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Genetic variability and fingerling quality in wild and reared stocks of European sea bass, *Dicentrarchus labrax*

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SUMMARY - The development of sustainable aquaculture models requires an increasing knowledge of hatchery production practices. This preliminary study has aimed at investigating the domestication process in the European sea bass, *Dicentrarchus labrax.* Genetic variability and frequencies of anatomical abnormalities were inspected in five hatcheries and in one sample of wild sea bass juveniles. Gene-enzyme analysis (carried out through starch gel electrophoresis on about 300 individuals) revealed low genetic distances among groups, and allelic and genotypic frequency shifts in the hatchery groups when compared to the wild one. The analysis of differences in meristic counts and physical anomaly types and frequencies (evaluated on more than 430 juveniles) revealed a wide morphological variation among the hatchery groups and also between these and the wild group, with some hatchery-specific trends.

Key words: *Dicentrarchus labrax*, genetic variability, skeletal anomalies, sustainability.

RESUME *- "Variabilité génétique et qualité des alevins chez des populations sauvages et cultivées de bar,* Dicentrarchus labrax*". Le développement des modèles d'aquaculture durable demande une connaissance croissante des pratiques de production d'écloserie. Dans ce cadre, cette étude a utilisé une approche intégrée pour évaluer la situation actuelle du loup,* Dicentrarchus labrax*. La variabilité génétique et les fréquences des malformations anatomiques ont été comparées entre lots d'alevins de différentes écloseries et avec des alevins sauvages. L'analyse gène-enzyme (exécutée par électrophorèse sur gel d'amidon appliquée à environ 300 alevins) a mis en évidence des distances génétiques courtes entres les groupes, et un déplacement des fréquences alléliques et génotypiques dans les alevins d'écloserie comparées à celles des sauvages. L'analyse des différences des comptes méristiques et les types et fréquences des anomalies (observées sur plus de 430 alevins) a montré une grande variation morphologique entre les différents groupes d'écloserie et avec le groupe sauvage.*

Mots-clés : Dicentrarchus labrax, *variabilité génétique, anomalies squelettiques, durabilité.*

Introduction

The European sea bass, *Dicentrarchus labrax,* is a marine and brackish water finfish. Aquaculture production has drastically increased in Mediterranean countries

during the last decade; production in the region increased from 285 mt in 1984 to over 18.500 mt in 1995 (FAO, 1995a, and unpublished data). Artificial propagation was introduced to meet the requirement of seed, and all reared sea bass fingerlings are now produced in hatcheries.

This preliminary study tries to assess the impact of artificial conditions on cultured juveniles of the European sea bass, using genetic and morphological descriptors. The application of the "Code of Conduct for Responsible Fisheries" (CCRF) (FAO, 1995 b) demands an increasing knowledge of the effects of human activities on both wild populations and the environment. This code implies that "wild" genetic resources must be documented in order to establish a benchmark to measure the impact of human intervention and that the genetic diversity of farmed species must be periodically assessed (CCRF, Article 9.3, FAO, 1995b). In fact, the large differences between natural spawning and hatchery conditions, i.e., the practice of artificial selection, the hybridisation of different stocks and strains, the transfer of broodstocks, eggs, larvae and fry over large distances, involve the risk of either reducing the effective population size (Ne), so increasing inbreeding, or causing artificial gene flow, which could induce a biodiversity decline or outbreeding depression (see discussion in Campton, 1995). Fish farmers demand systems to assess the quality of finfish seed, taking into account the diversified rearing techniques, from land-based extensive ponds to off-shore intensive farming.

This study used an integrated range of techniques which are usually applied for basic research, as a tool to monitor the impact of hatchery rearing on the European sea bass. One wild and five hatchery groups of sea bass juveniles were analysed. In addition to the analysis of morphological descriptors (meristic characters and developmental anomalies), previously adopted in order to assess fry quality criteria (Boglione *et al.*, 1993), a gene-enzyme systems analysis was undertaken in order to (i) test the sensitivity of the allozyme markers in assessing the present genetic situation of hatchery juveniles and in detecting any of the possible processes, above mentioned, that could affect genetic diversity; and (ii) compare patterns of variation in wild and hatchery stocks for the two sets of genetic and morphological data.

