Journal of Sea Research 68 (2012) 41–48

Contents lists available at SciVerse ScienceDirect

Journal of Sea Research

journal homepage: www.elsevier.com/locate/seares

Genetic considerations on the introduction of farmed fish in marine protected areas: The case of study of white seabream restocking in the Gulf of Castellammare (Southern Tyrrhenian Sea)

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ARTICLE INFO

Article history:
Received 27 July 2011
Received in revised form 18 November 2011
Accepted 14 December 2011
Available online 22 December 2011

Keywords:
Introgression
Biodiversity
Fisheries Management
Fishery Reserve
Microsatellites
Sparidae

ABSTRACT

Human exploitation has drastically reduced the abundance and distribution of several marine fish and invertebrate populations through overfishing and habitat destruction. Restocking can potentially mitigate these impacts and help to reconstitute depleted stocks but genetic repercussions must be considered. In the present study, the degree of genetic similarity between white seabream (*Diplodus sargus* Linnaeus 1758) individuals reared for restocking purposes and the receiving population in the Gulf of Castellammare fishery reserve (Sicily, Italy) was assessed using microsatellites. We also inferred the spatial pattern of the genetic structure of *D. sargus* and connectivity along Sicilian coasts. The farmed population showed significant heterozygosity deficiency in 6 loci and an important reduction in the number of alleles, which could indicate an incipient inbreeding. Both the farmed population and the target one for restocking (Castellammare fishery reserve), showed high and significant values of genetic differentiation due to different allele frequencies, number of privative alleles and total number of alleles. These findings indicate a low degree of genetic similarity between both populations, therefore this restocking initiative is not advisable. The genetic connectivity pattern, highly consistent with oceanographic currents, identified two distinct metapopulations of white seabream around Sicily. Thus it is recommended to utilize broods from the same metapopulation for restocking purposes to provide a better genetic match to the wild populations.

1. Introduction

Overfishing and habitat destruction have drastically reduced the abundance, size and spatial distribution of several marine fish and invertebrates, also leading to changes in the reproductive potential of populations. Fishery reserves and marine protected areas represent tools for ecosystem-based marine spatial management (EB-MSM) (Katsanevakis et al., 2011) and one of their main goals is to prevent the collapse of fish stocks (Sale et al., 2005; Teske et al., 2010). In parallel, restocking programmes are tools to recover stocks of commercially overexploited marine fish in several protected and non-protected areas (Bell et al., 2006, 2008; Gardner et al., 2010; Pereira et al., 2010; Stottrup and Sparrevohn, 2007; Travis et al., 1998). However it is important to stress the need of responsible approaches in restocking programmes thorough understanding of the ecology of the stock, monitoring and evaluation of the progress of released fish and assessment of potential biological and genetic impacts on wild fish populations (Gardner et al., 2010). Therefore, careful consideration is needed in restocking initiatives in order to ensure the maintenance of their pristine level of genetic structure and adaptedness to the local environment especially in marine managed areas (Stottrup and Sparrevohn, 2007). A common concern among fisheries undergoing restocking is the need for information about the genetic identity of the existing fish populations. The management of wild species should be directed at selecting suitable source stock so that relocated individuals are adapted to local environment conditions, and are not a threat to the genetic integrity of native populations (Latch et al., 2006).

In general, most restocking programmes utilize fishes from aquaculture facilities, which are usually sustained for many generations (D'Anna et al., 2004; Madeira et al., 2005). Such breeding programmes lead to changes in the genetic composition of farmed stocks over time. Genetic changes can occur even without the application of selective breeding programmes as a result of the effects of random genetic drift (Bekkevold et al., 2006). The magnitude of such drift is related to the genetically effective size of the breeding population, with more drift taking place in small populations (Bekkevold et al.,

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doi:10.1016/j.seares.2011.12.005

2006; Crow and Kimura, 1970). Hatcheries commonly use a small number of breeders compared with wild population sizes, and this results in cultured stocks loosing genetic variation at a higher rate and having lower genetic diversity compared with their wild counterparts, an effect known as genetic erosion (Alarcón et al., 2004; Evans et al., 2004; Exadactylos et al., 1999; Groeneveld et al., 2010; Hansen et al., 2001; Säisä et al., 2003). The amount of genetic variation in a breeding population has a direct relationship to its evolutionary potential, and populations with low genetic variability are less capable of responding adaptively to changing selection pressures (Bekkevold et al., 2006; González-Wangüemert and Pérez-Ruzafa, 2011; González-Wangüemert et al., 2009, 2011; Richards et al., 2010; Sanford and Kelly, 2011; Vergara-Chen et al., 2010). Therefore, it is necessary to have adequate knowledge on the genetic population structure before carrying out any restocking (Cross, 2000; Groeneveld et al., 2010; Lipcius et al., 2008; Pereira et al., 2010) and the identification of admixed individuals from hatcheries to evaluate the risk of biodiversity losses due to genetic homogenization (Blano-Aguiar et al., 2008).

