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

Gilthead seabream - *Sparus aurata*



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A. Biology, ecology and genetics

Distribution and capture



The gilthead seabream, *Sparus aurata*, is a subtropical Sparidae distributed from 62°N - 15°N, 17°W - 43°E. It occurs naturally in the Mediterranean and the Black Sea (rare), and in the Eastern Atlantic, from the British Isles, Strait of Gibraltar to Cape Verde and around the Canary Islands (1). Gilthead sea bream are captured with traditional and sporting equipment, and sometimes with semi-professional systems (Spain, Sicily, Egypt and Cyprus); trawl nets, bottom set longline and hand line are commonly used. This species is regularly present on the markets in the Adriatic Sea, Greece, Turkey and the Maghreb. It is commercialised fresh, refrigerated and frozen. In 2004, the world capture production of gilthead sea bream was of 8.914 tonnes, i.e. less then 10% of aquaculture production (2).

Biology

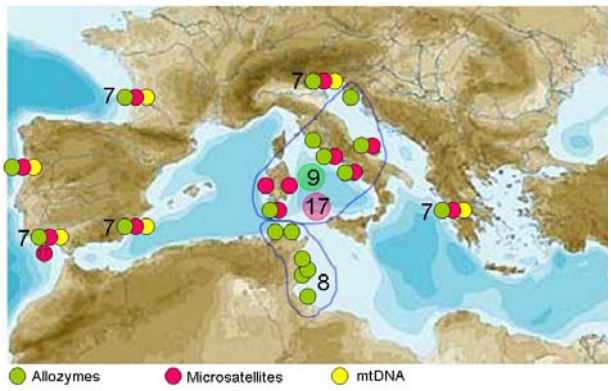
The gilthead seabream inhabits seagrass beds and sandy bottoms as well as the surf zone, commonly to depths of about 30 m, but adults may occur at 150 m depth. It is reported as a sedentary fish, though migrations are likely to occur on the Eastern Atlantic coast, from Spain to British Isles. It occurs either solitary or in small aggregations. It is an euryaline species and moves in early spring towards protected coastal waters in search for abundant food and milder temperatures (trophic migration). In late autumn it returns to the open sea for breeding purposes, being very sensitive to low temperatures (lower lethal limit is 2°C). It is mainly carnivorous (shellfish, including mussels and oysters), accessorily herbivorous (1).

The sea bream is a protandrous hermaphrodite: it is a functional male in the first two years and at over 30 cm in length becomes female. During the male phase, the bisexual gonad has functional testicular, with asynchronous spermatogenesis, and non functional ovarian areas (3, 4). Ovarian development is also asynchronous, and females are batch spawners that can lay 20.000-80.000 eggs per day for a period of up to 3 months. In the Mediterranean, they reproduce between October and December. The eggs are spherical and pelagic, with a diameter slightly lower than 1 mm and a single large oil droplet. The planktonic larval stage lasts about 50 days at 17-18° C.

Population genetics

Studies on gilthead sea bream have been carried out through gene-enzyme systems (5-10), AFLP (11) and mtDNA (7, 12, 13) analyses. In recent years, approximately 25 microsatellite loci have been reported (14-16) and several studies (6, 7, 11, 17, 18) have been performed by using these molecular markers. Additional 200 microsatellites and more than 3000 ESTs (Expressed Sequence Tags) have recently been produced within Bridgemap, an EU funded project (19).