Material and methods

Six groups of sea bass juveniles were genetically and morphologically analysed. Five samples were obtained by hatchery reproduction under controlled conditions in five Italian commercial hatcheries (located in Friuli, Veneto, Toscana, Abruzzo and Puglia), while the sixth group was composed of wild juveniles collected in the North Adriatic Sea. Commercial hatcheries are simply designated as M,S,Z,U, and V in order to preserve their anonymity. Gene-enzyme analysis was carried out through starch gel electrophoresis on about 300 specimens. Morphological analysis, through meristic counts (number of vertebrae and number of pectoral, dorsal, anal, and caudal fin rays) and observation of skeletal anomalies (Table 1), was carried out on more than 430 specimens, most of them X-rayed, and the smallest ones, lightly ossified, stained *in toto* with alizarin S red (modified from Dingerkus and Uhler, 1977).

Table 1. Legend of physical anomalies (modified from Boglione *et al.*, 1993) examined on specimens of European sea bass.

Code	Region	Anomaly
A	Cranial vertebrae	Axis: kiphosis, lordosis
		Vertebrae: fusion, malformation
		Neural arches: malformation
B	Pre-hemal vertebrae	Axis: kiphosis, lordosis
		Vertebrae: fusion, malformation
		Neural arches: malformation
C	Hemal vertebrae	Axis: kiphosis, lordosis
		Vertebrae: fusion, malformation
		Neural and hemal arches: malformation
D	Caudal vertebrae	Axis: kiphosis, lordosis
		Vertebrae: fusion, malformation
		Neural and hemal arches: malformation
F	Anal fin	Fusion, absence, supernumerary or malformation of rays and pterygiophores
G	Caudal fin	Hypuralia and epuralia: fusion or malformation
		Principal rays: fusion, absence or malformation
H	1st dorsal fin	Fusion, absence, supernumerary or malformation of rays and pterygiophores
I	2nd dorsal fin	Fusion, absence, supernumerary or malformation of rays and pterygiophores
L	Splanchno-cranium	Elongation, reduction or malformation of pre-maxillary, maxillary, dental.
M	Swim bladder	Not inflated
N	Urinary duct	Presence of *calculi* in the terminal tract

Results and discussion

Sixteen loci were found to be polymorphic among the thirty-five gene loci analysed. Levels of genetic variability (Ho and P%) were similar in both reared and wild groups. Correspondingly, genetic distances (Nei, 1978) (see Table 2) were very low, ranging from D=0.006 to D=0.047 in all six groups. Allelic and genotype frequencies in the hatchery groups were different from the wild group.

Allelic assortment at the most interesting loci is shown in Fig. 1. The locus for alcohol dehydrogenase, *ADH*,* as well as those for glucose-6-phosphate dehydrogenase, *G6PD-2*,* and for mannose-6 phosphate isomerase *MPI**, were found to be monomorphic in the five hatchery groups and polymorphic in the wild population. On the contrary, other loci, such as pyruvate kinase, *PK-1*,* were

monomorphic in the wild sample, but polymorphic in the hatchery ones. Moreover, for the malic enzyme locus, *ME**, the wild-allele was rare or totally absent in the hatchery groups. At least at two of these loci, *G6PD-2** and *ME**, non neutral alleles were detected in freshwater acclimated groups (Allegrucci at al. 1994) and in natural populations (Allegrucci *et al.*, 1997) of European sea basses. Nevertheless, these results might also reflect a certain degree of genetic structuring, proposed for the species after mtDNA (Patarnello *et al.*, 1993), microsatellite DNA (García de León *et al.*, 1997, Castilho and McAndrew, this volume) and allozymic (Allegrucci *et al.*, 1997) studies.

Table 2. Unbiased genetic distances (Nei, 1978) between the six groups of European sea bass. Calculations are based on 35 protein loci

Group	M	S	Z	U	V	W
M	-					
S	.007	-				
Z	.007	.015	-			
U	.007	.014	.016	-		
V	.006	.007	.020	.011	-	
W	.033	.045	.047	.027	.038	-

