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Genetical Improvement of Marine Fish and Shellfish: a French Perspective

Pierre Boudry[1], Béatrice Chatain[2], Yamama Naciri-Graven[1], Christophe Lemaire[2] and Andrérard[1]

[1] *Laboratoire "Génétique Aquaculture Pathologie" IFREMER BP 133 17390 LA TREMBLADE FRANCE*
[2] *Station d'Aquaculture Expérimentale IFREMER Route de Maguelone 34250 PALAVAS-LES-FLOTS FRANCE*

Abstract

In France, marine fish and shellfish farming is based mainly on the breeding of wild species, whose natural populations are traditionally exploited. Research programs are conducted by IFREMER, in co-operation with INRA and Universities. French research programs for the genetical improvement of marine fish and shellfish concern primarily seabass (*Dicentrarchus labrax)* and oysters (*Ostrea edulis and Crassostrea gigas*). The following programs are developed:

- The knowledge of wild and farmed populations. The most recent prodjects involve the highly variable microsatellite markers. Seven markers are presently available for seabass and the work is now initiated on turbot (*Scophthalmus maximus*). In molluscs, microsatellite markers have been developed for the European flat oyster (*O.edulis*).
- The control of sex-ratios. Research programs in physiology and developmental genetics are initiated in order to control sex-ratios in seabass.

  These programs include the production of gynogenetic progenies and the study of the sex-ratios of progenies issued from the crossing of hormonally feminised or masculinised breeders with normal ones.
- The production of polyploids. Triploid seabass have been produced using high pressure and thermal shocks. The results after 6 months show a lower survival and growth compared with diploid controls. Triploid oysters, produced using chemical treatments (cytochalasin B or 6-DMAP), show better performances compared to doploids.
- The establishment of breeding programs for traits of commercial interest. For marine fish, selection programs are initiated by industries and are scientifically supported by research institutes. The transfer to the industries is ensured by SYSAAF, a specialised structure that eases the

adaptation of the professional network for the development of genetical improvement programs. In the flat oyster, a breeding program for the resistance against *Bonamia osteae* has been initiated in 1985 by IFREMER. Results show a better survival of selected populations compared with wild controls.

1. Fish Genetics

1.1. Introduction

Fish farming in France started in the seventies when most effort was devoted to doing the spadework om larval rearing techniques of several species. Among these the follwing 3 species of high commercial value are presently industrially produced:

- the sea bass, *Dicentrarchus labrax,*
- the sea bream, *Sparus auratus,*
- and the turbot, *Scophtalmus maximus.*

In 1982, 3 farms were producing about 3 to 400,000 fry and 40 tons of commercial size fish. Today, 40 farms produce 12 million fry and roughly 1,600 tons of commercial size fish.

The basic zootechny of these species are presently considered to be mastered and research efforts are nowadays concentrated on genetic improvement. Three aims are being developed which are:

- the knowledge of wild and farmed populations
- the control of the sex-ratios
- and the control of sexual maturation through production of triploids.

1.2. The Knowledge of Wild and Farmed Populations

Up 1993, the broodstock utilised for industrial production of sea bass, sea bream and turbot originated mainly from domestic groups, randomly chosen onexternal "beauty aspect". During the last 3 years, most farms started domestication programs and several research institutes such as IFREMER (French Institute for Exploitation of the Sea), INRA (National Institute for Agronomic Research), CNRS (National Centre for Scientific Reaearch) and Universities developed programs to help to acquire some knowledge of wild and domestic populations.

Hypervariable markers such as microsatellites are, for example, available for sea bass (Garcia De Leon *et al.*, 1995a) and turbot (unpublished results) and these

species are undergoing studies on the variability of wild and domestical stocks. The same kind of work is being done on sea bream in Greece and Italy.

Such markers are not only useful for the broodstocks management but also for testing in original selection programs. They allowed, for example, to assess the parentage of all the individuals mass-reared in the a single tankthough issued from different progenies obtained from several couples. In this way, it was possible to realise a genetic analysis of quantitative characters (survival, body weight, length and body malformation, condition coefficient) on larvae (Garcia De Leon *et al.*, 1995b). Male and/or female effects could be pointed out in the very early life stage which is 《*a first*》 in the fish quantitative genetic history.

1.3 The Control of Sex-Ratio

The main objective of the sex-ratio control is to produce *monosexe* populations when the performances of one sex is superior to the other sex. Among the fish which interest us, the female sea bass are twice the weight of the male sea bass at 3 years old (1kg instead of 0.5kg) (Chatain, unpublished results).

Unfortunately, the sex-determinism of the species is unknown, its caryotype doesn't show any heterochromosome (Cataudella *et al.*, 1973) and the sex ratio of reared populations is unbalanced: 70 to 100% of reared sea bass are males (Pacallet, 1993). Such a unbalance cannot be explained by a simple heterogametic model such as XY or ZW. The major aim of the program concerning the sea bass is therefore devoted to the understanding of the sex determinism processes by studying the sex-ratio of gynogenetic fish or the sex-ratio of progenies issued from the crossing of normal with homonally inverted breeders.

