

**FISHERIES AND GENE DIVERSITY CONSERVATION:
THE CASE OF SWORDFISH IN THE MEDITERRANEAN SEA**

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Introduction

Fisheries management has been mainly based on data drawn from ecology and population dynamics, because the abundance and size of fish available for harvesting were considered as the most relevant information to address exploitation (Allendorf *et al.*, 1987). More recently, a new approach in fisheries management was needed, due to the global crisis of the most important fisheries in the world. Tools and methods of population genetics have been fruitfully used to produce a bulk of empirical data frequently allowing the estimation of parameters having management implications (Avisé, 1996) such as stocks identification, effective population size assessment, evaluation of biodiversity levels, investigation of the relationships occurring between over-exploitation and genetic erosion, estimation of extinction risks (Carvalho and Pitcher, 1995). In particular, population genetics background can provide quick and reliable tools in assessing the status of fish stocks: biomass exploitation can indeed cause the loss of genotypic arrays, reducing genetic diversity within populations. This process, called genetic erosion, can accomplish in few years, with a loss of genetic variation even more heavy and faster than biomass decline (Gharret and Thomason, 1987; Nielsen *et al.*, 1997; Smith *et al.*, 1990; Vuorinen *et al.*, 1991). The investigation of genetic variation levels is therefore a quick and reliable method to monitor the status of fish stocks.

Another innovative methodology to identify fish stocks and assess their status, is the study of fish parasites. Considering pathogens in exploited fish species, it has been suggested that in host-parasite systems, high levels of polymorphism in the host seem to be necessary to recognise and resist to parasite infection (Frank, 1997), by a "arms race" by which the parasite, that becomes adapted to the host most common genotypes, must face the rise and selective advantage of new genotypes in the host. If the genetic variability of the host is impoverished, this dynamic frequency-dependent selection process can no more operate, thus leading to a drastic enhancement of the host susceptibility to its parasites.

The combined assessment of genetic diversity and parasite infection levels, can be a sensitive and friendly method to provide reliable information about fish stocks state.

The results of a genetic diversity survey of Mediterranean and Atlantic samples of swordfish, carried out by means of allozyme electrophoresis, are here reported and their implication for the management of this species are discussed.

Materials and methods

Swordfish samples have been collected from four localities of the Mediterranean Sea and from Southern Atlantic Ocean offshore South-Africa, giving 407 specimens (Table 1). For each captured specimen a sample of muscle, liver and heart has been collected and frozen at -80°C.

Standard horizontal starch gel electrophoresis has been performed at 5°C at 7-9 V/cm for 3-7 hours, following the procedures reported in Cimmaruta *et al.*, 1996. Twenty-nine putative gene loci have been scored: α -Gpdh, Ldh-1, Ldh-2, Mdh-1, Mdh-2, Mdhp-1, Mdhp-2, Idh-1, Idh-2, 6Pgdh, Xdh, NADH-dh, Sod, Aar-1, Aar-2, Adk-1, Est-1, Est-2, Acph-2, Ap-1, Ap-2, Pep, PepD-1, PepD-2, Ada, Ca-2, Mpi, Gpi, Pgm-1.

Chi-square test (χ^2) and *F*-statistics (Wright, 1943, 1951) were used to detect significant departures from predictions of Hardy-Weinberg equilibrium. Contingency chi-square analysis has been performed to determine homogeneity of allozyme frequencies among populations (Sokal and Rohlf, 1981). Genetic variability of populations has been estimated using the following parameters: proportion of polymorphic loci (*P*), with a locus considered polymorphic if the frequency of the most common allele does not exceed 0.95, observed and expected mean heterozygosity per locus (H_o and H_e).

Table 1 - Collection localities listed with the catching gear used and the number of specimens analysed.

	Collection locality	Catching gear	<i>N</i>
1	Ligurian Sea	Long line	75
2	Northern Tyrrhenian Sea	Non professional equipment Long line	43
3	Southern Tyrrhenian Sea	Trolling gear Long line	66
4	Ionian Sea	Non professional equipment Long line	172
5	Southern Atlantic Ocean	Long line	51

The partitioning of genetic diversity within and among samples has been analysed for variable loci using Wright's F_{ST} (1943, 1951). Gene flow between conspecific populations has been estimated by the parameter Nm (where *m* is the fraction of migrants in a population of effective size *N*) calculated from F_{ST} following Crow and Aoki method (1984).

Results

18 out of the 29 scored loci are polymorphic: α -Gpdh-1, Ldh-1, Ldh-2, Mdh-1, Mdh-2, Mdhp-1, Mdhp-2, Idh-1, 6Pgdh, Sod, Adk-1, Aar-1, Ap-2, PepB-1, Ada, Ca, Pgi-1, Pgi-2. Two of them, α -Gpdh-1 and Mdhp-2, become monomorphic if the sample from Atlantic Ocean is not considered. From two to four allozymes per locus were observed, with a maximum of three per sample. In the Mediterranean samples the most common allele is always observed at very high frequencies, while in the Atlantic sample it has lower frequencies, indicating more stable polymorphisms in the oceanic population.

The analysis of the genetic variability parameters shows a scarce genetic variation in the Mediterranean samples, that is sensitively higher in the South-African population (Table 2). The observed heterozygosity H_o ranges indeed between 0.042 and 0.050 in the Mediterranean and reaches a value of 0.091 in the Atlantic Ocean. The percentage of polymorphic loci *P* as well has lower values in the Mediterranean (from 17.2 to 24.1) than in the Atlantic samples (37.9).

Table 2 - Genetic variability parameters for the samples of *X. gladius*.

