



## GENIMPACT

Evaluation of genetic impact of aquaculture activities on native populations.

A European network

WP1. Genetics of domestication, breeding and enhancement of performance of fish and shellfish

### European sea bass - *Dicentrarchus labrax*



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## A. Biology, ecology and genetics

### Distribution

The European (or common) sea bass, *Dicentrarchus labrax* L. (Moronidae, Perciformes) is found in coastal waters of the Atlantic Ocean from South of Norway (60°N) to Western Sahara (30°N) and throughout the Mediterranean Sea and the Black Sea. It has been introduced for culture purposes in Israel, and more recently in Oman and the United Arab Emirates.

### Capture

It is a fish with high commercial value both from capture from wild stocks, and in the last 25 years from aquaculture production. In 2004, the global sea bass capture fisheries production was of 11,481 tonnes (1) with France and Italy accounting respectively for 42% and 29% (Fig.1).

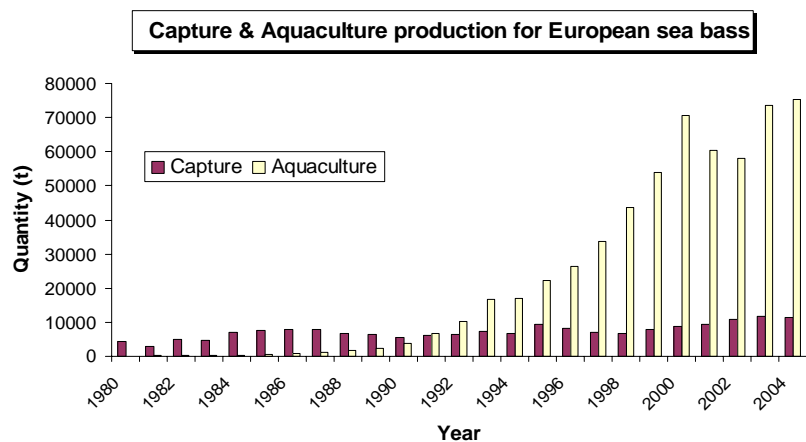


Fig 1. Capture fisheries (1) and aquaculture production (2) for European sea bass in Europe and in the Mediterranean

### Biology

European sea bass is a gonochoristic species. Females spawn in winter in the Mediterranean Sea (December to March) and up to June in the Atlantic Ocean. They present a high fecundity (on average 200,000 eggs / kg of female), start to reproduce over 2 kg and can reach 6 to 7 years in the wild (3). Eggs and larvae have a great dispersal during the 3 first months of life and adults migrate over several hundreds of kilometers.

### Population genetics

European sea bass population differentiation is one of the best studied among European marine fish. There are numerous genetic studies based on allozymes, mitochondrial DNA or RAPDs (4-8), microsatellites (9-14) at different geographic scales. These studies have led to the identification of three genetically distinct zones: the north-eastern Atlantic Ocean, the western Mediterranean and the eastern Mediterranean. On the basis of microsatellite loci the transition zones have been localized at the Almeria-Oran oceanic front between the Atlantic (the Alboran Sea included) and the western Mediterranean and somewhere around the Siculo-Tunisian strait between eastern and western Mediterranean. This was surprising for this euryhaline and eurythermic demersal species, since adult migratory behaviour has been reported as reaching several hundred kilometres. Nevertheless, this might be related to climatic changes and sea water fluctuations during the Pleistocene that undoubtedly had a strong influence on the distribution of the species in the Atlantic Ocean and the Mediterranean Sea.

There was no significant population structure found either in the Atlantic or in the western Mediterranean. On the contrary, the genetic structure of eastern Mediterranean sea bass populations is consistent with the subdivision of the region into several basins, e.g. the Adriatic, Ionian and Aegean Seas, the Libyco-Tunisian Gulf and the Levantine basin (13). Cases in which populations do not cluster with other samples belonging to the same geographical origin are not surprising, since eggs or fingerlings originating from the western basin were most likely used to seed many hatcheries around the Mediterranean, when sea bass aquaculture began.

It was suggested that some allozyme loci in *D. labrax* exhibit patterns of allele frequencies shaped by adaptation in different environments. When data from microsatellite and allozyme markers in Mediterranean lagoon and marine populations were compared (14), there was evidence that half of the allozymes used in the analysis undergo some sort of selection and only few allozyme loci seemed to be implicated in the differentiation between marine and lagoon samples.