Until a few years ago very little was known on the genetic structure of *S. aurata* and the first studies reported conflicting data concerning the existence of panmictic (5) or subdivided populations (12). More recent studies (7, 8, 9, 17, see figure for sampling localities) have depicted a picture of species subdivision that still needs to be clarified. A strong differentiation has been detected through allozymes between samples from the Tunisian coasts (8), but only a slight, though significant, genetic differentiation has been detected on a large-scale area, in several wild sample sets from the European Atlantic and Mediterranean coasts, both through allozymes (7, 9) and microsatellites (7, 17). The allozyme (21 loci) analysis of *S. aurata* from six localities along the Tunisian coast evidenced population sub-structuring (total F_{ST} = 0.093, highest pairwise F_{ST} = 0.265) in southern and northern populations, with a strong geographic trend (8). The analysis of gilthead seabream from three Atlantic and nine Mediterranean localities with a total of five different microsatellite loci and 37 different enzymatic loci, produced considerably lower F_{ST} values, similar in different studies, i.e., F_{ST} = 0.031 (7) or F_{ST} = 0.017 (9) for allozymes and F_{ST} = 0.036 (7) or F_{ST} = 0.010 (17) for microsatellites, suggesting a lower, but still significant, genetic differentiation and a weaker structuring pattern. Though some main assemblages (9, 17) can be identified, reflecting the collecting areas, the pattern of population subdivision is not reconcilable to an isolation-by-distance model.



Genomics

Since 2004, two European projects, Marine Genomics Europe (MGE) (20) and Aquafirst (21), have the ambition to render the genome of the gilthead sea bream as one of the best characterised among teleost species, apart from classical model fishes. Through the MGE data production pipeline (20), a medium-scale EST sequencing project has already been completed, with more than 18.000 ESTs from several sea bream tissues, in which hundreds of single nucleotide polymorphisms (SNPs) and sequences containing tandem repeats (SSR-ESTs) were identified.

The 200 microsatellite markers and the 3.000 ESTs produced within the Bridgemap project (19) lead to the construction of a first-generation genetic linkage map (22) and an RH map (23). Comparison between the two maps reveals a good concordance, as all markers in a single linkage group (LG) are also located in the same RH group. Moreover, a parentage analysis and a pilot QTL analysis are in progress toward the identification of genetic loci involved in the determination of economically important traits.

B. Breeding and culture practices

Production

The gilthead seabream has traditionally been cultured in Mediterranean coastal lagoons and brackish/salt water ponds, especially in the northern Adriatic *valli* in Italy and the Egyptian *hosha*. These extensive fish rearing systems acted as like natural fish traps, taking advantage of the natural trophic migration of juveniles from the sea. Restocking was usually performed with wild fry and juveniles, collected by specialised fishermen. By the late 1970s, the reduced availability of wild fry and the increasing demand from intensive farms enhanced the development of induced spawning techniques, establishing by the end of the 1980s a production scheme based on a reliable and programmed quantity of fry (24).

Sea breams are farmed extensively in lagoons, or intensively in tanks or cages. At present, most production is from intensive farming, with average densities of 20-100 kg m^3 and a FCR is 1,5-2: 1. Extensive farming still remains a traditional activity in some regions, but with a very low impact on the market. In 2004, the global aquaculture production was of 90,995 tonnes (Fig. 1). In the Mediterranean the main producers are Greece, Turkey, Spain and Italy (Fig. 2). At the beginning of the 1990s, twenty sea bream hatcheries were operating in the Mediterranean; at present over 65 hatcheries are distributed in Croatia, Cyprus, France, Greece, Italy, Morocco, Portugal, Spain and Tunisia.

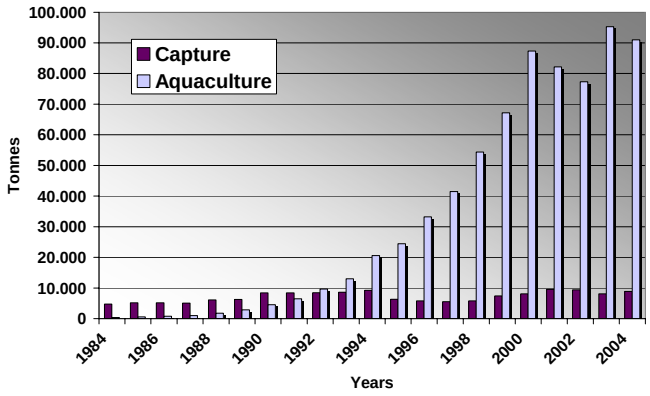


Fig. 1. Global capture fisheries and aquaculture production of sea bream (2)