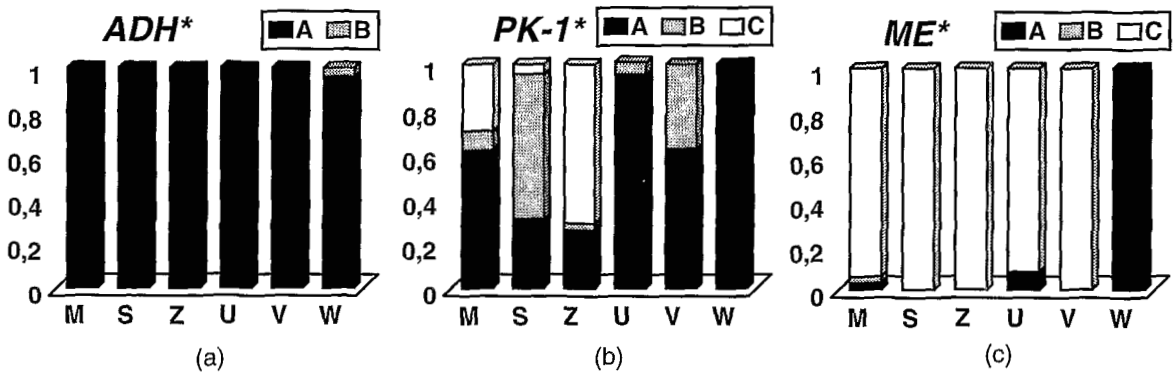

Fig. 1. Allelic frequencies at some loci in the six analysed samples of *D. labrax*. M, S, Z, U and V: hatchery groups. W: wild group.

Also genotypic frequencies showed differences between natural and reared samples. Indeed, although in the wild population all investigated loci conformed to Hardy-Weinberg equilibrium, in the hatchery ones many loci showed significant deviations from these proportions. Among these, loci *AAT-1**, *GAPDH-1**, *GDA**, *ME** and *PK-1** deviate from HWE in all the reared groups in which they are polymorphic and loci *ADA**, *LDH-1** and *SOD** only in some of them. In several cases, these deviations occurred as heterozygote deficiencies.

Genetic changes were observed that may be related to aquaculture practices. However it should be emphasised that when analysing hatchery stocks with technical tools usually used to analyse natural populations or experimentally controlled samples, data interpretation has to be performed from a different perspective. In fact the origin and recent history of hatchery stocks are uncertain, while the origin of natural populations and the recent history of experimental samples are better known. Moreover, farmed fish might have been more or less heavily subjected to human interventions such as mass selection, addition of F1 or F2 individuals to the original wild-spawner pool or mixing of juveniles from different subpopulations. Any of these interventions may have a consequence on the genetic composition of samples to be analysed. Present results, in particular the heterozygote deficit, suggest that one or more among the above mentioned interventions may have occurred.

The results suggest that allozyme analysis is an effective tool to study genetic resources in European seabass and to detect some genetic changes due to aquaculture practices. Genetic analysis on hatchery and wildgroups is continuing in order to understand better the causes and implications of these results.

Significant differences were found for some of the meristic characters we counted. Indeed each reared sample showed differences in the number of the second dorsal fin rays (Fig. 2a) when compared to wild population. Moreover, the U group also showed differences in the number of both the anal (Fig. 2b) and the first dorsal (Fig. 2c) fin rays.

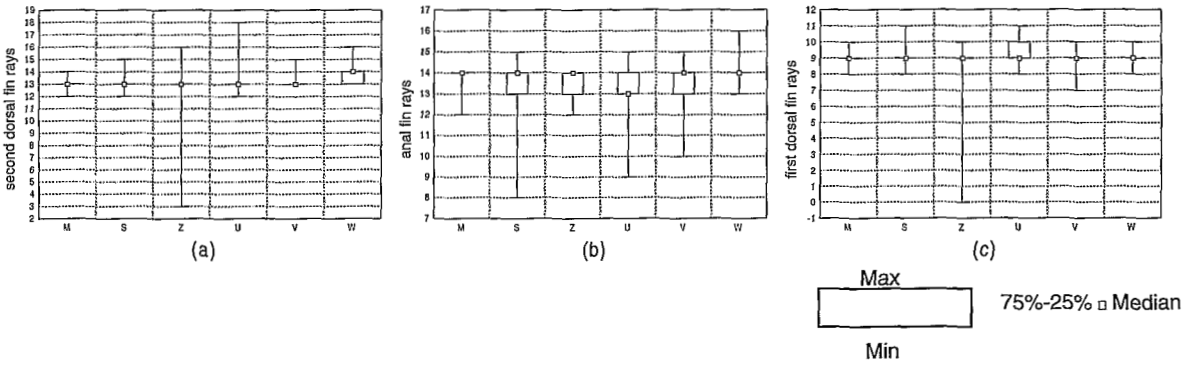

Fig. 2. Box-whisker plots (StatSoft Inc.,1996) of some meristic characters in the six analysed samples of *D. labrax.* M, S, Z, U and V: hatchery groups. W: wild group.

