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Effects of fishing protection on the genetic structure of fish populations

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ARTICLE INFO

Article history:

Received 2 June 2005

Received in revised form

26 October 2005

Accepted 31 October 2005

Available online 15 December 2005

Keywords:

Biodiversity

Conservation

Genetic resources

Marine reserves

Connectivity

Diplodus sargus

ABSTRACT

Marine reserves have been identified as an important tool in the management of fishery resources and their number is increasing rapidly, most of them being on islands. However, knowledge on the real effect of protection from fishing on the genetic structure of populations, the spatial scales involved, or the suitability of islands as reserves in terms of connectivity, is scarce. This paper analyses the effects of fishery protection on the genetic structure of populations of *Diplodus sargus*, a target species, in protected and non-protected areas of the western Mediterranean. Populations studied showed high genetic variability at spatial scales from 10¹ to 10³ km. Protected areas have significantly higher allelic richness. The lower levels of heterozygosity and higher heterozygote deficit showed by islands compared with coastal areas makes clear the importance of considering the connectivity processes when designing a MPA.

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1. Introduction

Increased fishing pressure has resulted in widespread overexploitation of populations (Plan Development Team, 1990; Roberts and Polunin, 1991; Bohnsack and Ault, 1996; Roberts, 1997; Roberts et al., 2001), with declines in overall abundance of stocks and average fish size; adverse genetic selection leading to loss of potential fecundity; reduced average spawning size; change of sex-ratio and interspecific equilibrium; and loss of genetic diversity (Wilson and Clarke, 1996).

After the failure of traditional management measures (Waters, 1991), marine reserves have been strongly advocated as an ideal tool for the management of coastal fisheries (Plan Development Team, 1990; Roberts and Polunin, 1991; Dugan and Davis, 1993; Agardy, 1994; Gerber et al., 2002) and a large

number of marine protected areas (MPAs) have been established around the world over recent decades (Lubchenco et al., 2003), offering protection to natural communities, in an attempt to halt further deterioration of sensitive habitats, or serving as fisheries management tools (Jones, 2002).

Marine fishery reserves are intended to protect critical spawning stock biomass, intraspecific genetic diversity, population age structure, recruitment supply and ecosystem balance, while maintaining fisheries (Plan Development Team, 1990).

The effect of fishing restrictions on the density, size structure and biomass of fish populations has been thoroughly investigated, both in the Mediterranean and other marine areas (see reviews by García-Charton et al., 2000; McClanahan and Mangi, 2000; Russ, 2002; Halpern, 2003). However, in the case of genetic resources, although MPAs have been consid-

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doi:10.1016/j.biocon.2005.10.040

ered the most efficient and logical means of maintaining them (Polunin, 1983; Palumbi, 2003), no data are available on the effects of protection on the genetic structure of marine populations and the mechanisms involved. Therefore, although some proposed benefits have been demonstrated, others require verification or further testing (Bohnsack, 1998; Sale et al., 2005).

In any case, although bearing in mind that the mechanisms underlying the process of stock replenishment are still not well understood (Roberts and Polunin, 1991), marine reserves have been identified as an important tool in the precautionary management of fishery resources and their number is increasing rapidly, most of them being on islands (Badalamenti et al., 2000). In the meanwhile, some questions arise which have not yet been answered, such as: what are the real effects of protection from fishing on the genetic structure of populations? On what spatial scales does it operate? Are islands, as opposed to other coastal locations, a good choice for establishing MPAs?

To test the hypothesis that MPAs preserve intraspecific genetic diversity, this paper analyses the effects of fishery protection on the genetic structure of populations of *Diplodus sargus* (Linneo), a target species, in protected and non-protected areas of the western Mediterranean and discusses the implication of the results on the selection criteria for MPAs.

2. Materials and methods

2.1. Study area, sampling design and technique

To study the effects of protection from fisheries on the genetic structure of *D. sargus* populations in the western Mediterranean, five localities were sampled in 1999–2000 in the SE of

Spain, two of them being marine fishing reserves: Tabarca island (established in 1989) and Cape Palos-Hormigas Islands (established in 1995), and three neighbouring non-protected areas (Águilas, Mazarrón and Guardamar) (González-Wangüemert et al., 2002, 2004). The data gathered were jointly analysed and compared with those from Lenfant and Planes (1996) and Lenfant (1998) at six NW Mediterranean localities including one marine reserve (Banyuls, established in 1974) and the islands Elba and Giglio (Fig. 1). Only allelic frequencies of polymorphic loci screened in both studies were used. In order to establish the allele 100, a calibration was performed at the University of Perpignan using samples from both studies (González-Wangüemert et al., 2004).

Populational genetic structure analyses were carried out on data corresponding to a total of 1804 fishes: 1249 individuals obtained from local fishermen in the SW Mediterranean (González-Wangüemert et al., 2004), and data on 555 individuals from NW Mediterranean populations as per Lenfant (1998).

The fish were frozen after capture and kept at -40°C until dissection. Liver, eye and muscle were extracted from each specimen, and kept at -70°C . Electrophoresis and staining protocols were performed according to Aebersold et al. (1987) and Harris and Hopkinson (1976).

2.2. Data analysis

Genetic variability was recorded as observed (H_o) and expected (H_e) mean heterozygosity *per locus*, as a proportion of polymorphic loci at the 5% criterion, and as the mean number of alleles *per locus*. Heterozygote deficit was calculated as $HD = (H_o - H_e)/H_e$. *F*-statistics were calculated in order to detect non-random mating within populations (F_{IS}) using the

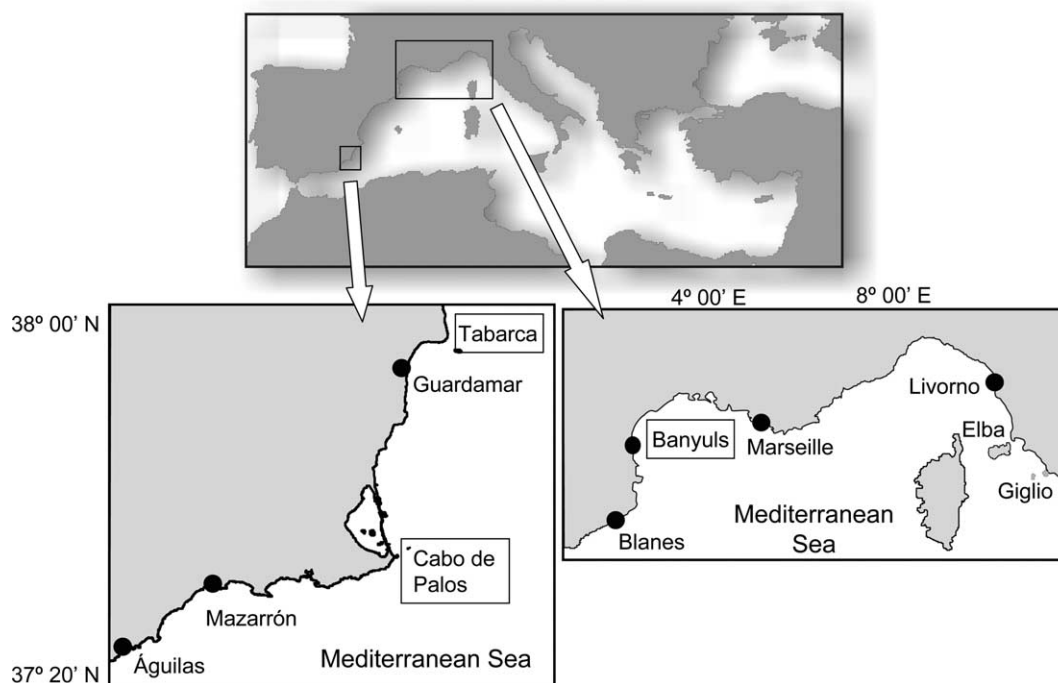


Fig. 1 – Location of sampling sites in the western Mediterranean.

Weir and Cockerham (1984) method. The significance of F -values was assessed via permutations (using Genetix-software, Bonhomme et al., 1993; www.univ-montp2.fr/genome-pop/genetix.htm) and Fisher's exact test (GENEPOP-vs.3.3-software, Raymond and Rousset, 1995).