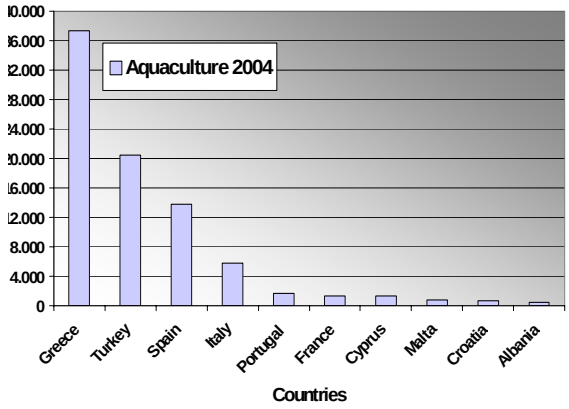


Fig. 2. Major aquaculture producing countries in the Mediterranean in 2004 (2)

Hatchery practices

At first, broodstocks were all from the wild, with a substantial division among two different stocks, the Mediterranean and the Atlantic ones. Today, after twenty years of hatchery practices and, most importantly, fry market all over Europe and the Mediterranean, strains are mixed and even the large distinctions among Mediterranean and Atlantic stocks are dispersed. Most broodstock are kept for spawning for several years. New males must be added to the broodstock every year, as they turn into females at 2 years old, so that a 5 to 20 % per year renewal occurs, whenever possible with wild fishes. However, often males are taken from F1 and farmers select among their stock the best performing specimens for reproduction. One female can produce up to 1 million eggs and the normal fertilization ratio is 90 – 95 %. Fertility and egg quality are strictly related to a calm environment and a balanced diet. Males range around 150 – 300 g, while the optimal female weight is around 1,5 kg. Hormones (HCG and, later, luteinic hormones) were used at first to induce spawning, then replaced by modulation of environmental rearing conditions, in particular temperature and photoperiod, that enabled to extend the spawning season to all year round. Larval rearing is performed in cylindroconical tanks 3 to 6 m in diameter, at a stocking density of 100 to 250 individuals per liter and first feeding starts at day 4 (at 19 °C). Green water is the most used system and larvae are fed with enriched rotifers for 25 days, then artificial feed and Artemia nauplii, and finally artificial feed only.

Optimization and control of the major environmental parameters have been the first steps to improve fry production, in particular temperature, salinity, dissolved oxygen, light intensity and photoperiod (25, 26). The fry quality is directly linked to the later performances in grow out. Morpho-anatomic quality principally refers to frequent malformations affecting swim bladder, opercula and skeleton. The genetic origin of such anomalies has not been demonstrated, and is presently being studied within an EU project, FINE FISH (27).

Large volumes technique is an alternative technique for fry rearing, mainly performed at experimental level, and conjugates the green water and semintensive mesocosms techniques. Its use is under debate, especially as far as the number of malformations is concerned.

Restocking programmes

In Italy and Greece, restocking of coastal lagoons with wild fry of gilthead sea bream has been carried out for many years, in order to increase the production in these confined brackish water bodies. Specialized fishermen (in Italy called *pescenovellanti*) had fishing rights for a definite site on the coast line. After capture, fry were then carefully transported from all over Italy to the *valli* and coastal lagoons for restocking purposes, paying no attention of fry origin. Today restocking is performed mostly with hatchery fry, though broodstock origin is seldom known. Since 1994, more than 1.100.000 juveniles have been released in various Greek lagoons (24). In Orbetello Lagoon (Central Italy) restocking with hatchery fry started in 1995 and the production of gilthead sea bream increased 9 folds from 1995 to 2000. Sea ranching is not performed in the Mediterranean.