Thirty-four skeletal anomalies were detected in different body regions, such as vertebral axis, vertebrae, fins, and splanchno-cranium (Table 1). The absence of a functional swim bladder, as well as the presence of *calculi* in the terminal tract of the urinary ducts were also considered and computed among the developmental anomalies.

The inspection of anomalies revealed a high percentage of affected individuals in each group corresponding to 82% of affected individuals in the wild group and minimum 95% in the farms groups. When considering the frequency of anomalies in

each body region for each group (Fig. 3), it is possible to notice that some farm specific trends in the anomalies pattern distribution characterise the five hatchery samples. In fact, for example, both groups S and M showed a high percentage of anomalies in the hemal region, but, only group M showed an altered caudal fin. Group Z showed a high percentage of anomalies in the dorsal fin, but showed almost no anomalies either in the skeletal axis or in the vertebrae. In the wild group, the percentage of fish with multiple anomalies in the hemal region was higher than that reported in other wild samples (Boglione *et al.*, 1993; Boglione *et al.*, 1994; Marino *et al.*, 1993). Moreover, the latter samples showed more anomalies in the pre-hemal than in the hemal vertebrae and no axis deformation.

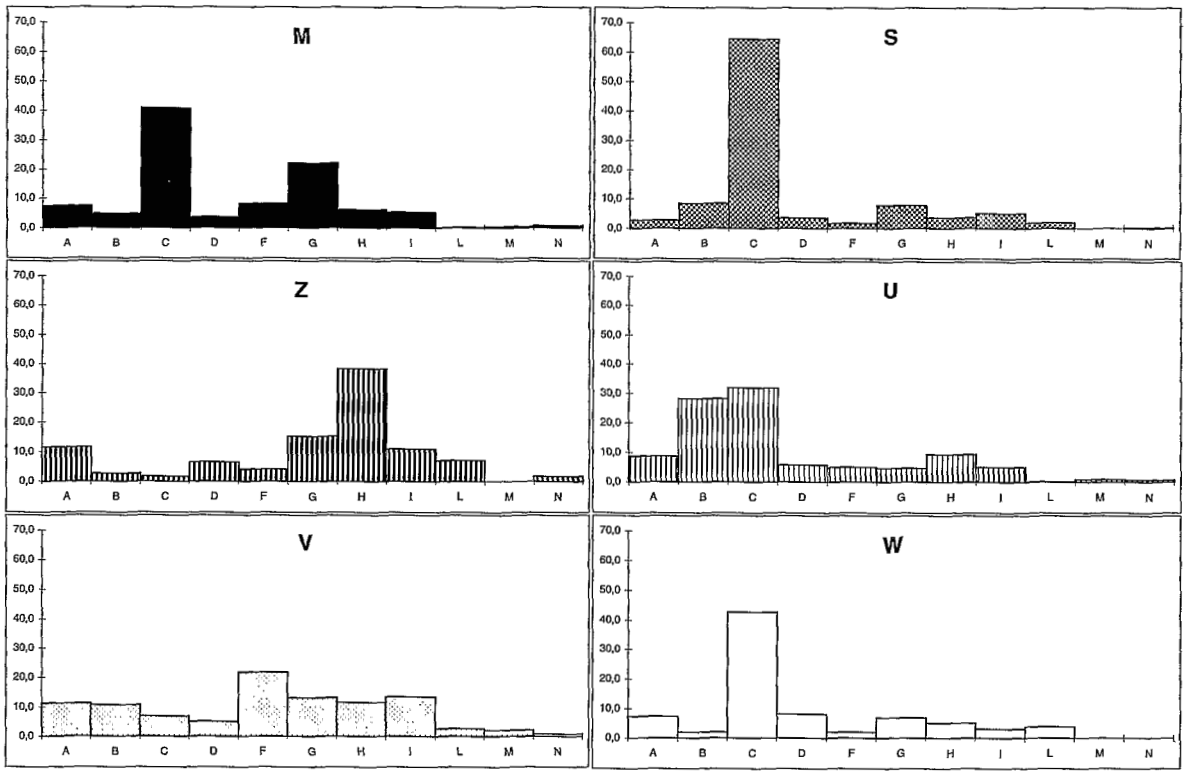

Fig. 3. Relative frequencies of anomalies in different body regions for each group of *D. labrax*. M, S, Z, U, V: hatchery groups; W: wild group. A, B, C, D: cranial, pre-hemal, hemal and caudal regions of vertebral axis; F, G, H, I: anal, caudal, first dorsal and second dorsal fins; L: splanchno-cranium; M: swim bladder; N: urinary ducts.

