td>0.742</td>
<td>0.395*</td>
<td>0.843</td>
<td>0.810</td>
<td>0.787</td>
<td>0.875</td>
<td>0.788</td>
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<tr>
<td>Mb15</td>
<td>n</td>
<td>24</td>
<td>20</td>
<td>20</td>
<td>42</td>
<td>45</td>
<td>60</td>
<td>23</td>
<td>68</td>
<td>41</td>
<td>53</td>
<td>58</td>
<td>47</td>
<td>47</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>H*</td>
<td>0.667</td>
<td>0.700</td>
<td>0.722</td>
<td>0.342*</td>
<td>0.667</td>
<td>0.685</td>
<td>0.727</td>
<td>0.564*</td>
<td>0.625*</td>
<td>0.640</td>
<td>0.672</td>
<td>0.638</td>
<td>0.750</td>
<td>0.848</td>
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<tr>
<td>Mb26</td>
<td>n</td>
<td>24</td>
<td>20</td>
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<td>58</td>
<td>47</td>
<td>48</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>H*</td>
<td>0.818</td>
<td>0.550*</td>
<td>0.833</td>
<td>0.414*</td>
<td>0.659</td>
<td>0.533*</td>
<td>0.800</td>
<td>0.778</td>
<td>0.643</td>
<td>0.736</td>
<td>0.810</td>
<td>0.766</td>
<td>0.708</td>
<td>0.848</td>
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<td>Mb31</td>
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<td>47</td>
<td>48</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>H*</td>
<td>0.783</td>
<td>0.947</td>
<td>0.833</td>
<td>0.595*</td>
<td>0.704</td>
<td>0.728</td>
<td>0.739</td>
<td>0.851</td>
<td>0.643</td>
<td>0.837</td>
<td>0.827</td>
<td>0.957</td>
<td>0.875</td>
<td>0.848</td>
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<td>Mb39</td>
<td>n</td>
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<td>58</td>
<td>47</td>
<td>48</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>H*</td>
<td>0.591*</td>
<td>0.600*</td>
<td>0.889</td>
<td>0.486*</td>
<td>0.475*</td>
<td>0.463*</td>
<td>0.900</td>
<td>0.838*</td>
<td>0.345*</td>
<td>0.750</td>
<td>0.845</td>
<td>0.723*</td>
<td>0.854</td>
<td>0.757*</td>
</tr>
</tbody>
</table>

n, number of individuals; AS, allele size; H*, observed heterozygosity; H* expected heterozygosity.

*Test significant (p < 0.0005) after the Bonferroni correction.
The likelihood of the data was lowest at $K = 1$ ($\log = -16.828$), indicating a lack of genetic homogeneity, but two modes of $\Delta K$ were observed, for $K = 2$ ($\log = -15.287$) and $K = 5$ ($\log = -14.438$). The average proportion of membership ranged from $q = 0.422$ to $q = 0.988$ (Table 4). For $K = 2$, AD samples grouped in one cluster, and GL, TIR, SS, and IO samples formed the other cluster. When $K = 5$, AD samples grouped in a cluster with a large proportion of membership, and GL, TIR, SS, and IO samples were distributed in the other four clusters, devoid of any geographical correspondence (Table 4).

An AMOVA was performed in different ways, clustering samples together, clustering samples following a geographical criterion according to FAO geographical subareas (Table 1), including and excluding the Adriatic samples (analyses N2 and N3), and by clustering the samples following a genetic criterion according to the STRUCTURE results (analyses N4 and N5). The partitioning of genetic variance among and within the 14 populations, calculated as $F$-statistics, yielded a mean $F_{ST}$ value of 0.003 (Table 5).

According to the second analysis, there were significant differences among groups ($F_{CT} = 0.0018; p < 0.001$), among populations within groups ($F_{SC} = 0.0011; p < 0.001$), and within populations ($F_{ST} = 0.0029; p < 0.001$). In analysis N3 (AD samples excluded), there were no significant differences among groups ($F_{CT} = -0.0003; p > 0.001$), but among populations within groups and within populations, fixation indices were significantly different from zero. With $K = 2$, analysis N4 confirmed a significant difference between the AD samples and the others. Analysis N5, with a genetic cluster of $K = 5$, revealed that 99.7% of the genetic variation resided within populations, 0.12% was distributed among populations within the cluster, and 0.22% depended on differences among clusters. All fixation indices were statistically significant, although the proportion of genetic variation was small (Table 5).