Genetic distance (D) (Nei, 1978) was computed between pairwise samples and the resulting matrix was clustered using the Euclidean distance and UPGMA algorithm (Statistica vs. 6.0). Significance for Nei's D values was tested via permutation and D was plotted against geographic distances measured as the shortest marine route between locations. Correlation between geographic and genetic distances was tested using Mantel's test (Genetix-software). Genetic diversity using the Shannon index (H') on the allelic frequency matrix and the Pielou evenness index (J'), were also estimated (Magurran, 1988). The spatial variation of the genetic structure of populations was explored using Principal Components Analyses (PCA), performed on the allele frequency matrix after square root transformation.

Comparisons of the genetic structure of populations were performed for allelic richness, observed and expected heterozygosity, heterozygote deficit, allelic diversity (H') and Pielou's evenness using ANOVA. As the number of case studies was not enough to perform a mixed, trifactorial analysis, an independent analysis has been performed for the following factors: Region (on two levels: south-western/north-western), Protection (protected/non-protected) and Location (coastal/island), using a Bonferroni corrected α -level ($\alpha = 0.167$) for the interpretation of results. As allelic richness showed significant differences between regions, in further analyses a standardization of this variable was performed using: allelic richness in population_{*i*}/total allelic richness in Region_{*j*}.

As the number of fish studied at each locality was unequal, rarefaction techniques using Primer-5 software were applied to the absolute allele frequency matrix to determine the minimum sample size required to be representative of each population's genetic structure. The results showed that the minimum sample size for 90% of the expected total allelic richness ranges between 10 individuals at Blanes to 37 individuals at Mazarrón. Only Banyuls required a higher number of individuals (76). All the populations sampled exceeded the number of individuals required to reach 100% of the total expected allelic richness.

3. Results

3.1. Geographical variability of the genetic structure of populations

In total, we found 74 alleles among the nine shared loci. All loci were polymorphic ($p \leq 0.95$) and the mean observed heterozygosity (H_o) ranged from 0.3064 for the Elba population to 0.5075 at Cape Palos (Table 1).

Seventeen alleles were present at all localities. These included all alleles 100 (except $IDH1^*100$ at Livorno) and $AAT-2^*110$, ADA^*70 , ADA^*80 , ADA^*120 , GDA^*90 , GDA^*110 , GDA^*120 , $IDH-1^*60$, $MDH-1^*80$.

Some populations were characterised by unique alleles: Tabarca ($PGI-2^*40$), Cape Palos ($MDH-1^*90$), Banyuls ($PGI2^*85$,

$PGI-2^*105$, $AAT-1^*95$, and ADA^*50) and Blanes ($AAT-2^*115$). $AAT-1^*120$ were common only to Banyuls and Tabarca as was PGM^*140 for Banyuls and Elba. In total, 15 alleles were exclusive to the SW Mediterranean and 11 to the NW region (Table 1).

The ANOVA performed on the 11 localities showed significant differences for the factor Region. This factor had a significant effect on mean allelic richness ($p < 0.005$), genetic diversity ($p = 0$) and mean observed ($p < 0.005$) and expected heterozygosity, as well as on heterozygote deficit ($p = 0$) (Table 2). The SW region showed higher allelic richness and diversity, higher heterozygosity (both observed and expected) and a strong heterozygote deficit in comparison with the NW region.

Allelic richness in the five populations in SE Spain ranged between 52 and 60, with a mean of 55.8 ± 1.36 alleles (mean \pm s.e.) which was higher than values observed in the north-western Mediterranean populations (32.83 ± 4.88) (Lenfant, 1998), except for the Banyuls population at 56 alleles.

There was a significant heterozygote deficit for all loci in all populations, mainly in south-western localities. F_{IS} values per locus ranged from 0.0028 to 0.7941 in this area. The highest multilocus heterozygote deficit was at Tabarca island ($HD = -0.447$; $F_{IS} = 0.450$) and the highest heterozygote deficit is shown by the locus $AAT-1$, also at Tabarca ($HD = -0.76442$; $F_{IS} = 0.7652$). Populations from the NW Mediterranean tended to also show heterozygote deficit in the localities of Banyuls ($F_{IS} = 0.07016$) and Giglio ($F_{IS} = 0.06361$), although most of the loci provided insignificant results (Lenfant, 1998).

The average heterozygosity estimated for loci common to the five populations from the south-western Mediterranean ($H_o = 0.4339 \pm 0.0217$) was significantly higher ($p < 0.005$) than those found in north-western populations ($H_o = 0.3465 \pm 0.0099$).

The dendrogram constructed using genetic distances reflects the differentiation among regions (Fig. 2), but no clear geographic pattern is observed within each group. In the SW Mediterranean, the Guardamar and Tabarca populations clustered together, as expected in accordance with their geographic proximity, although the Águilas population joins this group despite being the farthest. On the other hand, in the NW Mediterranean, excepting Livorno which is separate from both groups, all localities constitute a more homogeneous cluster, irrespective of geographic distance.

3.2. Effects of protection on the genetic structure of *D. sargus* populations

Protected areas considered in this study showed slightly higher mean allelic richness and diversity, lower expected and observed heterozygosity and a higher heterozygote deficit than non-protected zones. However, most of these results are probably influenced by geographic variability, and the Protection factor was only significant for allelic richness, both absolute ($p < 0.005$) and standardised ($p = 0.05$), and marginally so for evenness ($p = 0.16$), which did not follow any geographical pattern (Fig. 3).

MPAs include 97.3% of the total number of alleles found in the populations studied. Seven alleles ($PGI-2^*40$, $PGI-2^*85$,

Table 1 – Genetic structure descriptors and allelic frequencies for the grouping factors (region, protection and location) considered in the study of *Diplodus sargus* populations at western Mediterranean