In the Gulf of Castellammare (NW Sicily, Mediterranean Sea), several attempts have been made since the mid-1980s by the Sicilian Regional Government in order to rebuild the severely depleted stocks. These initiatives included the deployment of a large artificial reef (AR) system of about 20000 m^3 (Badalamenti et al., 2000) and the establishment of a 20000-ha fishery reserve (Pipitone et al., 2000). Under this framework, a project involving a release of reared white seabreams on artificial reefs was planned by a Consortium aggregating several municipalities in the Gulf of Castellammare. The source of reared fish was an aquaculture facility in the southeastern coast of Sicily (Acqua Azzurra S.P.A) and the target population for restocking was that of the Castellammare fishery reserve in the northwest coast of the island.

In the present work, genetic considerations related to fish restocking initiatives are explored through a case study: white seabreams reared for restocking the local population at Castellammare fishery reserve in Sicily (Italy). The specific objectives of this paper were (1) to assess the degree of genetic variability of cultivated stock of *Diplodus sargus* comparing it with that of geographically close wild stocks; (2) to assess the genetic diversity of receiving population, Castellammare fishery reserve; (3) to assess the degree of genetic similarity between the cultivated population (Acqua Azzurra) and the wild receiving population (Castellamare); (4) to infer the spatial pattern of genetic diversity in the Sicilian populations of white seabream to detect the best option to restocking at Castellamare fishery reserve and to improve the management of *D. sargus* stocks in Sicily.

2. Materials and methods

2.1. Study species

The white seabream, *D. sargus* (Linnaeus 1758), is a commercial species which includes seven subspecies (Bauchot and Hureau, 1986) in the Atlantic and Indian Oceans, the Mediterranean Sea and the Persian Gulf. Two subspecies *Diplodus sargus cadenati* De la Paz et al. (1973) and *Diplodus sargus sargus* Linnaeus (1758) have been reported on Atlantic coasts and from the Mediterranean to the Black Sea respectively (Fisher et al., 1987). White seabream is successfully bred but the slow growth rate, after the first year of rearing, makes it inappropriate for intensive aquaculture (Abellán et al., 1994). Recently, hatchery-reared juveniles of *D. sargus* have been released for stock enhancement purposes in artificial habitats (D'Anna et al., 2004; Pereira et al., 2010; Santos et al., 2006). The life history of the white seabream shows a pattern consistent with digynic hermaphroditism achieving sexual maturity during the second or third year of life. Spawning occurs from March to June and the onset and duration of spawning season in the seabream appear to be influenced by sea water temperatures (Morato et al., 2003). *D. sargus* larvae spend from 3 to 4 weeks in the open sea before reaching favourable sites for recruitment (González-Wangüemert et al., 2004). Juveniles live close to the coast and shift to neighbour adult habitats after the first year of life (Macpherson, 1998). Subadult and adult fish display a limited home range (Morato et al., 2003). Therefore the most likely time for migration is when larval dispersal is likely to be influenced most dominantly by prevailing currents (González-Wangüemert et al., 2007).

2.2. Sampling and DNA extraction

Fishes were sampled by trammel and hand nets in five localities around Sicily (Fig. 1): the source of reared fish at Acqua Azzurra aquaculture facilities (AQ) in the eastern coast of the island (progeny); the wild population in the neighbourhood of the fish-farm at Pachino (PC); the target population for restocking at Castellammare fishery reserve (CT) in the northwest coast; and two other wild populations, Messina (MS) and Marsala (MA) in the northeast and west coast, respectively.

According to the supplied information by Acqua Azzurra company, the aquaculture stock (AQ) has been held in culture only for one generation and it came from wild stocks. In particular, the broodstock (sampled individuals) was composed of wild specimens which had been held in culture for a period ranged from 4 to 2 years and selective breeding of the stock has not been adopted. Acqua Azzurra held the same wild broodstock which has periodically enriched with other wild specimens and this broodstock is used only in the presence of a convenient demand of juvenile production. However, due to its slow growth in intensive aquaculture, the demand of *D. sargus* juveniles is aimed only for some trials of sea cages rearing or for restocking initiative supported by regional or local funds.