The putative migrants identified by the assignment-based approaches are listed in Table 6. A Bayesian-based assignment test identified fewer migrants than the frequency-based method of GENECLASS owing to the different threshold value used; various fish were placed in the same sampling region, even if the probability value $q$ was low ($q < 0.90$). Those samples represented fish with mixed or ambiguous ancestry, and some were identified as migrants by the assignment test conducted with GENECLASS.

The latter identified 25 migrants with an assignment probability of $<0.10$. Only eight fish were consistently identified as migrants by both tests, and three of these were assigned to the same source population.

### Discussion

#### Hardy–Weinberg departure

The levels of genetic variation observed in *M. barbatus* are similar to those identified in other marine teleosts, assayed by microsatellite markers (De Woody and Avise, 2000). The test for Hardy–Weinberg equilibrium revealed departures with an overall tendency to heterozygote deficiency. A deficit in heterozygote genotypes has been reported for many invertebrates (Zourous and Foltz, 1984; Shaw *et al.*, 1999) and fish (Waldman and McKinnon, 1993). This could generally be caused by null alleles, the Wahlund effect, or processes such as inbreeding and selection. The presence of null alleles (alleles that are not amplified because of the absence of a matching primer sequence) can be suspected for microsatellite analysis conducted with heterologous primers.
but it should not occur when the primers used have been identified from the species studied. Tests carried out with MICROCHECKER allowed us to exclude the presence of null alleles. The cases of inbreeding or mating among relatives were also rejected because no significant heterozygote deficiency was scored over all six loci. Therefore, the Wahlund effect should be the most consistent explanation for the observed heterozygote deficiency. This occurs when two or more genetically differentiated populations are inadvertently sampled as a single population.

For red mullet, an apparent deficit of heterozygotes could be caused by differences in the sampling period, given that there is regular seasonal movement of cohorts. Ontogenetic seasonal migrations of juvenile red mullet from shallow to deeper water and from inshore to offshore have been documented (Machias and Labropoulou, 2002). Such migrations might determine the mixing of different demes, and hence cause a sampling bias and a Wahlund effect. Life history characteristics such as ontogenetic migration, plus ecological factors, could have determined local and partial separation among the sample sites, supporting the hypothesis of the admixture of demes.

**Isolation of the Adriatic Sea**

The Bayesian approach detected a significant genetic structure, so confirming the results obtained from the assessment of divergence. Both methods displayed a clear difference between the Adriatic Sea (AD) and the other samples, i.e. from the Gulf of Lions (GL), the Tyrrhenian Sea (TIR), the Strait of Sicily (SS), and the Ionian Sea (IO). This difference was evident from the AMOVA results as well as from the PCA graph, in which AD samples represented a distinct group. The genetic differences between the AD and the GL, TIR, SS, and IO samples could be explained by a correlation of biological and ecological factors. First, the GL, TIR, SS, and IO populations of red mullet do not mix easily with the AD populations because of physical separation, determined by the topography of the land masses in the Otranto Strait, which connects the southern Adriatic with the western Ionian Sea (Figure 1). The area is crucial in defining circular currents of the upper water masses. Moreover, the Ionian Sea, the deepest part of the Mediterranean, could represent a bathymetric constraint to pelagic larvae, juvenile retention, and the adult migration of red mullet, as previously suggested by Mamuris et al. (1998a).

As a semi-closed area with its own characteristics (Astraldi et al., 1999) in term of salinity, temperature, and depth, the Adriatic Sea seems to have influenced the genetic pattern across fish species. Several studies have shown geographical partitioning across multiple co-distributed fish species. Bembo et al. (1996) found evidence of a different stock of anchovy Engraulis encrasicolus in the Adriatic, separate from adjacent areas. Arculio et al. (2003) demonstrated the existence of different distributions of allelic frequencies in the Adriatic and Tyrrhenian samples of two sparids, Diplodus vulgaris and Lithognatus mormyrus. Data on Sparus aurata collected by

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**Table 4. Bayesian clustering results of STRUCTURE, where K is the number of genetic clusters.**

<table>
<thead>
<tr>
<th>k</th>
<th>Log p (K/x)</th>
<th>Variance log p (K/x)</th>
<th>Proportion of membership of subpopulation cluster</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-16 828</td>
<td>51.8</td>
<td>1 2 3 4 5</td>
</tr>
<tr>
<td>2</td>
<td>-15 012</td>
<td>408</td>
<td>- - - - -</td>
</tr>
<tr>
<td>3</td>
<td>-14 567</td>
<td>921.7</td>
<td>- - - - -</td>
</tr>
<tr>
<td>4</td>
<td>-14 438</td>
<td>807.7</td>
<td>0.740 0.422 0.963 0.988 0.552</td>
</tr>
<tr>
<td>5</td>
<td>-15 193</td>
<td>1 571</td>
<td>- - - - -</td>
</tr>
<tr>
<td>6</td>
<td>-15 092</td>
<td>1 560</td>
<td>- - - - -</td>
</tr>
</tbody>
</table>