# Localities	Region				Protection				Location			
	SW		NW		Protected		Non-Protected		Island		Coastal	
	5		6		3		8		3		8	
Total richness	62		59		3		8		3		8	
	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.
Richness	55.80	1.36	32.83	4.87	56.67	1.76	38.25	5.05	38.67	10.67	45.00	5.03
Standard richness					0.93	0.03	0.63	0.08	0.64	0.16	0.74	0.08
Evenness	0.87	0.00	0.83	0.02	0.82	0.05	0.86	0.01	0.86	0.01	0.85	0.02
Diversity (H')	5.07	0.03	4.12	0.03	4.77	0.28	4.47	0.18	4.44	0.34	4.59	0.18
H _o	0.4339	0.0217	0.3465	0.0099	0.4147	0.0465	0.3756	0.0174	0.3428	0.0194	0.4026	0.0203
H _e	0.6513	0.0057	0.3807	0.0077	0.5594	0.0859	0.4828	0.0509	0.4717	0.0918	0.5156	0.0513
D	-0.3413	0.0354	-0.0622	0.0199	-0.2431	0.1130	-0.1688	0.0536	-0.2286	0.1099	-0.1743	0.0552
PGI-2 40*	0.0007	0.0007	0	0	0.0012	0.0012	0	0	0.0012	0.0012	0	0
PGI-2 60*	0.0500	0.0202	0	0	0.0263	0.0263	0.0214	0.0139	0.0263	0.0263	0.0214	0.0139
PGI-2 70*	0	0	0.0075	0.0046	0.0097	0.0097	0.0020	0.0013	0	0	0.0056	0.0036
PGI-2 80*	0.1667	0.0231	0.0022	0.0019	0.1218	0.0614	0.0602	0.0309	0.0531	0.0531	0.0859	0.0342
PGI-2 85*	0	0	0.0021	0.0021	0.0042	0.0042	0	0	0	0	0.0016	0.0016
PGI-2 90*	0	0	0.0331	0.0044	0.0139	0.0139	0.0196	0.0065	0.0219	0.0117	0.0166	0.0069
PGI-2 93*	0.0187	0.0055	0	0	0.0098	0.0098	0.0081	0.0042	0.0098	0.0098	0.0081	0.0042
PGI-2 100*	0.5923	0.0084	0.9528	0.0092	0.7030	0.1170	0.8212	0.0663	0.8259	0.1269	0.7751	0.0676
PGI-2 105	0	0	0.0001	0.0001	0.0002	0.0002	0	0	0	0	0.0001	0.0001
PGI-2 110*	0	0	0.0030	0.0017	0.0008	0.0008	0.0020	0.0013	0.0033	0.0033	0.0010	0.0007
PGI-2 120*	0.1136	0.0153	0.0036	0.0033	0.0638	0.0387	0.0498	0.0226	0.0250	0.0160	0.0644	0.0243
PGI-2 140*	0.0262	0.0154	0	0	0.0270	0.0241	0.0062	0.0062	0.0250	0.0250	0.0070	0.0062
PGI-2 167*	0.0219	0.0090	0	0	0.0221	0.0162	0.0054	0.0036	0.0043	0.0043	0.0121	0.0069
PGI-2 180*	0.0099	0.0051	0	0	0.0049	0.0040	0.0044	0.0035	0.0043	0.0043	0.0046	0.0035
PGM 40*	0.1817	0.0111	0.0011	0.0009	0.1151	0.0583	0.0713	0.0345	0.0645	0.0645	0.0902	0.0341
PGM 60*	0.1927	0.0141	0.0047	0.0041	0.1204	0.0603	0.0788	0.0367	0.0519	0.0519	0.1045	0.0375
PGM 80*	0	0	0.1736	0.0042	0.0539	0.0539	0.1100	0.0323	0.1208	0.0607	0.0849	0.0321
PGM 100*	0.4496	0.0129	0.8116	0.0069	0.5841	0.1218	0.6707	0.0677	0.6973	0.1044	0.6283	0.0715
PGM 120*	0.1760	0.0106	0.0065	0.0022	0.1258	0.0614	0.0677	0.0301	0.0613	0.0499	0.0919	0.0337
PGM 140*	0	0	0.0025	0.0021	0.0008	0.0008	0.0016	0.0016	0.0043	0.0043	0.0003	0.0003
PGDH 40*	0.0375	0.0129	0	0	0.0268	0.0135	0.0134	0.0101	0.0126	0.0126	0.0187	0.0105
PGDH 60*	0.1997	0.0174	0.0001	0.0001	0.1174	0.0584	0.0809	0.0404	0.0571	0.0571	0.1035	0.0402
PGDH 70*	0	0	0.0837	0.0096	0.0236	0.0236	0.0539	0.0172	0.0663	0.0352	0.0379	0.0150
PGDH 80*	0.0131	0.0056	0.0138	0.0088	0.0212	0.0094	0.0106	0.0062	0.0080	0.0080	0.0155	0.0066
PGDH 90*	0	0	0.1234	0.0136	0.0324	0.0324	0.0804	0.0253	0.0816	0.0428	0.0620	0.0251
PGDH 100*	0.4312	0.0179	0.7741	0.0145	0.5657	0.1124	0.6379	0.0663	0.6679	0.1038	0.5996	0.0679
PGDH 110*	0.0134	0.0065	0.0048	0.0025	0.0065	0.0046	0.0096	0.0044	0.0095	0.0048	0.0084	0.0044
PGDH 120*	0.0059	0.0036	0.0002	0.0002	0.0049	0.0044	0.0020	0.0020	0.0046	0.0046	0.0021	0.0020
PGDH 140*	0.2122	0.0159	0	0	0.1450	0.0738	0.0782	0.0390	0.0645	0.0645	0.1084	0.0420
PGDH 160*	0.0097	0.0025	0	0	0.0072	0.0043	0.0033	0.0021	0.0023	0.0023	0.0052	0.0024
PGDH 180*	0.0697	0.0040	0	0	0.0492	0.0247	0.0251	0.0124	0.0257	0.0257	0.0339	0.0130
PGDH 210*	0.0077	0.0033	0	0	0	0	0.0048	0.0024	0	0	0.0048	0.0024
GDA 70*	0.0028	0.0023	0.0002	0.0002	0.0044	0.0038	0.0002	0.0002	0.0040	0.0040	0.0004	0.0003
GDA 80*	0.0609	0.0218	0.0516	0.0330	0.0898	0.0313	0.0431	0.0238	0.0349	0.0349	0.0636	0.0244
GDA 90*	0.2096	0.0181	0.1955	0.0205	0.2178	0.0209	0.1959	0.0169	0.2210	0.0302	0.1947	0.0151
GDA 100*	0.3711	0.0198	0.2934	0.0142	0.3360	0.0299	0.3260	0.0210	0.3301	0.0423	0.3282	0.0187
GDA 110*	0.1562	0.0188	0.4276	0.0490	0.2194	0.0681	0.3361	0.0630	0.3414	0.1343	0.2903	0.0545
GDA 120*	0.1088	0.0186	0.0316	0.0072	0.0926	0.0218	0.0569	0.0185	0.0461	0.0294	0.0744	0.0178
GDA 130*	0.0906	0.0177	0.0002	0.0002	0.0401	0.0199	0.0418	0.0216	0.0223	0.0223	0.0484	0.0207
IDH-1 60*	0.2104	0.0164	0.1626	0.0334	0.2086	0.0059	0.1752	0.0275	0.1956	0.0396	0.1801	0.0250
IDH-1 80*	0.0532	0.0030	0.0307	0.0307	0.0375	0.0188	0.0422	0.0222	0.0181	0.0181	0.0494	0.0214
IDH-1 100*	0.5448	0.0033	0.6666	0.1348	0.6230	0.0768	0.6068	0.0991	0.7104	0.0909	0.5741	0.0941
IDH-1 110*	0.0260	0.0034	0.1317	0.1317	0.0174	0.0090	0.1085	0.0975	0.0099	0.0099	0.1113	0.0971
IDH-1 120*	0.1359	0.0151	0.0029	0.0026	0.0965	0.0474	0.0509	0.0250	0.0549	0.0473	0.0665	0.0265
IDH-1 130*	0.0094	0.0042	0	0	0.0037	0.0022	0.0045	0.0032	0.0012	0.0012	0.0054	0.0031
IDH-1 140*	0.0204	0.0039	0.0023	0.0019	0.0133	0.0058	0.0095	0.0044	0.0098	0.0052	0.0108	0.0045
AAT-1 80*	0.0201	0.0101	0.0113	0.0082	0.0066	0.0038	0.0186	0.0083	0.0046	0.0046	0.0193	0.0081
AAT-1 90*	0.1557	0.0148	0.0349	0.0073	0.1117	0.0426	0.0816	0.0242	0.0884	0.0491	0.0903	0.0237
AAT-1 95*	0	0	0.0001	0.0001	0.0002	0.0002	0	0	0	0	0.0001	0.0001
AAT-1 100*	0.7219	0.0150	0.9496	0.0053	0.8140	0.0708	0.8582	0.0448	0.8785	0.0823	0.8340	0.0426
AAT-1 110*	0.0307	0.0110	0.0024	0.0016	0.0322	0.0203	0.0089	0.0043	0.0051	0.0051	0.0191	0.0086