	Sampling locality	P_{95}	H_o	H_e
1	Ligurian Sea	24.1	0.042 (0.014)	0.043 (0.015)
2	Northern Tyrrhenian Sea	21.4	0.044 (0.014)	0.045 (0.016)
3	Southern Tyrrhenian Sea	17.2	0.050 (0.020)	0.048 (0.019)
4	Ionian Sea	24.1	0.048 (0.015)	0.050 (0.015)
5	Atlantic Ocean	37.9	0.091 (0.025)	0.090 (0.024)

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Table 4 - Number of swordfish specimens analysed by RFLP and of genotypes found per studied locality (From Chow *et al.*, 1997)

Sample	Nspecimens	Ngenotypes
Japan	45	20
Hawaii	51	22
Mexico	34	13
Ecuador	21	15
Peru	53	20
S-W Pacific	31	14
Sri Lanka	27	16
S Java	35	16
South Africa	11	7
Brazil	59	21
N-W Atlantic 90	26	12
N-W Atlantic 93	29	10
Mediterranean	34	5

A possible scenario explaining these data is that the gene pool of the Mediterranean swordfish has been depleted, probably by over-exploitation, and the strong isolation of this stock has prevented its restoration through gene exchange with extra-Mediterranean populations. Being the swordfish exploited since long time, it is impossible to prove the depletion of its genetic variability by overfishing today. It is however possible to find indirect evidences supporting the previously depicted scenario. A recent study carried out on a number of large pelagic fishes in the Mediterranean Sea has shown that they all exhibit comparable levels of genetic variation except the swordfish and bluefin tuna (Cimmaruta *et al.*, 1996, 1997, 1998). This could be due to the different life history, but also to the different fishing pressure they suffered. Another indication comes from a preliminary parasitological survey of Mediterranean swordfish, revealing a higher prevalence of the copepods *P. ennella* spp., of the nematodes *Hysterothylacium corrugatum* and *H. incurvum* and of monogeneans *Tristoma coccineum* and *T. integrum*, with respect to the observations for Atlantic swordfish (Hogans *et al.*, 1983; Nascetti *et al.*, 1998; IMattiucci *et al.*, unpublished data).

The data examined seem to indicate that the Mediterranean stock of swordfish is over-exploited and that conservation measures are needed. However, in spite of the great amount of scientific data produced on economically relevant fishes, only a few gave a real contribution in planning a more sustainable yield policy. This was because the socio-economic implications of the obtained results were poorly considered, and now both scientists and politicians admit that it is mandatory to take account of the consequences of reduced fishing (the only possible solution for over-exploited stocks) in the social and economic field, to have a real impact on fisheries management (Anon., 1997). This is why the European Community fishery policy has, for example, focused the Multiannual Guidance Programmes on size classes management, among other topics. In the case of Mediterranean swordfish, being the conservation of its residual genetic variation a priority, it could be worth to test if, as for other species, there is a correlation between size and individual heterozygosity. In this case the larger specimens should retain the more heterozygous genotypes and therefore their conservation should be a priority in preventing the complete depletion of the genetic resources of the Mediterranean stock.

The number of specimens sampled per population is not the same in all the studied localities, but the sample showing the higher genetic variation (Atlantic Ocean) has the lower number of specimens analysed (Table 1), strongly supporting the idea that different levels of variability characterise Mediterranean and Atlantic samples

The examination of the F_{ST} parameter per locus (Table 3) shows a remarkable differentiation at locus *Mdhp-2*, ($F_{ST} = 0.350$), mainly due to a inter-population differentiation ($F_{IT} = 0.415$ vs. $F_{IS} = 0.101$).

Table 10 - Genetic diversity parameters for the studied *X. gladius* samples.

LOCUS	F_{IS}	F_{IT}	F_{ST}
α -Gpdh-1	-0.032	0.008	0.024
Ldh-1	-0.005	-0.001	0.004
Ldh-2	-0.144	-0.105	0.033
Mdh-1	-0.030	-0.029	0.001
Mdh-2	-0.053	-0.013	0.038
Me-1	-0.043	-0.017	0.025
Me-2	0.101	0.415	0.350
Idh-1	-0.001	0.020	0.021
6Pgdh	-0.049	-0.028	0.020
Sod	-0.119	-0.059	0.054
Adk-1	0.023	-0.045	0.022
Aat-1	-0.165	-0.045	0.103
Ap-2	-0.093	-0.041	0.047
PepB-1	-0.102	-0.048	0.049
Ada	0.233	-0.235	0.002
Ca	0.036	0.095	0.062
Pgi-1	-0.031	-0.011	0.020
Pgi-2	-0.019	0.007	0.012
Mean	-0.012	0.060	0.071

The estimated gene flow is $Nm = 1.84$, quite low for a large pelagic fish. The value of the Nm parameter however grows up to 10.66 when the Atlantic sample is removed from the analysis (at the same time F_{ST} lowers to 0.013), showing that the Mediterranean sample has a gene pool not only characterised by a low genetic variability, but also partially isolated from the oceanic population.

Discussion and conclusions

A sensitive difference in the levels of genetic variation has been found comparing swordfish samples from Mediterranean Sea and Southern Atlantic Ocean. The samples from the Mediterranean basin are characterised by a low level of genetic variability while the Atlantic sample shows a genetic variation nearly twice greater ($H_o = 0.091$). These data are in agreement with those reported by Chow *et al.* (1997), who investigated the genetic structure of swordfish by PCR-RFLP analysis. In their world-wide survey they have recorded the lowest number of genotypes in the Mediterranean Sea than in any other geographic area (Table 4). Allozymes and RFLP both agree also in finding the Mediterranean swordfish population nearly completely isolated from the others. Our estimation of the gene flow parameter gave a Nm value of 1.84, very close to a total absence of gene exchange ($Nm = 1$).

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