For each most probable value of K (2 or 5), the average proportion of membership, q, is reported. For K = 2, cluster 1 groups AD1, AD2, AD3, and AD4, and cluster 2 groups GL1, TIR1, TIR2, TIR3, TIR4, TIR5, SS1, SS2, SS3, and IO1. For K = 5, cluster 1 groups GL1, TIR2, and SS3, cluster 2 groups TIR1 and SS2, cluster 3 groups SS1, TIR4, and TIR5, cluster 4 groups AD1, AD2, AD3, and AD4, and cluster 5 groups TIR3 and IO1.
Table 5. Results of AMOVA conducted on 14 samples of *M. barbatus* from the Mediterranean Sea.

<table>
<thead>
<tr>
<th>Sample clustering</th>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Variance components</th>
<th>Variation (%)</th>
<th>Fixation indices</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. All together</td>
<td>Among populations</td>
<td>13</td>
<td>0.001</td>
<td>0.019</td>
<td><em>F_{ST} = 0.003</em></td>
</tr>
<tr>
<td></td>
<td>Within population</td>
<td>154</td>
<td>–</td>
<td>99.73</td>
<td></td>
</tr>
<tr>
<td>2. FAO geographical subareas</td>
<td>Among groups</td>
<td>4</td>
<td>0.0005Va</td>
<td>0.11</td>
<td><em>F_{CT} = 0.0018</em></td>
</tr>
<tr>
<td></td>
<td>Within groups</td>
<td>9</td>
<td>0.0009Vb</td>
<td>0.18</td>
<td><em>F_{SC} = 0.0011</em></td>
</tr>
<tr>
<td></td>
<td>Within populations</td>
<td>154</td>
<td>0.4978Vc</td>
<td>99.71</td>
<td><em>F_{ST} = 0.0029</em></td>
</tr>
<tr>
<td>3. FAO geographical subareas, excluding Adriatic samples</td>
<td>Among groups</td>
<td>2</td>
<td>–0.0002Va</td>
<td>–0.03</td>
<td><em>F_{CT} = 0.003</em></td>
</tr>
<tr>
<td></td>
<td>Within groups</td>
<td>7</td>
<td>0.0041Vb</td>
<td>0.83</td>
<td><em>F_{SC} = 0.0083</em></td>
</tr>
<tr>
<td></td>
<td>Within populations</td>
<td>786</td>
<td>0.4914Vc</td>
<td>99.20</td>
<td><em>F_{ST} = 0.0080</em></td>
</tr>
<tr>
<td>4. Genetic cluster K = 2</td>
<td>Among groups</td>
<td>1</td>
<td>0.0007Va</td>
<td>0.14</td>
<td><em>F_{CT} = 0.0014</em></td>
</tr>
<tr>
<td></td>
<td>Within groups</td>
<td>12</td>
<td>0.0010Vb</td>
<td>0.21</td>
<td><em>F_{SC} = 0.0021</em></td>
</tr>
<tr>
<td></td>
<td>Within populations</td>
<td>154</td>
<td>0.4978Vc</td>
<td>99.65</td>
<td><em>F_{ST} = 0.0035</em></td>
</tr>
<tr>
<td>5. Genetic cluster K = 5</td>
<td>Among groups</td>
<td>4</td>
<td>0.0011Va</td>
<td>0.22</td>
<td><em>F_{CT} = 0.0022</em></td>
</tr>
<tr>
<td></td>
<td>Within groups</td>
<td>8</td>
<td>0.0006Vb</td>
<td>0.12</td>
<td><em>F_{SC} = 0.0012</em></td>
</tr>
<tr>
<td></td>
<td>Within populations</td>
<td>1049</td>
<td>0.4978Vc</td>
<td>99.66</td>
<td><em>F_{ST} = 0.0034</em></td>
</tr>
</tbody>
</table>

Analysis 1, ungrouped samples; Analysis 2, samples grouped according to FAO geographical subareas (including the Adriatic Sea); Analysis 3, samples grouped according to FAO geographical subareas, but excluding the Adriatic Sea; Analyses 4 and 5, samples grouped according to the K values obtained from Bayesian analysis.

Fixation indices are *F_{CT}*, *F_{SC}*, and *F_{ST}*, among groups, populations within groups, and between populations, respectively.