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Table 1 – continued

# Localities	Region				Protection				Location			
	SW		NW		Protected		Non-Protected		Island		Coastal	
	5	6	59	6	3	8	3	8	3	8	3	8
Total richness	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.
AAT-1 120*	0.0014	0.0014	0.0001	0.0001	0.0025	0.0022	0	0	0.0023	0.0023	0.0001	0.0001
AAT-1 125*	0.0701	0.0104	0	0	0.0329	0.0183	0.0315	0.0156	0.0211	0.0211	0.0359	0.0149
AAT-2 80*	0.0263	0.0037	0.0001	0.0001	0.0215	0.0107	0.0085	0.0044	0.0120	0.0120	0.0120	0.0048
AAT-2 90*	0.2183	0.0047	0.0015	0.0015	0.1481	0.0695	0.0820	0.0401	0.0713	0.0713	0.1108	0.0411
AAT-2 100*	0.5603	0.0109	0.6405	0.0333	0.6089	0.0496	0.6022	0.0261	0.6079	0.0559	0.6026	0.0248
AAT-2 110*	0.1400	0.0058	0.3557	0.0349	0.1804	0.0528	0.2866	0.0468	0.2968	0.1028	0.2430	0.0410
AAT-2 115*	0	0	0.0010	0.0010	0	0	0.0007	0.0007	0	0	0.0007	0.0007
AAT-2 120*	0.0552	0.0089	0.0003	0.0003	0.0411	0.0243	0.0193	0.0097	0.0120	0.0120	0.0302	0.0121
ADA 50*	0	0	0.0001	0.0001	0.0002	0.0002	0	0	0	0	0.0001	0.0001
ADA 70*	0.0425	0.0067	0.0779	0.0240	0.0390	0.0127	0.0704	0.0182	0.0621	0.0268	0.0618	0.0176
ADA 80*	0.1831	0.0347	0.3345	0.0172	0.1963	0.0605	0.2917	0.0309	0.2876	0.0507	0.2575	0.0373
ADA 90*	0.0943	0.0217	0.0002	0.0002	0.0676	0.0409	0.0337	0.0194	0.0474	0.0474	0.0413	0.0189
ADA 100*	0.3862	0.0299	0.4668	0.0168	0.4459	0.0329	0.4243	0.0255	0.4205	0.0235	0.4338	0.0268
ADA 110*	0.0453	0.0111	0.0006	0.0006	0.0254	0.0175	0.0192	0.0104	0.0200	0.0200	0.0213	0.0099
ADA 120*	0.1788	0.0274	0.1156	0.0274	0.1810	0.0396	0.1306	0.0245	0.1430	0.0384	0.1448	0.0266
ADA 130*	0.0532	0.0123	0.0010	0.0010	0.0395	0.0169	0.0192	0.0118	0.0177	0.0177	0.0273	0.0122
ADA 140*	0.0166	0.0047	0.0004	0.0004	0.0068	0.0032	0.0081	0.0044	0.0017	0.0017	0.0100	0.0042
MDH-1 40*	0.0158	0.0040	0	0	0.0153	0.0089	0.0042	0.0021	0.0103	0.0103	0.0060	0.0024
MDH-160*	0.1192	0.0105	0.0088	0.0021	0.0646	0.0295	0.0569	0.0233	0.0380	0.0256	0.0668	0.0231
MDH-1 80*	0.2845	0.0136	0.3524	0.0141	0.2977	0.0176	0.3304	0.0180	0.3354	0.0271	0.3163	0.0174
MDH-1 90*	0.0004	0.0004	0	0	0.0006	0.0006	0	0	0	0	0.0002	0.0002
MDH-1 100*	0.5047	0.0103	0.6361	0.0145	0.5545	0.0530	0.5846	0.0256	0.5743	0.0467	0.5771	0.0275
MDH-1 120*	0.0754	0.0119	0.0003	0.0003	0.0670	0.0333	0.0222	0.0113	0.0372	0.0372	0.0334	0.0133

H_o : observed heterozygosity; H_e : expected heterozygosity; D: heterozygote deficit; s.e.: mean standard error.

Table 2 – ANOVA table for the genetic variables considered in the study of *Diplodus sargus* populations at western Mediterranean (mean allelic richness, H' genetic diversity, observed (H_o) and expected (H_e) heterozygosity and deficit of heterozygotes (D)) analyzed for the grouping factor Region (north-western vs. south-western region)

Variable	Source	Sum-of-squares	df	Mean-square	F-ratio	P
Allelic richness	Treatment	1438.548	1	1438.548	17.271	0.002
	Error	749.633	9	83.293		
Allelic diversity (H')	Treatment	2.427	1	2.427	418.447	0.000
	Error	0.052	9	0.006		
H_o	Treatment	0.021	1	0.021	15.214	0.004
	Error	0.012	9	0.001		
H_e	Treatment	0.200	1	0.200	741.357	0.000
	Error	0.002	9	0.000		
D	Treatment	0.212	1	0.212	51.757	0.000
	Error	0.037	9	0.004		

PGI-2*105, AAT-1*95, AAT-1*120, ADA*50, MDH-1*90) are present exclusively in MPAs and only two (PGDH*210, AAT-2*115) are absent.

3.3. Effects of insularity on genetic structure

The Location factor (island vs. coastal) did not show significant differences for most variables and only did so marginally for mean observed heterozygosity ($p = 0.1$) (Fig. 4). We also explored the effects of insularity on genetic structure, comparing populations from the three islands, Tabarca, Elba and Giglio, with populations from three coastal localities selected

at random (Águilas, Banyuls and Blanes). No differences were found for any variable. However, when comparing island to coastal locations among north-western localities only, excluding the Banyuls coastal marine reserve in order to avoid the mixed effects of Region and Protection on Location, the mean heterozygote deficit turned out to be significant ($p < 0.05$) (Fig. 4).

Across the entire genetic pool, eight alleles (10.8%) were not found on islands, six of these were exclusive to the NW Region, the other two being exclusive to the SW region, as opposed to only one (exclusive to the SW region) that is absent in coastal zones.

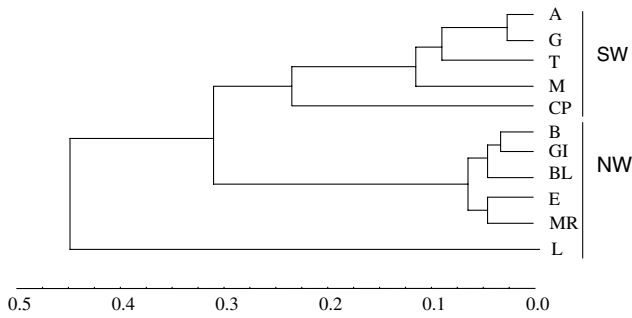


Fig. 2 – Cluster of studied populations according to their genetic distances.

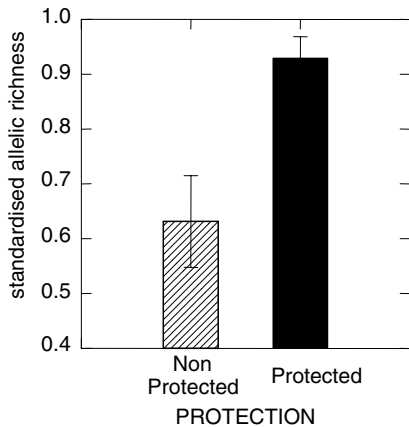


Fig. 3 – Mean standardised allelic richness in protected and non-protected populations.

3.4. The combined effect of factors

The results of the PCA applied to allele frequency data show the influence of geographical differentiation. The first axis explains 54.1% of total variation, separating the SW Mediterranean localities from the NW ones (Fig. 5). The second axis explains an additional 8.9%, separating in their negative part the two oldest MPAs (Banyuls and Tabarca) and their closest

respective localities (Blanes and Guardamar). The combination of the Principal Components II and III, accounting for 16.7% of the variation, tends to separate the MPAs from the other localities.

When performing a PCA on the mean allele frequencies and mean values for genetic structure variables (Fig. 6), the space for variation is reduced and the three first axes account for 96.9% of the total variance in data. The first axis explains 71% and corresponds to the Region factor, with the southwestern region in the positive part and the north-western one in the negative. Evenness, observed and expected heterozygosity and genetic diversity are the variables related to the positive part of this axis.

The second axis explains an additional 15.1% and is related to the Protection factor, with protected zones in the negative part and non-protected ones in the positive, the variables responsible being allelic richness and evenness, respectively. The third axis explains the remaining 10.7% and corresponds to the Location factor. Coastal areas presented higher allelic richness, higher observed heterozygosity and lower heterozygote deficit than islands.

4. Discussion

4.1. Genetic differentiation and spatial heterogeneity of *D. sargus* populations

Electrophoretic data show that *D. sargus* populations in the western Mediterranean are not genetically homogeneous. Allelic frequencies, Nei's distances and *F*-statistics suggest a high genetic differentiation between groups at spatial scales from 10² to 10³ km.

As Fig. 7 shows, there is a lack of a positive relationship between genetic and geographic distance at small spatial scales while such relationship is positive and significant at Western Mediterranean scale. It suggests that the interchange of individuals between close populations probably responds to complex paths through oceanographic currents (González-Wangüemert et al., 2004). At higher spatial scales the existence of exclusive alleles in each Region determines most of the genetic distances.

Such differences in genetic structure are probably related to speciation processes and low connectivity among

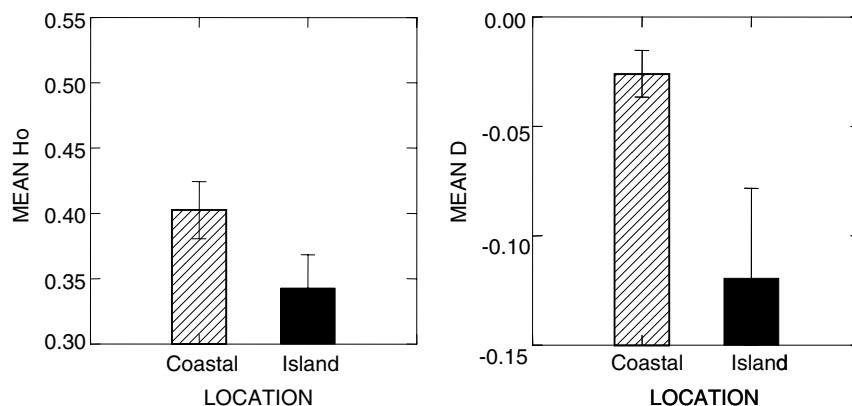


Fig. 4 – Mean observed heterozygosity (*H_o*) and mean deficit of heterozygotes (*D*) in coastal and island populations.