A total of 155 individuals were collected and analysed (Fig. 1, Table 1). Fin samples were removed from fresh fish and stored in absolute ethanol at room temperature. Total genomic DNA was extracted from small (3–5 mg) sections of tissue following a protocol based on Sambrook et al. (1989) and suspended in elution buffer.

2.3. PCR amplification and screening

Samples were screened for variation at each of eight microsatellite loci, previously isolated and characterized for *Diplodus vulgaris* by Roques et al. (2006) and one microsatellite (SAI19) for *Sparus aurata* by Brown et al. (2005) (Table 1). Polymorphisms of eight microsatellite loci (Roques et al., 2006) were tested by multiplex PCR performed in 20 μl total volume, which include 50 ng of DNA, 2 mM of MgCl2, 0.25 μM of each primer, 200 μM dNTP's, buffer and 0.5 U Taq polymerase. Reaction conditions were as follows: an initial denaturation step of 5 min at 95 °C, eight cycles consisting of 45 s at 92 °C, 45 s at 53 °C annealing temperature, 45 s at 72 °C followed by an additional 24 cycles consisting of 30 s at 92 °C, 30 s at 55 °C annealing temperature, and 30 s at 72 °C.

An extra locus (SAI19) was analysed that can be amplified in 10 μl total volume, which included 40 ng genomic DNA, 0.75 μM forward and reverse primers, 130 μM dNTPs, 1.5 mM MgCl2, buffer and 0.5 U Taq polymerase. The PCR reactions were performed with the following programme: initial denaturation step of 95 °C for 3 min, followed by 30 cycles of 50 s at 95 °C, 50 s at 60 °C and 1 min at 72 °C, then a final extension step of 30 min at 60 °C.

Individuals were genotyped by assessing allele size on an ABI 3700 automated sequencer, using forward primers labelled with FAM (SIGMA), HEX (SIGMA) and NED (Applied Biosystems). Allele scoring was carried out using STRand software (v. 2.3.94).

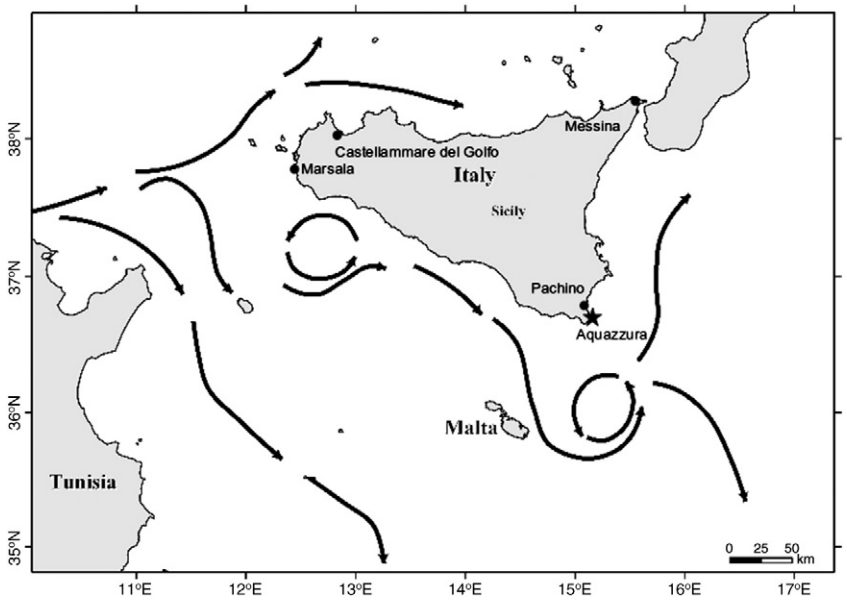

Fig. 1. Location of the sampling sites and schematic representation of surface circulation as synthetized from Lermusiaux (1999), Lermusiaux and Robinson (2001), Poulain and Zambianchi (2007) and Fortibuoni et al., (2010). Circles represent the wild samples, while star indicates the farmed sample.

2.4. Statistical analyses

Allele frequencies, mean allelic richness, expected (He) and observed (Ho) heterozygosity were calculated using Genetix v.4.1 software. Population genetic diversity was evaluated considering the total number of alleles, the number of private alleles, mean allelic richness and heterozygosity.