Considering the two sets of data, it can be concluded that there is a wide morphological variation in terms of meristic and anomalies descriptors, both among the hatchery groups and when these latter are compared to the wild group. Additional analyses are being carried out to evaluate the relationships among the genetic and the anomalies patterns.

References

Allegrucci, G., Fortunato, C., Cataudella, S. and Sbordoni, V. (1994). Acclimation to freshwater of the sea bass: Evidence of selective mortality of allozyme genotypes. In: *Genetics and Evolution of Aquatic Organism,* Beaumont, A.R. (ed). Chapman & Hall, New York, NY, pp.486-502.

Allegrucci, G., Fortunato, C. and Sbordoni, V. (1997). Genetic structure and allozyme variation of sea bass (*Dicentrarchus labrax* and *D. punctatus*) in the Mediterranean Sea. *Mar. Biol.,* 128, 347-358.

Boglione, C., Marino, G., Bertolini, B., Rossi, A., Ferreri, F. and Cataudella, S. (1993). Larval and post larval monitoring in sea bass: Morphological approach to evaluate finfish seed quality. In: *Production, Environment and Quality,* Bordeaux Aquaculture, 1992, Barnabé, G. and Kestemont, P. (eds). European Aquacolture Society. Special publication no. 18, Ghent, Belgium, pp. 189-204.

Boglione, C., Marino, G., Ferreri, F., Finoia, M.G., Scardi, M., Fresi, E. and Cataudella, S. (1994). Anatomical aspects for seed quality assessment in sea bass (*Dicentrarchus labrax*): Hatchery and wild populations. In: *Measures for success: Metrology and instrumentation in aquaculture management,* Bordeaux Aquaculture, 1994, Kestemont, P., Muir, J., Sévila, P., Williot, P. (eds), CEMAGREF-Edition, pp 191-197.

Campton, D.E. (1995). Genetic effects of hatchery fish on wild population of Pacific salmon and steelhead: What do we really know? In: *Uses and Effects of Cultured Fishes in Aquatic Ecosystem,* Schramm, H.L. and Piper, R.G. (eds.) American Fisheries Society Symposium 15, Bethesda, MD, pp 337-353.

Dingerkus, G. and Uhler, L.D. (1977). Enzyme clearing of Alcian blue stained whole small vertebrates for demonstration of cartilage. *Stain Technology,* 52: 229-232.

FAO (1995a). *Aquaculture production statistics. 1985-1994.* FIDI/C815 (Rev. 8). Food and Agriculture Organization of the United Nations, Rome, Italy.

FAO (1995b). *Code of Conduct for Responsible Fisheries.* Food and Agriculture Organization of the United Nations, Rome. ISBN 92.5.103834-1.

García de León, F.J., Chikhi, L. and Bonhomme, F. (1997). Microsatellite polymorphism and population subdivision in natural populations of European sea bass *Dicentrarchus labrax* (Linnaeus, 1758). *Mol. Ecol.,* 6, 51-62.

Marino, G., Boglione, C., Bertolini, B., Rossi, A., Ferreri, F. and Cataudella, S. (1993). Observations on development and anomalies in the appendicular skeleton of sea bass (*Dicentrarchus labrax*, L. 1758) larvae and juveniles. *Aquacult. Fish. Manag.*, 24: 445-456.

Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89: 583-590.

Patarnello, T., Bargelloni, L., Caldara, F. and Colombo, L. (1993). Mitochondrial DNA sequence variation in the European sea bass, *Dicentrarchus labrax* L. (Serranidae): Evidence of differential haplotype distribution in natural and farmed populations. *Mol. Mar. Biol. Biotech.* 2: 333-337.

StatSoft Inc. (1996). STATISTICA for Windows [Computer program manual]. Tulsa, OK, USA.