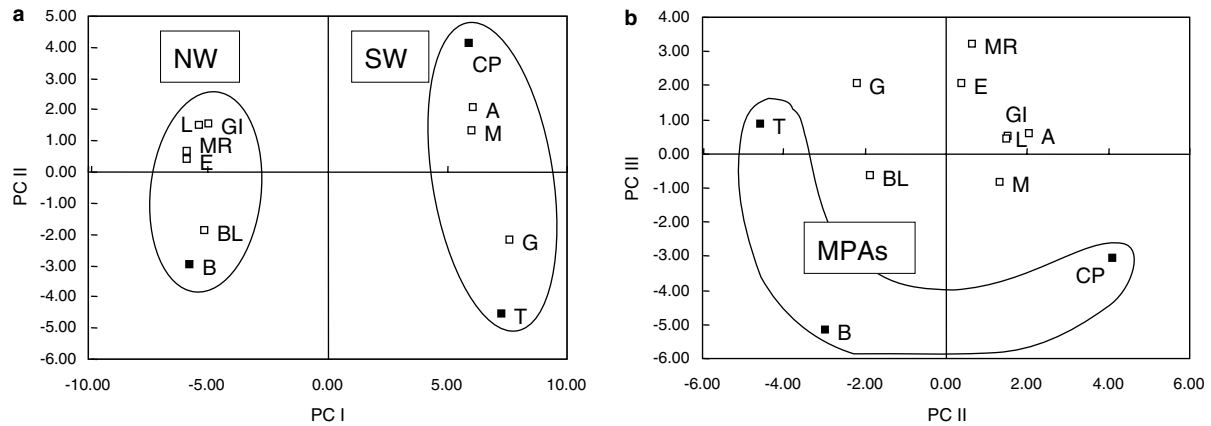


Fig. 5 – Results of the ordination analysis (PCA) displayed in a biplot, scaling the axes and adjusting the alleles scores to the alleles variance: the resulting scores are correlations between alleles and eigenvectors. (a) Components I and II segregate north-western regions from south-western ones and (b) components II and III segregate MPAs from other populations.

geographic regions in the Mediterranean. In a recent study, González-Wangüemert et al. (unpublished data) found that among the 15 exclusive alleles in the SW *D. sargus sargus* populations, 33% are shared with the Atlantic subspecies *D. sargus cadenati*, while none of the 11 exclusive alleles reported by Lenfant (1998) in NW populations are present in the Atlantic subspecies, thus suggesting a more ancient separation. On the other hand, as the calculation of Nei's *D* is based upon comparisons of allele frequencies and is not strongly affected by the presence of unique alleles at low frequencies in particular populations (Avice, 1994), the genetic distinction of relatively close localities (as for example Mazarrón and Aguilas or Marseille and Banyuls) must be attributed to differences in the more common allele frequencies. Thus, in order to explain the heterogeneity in the genetic structure of intraspecific fish populations, the importance of both historical biogeography and contemporary gene flow should be considered, probably being shaped by differential fishing pressure.

Furthermore, the western Mediterranean populations studied are characterised by high levels of heterozygosity but also show high variability between regions, as well as other factors considered. The average heterozygosity for the five populations of *D. sargus* from the south-western Mediterranean ($H_o = 0.4333$) was significantly higher ($p < 0.005$) than those found in the north-western Mediterranean ($H_o = 0.3465$), and both are higher than the values for this species in the southern Adriatic ($H_o = 0.1925$) (Cervelli, 1999) and those reported for many marine fish (Mamuris et al., 1998). High levels of heterozygosity have been associated with having large populations and a long, unbroken history free of population bottlenecks (Gyllensten, 1985; Mamuris et al., 1998). However, we have detected bottlenecks in the Cape Palos and Mazarrón populations ($p < 0.05$; Infinite Allele and Stepwise Mutation Models; Cornuet and Luikart, 1996). On the other hand, the mean heterozygote deficit ($HD = -0.3413 \pm 0.0354$ for the SW Mediterranean vs. -0.0622 ± 0.0199 for the NW region) also shows significant differences ($p = 0.001$).

Finding exclusive alleles in the south-western Mediterranean populations of *D. sargus* with regard to north-western populations, although shared with Atlantic subspecies (González-Wangüemert et al., unpublished data) could mean that some allelic input from the Atlantic gene pool could currently be taking place in the south-western Mediterranean region. This could also explain the higher allelic richness and the higher values of heterozygosity and the heterozygote deficit found in south-western populations, as expected when mixing of distinct genetic stocks takes place (Walhund effect).

4.2. Effect of protection from fishing on the genetic structure of populations

The western Mediterranean reef fish assemblage is patchy at a variety of spatial scales, from tens of metres to hundreds of kilometres. Such spatial heterogeneity makes it difficult to find significant reserve effects at high spatial scales (García-Charton et al., 2004).

In this study, although spatial genetic heterogeneity also makes it difficult to find differences between factors (Protection or Location), the results show the importance of protection from fishing in the preservation of genetic biodiversity.

Both total and standardised allelic richness showed significant differences for the Protection factor ($p = 0.05$) and although the allelic richness of the five populations analysed in the SE of Spain was higher than that of the north-western Mediterranean populations (Lenfant, 1998), the Banyuls MPA population showed similar values to southern populations.

The three MPAs together provided 97.3% of the total number of alleles found in all the western Mediterranean populations studied and 9.5% of this area's genetic pool is shut away in these marine reserves. It is clear that fish sanctuaries act as reservoirs for rare alleles, thus precluding their extinction.

These alleles are also important because they may increase fitness under unusual conditions. Natural selection can act on an allele when it is present but, of course, can not act if the allele does not occur in the gene pool (Ryman et al., 1995).