Linkage disequilibrium was tested for each locus–population combination using Genepop v.3.1 (Raymond and Rousset, 1995), which employed a Markov chain method with 10000 iterations, following the algorithm of Guo and Thompson (1992). Deviations from Hardy–Weinberg (HWE) genotype proportions were characterized by F_{IS} and tested using exact tests in the software Genepop v.3.1. In instances where the observed genotype frequencies deviated significantly from HWE, the programme Micro-Checker v.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, 2006).

Spatial genetic structure was studied by a set of statistical approaches because the microsatellite markers are very variable, so that several methods are necessary to detect the minimal and significant genetic differentiation between populations and to corroborate it. First, the differentiation among locations was assessed by F_{ST} (using the estimator θ of Weir and Cockerham, 1984) and the estimation of genic and genotypic differentiation using Genepop software (v. 3.1) and tested for allele-frequency heterogeneity using an exact test. The null hypothesis of no genetic differentiation between populations was tested by permutation of individuals, using the Genetix software (Belkhir et al., 1996–2004). 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 pair-wise comparisons. Second, we performed an assignment test with a Bayesian approach using the Geneclass v.2.0 software (Cornuet et al., 1999). In this test, each individual fish was assigned to its most likely geographical origin on the basis of its multi-locus genotype. Third, Cavalli-Sforza distances were computed between pairwise samples. Fourth, genetic differences were also compared using a principal component analysis (PCA) on the allelic frequencies of samples. These analyses have been described by She et al. (1987) and were calculated using the "ade4" package (Chessel, 1992) of R statistical software (R Development Core Team, 2007).

To check the existence of an isolation by distance, the correlation between genetic and geographic distances was assessed in *D. sargus* populations using the Mantel permutation test (10000 permutations; Mantel, 1967) implemented in Genetix software. The geographical distance (km) was estimated as the coastline distance between sample locations.

Gene flow between samples was estimated as the number of migrants exchanged between populations per generation at equilibrium (N_em). N_em values were derived from one approach with F_{ST} values, following the island model of Wright (1951) with a small level of migration, whereby,

$$N_em = (1-F_{ST})/4\ F_{ST}$$

Considering the farmed origin of the AQ sample, we tested the presence of bottlenecks at studied samples. Bottlenecks can be detected by the depletion of allele number and heterozygosity excess. To determine whether a population exhibits a significant number of loci with heterozygosity excess, we used the Sign and Wilcoxon tests (S–W) implemented in the programme Bottleneck v.1.2.2 (Piry et al., 1999). Computations were based on the infinite allele model (IAM) and two-phased model of mutation (TPM). The TPM is intermediate to the stepwise mutation model (SMM) and IAM. Most microsatellite data sets better fit the TPM than the SMM or IAM (Di Rienzo et al., 1994).

To assess the possible selection in aquaculture population, a selection detection workbench LOSITAN (Antao et al., 2008) based on the FDIST F_{ST} outlier methods of Beaumont and Nichols (1996) was used to evaluate the neutrality of the microsatellites in AQ and PC populations. For all runs 50000 simulations were generated with 'neutral mean F_{ST}' and 'force mean F_{ST}', to increase the reliability of the mean F_{ST} and IAM model was used.

3. Results

3.1. Genetic diversity and equilibrium

The total number of alleles by locus varied from 8 (Dvul11 and Sal19) to 41 (Dvul61). Levels of genetic variability were high across

Table 1
Levels of genetic variation observed at nine microsatellite DNA loci within 5 *Diplodus sargus* samples (AQ: Acqua Azzurra fish-farm; PC: Pachino; MA: Marsala; CT: Gulf of Castellammare; MS: Messina; Ho: observed heterozygosity; He: expected heterozygosity; H–W: Hardy–Weinberg equilibrium).