In order to masculinised or feminised fishes, 17 a-methyltestosterone and oestradiol treatments have been tested. The best treatments show 100% masculinisation but in 10% of these there was abnormal testis development (testis nodules and/or spermiduct atrophy). Nevertheless, testis crushing of such fish allowed fertilisation rates similar to control fertilisation realised with normally ejaculated sperm. Oestradiol treatments ended in 100% inversion and nerver induced any ovary deformity

21 crosses between inverted breeders and normal ones were done in 1994 and the sex-ratios of their progenies are awaited for beginning of 1997. Results cannot be obtained earlier as the phenotypic sex cannot be recognised (evenunder histology

examination) before the fish reach about 100 g:it was indeed demonstrated that 30% of the fish are still sex-undertermined at 50 g (Cauvin, 1993; Saillant, 1995).

Early sexing methods are under investigation and some hopeful results have recently been obtained by measuring levels of 17 β-oestradiol level in blood after stimulation with gonadochorionic hormone (Sallant, 1995). Gonad echographic recognition was tested without success (Saillant, 1995).

The knowledge of the gynogenetic fish sex-ratio is also awaited at beginning of 1997. Four methods were utilised to produce such fish:U.V.irradiation (8 min) of homologous or heterologus (sea bream) spem with restoration of diploidy by high pressure or thermal shock (8,000psi during 2 min, or 2℃ during 20 min, 6 min after fertilisation). Fertilisation rates varied from 30 to 50% and hatching rates, the non-participation of the paternal DNA is verified with microsatellite markers (Peruzzi *et al.*, unpublished results).

The zootechnical performances of the 6 gyongenetic fish groups we are presently rearing, are nearly the same as their controls in the following ways:

- significant decrease in survival during the larval rearing and nursery
- no difference in growth (between-25 to +54%) in 12 month old fish (50~60 g)

An enormous variability in the results is due to the between-females variability.

1.4 The Control of Sexual Maturation

In Salmon, sterility can be obtain by triploidisation. It induces growth gain and greatly improves flesh quality by supperssing the gonad maturation and/or development (Chourrout, 1980).

Such a result is awaited for sea bass where meiotic triploids were produced in 1994 using, as for gynogenesis, hyper pressure or thermal shocks to double the ovule DNA content. The best ferilisation rates are obtained by pressure shock and are about 70% compared to the control. The triploidy rates, verified by flux cytometry, are always 100% (Peruzzi *et al.*, unpublished results).

The zootechnical performances of the triploid fish we are presently rearing are the following:

- a drop of of 4 to 12% in survival during the larval rearing
- no difference in survival during nursery
- and a 25% difference in growth at 6 months old (10~25 g)

As for gynogenetic fish, variability in the results is due to the between-females

variability. It is expected that, as in Salmon, the growth delay will be compensated and transformed to gain after the first maturation.

2. Shellfish Genetics

In France, shellfish farming is an important activity in terms of economics and employment. It is mainoy based on the collection of wild spat of two species:

- the flat oyster, *Ostrea edulis*, whose production is strongly reduced due to two protozoan parasites (Meuriot and Grizel, 1984) (production in 1995 : 1,800t).
- the cupped oyster, *Crassostrea gigas*, introduced into France after massive mortality of *C. angulata* (Grizel and Héral, 1991) (production in 1995 : 150,000 t). The two main sites for spat collection are Arcachon and Marennes-Oléron.

2.1 Production and Performance of Triploids

In the cupped oyster *C. gigas*, as in most bivalves, the resources allocated the reproduction are known to be very high : 60~56% of resources in 2-year old oysters (Deslou-Paoli and Héral, 1983). As gonadogenesis is strongly reduced in triploids (Allen and Downing, 1986), triploid oysters are expected to allocate resources towards growth, that would otherwise be allocated to reproduction. In molluscs, ovocytes are expelled at a earlier stage than in fish (i.e. at the end or the prophase stage ot the beginning of the metaphase of the fist mitotic division). Therefore, both first or second ploar body expulsion can be targeted to produce triploid molluscs.

The production of triploids hsa been successfully performed in *C. gigas* (Desrosier *et al.*, 1993; Géard *et al.*, 1994a), *O. edulis* (Gendreau and Grizel, 1990, Hawkins *et al.*, 1994), *Ruditapes philippinarum* (Dufy and Diter, 1990) and *R. decussatus* (Gérard *et al.*, 1994b). A new method, based on treatment of ovocytes using 6-Dimethylaminopurine (6-DMAP) has been developed (Gérard *et al.*, 1994a). This method hasproved to be easier to use (6-DMAP is solved in sea water, no DMSO is used), less toxic and equally efficient as the one based on Cytochalasine B (CB) (Downing and Allen, 1987). Percentage of triploids among larvae is assessed usingimage-analysis techniques (Gérard *et al.*, 1994c). This test allowed the optimisation of triploid production, the triploid percentage being usually above 80%.