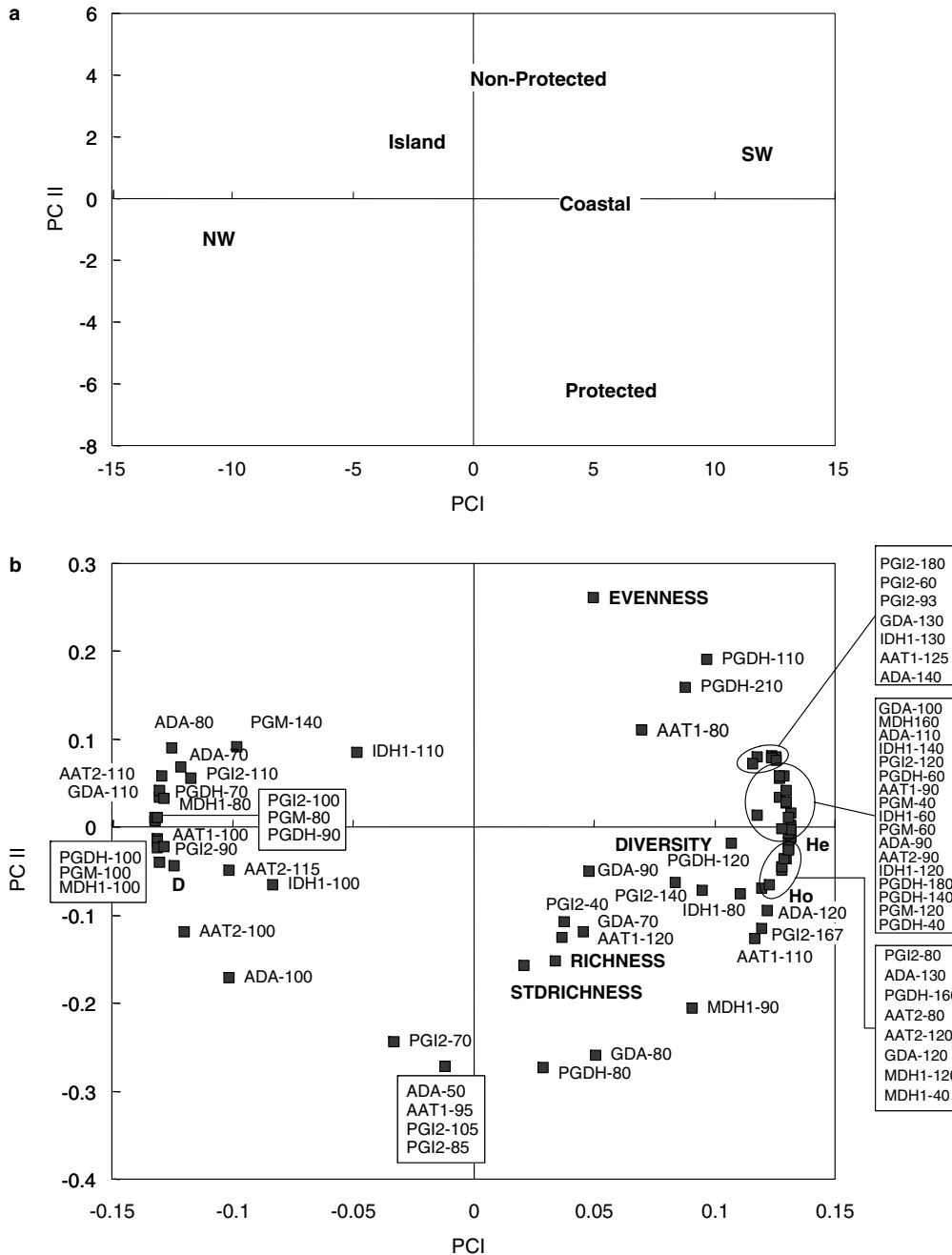


Fig. 6 – Representation of the first two components of the ordination analysis (PCA) performed on populations studied and grouped by factors (a) and the main genetic variables and allelic frequencies explaining it (b).

Facilitating richness seems to be a constant of the reserve effect at higher biological levels, this being the main and almost sole significant effect detected at a species level by meta-analysis in a series of MPAs around the world (Coté et al., 2001).

The fact that evenness was marginally significant ($p = 0.16$) for the Protection factor and correlated with non-protected zones in PCA analyses, shows that fishing acts by increasing evenness. Fishing is selective with regard to phenotypic variation within species (Law, 2000) as fishing gears selects the largest individuals from a population. Natural selection will preserve individuals with higher fecundity and growth rates, making

their phenotypes more frequent, but in an exploited population, these are the very same fish selected by fishing, leading to a reduction in the dominance of their alleles, thus increasing evenness, a measure of the degree to which the alleles are equally represented within the population (Gregorius, 1990).

4.3. Are islands the best choice when establishing MPAs?

Apart from the biological and ecological aspects, a number of geographical factors will affect the success of MPAs and are taken into account in the selection of sites (Badalamenti et al., 2000). Many socio-cultural concerns determine the

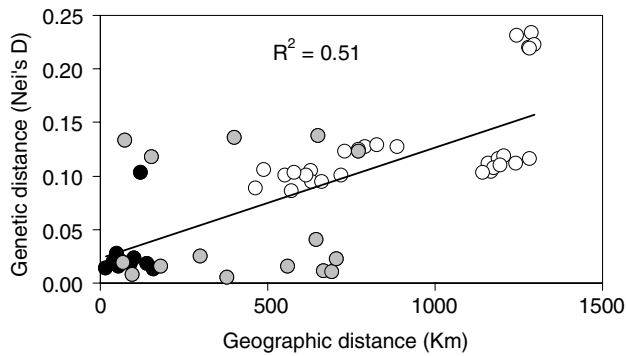


Fig. 7 – Representation of Nei's D genetic distances relative to geographic distances for north-western (grey), south-western populations (black) and between populations of both regions (white) using the shared loci in both studies.

weight of these factors (Soulé and Simberloff, 1986), one of them being the facilities available to ensure vigilance and reduce human pressure.

Of all the seas in the EU, the Mediterranean holds the largest number of marine reserves (17 in number by 1995) (Boudouresque and Ribera, 1993) and this is increasing at a significant rate. In 1999, Badalamenti et al. (2000) inventoried 33 MPAs in Spain, France, Italy and Greece alone. Of these, 58% were located on islands, comprising 88% of the total protected surface. As such, the relevance of the question as to whether islands are a good choice for establishing MPAs is evident and requires adequate answers.

Although geographic variability makes detecting significant differences difficult using ANOVA, there is some evidence of the island effect on the genetic structure of populations which invites discussion.

PCA performed on mean values grouped by factors suggests that islands showed lower allelic richness than coastal zones. Furthermore, 10.8% of the total allelic pool was not found on islands, as opposed to only one allele being absent in coastal zones. Although this result is not conclusive as the sampling effort is not balanced, in comparison with the reserve effect which accounts for 97% of the total allelic richness with the same sampling effort, it reinforces the idea that island populations tend to display an impoverished genetic structure.

Islands also showed lower mean observed heterozygosity ($p = 0.1$) and when avoiding the effects of Region and Protection on Location, the mean heterozygote deficit turned out to be significant ($p < 0.05$).

South-western Mediterranean populations showed the highest heterozygosity among the populations studied and despite this, they also presented a heterozygote deficit, this tendency becoming more marked at Tabarca Island (González-Wangüemert et al., 2004).

Factors causing heterozygote deficit against the Hardy-Weinberg model include: (i) scoring artefacts due to electrophoretic problems such as null alleles; (ii) selection against heterozygotes; (iii) non-random mating or intrasample structure (i.e. Walhund effect) (Meffe, 1986; Mamuris et al., 1998; Rossi et al., 1998).

The effect of null alleles on heterozygote deficiencies has generally been rejected since it requires either extremely high

mutation rates or strong selection in favour of null heterozygotes (Zouros and Foltz, 1984).

If inbreeding were the cause of the heterozygote deficiency in the *D. sargus* populations studied, then one would expect the same effect at all variable loci (Zouros and Foltz, 1984), and this has not been the case. Usually, for organisms which have external fertilization and extended dispersal of planktonic larvae, as is the case for *Diplodus* species, mating between relatives is generally considered to occur with negligible frequency (Gaffney et al., 1990). However, it could be a problem on small islands.

In the case of south-western populations, and in particular that of Tabarca island, the Walhund effect has been proposed as an explanation for the heterozygote deficit on the basis that this locality showed the highest rates of interchange of individuals with the other populations ($N_e m \geq 36$; $F_{ST} \leq 0.005$), as well as the possible connection of this area with Atlantic subspecies which would reinforce the effect (González-Wangüemert et al., 2004).

Another possible explanation for the heterozygote deficit on islands would be selection against heterozygotes. Two modes of selection could account for slight deficiencies: underdominance for viability (selection against heterozygotes) and dominance of the unfavourable allele (Zouros and Foltz, 1984), however, it is difficult to substantiate that this occurs with higher intensity on islands than it does in coastal areas.

Fishing can also reduce heterozygosity (Bergh and Getz, 1989) however there were no significant differences when comparing the heterozygote deficit in protected and non-protected populations.

Therefore, accepting the Walhund effect as a possible explanation for Tabarca Island, but not necessarily for other islands, a combination of factors (isolation, smaller population size and fishing pressure) could be responsible for heterozygote deficit trends on islands.

4.4. The role of MPAs as fisheries management tools from the perspective of population genetics

Avoiding extinction of heavily exploited populations is the first goal of any conservation effort (Man et al., 1995), but since all environments ultimately change and will probably change at an ever-increasing rate due to human influence, then conservation programs must also maintain the capacity of fish to adapt genetically, preserving genetic variability (Meffe, 1986). There is little doubt that increased homozygosity can lower an individual's fitness. There exist numerous data sets and a general consensus that fitness (growth rates, scope for growth, viability, longevity, metabolic efficiency, frequency of disease, survival during stress, variability, vigour, fecundity, fertility, etc.) is enhanced by heterozygosity, and that any decrease in genetic variation, both at an individual or population level, will be paralleled by increasing the mortality rate and a decrease in fitness and evolutionary potential (Polunin, 1983; Meffe, 1986; Soulé and Simberloff, 1986). Heterozygosity is primarily a descriptor of the degree of genetic variation represented by genes occurring relatively frequently in the population so that alleles occurring at low frequencies contribute very little to heterozygosity. However, the reservoir of genetic variation represented by such low-frequency alleles

is also important, because they may increase fitness under unusual conditions. Therefore, our major concern should be the maintenance of existing genetic variance, both within and among different populations, maintaining high levels of heterozygosity and preserving allelic richness (Meffe, 1986).