## **B. Breeding and culture practices**

### **Production**

Aquaculture production reached 80,161 tonnes in 2005 (2), with Greece producing 35,000 tonnes, followed by Turkey (20,900 t), Italy (9,800 t), Spain (6,130 t), and France (4,300 t).

### **Hatchery practices**

Eggs are produced all year around using adequate temperature and photoperiod. Sea bass spawn naturally in tanks and buoyant eggs are collected at the water outlet of the spawning tanks. In captivity, first sexual maturation occurs in 1-2 years-old males and in 3-5 years-old females. A generation interval of 2 years can be obtained in controlled rearing conditions but in practice it is longer and ranges between 4 and 6 years. Eggs and sperm can be collected by a gentle pressure on the flanks of anaesthetized fish. Hormonal stimulation of ovulation by LHRH-a or GnRH-a is needed to collect eggs (15). Sperm can be frozen with several types of protocols.

### **Grow-out**

Most of the sea bass production is achieved in sea cages at 10 to 20 kg/m<sup>3</sup>. Sea bass is also reared in concrete raceways in France, Italy and Spain (30 to 80 kg/m<sup>3</sup>), in ponds (in extensive systems in Portugal, Greece and Egypt, < 2 kg/m<sup>3</sup>) or in closed systems. Restocking of juveniles in lagoons is traditionally performed in a limited way in Greece and in Italy. The genetic impact of these releases is uncertain. Farming has been in progress since the mid 1980s (20) when the mastering of early survival until weaning (from 0 - 10% to 35 - 65%) was achieved with better management practices. The fry quality variability remains one of the main issues. Other important issues are related to the species slow growth, its susceptibility to viral diseases in warm waters and to its poor conversion efficiency (>1.6 in large size fish). Some of these critical aspects can be linked to the fact that in farming conditions there is an excess of males (70 to 95%) that present a precocious sexual maturation (<100 g) and a slower growth.

### **Selective breeding**

Domestication of the European sea bass has been initiated in the mid 1980s by some pioneering companies in France, Spain, Italy and Israel and some strains are now kept in captivity and selected since 5 to 6 generations. However, most of the hatcheries still maintain their own broodstocks, often scarcely recruiting from wild populations or with juveniles

bought in the market. Deviations from HW equilibrium are documented in some hatchery populations compared to wild ones, mainly as heterozygote deficiencies (6, 10, 13), less alleles, and differences in allele and genotype frequencies. This may be due to mass selection practices, the addition of F1 or F2 individuals to the original breeders' pool or small founder effects. There were also cases in which some of these aquaculture stocks were found largely outbred and open to fishes coming from the wild (12).

Only two studies report the performance evaluation of different farmed strains (16) although differences in growth performance in seed originating from different hatcheries are well known and recognized by growers. A first evaluation of genetic parameters for growth has been reported (17) indicating the real potential of selection to efficiently improve this trait. Recent advance achieved in the EU CRAFT project (18) has also provided first genetic parameters for quality traits, sex determinism and genotype\*environment (G\*E) interactions. All the measured traits (gutted yield, fat in the fillet, filet yield, sex-ratio) present an intermediate to high additive genetic determinism indicating that genetic progress by selection can be obtained quickly. A low level of G\*E interaction was quantified for all the traits in the different environments tested (pond, cage, race-way, re-circulating system).

Several selective breeding programmes have been initiated in France, Israel, Greece and Spain (19, 20). Traits selected are growth, morphology or carcass yield. These programs will likely play a major role for dissemination of genetically improved seed in the future. The high fecundity of the species allows high selection intensity and facilitates rapid genetic gain. It is assumed that as much as 80% of the sea bass production comes currently from commercial populations which have undergone some level of genetic improvement. In some cases, DNA fingerprinting is used in order to optimize mating schemes and limit inbreeding. At least two commercial laboratories propose their services to perform parentage assignment for this species using fingerprints.

Females are 10 to 40 % larger than males. The farming of all female populations is one of the potential option to increase the overall production efficiency. According to the EU regulation (Directive 96/22/CE), the phenotypic sex of the breeders can be controlled by hormone administrated in the diet after weaning and 100% of males or females can be achieved depending on steroids used . Sex ratio was proven to have a genetic variability between sires, a positive genetic correlation with growth and an interaction with temperature was also found (21, 22). However, applied and economically beneficial protocols are lacking for the industry.