Selective breeding

KEGO S.A., a leading livestock company in Greece, initiated a large scale, family based selective breeding programme for sea bream in 2002 (28). The base material was collected broadly from locations in Greece and elsewhere, and 50 full- and half-sib families were produced and performance tested annually. Re-use of a limited number of breeders in subsequent year-classes allows for testing of approximately 150 families per generation. The estimated selection response for growth rate resulting from the first cycle of selection was approx. +20% (Rye, pers. comm.). The first genetically improved eggs were marketed in 2005. Recently, the principal objective of a new EU project (29) are to identify genes whose expression is associated with disease and stress resistance and, from this information, to develop genetic approaches that should allow characterisation of genetic markers for marker-assisted selective breeding of disease and/or stress resistant individuals. Therefore, a new QTL analysis in the species will be carried out and the density of the sea bream genetic map will be increased with the addition of more markers to determine the relative map position of candidate genes.

C. Interaction studies

Little is known on the success of restocking practices and their impact on wild local populations. Similarly, very few data are available on the escapes of fish from accidental cage breaks or poor management practices, as there is no legislation/regulation requiring mandatory reporting of these events. The intentional release of fry of unknown origin in restocking programmes in more or less confined coastal lagoons, or the accidental escape of fish from farms, have certainly contributed to a mix of all gilthead sea bream genetic stocks. This is particularly important in the last decades, with the development of transport means which enabled long distance transport of eggs, fry and broodstock.

Genetic analysis (30) of 13 Italian broodstocks revealed that the number of microsatellite alleles was not significantly different from those from natural populations and no decline of genetic variability parameters has been observed. However, geographic assignment of breeders (18) revealed a mixed and highly heterogeneous origin of broodstocks, with a high percentage of Atlantic individuals among breeders, and a significant genetic divergence between cultured samples and local wild populations (7). On the other hand, parental assignment of offspring from several mass-spawning events (31) demonstrated the consistently low effective population size, and the influence of the differential male and female average contribution, thus evidencing the needs to increase effective population size in

order to manage the genetic variation within the farm population. A simulation study (11) demonstrated that molecular tags (AFLP, microsatellites) allow the identification of hatchery escapees of both Atlantic and Mediterranean origin among wild fishes. Hence, genetic tagging of sea bream broodstocks in commercial hatcheries might be a suitable tool to monitor the genetic impact of fish farm escapes/releases.

D. Conclusions / Implications

There is a first need to investigate on the life cycle and ecology of the species in the wild, as little is known on its biology, in particular the effective and census sizes, spatial distribution, spawning grounds and behaviour, and eventual migration. This basic biological information is needed to understand how the farmed individuals, depending on the life stages and places where they are intentionally or accidentally released, may interact with the wild ones.

The species subdivision within the Mediterranean Sea, suggested by all studies, discloses a potential impact of aquaculture on natural populations. Indeed, the common practice for farmers to use breeders of different geographic origin could determine changes in allelic frequencies and/or the introduction of non native alleles into local populations in case of escapees from culture facilities, in particular from floating cages, or restocking programmes.

As molecular genetic markers represent a suitable tool for genetic tagging of both wild, broodstock and cultured gilthead seabream (11, 18, 20-23, 29-31), it is urgent to genetically investigate additional natural populations, in order to cover the whole geographical range of the species and to fill the sampling gaps (even using historical collection of samples - scales, otoliths, pickles), to i) qualify the species fragmentation in terms of the genetic differentiation underlying it; ii) extend the baseline for more sensitive monitoring tool and iii) investigate whether the molecular markers are connected to peculiar biological traits in different regions of the species distribution range.

As far as hatchery broodstocks are concerned, further investigation is required in order to determine broodstock origin and entity in all main hatcheries, their subdivision in different spawning stocks and their genetic variability. Consequently, it would be important to trace, record and quantify ”gene flow” in the industry through the exchange of fry and broodstock among producing countries.

Last, studies should be performed to determine to what extent spontaneous spawning, and consequent egg and fry release, occurs in cages (an event which has been reported by farmers) and to develop technical solutions to eliminate it.

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