As our results show, marine reserves preserve the gene pool and genetic diversity, and both are important, not only for the preservation of the structure of populations where fishing is prohibited, but also for ensuring the gene flow between more or less distant populations. However, not all coastal areas would be adequate, in terms of connectivity, for establishing a reserve.

The dispersal of marine fish can take place via three mechanisms; larval drift (Cowen et al., 2000), trophic or reproductive migrations of adults and home range (Rakitin and Kramer, 1996; Russ and Alcala, 1996; Kramer and Chapman, 1999). Difficulties in detecting the exportation of biomass from MPAs to surrounding areas (McClanahan and Mangi, 2000; Gerber and Heppell, 2004) suggest that these mechanisms are not as effective as expected for many littoral demersal fish. Therefore, although the dispersal of marine organisms occurs commonly by means of free-drifting larvae (Gerber et al., 2002) the mortality in these phases is high enough to preclude high rates of exportation over significant distances. Furthermore, many species, such as *D. sargus*, do not show migratory behaviour and juveniles stay close to adult population sites, also, the colonization of empty habitats throughout the home range probably operates at very low spatial scales and excessively long time scales.

Our analyses shows spatial heterogeneity as exists at different scales, suggesting relatively low gene flow, and underlining the role of fishing reserves in the preservation of genetic biodiversity and the importance of maintaining connectivity between populations and MPAs in order to minimize the genetic deterioration of a stock.

At present, marine conservation is very much based on the demarcation of protected sites, but certain ecological processes depend on horizontal dynamics connecting some areas with others (Pineda and Schmitz, 2003) and it is need to guarantee the genetic exchange between subpopulations.

Connectivity depends on the habitat's characteristics and its fragmentation, the distance between patches and species-dispersal capability, being therefore scale dependent (Söndgerath and Schröder, 2002).

In general, it is assumed that in the marine environment, spatially separated areas are more likely to be functionally connected than in terrestrial ecosystems (Jones, 2002). However, this is not always as evident as expected (Palumbi et al., 2003), and the importance of recognising the connectivity between sources and sinks of recruits in designing MPAs for fisheries management has been stressed (Roberts et al., 2001). Protection of dispersal and migratory patterns should be based on the recognition of their spatial connections and, as supported by the spatial heterogeneity displayed by fish populations in the present study, in marine ecosystems, local measures are insufficient when the scale of the connections encompasses large areas of territory. The notion of a critical distance representing an organism's

ability to travel between habitat patches, sensu D'Eon et al. (2002) is a fundamental element to be considered when establishing an MPA, whether coastal or island, and a relationship between mean dispersal and reserve size could determine the persistence of species within a reserve (Lockwood et al., 2002) and the effectivity of protection as fisheries management tool.

Here, development time in larval phases, the pattern and velocity of currents and water mass characteristics involved, are factors to be considered when pelagic dispersal occurs, and as our results suggest, for *D. sargus* medium and large scale connectivity depends more on the main currents in the water column than on habitat characteristics in some potential coastal corridors.

5. Conclusions

Fishing protection is a necessary and effective tool for protecting the genetic biodiversity of target species. The main effect of protection consists in the conservation of allelic richness preserving rare alleles. However, considering the high spatial variability showed by *D. sargus* populations at scales from 10^1 to 10^3 km, the design of MPAs must take into account the spatial heterogeneity in the genetic structure of populations and the connectivity between protected and non-protected populations as well as between MPA network constituents (Cowen et al., 2000; Hellberg et al., 2002). In this sense, a multiscaled approach in detecting connectivity processes is necessary and we agree with Keitt et al. (1997) in that incorporating regional-scale habitat analyses in conservation ecology represents an important step forward, as it places management decisions within the seascape/biogeographic context in its wider meaning and thus avoids local policies that fail to recognize critical links between populations.

Acknowledgements

We thank Dr. Serge Planes from the Laboratoire d'Ichtyoécologie Tropicale et Méditerranéenne (EPHE-UMR CNRS, Université de Perpignan), by its facilities for electrophoresis calibration. Many thanks are also given to fishermen and, particularly, to Toni, whose enthusiastically helped in collecting the samples. We also thank F. Cánovas for his continued support. This research was partially supported by projects MAR98-0449-CO2-01 (Plan Nacional de I+D) and PB/56/FS/02 (Programa SÉNECA).

REFERENCES

- Aebersold, P.B., Winans, G.A., Teel, D.J., Miller, G.B., Utter, F.M., 1987. Manual for starch gel electrophoresis: a method for detection of genetic variation. NOAA Technical Report NMFS 61, pp. 1–19.
- Agardy, T., 1994. Advances in marine conservation: the role of marine protected areas. *Trends in Ecology and Evolution* 9 (7), 267–270.
- Avise, J.C., 1994. *Molecular Markers, Natural History and Evolution*. Chapman and Hall, New York.