*Fixation index significant (p < 0.001).

Rossi et al. (2006) also suggested a differentiation between samples taken from the Tyrrhenian Sea and the Adriatic Sea. Stefanni and Thorley (2003) have demonstrated the existence of an evolutionary significant unit in the Adriatic for *Pomatoschistus minutus*. Recently, Kristoffersen and Magoulas (2008) found significant differences between the Adriatic and the Tyrrhenian Seas for *E. encrasicus*, using different methods (introns and mtDNA analysis; otolith and body shape).

The isolation of Adriatic populations of *M. barbatus* was identified, despite opportunities for the genetic homogenization of the species, for instance, through the passive drift between areas of pelagic eggs and larvae, the pelagic movement of juveniles, and adult migration. However, differences in dispersal capability may affect gene flow, pelagic dispersal being influenced by ecological factors such as temperature, salinity, or currents. A common response to environmental factors seems to delineate a geographical barrier in the population structure of red mullet and other species, suggesting a scenario of limited gene flow attributable to limited adult migration and a reduced passive drift of pelagic larvae from and to the Adriatic Sea.

Although environmental factors cannot be excluded in shaping intraspecific genetic patterns, other causes strictly linked to the life history of the species need to be further researched. Recorded fluctuations in the population size of red mullet could support the differentiation between the AD and the GL, TIR, SS, and IO populations at microsatellite loci, because it has been demonstrated that a reduction in population size or a bottleneck can produce a change in the distribution of allele frequency and give rise to genetic differentiation among samples (Hedrick, 1999; Patarnello et al., 2007). Large fluctuations in the population size of *M. barbatus* have been reported. A disappearance of the stocks from fishing grounds in various areas of the Mediterranean from May to August has been well documented (Haidar, 1970), generally attributed to adults migrating offshore. These migrations could be linked to water temperature and reduced interspecific competition (Machias and Labropoulou, 2002). On the other hand, red mullet catches increased after a trailing closure; from this, it has been hypothesized that rapid stock recovery depends on characteristics regarding life history, such as the young age at first maturity (Pipitone et al., 2000).

**Differentiation among sites**

Our results also demonstrated that the Gulf of Lions, Tyrrhenian Sea, Strait of Sicily, and Ionian Sea samples cannot be considered as a single homogeneous population. Rather, the samples should be considered as a group of single demes locally and partially separated, in which each sample site seems to have subtle but significant differences. GL, TIR, SS, and IO heterogeneity was confirmed by Bayesian analysis with *K* = 5, with AD samples belonging to a single genetic cluster with a large proportion of membership, and GL, TIR, SS, and IO samples distributed in four separate genetic clusters with lower proportions of membership. The five genetic clusters obtained by Bayesian analysis are characterized by a different proportion of membership; three have *q* values < 0.90, showing admixture among SS1, SS2, and IO1 samples. The presence of two modes in the ΔK graph (*K* = 2 and 5) allowed us to hypothesize a model of isolation referred to as the “contact zone” by Evanno et al. (2005). The model implies relative genetic isolation among the two groups of populations when *K* = 2 (AD and GL, TIR, SS, and IO in our dataset), and occasionally also a pattern of isolation within each group (*K* = 5), for instance in the GL, TIR, SS, and IO groups. Another demonstration of local and partial separation among the GL, TIR, SS, and IO samples was derived from the PCA graph, in which the samples were not grouped as closely as the AD samples.
Table 6. Migrants of M. barbatus identified by two different assignments approaches.