Locus	AQ	PC	MA	CT	MS
Size sample	37	35	24	30	36
Dvul11					
Allele number	4	6	5	3	4
Ho	0.7027	0.7143	0.6957	0.3333	0.6111
He	0.5570	0.6339	0.6257	0.4575	0.6516
H–W (P-values)	0.1347	0.0622	0.8794	0.2373	0.0001
Dvul12					
Allele number	6	7	5	4	7
Ho	0.4857	0.2000	0.1905	0.3182	0.4000
He	0.7535	0.7416	0.7245	0.6539	0.7804
H–W (P-values)	0.0000	0.0000	0.0000	0.0000	0.0000
Dvul38					
Allele number	5	4	4	5	9
Ho	0.4857	0.4571	0.4783	0.6250	0.7780
He	0.5690	0.6073	0.7250	0.6111	0.7681
H–W (P-values)	0.0000	0.0015	0.0004	0.0003	0.0001
Dvul4					
Allele number	8	17	13	16	15
Ho	0.7429	0.8235	0.7727	0.6667	1.0000
He	0.7935	0.9040	0.8864	0.8976	0.9004
H–W (P-values)	0.0000	0.0143	0.0417	0.0000	0.0004
Dvul84					
Allele number	7	11	8	8	8
Ho	0.8571	0.9143	0.6957	0.7500	0.7780
He	0.7878	0.8449	0.8233	0.7891	0.8245
H–W (P-values)	0.0030	0.3983	0.1158	0.6457	0.0007
SAI19					
Allele number	5	4	1	2	3
Ho	0.0541	0.1143	0.000	0.0417	0.1111
He	0.1538	0.1098	0.000	0.0408	0.1559
H–W (P-values)	0.0090	1.0000	-	1.0000	0.0977
Dvul33					
Allele number	6	13	9	9	10
Ho	0.8286	0.8000	0.7391	0.8182	0.9412
He	0.7514	0.8531	0.8289	0.8337	0.8361
H–W (P-values)	0.0472	0.2776	0.3035	0.1602	0.5086
Dvul61					
Allele number	12	15	13	11	12
Ho	0.7297	0.7429	0.8261	0.6087	0.6176
He	0.7425	0.8837	0.8979	0.8318	0.8270
H–W (P-values)	0.0108	0.0010	0.3138	0.0341	0.0000
Dvul6					
Allele number	16	28	21	23	31
Ho	0.7297	0.8235	0.8500	0.5217	0.8462
He	0.8674	0.9472	0.9337	0.9301	0.9586
H–W (P-values)	0.0000	0.0000	0.0000	0.0003	0.0008
All loci					
Total allele number	69	105	79	81	99
Mean allele number	7.6667	11.6667	8.7778	9.0000	11.0000
Mean Ho	0.6240	0.6211	0.5831	0.5204	0.6759
Mean He	0.6640	0.7251	0.7162	0.6717	0.7447

all loci and within all samples except for SaI19 locus at MA. Across all nine microsatellites combined, the mean number of alleles per locus ranged from 7.6 (AQ) to 11.6 (PC), and mean expected heterozygosity (He) ranged from 0.66 (AQ) to 0.74 (MS) (Table 1). AQ population showed the lowest allelic richness (69 alleles) with an important loss of alleles (10) which were found in all the other four studied populations at Dvul61, Dvul6, Dvul33, Dvul84 and Dvul4 loci. Also three alleles (Dvul4 58, Dvul6 430 and Dvul6 490) only found in south populations (MA and PC), were not detected at AQ population which however showed 7 exclusive alleles in SaI19, Dvul61 and Dvul84 loci.

Within samples, mean observed heterozygosity (Ho) ranged from 0.00 (SAI19/MA) to 1.00 (Dvul4/MS) (Table 1). The highest level of genetic diversity, in terms of observed and expected heterozygosity, as well as exclusive allele number, was found at MS.

Linkage disequilibrium was not observed although some loci showed single significant values for some populations: Dvul6/SaI19, Dvul61/Dvul33, Dvul84/Dvul4, and Dvul84/Dvul33 in AQ; Dvul12/Dvul38 in PC; and Dvul11/Dvul33, Dvul11/Dvul84, Dvul11/Dvul4 and Dvul33/Dvul12 in MS.

Significant departures from Hardy–Weinberg equilibrium were observed in most localities, mainly due to Dvul12, Dvul61, Dvul6 and Dvul38 loci (Table 1). The Micro-Checker software detected the presence of null alleles in loci Dvul6 and Dvul61 in some localities. However, we did not find an overestimation of F_{ST} values, being some values even higher when we recalculated these parameters eliminating the microsatellite loci with null alleles (data not shown). However, the presence of null alleles could be problematic in the estimate of Nei's genetic distances (Nei, 1978), so that we used Cavalli-Sforza distance (Cavalli-Sforza and Edwards, 1967) because of this distance is less affected by null alleles (Chapuis and Estoup, 2006).

To test the possibility of selection events on AQ population, coalescent simulations (performed by LOSITAN software), using overall F_{ST} ($F_{ST} = 0.0416$), as an expected value for neutral markers and infinite allele model, found the upper and lower F_{ST} limits at the 0.95 probability level (Fig. 2). Among the 9 polymorphic loci, Dvul38 appeared to be a candidate for balancing selection ($He = 0.5922$, $F_{ST} = -0.0075$, $P = 0.0000$).