- Badalamenti, F., Ramos, A.A., Voultziadou, E., Sánchez-Lisaso, J.L., D'Anna, G., Pipitone, C., Mas, J., Ruiz-Fernández, J.M., Whitmarsh, D., Riggio, S., 2000. Cultural and socio-economic impacts of Mediterranean marine protected areas. *Environmental Conservation* 27, 110–125.
- Bergh, M.O., Getz, W.M., 1989. Stability and harvesting of competing populations with genetic variation in life history strategy. *Theoretical Population Biology* 36, 77–124.
- Bohnsack, J.A., 1998. Applications of marine reserves to reef fisheries management. *Australian Journal of Ecology* 23, 298–304.
- Bohnsack, J.A., Ault, J.S., 1996. Management strategies to conserve marine biodiversity. *Oceanography* 9, 72–82.
- Bonhomme, F., Belkhir, K., Borsa, P., Mathieu, E., Roux, M., 1993. GENETIX-Logiciel D'analyse Des Données Du Groupe de Génétique Des Populations de Montpellier, V.0.1. Université Montpellier II, France.
- Boudouresque, C.F., Ribera, M.A., 1993. Les espèces et les espaces protégés marins en Méditerranée, situation actuelle, problèmes et priorités. Les zones protégées en Méditerranée. Actes de colloque, (C.E.R.P./C.E.M.), Tunis, pp. 94–141.
- Cervelli, M., 1999. *Diplodus sargus*: genetic analysis of a population from the Adriatic Sea. *Biology Marine Mediterranean* 6 (1), 302–305.
- Cornuet, J.M., Luikart, G., 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics* 144, 2001–2014.
- Côté, I.M., Mosqueira, I., Reynolds, J.D., 2001. Effects of marine reserve characteristics on the protection of fish populations: a meta-analysis. *Journal of Fish Biology* 59 (Suppl. A), 178–189.
- Cowen, R.K., Lwiza, K.M.M., Sponaugle, S., Paris, C.B., Olson, D.B., 2000. Connectivity of marine populations: open or closed? *Science* 287, 857–859.
- D'Eon, R.G., Glenn, S.M., Parfitt, I., Fortin, M.-J., 2002. Landscape connectivity as a function of scale and organism vagility in a real forested landscape. *Conservation Ecology* 6 (2), 10.
- Dugan, J.E., Davis, G.E., 1993. Applications of marine refugia to coastal fisheries management. *Canadian Journal of Fisheries and Aquatic Sciences* 50, 2029–2042.
- Gaffney, P.M., Scott, T.M., Koehn, R.K., Diehl, W.J., 1990. Interrelationships of heterozygosity, growth rate and heterozygote deficiencies in the Coot clam, *Mulinia lateralis*. *Genetics* 124, 687–699.
- García-Charton, J.A., Williams, I., Pérez-Ruzafa, A., Milazzo, M., Chemello, R., Marcos, C., Kitsos, M.S., Koukouras, A., Riggio, S., 2000. Evaluating the ecological effects of Mediterranean marine reserves: habitat, scale and the natural variability of ecosystems. *Environmental Conservation* 27, 159–178.
- García-Charton, J.A., Pérez-Ruzafa, A., Sánchez-Jerez, P., Bayle-Sempere, J.T., Reñones, O., Moreno, D., 2004. Multi-scale spatial heterogeneity, habitat structure, and the effect of marine reserves on Western Mediterranean rocky reef fish assemblages. *Marine Biology* 144, 161–182.
- Gerber, L.R., Heppell, S.S., 2004. The use of demographic sensitivity analysis in marine species conservation planning. *Biological Conservation* 120, 121–128.
- Gerber, L.R., Kareiva, P.M., Bascombe, J., 2002. The influence of life history attributes and fishing pressure on the efficacy of marine reserves. *Biological Conservation* 106, 11–18.
- González-Wangüemert, M., Marcos, C., García-Charton, J.A., Pérez-Ruzafa, A., 2002. Importance of population genetics in the management of marine protected areas. In: Aragonés, E. (Ed.), *Mediterranean Symposium on Protected Marine and Coastal Areas. Action Plan of the United Nations Environment Programme*. Thau, S.L., Barcelona, pp. 339–348.
- González-Wangüemert, M., Pérez-Ruzafa, A., Marcos, C., García-Charton, J.A., 2004. Genetic differentiation of *Diplodus sargus* (Pisces: Sparidae) populations in southwest Mediterranean. *Biological Journal of the Linnean Society* 82, 249–261.
- Gregorius, H.R., 1990. A diversity-independent measure of evenness. *American Naturalist* 136, 701–711.
- Gyllensten, U., 1985. The genetic structure of fish: differences in the intraspecific distribution of biochemical genetic variation between marine, anadromous and freshwater species. *Journal of Fisheries Biology* 26, 691–699.
- Halpern, B.S., 2003. The impact of marine reserves: do reserves work and does reserve size matter? *Ecological Applications* 13 (Suppl. 1), S117–S137.
- Harris, H., Hopkinson, D.A., 1976. *Handbook of Enzyme Electrophoresis in Human Genetics*. North Holland, Amsterdam.
- Hellberg, M.E., Burton, R.S., Neigel, J.S., Palumbi, S.R., 2002. Genetic assessment of connectivity among marine populations. *Bulletin of Marine Science* 70 (1), 273–290.
- Jones, P.J.S., 2002. Marine protected area strategies: issues, divergences and the search for middle ground. *Reviews in Fish Biology and Fisheries* 11, 197–216.
- Keitt, T.H., Urban, D.L., Milne, B.T., 1997. Detecting critical scales in fragmented landscapes. *Conservation Ecology* [online] 1 (1), 4.
- Kramer, D.L., Chapman, M.R., 1999. Implications of fish home range size and relocation for marine reserve function. *Environmental Biology of Fishes* 55, 65–79.
- Law, R., 2000. Fishing, selection, and phenotypic evolution. *ICES Journal of Marine Science* 57, 659–668.
- Lenfant, P., 1998. Influence des paramètres démographiques sur la différenciation génétique intra-et inter-populations: le cas du poisson marin, *Diplodus sargus* (Linné, 1758). Thesis, Université Pierre et Marie Curie et de l'Ecole Pratique des Hautes Etudes.
- Lenfant, P., Planes, S., 1996. Genetic differentiation of white sea bream within the Lion's Gulf and the Ligurian Sea (Mediterranean Sea). *Journal of Fish Biology* 49, 613–621.
- Lockwood, D.R., Hastings, A., Botsford, L.W., 2002. The effects of dispersal patterns on marine reserves: does the tail wag the dog? *Theoretical Population Biology* 61, 297–309.
- Lubchenco, J., Palumbi, S.R., Gaines, S.D., Andelman, S., 2003. Plugging a hole in the ocean: The emerging science of marine reserves. *Ecological Applications* 13 (Suppl. 1), S3–S7.
- Magurran, A., 1988. *Ecological Diversity and its Measurement*. Princeton University Press, New Jersey, USA.
- Mamuris, Z., Apostolidis, A.P., Trianta-Phyllidis, C., 1998. Genetic protein in red mullet (*Mullus barbatus*) and striped red mullet (*M. surmuletus*) populations from the Mediterranean Sea. *Marine Biology* 130, 353–360.
- Man, A., Law, R., Polunin, N.V.C., 1995. Role of marine reserves in recruitment to reef fisheries: a metapopulation model. *Biological Conservation* 71, 197–204.
- McClanahan, T.R., Mangi, S., 2000. Spillover of exploitable fishes from a marine park and its effect on the adjacent fishery. *Ecological Applications* 10 (6), 1792–1805.
- Meffe, G.K., 1986. Conservation genetics and the management of endangered fishes. *Fisheries* 11 (1), 14–23.
- Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89, 583–590.
- Palumbi, S.R., 2003. Population genetics, demographic connectivity and the design of marine reserves. *Ecological Applications* 13 (1), 146–158.
- Palumbi, S.R., Gaines, S.D., Leslie, H., Warner, R.R., 2003. New wave: high-tech tools to help marine reserve research. *Frontiers in Ecology of the Environment* 1 (2), 73–79.
- Pineda, F., Schmitz, M.F., 2003. Spatial meshes of the landscape. Concepts applicability and urgent themes related to land planning. In: García-Mora, M.E. (Ed.), *Environmental*

- Connectivity: Protected Areas in the Mediterranean Basin. Junta de Andalucía, pp. 9–27.
- Plan Development Team, 1990. The potential of marine fishery reserves for reef fish management in the US southern Atlantic. NOAA Technical Memorandum, NMFS-SEFC 261, Miami.
- Polunin, N.V.C., 1983. Marine genetic resources and the potential role of protected areas in conserving them. *Environmental Conservation* 10 (1), 31–41.
- Rakitin, A., Kramer, D.L., 1996. Effect of a marine reserve on the distribution of coral reef fishes in Barbados. *Marine Ecology Progress Series* 131, 97–113.
- Raymond, M., Rousset, F., 1995. An exact test for population differentiation. *Evolution* 49, 1280–1283.
- Roberts, C.M., 1997. Ecological advice for the global fisheries crisis. *Trends in Ecology and Evolution* 12, 35–38.
- Roberts, C.M., Polunin, N.V.C., 1991. Are marine reserves effect in management of reef fisheries? *Reviews in Fish Biology and Fisheries* 1, 65–91.
- Roberts, C.M., Bohnsack, J.A., Gell, F., Hawkins, J.P., Goodridge, R., 2001. Effects of marine reserves on adjacent fisheries. *Science* 294, 1920–1923.
- Rossi, A.R., Capula, M., Crosetti, D., Sola, L., Campton, D.E., 1998. Allozyme variation in global populations of striped mullet, *Mugil cephalus* (Pisces: Mugilidae). *Marine Biology* 131, 203–212.
- Russ, G.R., 2002. Yet another review of marine reserves as reef fishery management tools. In: Sale, P.F. (Ed.), *Coral Reef Fishes. Dynamics and Diversity in a Complex Ecosystem*. Academic Press, San Diego, pp. 421–443.
- Russ, G.R., Alcalá, A.C., 1996. Do marine reserves export adult fish biomass? Evidence from Apo Island, central Philippines. *Marine Ecology Progress Series* 132, 1–9.
- Ryman, N., Utter, F., Laikre, L., 1995. Protection of intraspecific biodiversity of exploited fishes. *Reviews in Fish Biology and Fisheries* 5, 417–446.
- Sale, P.F., Cowenb, R.K., Danilowicz, B.S., Jonesd, G.P., Kritzer, J.P., Lindemanf, K.C., Planes, S., Poluninh, N.V.C., Russ, G.R., Sadovyi, Y.J., Steneckj, R.S., 2005. Critical science gaps impede use of no-take fishery reserves. *Trends in Ecology and Evolution* 20 (2), 74–80.
- Söndgerath, D., Schröder, B., 2002. Population dynamics and habitat connectivity affecting the spatial spread of populations – a simulation study. *Landscape Ecology* 17 (1), 57–70.
- Soulé, M.E., Simberloff, D., 1986. What do genetics and ecology tell us about the design of nature reserves? *Biological Conservation* 35, 19–40.
- Waters, J.R., 1991. Restricted access vs. open access methods of management: toward more effective regulation of fishing effort. *Marine Fisheries Review* 53, 1–10.
- Weir, B.S., Cockerham, C.C., 1984. Estimating F-statistics for the analysis of population structure. *Evolution* 38, 1375–1370.
- Wilson, D.S., Clarke, A.B., 1996. The shy and the bold. *Natural History* 9/96, 26–28.
- Zouros, E., Foltz, D.W., 1984. Possible explanations of heterozygote deficiency in bivalve molluscs. *Malacologia* 25, 583–591.