<table>
<thead>
<tr>
<th>Migrant</th>
<th>Source population</th>
<th>Bayesian STRUCTURE assignment</th>
<th>GENECCLASS assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TIR1</td>
<td>–</td>
<td>TIR5</td>
</tr>
<tr>
<td>2</td>
<td>TIR2</td>
<td>AD4</td>
<td>SS1</td>
</tr>
<tr>
<td>3</td>
<td>TIR2</td>
<td>AD1</td>
<td>SS1</td>
</tr>
<tr>
<td>4</td>
<td>TIR2</td>
<td>AD1</td>
<td>AD1</td>
</tr>
<tr>
<td>5</td>
<td>TIR4</td>
<td>TIR6</td>
<td>TIR5</td>
</tr>
<tr>
<td>6</td>
<td>TIR4</td>
<td>–</td>
<td>AD4</td>
</tr>
<tr>
<td>7</td>
<td>TIR4</td>
<td>–</td>
<td>SS1</td>
</tr>
<tr>
<td>8</td>
<td>TIR5</td>
<td>–</td>
<td>IO1</td>
</tr>
<tr>
<td>9</td>
<td>TIR5</td>
<td>–</td>
<td>TIR1</td>
</tr>
<tr>
<td>10</td>
<td>TIR6</td>
<td>–</td>
<td>TIR1</td>
</tr>
<tr>
<td>11</td>
<td>TIR6</td>
<td>–</td>
<td>TIR4</td>
</tr>
<tr>
<td>12</td>
<td>TIR6</td>
<td>–</td>
<td>TIR5</td>
</tr>
<tr>
<td>13</td>
<td>TIR6</td>
<td>SS3</td>
<td>TIR5</td>
</tr>
<tr>
<td>14</td>
<td>TIR6</td>
<td>SS3</td>
<td>SS3</td>
</tr>
<tr>
<td>15</td>
<td>TIR6</td>
<td>–</td>
<td>SS1</td>
</tr>
<tr>
<td>16</td>
<td>SS3</td>
<td>IO1</td>
<td>TIR6</td>
</tr>
<tr>
<td>17</td>
<td>SS3</td>
<td>IO1</td>
<td>TIR3</td>
</tr>
<tr>
<td>18</td>
<td>SS3</td>
<td>TIR5</td>
<td>TIR6</td>
</tr>
<tr>
<td>19</td>
<td>SS3</td>
<td>SS2</td>
<td>–</td>
</tr>
<tr>
<td>20</td>
<td>SS3</td>
<td>TIR1</td>
<td>–</td>
</tr>
<tr>
<td>21</td>
<td>SS1</td>
<td>–</td>
<td>TIR6</td>
</tr>
<tr>
<td>22</td>
<td>SS2</td>
<td>–</td>
<td>TIR5</td>
</tr>
<tr>
<td>23</td>
<td>SS2</td>
<td>–</td>
<td>IO1</td>
</tr>
<tr>
<td>24</td>
<td>IO1</td>
<td>–</td>
<td>SS1</td>
</tr>
<tr>
<td>25</td>
<td>IO1</td>
<td>–</td>
<td>TIR5</td>
</tr>
<tr>
<td>26</td>
<td>IO1</td>
<td>–</td>
<td>SS1</td>
</tr>
<tr>
<td>27</td>
<td>AD1</td>
<td>–</td>
<td>TIR3</td>
</tr>
<tr>
<td>28</td>
<td>AD4</td>
<td>AD1</td>
<td>–</td>
</tr>
<tr>
<td>Total</td>
<td>putative migrants</td>
<td>11</td>
<td>25</td>
</tr>
</tbody>
</table>

Similar results for the two methods are emboldened.

Even in that case, as previously mentioned for the regional spatial scale, genetic differentiation on a finer scale can be explained by hydro-geographical and biological characteristics. *Mullus barbatus* is a demersal species, displaying a close relationship between habitat characteristics (depth, salinity, temperature, etc.) and life history. The active and passive movements of adults, juveniles, and larvae have been reported during the spawning, feeding, and recruitment periods. Larvae and juveniles are pelagic, and when they move they follow currents at the surface, but the adults inhabit deeper water (Haidar, 1970). Therefore, perhaps, local surface currents in various areas prevent larvae and juveniles from dispersing, causing a reduction in the gene flow and weak differentiation, detected on microsatellite loci. Moreover, adult *M. barbatus* inhabit a wide range of depths (10–300 m) and biotopes, and they can probably move over large areas and cover long distances. Consequently, the distribution pattern of red mullet can be one of continuous admixture.

Always, the dispersal capability has to be related to environmental conditions, such as currents, temperature, and salinity, and the extent of gene flow on a finer geographical scale is hard to predict *a priori* with a single molecular marker. Such a restricted geographical area needs to be investigated more thoroughly by referring also to the temporal scale; studies on other species, e.g. *Raja clavata* (Chevolot et al., 2008), identified a temporal variation in allele frequencies but a stable genetic diversity on a spatial scale, showing that patterns of population structure are not easy to describe.

**Fishery management perspectives**

The extent of gene flow between the Gulf of Lions, the Tyrrenian Sea, the Strait of Sicily, the Ionian Sea, and the Adriatic should be considered too low for the species to be managed as a single panmictic unit in terms of the Mediterranean stock complex. Failure to identify discrete populations has led elsewhere to the depletion of exploited fish species through the overexploitation of a single subpopulation. For this reason and to improve the management of this resource, prudence regarding the exploitation of red mullet should be exercised. Moreover, a more detailed understanding of the life history and ecology of the species, its spawning and nursery areas, adult movement patterns, and regional oceanography are necessary in supporting the management process.

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**References**