The species has 48 chromosomes. Triploids have been produced using different protocols and show a gonad sterility in females and gamete sterility in males. Growth is equivalent or 20 % lower than diploids growth. Nevertheless, triploid females' growth is almost the same than that of diploid males indicating that farming of such genotypes could be seen as a potential application to limit genetic risk potentially associated with escapees (23). Several other traits remain to be evaluated such as feed conversion efficiency during the reproductive season, disease resistance and performance in cages. Triploids are not grown by the farmers, for the fear of consumers and citizens reaction. It seems obvious that this technology will need to be associated in the future to the increase of female proportions by the use of environmental and/or genetic monosexing. Gynogenetic and homozygous clones were produced. Trials to produce tetraploids were not successful and no successful transgenesis is reported in sea bass in the literature.

## **Genomics**

European sea bass is gradually heading towards being one of the ten most genome rich teleosts. An EU funded project (24) produced a first generation linkage map with more than

250 microsatellite markers, which were added to those previously reported (25), few hundred ESTs, more than 200 AFLP markers (to be included in a 2<sup>nd</sup> generation linkage map) and a 6X coverage BAC library. QTL mapping of 14 commercially important features of sea bass is in progress. Since 2004, three other European projects (26-28) include sea bass genomics; in the context of the NoE Marine Genomics Europe (MGE), several cDNA libraries were constructed and a medium-scale EST sequencing project has been completed with more than 17,000 EST's, in which hundreds of SNPs and sequences containing tandem repeats (SSR-ESTs) have already been identified. Finally, Radiation Hybrid (RH) panel and BAC-ends sequencing projects are under way moving towards the construction of a physical map.

### **C. Interaction studies**

The entity of escapees is unknown. Escape events can theoretically occur at all the stages of the farming process: induced breeding, larval stage, grow-out. It can also occur during transport of fish from the hatcheries to the growing sites and from the growing sites to the processing plants. Risk of escape from the hatcheries seems limited as most of them operate in closed systems with effluent treatment.

Most of the data are mainly based on limited studies and may not precisely describe the current situation and thus the potential risks associated with mid- and long-term interaction between wild and farmed populations today. A phylogeographic analysis based on microsatellites (13) revealed that among the wild eastern samples, two samples from Greece and one from Egypt did not cluster according to their geographic origin, but rather with the western Mediterranean group. Furthermore, a wild population from the Gulf of Tunis, although clustered within the western Mediterranean group, probably originated from aquaculture. Within these particular samples, a lower allelic diversity was observed, indicating that they originated from a limited number of broodstock of foreign origin. These findings may be explained by the use of Western fingerlings to seed Eastern Mediterranean farms in the early 1980s, and escapes in the wild.

### **D. Conclusions / Implications**

Future work should include the genetic analysis of additional natural populations, mainly from the eastern Mediterranean where sea bass aquaculture production is the largest. In principle, there is no sound reason to suspect a reproductive barrier between local and domesticated stocks and the persistence in the wild of stocks genetically identified as of 'western origin' could imply the existence of behavioral (e.g. assortative mating) and/or physiological (e.g. shift in the reproduction period) mechanisms that limit interbreeding and should also be the subject of further study. Future works should also include evaluation of domestication effect on the fitness of domesticated fish and its ability to survive and breed in the wild.

It is obvious from genetic studies that the European sea bass consists of well-defined stocks throughout its distribution range. Practices such as the crossing of different strains and the eastward transfer of broodstocks, eggs, larvae and fry over large distances can involve the risk of causing artificial gene flow from escapees to local populations; this could induce a biodiversity decline or outbreeding depression.

The distribution, abundance, temporal change and life cycle (early survival, migration, and reproductive behavior) should be investigated to get a more precise view of how escapees can interact with their wild congeners. Moreover, the genetic impact of escapees is not known at all. Several factors can explain this lack of knowledge:

- The biology and the ecology of the European sea bass in the wild are not well documented regarding key aspects such as the juvenile phase or the species migration. Moreover, populations and sub-populations in the eastern Mediterranean and at the limit of the Atlantic distribution of the species (Morocco to Norway) need to be analyzed.
- The application of selective breeding in captive broodstocks is recent, and it can be assumed that the genetic differentiation between wild and farmed populations can be limited.
- Selective breeding may have already induced some change of fitness traits in the wild, which is expected. This may already have reduced the ability of domesticated fish to succeed to interbreed with wild congeners; however, this needs to be addressed.
- Selective breeding programmes depend not only on technical factors but also on business decisions, fusion, acquisition, concentration, stock exchange value. This changing and unpredictable evolution should be taken into account in the future in order to set up efficient tools to evaluate the genetic impact of escapes.

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