3.2. Genetic differentiation

Significant genic and genotypic differentiation was detected between the five sampled populations except for MA–PC and CT–MS pairs (data not shown). Considering F_{ST} values, the most relevant finding is the clear differentiation of CT and MS samples from the rest of populations with high and significant values of F_{ST} ranging from 0.1205 to 0.1613 (Table 2). AQ sample shows significant genetic differences with all other populations including PC, although with minor F_{ST} values. All these F_{ST} values remained significant after the sequential Bonferroni correction. The negative F_{ST} value between MA and PC implies that both samples are very similar and present an unlimited gene flow. CT and MS have low and non-significant genetic differentiation. Similar results were obtained using Cavalli-Sforza distances (data not shown). Non-significant associations between genetic differentiation (F_{ST}) and geographical distance in the white seabream samples were revealed by Mantel test ($r = -0.066$; $P = 0.71$), so that isolation by distance was rejected.

The first two first axes of the PCA represented respectively 59.7% and 11.03% of the total variation. The PCA plot discriminated the AQ population from the rest, grouping MA and PC populations in the second quadrant and those from MS and CT in the third quadrant (Fig. 3). Such ordination is in line with the genetic differentiation obtained by considering genic and genotypic features and F_{ST} values.

Assignment test showed that the 96% of individuals were assigned rightly; CT and MA samples revealed the highest percentage of correctly assigned individuals (100%).

Considering the farmed origin of the AQ sample, two tests to detect bottlenecks were carried out, but only a recent and significant bottleneck was detected under IAM ($P<0.05$) and TPM ($P<0.05$) models for MA locality using S–W test.

4. Discussion

4.1. Genetic diversity

Genetic diversity provides the raw material for the maintenance of species diversity over longer, evolutionary time-scales and may also confer the basis for adaptation to environmental change (Bell and Okamura, 2005; Sanford and Kelly, 2011). Populations analysed in this work showed high levels of polymorphism which is consistent

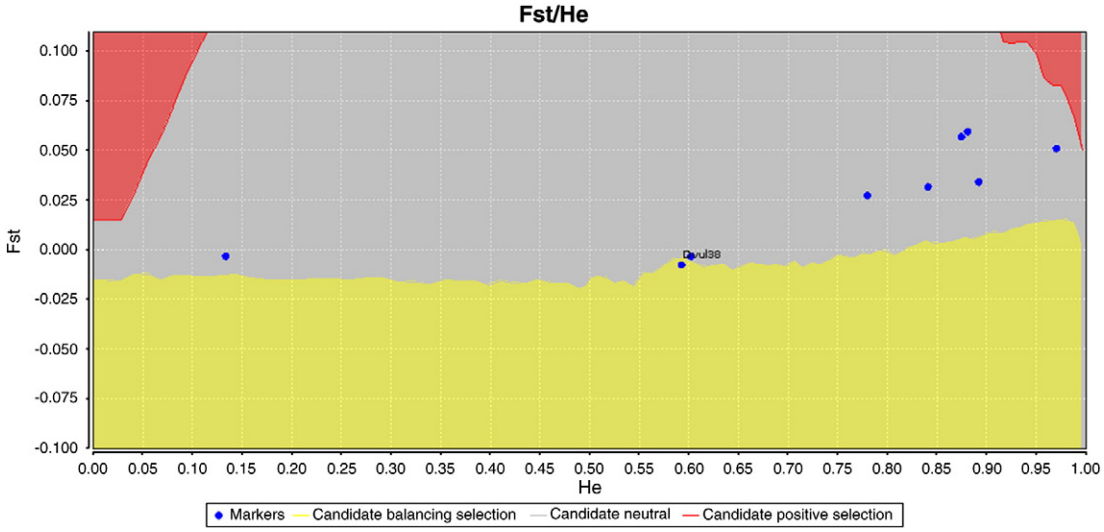

Fig. 2. Comparison of F_{ST} and He in polymorphic loci of *Diplodus sargus* to identify outliers and potential candidates for selection using LOSITAN software. Graphical output shows the simulated confidence area for neutral loci (pale grey shading), positive selection (red area) and balancing selection (yellow bottom area). Loci outliers are tagged with labels. Locus Dvul38 is candidate for balancing selection. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