The major parameters for triploid induction using 6-DMAP are the timing, length, and concentration of the treatment, as well as temperature of incubation.

In order to test the growth advantage of triploids against diploid controls, a multisite experiment was performed from January 1993 to January 1995 (unpublished data). Significant differences were found for total weight, body weight, condition index, glycogen and lipid content. Among the 4 experimental sites, the largest difference between triploid and dipliod controls was found in the warmest habitat: the Thau lagoon (Mediterranean Sea). Under these conditions, body weight of triploids was 38% higher than diploids. Under the coolest trophic conditions (Normandy) this value is equal to 20%

This experiment demonstrates that triploid oysters show better performance in terms of growth and quality (i.e giyicogen content) in all sites. Transfer to industry has been initiated for the commercial production of triploid oysters. This could be of great interest for the development of hatchery companies producing spat in France.

2.2 Selection for Resistance to Parasites

Farming of the flat oyster *O.edulis* had recently to face two successive epidemics. In the 70's, *Martelia refringens* affected the estuary production areas, limiting the farming to subtidal areas. Since the 80's, this production has been affected by *Bonamia ostreae*. These two parasites are directly responsible for a drastic drop in the French production of flat oysters from 20,000t/year in the 70's down to 1800 t in 1995. In 1985, a program was initiated in order to select oysters resistant to *B. ostreae*. Selection for *B. ostreae* resistance was favoured over selection for *Martelia* resistance since the techniques of isloation, purification and inoculation of *B. ostreae* were available (Mialhe *et al.*, 1988; Hervio *et al.*, 1995), while experimental inoculation of *M. refringens* is not yet available. The artificial inoculation of the parasite shortens the screening of resistant oysters from 3~4 years down to 1 year, allowing a generation of selection in only 2 years.

Two different strains have been developed since 1985 (Martin *et al.* 1993) They are now respectivel in their second and third generation of selection. They show significant resistance fot *Bonamia* when compared with contrlos, both in the case of experimental infection with the parasite and in field tests in areas where the parasite occurs naturally (Naciri-Graven *et al.*, in prep). In a field experiment that ran over

22 months, the survival of the most advanced strain was 59%, while survival in the control was only 13%. The first generations were based on mass selection, but the program is now based on biparental crosses in order to optimise the population effective size and to avoid inbreeding depression. These full-sib families should also provide an estimate of the genetic basis of the resistance. Additionally, the measurement of the growth of hybrids among strains shows better performance than the wild control.

The development of microsatellite markers has also been initiated in order to assess level of genetic variability in the selected strains and, in a second step, to establish a linkage map and to identify markers linked to the resistance. The first 3 loci (Naciri *et al.*, 1995) show contrasted levels of polymorphism : from 5 to 48 alleles per locus at the within population level (sample size: 75 individuals). Additional loci are under development.

2.3. Acclimation of New Species

Todsy, French oyster farmiog is mainly based on a single: species *C. gigas*. Its introduction from Japan took place in 1996, while massive mortality of *C. angulata* was occurring due to a viral disease. A genebank of cupped oysters of the genus *Crassostrea* has been established in order to test new species that would be of interest in case of development of new diseaes outbreaks in *C gigas*. The main objectives are as follows:

- to import new species and new strains of cupped oysters,
- to provide information about their ability to be produced in France,
- to study interspecific hybridization,
- to develop molecular markers to study the genetic differentiation within and among species.

Up to now, five species have been imported : *C. gigas, C. angulata, C. rivularis* (= *C. ariakensis*), *C. sikamea* and *C. virginica*. All experiments are performed under strict quarantine in agreement with local veterinary authorities and international recommendations. Within and among species crosses will be performed in order to compare performances of the different species and hybrids.

The first results concern the development of species specific markers, based on PCR-RFLP techniques on mitochondrial DNA. Each of the 5 species show specific restriction profiles fot at least one enzyme. Thos provides the first tool for

distinguishing between populations of *C. angulata* and *C. gigas*, uaually considered as conspecific (for review see Gaffney and Allen, 1993).

3. Conclusion

In France, the situation is rather contrasted between marine fish and shellfish. With marine fish, genetic improvement is performed by private industry while fundamental research programs (methodology, genetic markers…) are done by public institutes (IFREMER, INRA, Universities). The role of SYSAAF is to ensure technical assistance and the transfer of technologies from public institutes to industries. With shellfish however, both fundamental studies and selection programs are performed by the public institute (IFREMER). The transfer of genetically improved shellfish to industry will require more development of hatcheries.

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