with the mean number of allele observed in the same and another fish species using microsatellites (Aurelle et al., 2003; Carlsson et al., 2006; Carreras-Carbonell et al., 2006; DeWoody and Avise, 2000; González-Wangüemert and Pérez-Ruzafa, 2011; González-Wangüemert et al., 2010; Schunter et al., 2011). However, the values of expected heterozygosities from the studied white seabream populations were lower than those described by Carvalho and Hauser (1998) for several marine fishes, although similar to those obtained from *D. sargus* populations studied at other localities (González-Wangüemert and Pérez-Ruzafa, 2011; González-Wangüemert et al., 2010) and from other sparid fishes (Pérez-Enriquez and Taniguchi, 1999; Piñera et al., 2007; Yun-Guo et al., 2007). It is important to stress that low values of heterozygosity for sparid species are probably linked to the fact that proterandic and protogynous hermaphrodite species may present a lower genetic variability than dioecious ones because of a lower effective size (Nielsen and Kenchington, 2001).

The same biological feature further promotes inbreeding in the context of aquaculture facilities. The farmer can rely on the same individuals to act first as males and later as females, decreasing the effective population size of breeding stocks and favouring an inbreeding depression. We have not observed a deep depression at aquaculture sample (AQ), but a significant reduction in the number of microsatellite alleles was detected in farmed sample as regards wild samples; this loss of alleles may indicate bottleneck, inbreeding or both. Since bottleneck was not detected by the W–S test, the reduction of number of alleles in the AQ population, mainly the lost of alleles found in the other four populations (10 alleles), the presence of 7 exclusive alleles, the significant heterozygosity deficiency in 6 of 9 analysed loci, and a locus under balancing selection (Dvul38), could be interpreted as an incipient inbreeding print that would evolve to a detectable level in time. Therefore, the restocking of Castellammare population using massively broods from AQ facilities could provoke genetic impact because of several reasons: 1) the AQ population does not have 10 alleles which are found in Castellammare population; 2) the 7 private alleles for the AQ population are potential candidates for introgression into the CT gene pool, mainly two of them (Sal19 264 and Sal19 276, which have not been detected in other 11 localities from Mediterranean Sea and Atlantic Ocean (González-Wangüemert and Pérez-Ruzafa, 2011; González-Wangüemert et al., 2010); 3) Castellammare population showed only significant heterozygosity deficiency in 4 loci, while we found 6 loci with this significant deficiency in the AQ population.

Table 2
Pairwise estimates of multilocus F_{ST} (above) and number of migrants *per* generation (below) between samples of *Diplodus sargus*, with results of permutation testing of significant departures from zero. Bold F_{ST} values are significantly greater than zero at $P<0.05$. AQ: Acqua Azzurra fish-farm PC: Pachino; MA: Marsala; CT: Gulf of Castellammare; MS: Messina.

	AQ	PC	MA	CT	MS
AQ	–	**0.0325**	**0.0421**	**0.1613**	**0.1440**
PC	7.44	–	−0.0010	**0.1293**	**0.1134**
MA	5.69	999999	–	**0.1375**	**0.1205**
CT	1.30	1.68	1.57	–	0.0091
MS	1.49	1.95	1.82	27.07	–

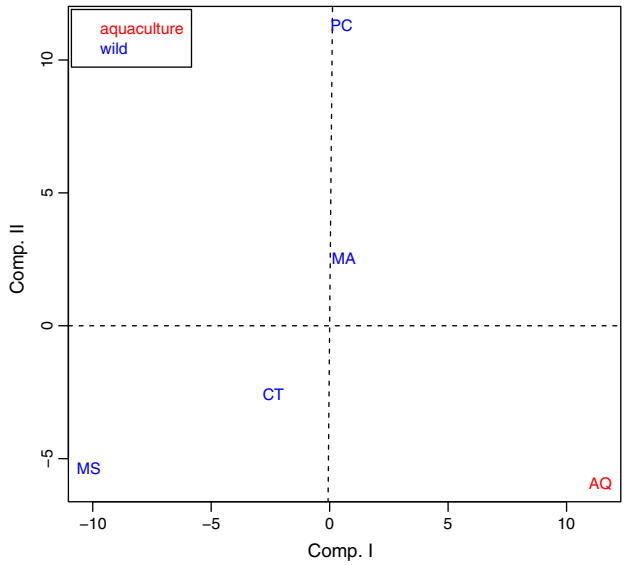

Fig. 3. Scaleless ordination of the first two axes of the principal component analysis on the allelic frequencies of *Diplodus sargus*, which jointly explained 70.73% of the variance in the global data set. PC: Pachino; MA: Marsala; CT: Gulf of Castellammare; MS: Messina; AQ: Acqua Azzurra fish-farm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4.2. Genetic structure

Significant genetic differences (Fst values, Cavalli-Sforza distances, genic and genotypic differentiation, PCA) were detected between the source of reared fish (AQ) and the target population for restocking (CT). In fact, the F_{ST} values among the AQ stock and the wild populations from CT and MS were even larger than those found among *D. sargus* populations across the western Mediterranean Sea and northeastern Atlantic Ocean (González-Wangüemert et al., 2010). In spite of the periodical incorporation of some individuals from neighbouring wild source (PC), the reduction in the effective size accounts for high differentiation in the farmed stock (AQ), which is consistent with the findings of many previous studies (Alarcón et al., 2004; Borrell et al., 2007; Chistiakov et al., 2006; Gardner et al., 2010; Hindar and Fleming, 2007; Pereira et al., 2010; Taniguchi and Pérez-Enríquez, 2000). Thus, restocking from farmed stocks must be meticulously assessed because potentially important impacts on the genetic structure of wild populations specially in spatially managed areas (Katsanevakis et al., 2011; Pérez-Ruzafa et al., 2006). These effects can be further magnified if the restocked population is very different from the reared one such as it is observed between populations from CT and AQ. The different geographic origin of these populations (CT *vs.* AQ) could explain this significant genetic differentiation, because we observed high F_{ST} and Cavalli-Sforza values among CT and PC, but this can be not the only reason: we also found significant F_{ST} values and Cavalli-Sforza distances between populations from AQ and PC and according to the results from principal component analysis, the AQ population appears separated from the other samples including the PC population. Therefore, AQ population is different to that from CT due to both geographic isolation and farming activities.

By contrast, MA and PC samples were very similar, showing negative F_{ST} values, non-significant Cavalli-Sforza genetic distances and high gene flow. Such high genetic similarity and gene flow imply a high degree of connectivity between both populations. Given that the geographic distance between MA and PC is in the order of hundreds of km, they exceed by far the migratory capabilities of adult and subadult individuals of *D. sargus* (Abecasis et al., 2009; Macpherson, 1998). Therefore the most likely mechanism providing such connectivity between the local populations is larval dispersal such as has been demonstrated in other genetic works, which seem reasonable given the duration of the pelagic phase in this species (González-Wangüemert et al., 2004, 2006, 2010, 2011) as well as the intensity and stability of the pattern of sea surface circulation in late Spring (Fortibuoni et al., 2010; Garofalo et al., 2011; Lermusiaux, 1999; Lermusiaux and Robinson, 2001). In fact, the floating eggs and the pelagic larval phase of this species allow the transport of substantial offspring by the prevailing currents (Poulain and Zambianchi, 2007). An analogous pattern is suggested for the populations from CT and MS, which also reflect a high level of connectivity. These patterns are highly consistent with that of the Atlantic Surface Water current that arrives to Sicily from the west and split in two main currents at the islands off Trapani (around 20 km north from MA) (Fortibuoni et al., 2010; Garofalo et al., 2011; Lermusiaux, 1999; Lermusiaux and Robinson, 2001; Poulain and Zambianchi, 2007, Fig. 1). These two distinct currents are separated by the landmass of the Sicily island and do not meet downstream due to the presence of the Strait of Messina between Sicily and the continental land-mass. The strait is very deep, the coasts drop abruptly and currents are turbulent providing an intense vertical mixing. All these features should preclude the survival of *D. sargus* larvae travelling across the strait (Azzaro et al., 2007; Hopkins, 1984).

Therefore the genetic connectivity pattern, highly consistent with oceanographic currents, identified two distinct metapopulations of white seabream around Sicily. This is an important result because it highlights the progressively accepted idea that stocks of many marine fish and invertebrates are structured at relatively small spatial scales (Evans et al., 2010; Gold et al., 2010; McCuster and Bentzen, 2010; O'Learly et al., 2007; Tripp-Valdez et al., 2010).

Thus, the main inferences from this work are that white seabream stocks should not be managed as a whole, instead, management should be aimed at the self-replenishing populations within stocks and that it is recommended to utilize broods from the same metapopulation for restocking purposes to provide a better genetic match to the wild populations.

Acknowledgements

Authors are very grateful to Dr. Garofalo for her precious indications about sea surface circulation in the study area. Dr. Borrero Pérez for helping with the DNA extractions and to G. Di Stefano for assistance in the field. M.G.W. was supported by a FCT postdoctoral grant (SFRH/BPD/70689/2010). The authors thank two anonymous referees for their helpful comments on earlier draft of the manuscript.

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