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# Genetic variation of growth and sex ratio in the European sea bass (*Dicentrarchus labrax* L.) as revealed by molecular pedigrees

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*"Ondine scrute l'océan où sa mère doit..., où son père doit chasser le congre ou le bar.*
*Le congre que le bar abhorre ou le bar que le congre hait.*
*Car Ondine a la dalle et la mère a les crocs.*
*Selon qu'il aura pris la barque à bars ou la barque à congres,*
*le père devra remplir la barque à bars à ras bord de bars ou*
*la barque à congres à ras bord de congres.*
*Or, il n'a pas pris la barque à congres ; Il a pris la barque à bars.*
*A l'arrière plan, le spectateur voit, au flanc de la montagne rouge feu, moutonner un maquis vert.*
*Il y serpente des chemins rares qui débouchent soudain sur des criques sauvages où nul imbécile,*
*cintré dans sa bouée Snoopy ne vient jamais ternir de son ombre grasse et populacière, l'irréelle clarté*
*des fonds marins mordorés, où s'insinue le congre que donc, le bar abhorre.*
*Oui : le bar abhorre le congre par atavisme. Le congre est barivore. Et donc le bar l'abhorre.*
*Le bar est fermé aux congres du même fait que le palais des congres est ouvert au bar."*

Pierre Desproges, 1986

Puisse ce travail contribuer à remplir la barque à bars à ras bord de beaux bars… d'élevage bien sûr !

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## Table of contents


*1 General introduction* 1

**1.1 Aquaculture: a fast-growing animal production deserving optimisation** 1

**1.2 Starting from the wild: domestication and selective breeding in fish** 2

**1.3 Selective breeding in fish: accessing the pedigree?** 4

1.3.1 Not using the pedigree: individual selection 4

1.3.2 Separate rearing of the families 5

1.3.3 *A posteriori* parentage assignment with genetic markers 6

**1.4 The genetics of European sea bass** 7

1.4.1 Population genetics of sea bass 7

1.4.2 Genetic variation for quantitative traits 8

**1.5 Sex ratio in the sea bass: a difficult trait to deal with** 8

**1.6 A two-steps approach for studying genetic variation and its application to sea bass culture 10**

*2 Genetic variation as revealed by between-family variation in common garden experiments* 15

**2.1 Genetic variation for body size** 15

2.1.1 Introduction 15

2.1.2 Material and methods 16

2.1.2.1 Animals 16

2.1.2.2 Data collection. 17

2.1.2.3 Parentage assignment 17

2.1.2.4 Statistical analyses 18

2.1.3 Results 19

2.1.4 Discussion 22

2.1.4.1 Deformities 22

2.1.4.2 Maternal effect 22

2.1.4.3 Heritability estimates 23

2.1.4.4 Genotype by environment interactions 23

2.1.4.5 Correlations between growth traits 24

2.1.5 Summary 26

**2.2 Genetic variation for growth rate** 27

2.2.1 Introduction 27

2.2.2 Material and methods 27

2.2.2.1 Animals 27

2.2.2.2 Data collection 28

2.2.2.3 Statistical analyses 29

2.2.3 Results and discussion 30

2.2.4 Summary 32

**2.3 Additional data: genetic correlations between initial weight, slaughter weight and growth rate 32**

**2.4 Genetic variation for sex ratio** 35

2.4.1 A polygenic hypothesis for sex determination 35
2.4.1.1 Introduction 35
2.4.1.2 Material and methods 36
2.4.1.3 Results 39
2.4.1.4 Discussion 41
2.4.1.5 Summary 45
2.4.2 Supplemental information 46
2.4.2.1 The threshold model for sex determination 46
2.4.2.2 Genetic and environmental correlations of sex tendency and body length at different ages 47

*3 Effects of domestication and directional selection for body length 48*

**3.1 Selection response for growth 48**
3.1.1 Introduction 48
3.1.2 Materials and methods 49
3.1.2.1 Selection of sires 49
3.1.2.2 Constitution of the experimental progeny 50
3.1.2.3 Rearing conditions and phenotyping 51
3.1.2.4 Parentage assignment 51
3.1.2.5 Statistical analyses 52
3.1.3 Results 53
3.1.3.1 Parentage assignment 53
3.1.3.2 Selection response in separate tanks 54
3.1.3.3 Selection response in mixed tanks 55
3.1.4 Discussion 57
3.1.4.1 Parentage assignment in mixed tanks 57
3.1.4.2 Response to selection 57
3.1.4.3 Effect of domestication 59
3.1.4.4 Possible application of the results at commercial scale 59
3.1.5 Summary 60

**3.2 Selection response for sex ratio 61**
3.2.1 A necessary baseline: sex ratios in wild sea bass populations 61
3.2.1.1 Introduction 61
3.2.1.2 Material and methods 62
3.2.1.3 Results 62
3.2.1.4 Discussion 63
3.2.1.5 Summary: 66
3.2.2 Sex ratio changes in domesticated and selected populations 67
3.2.2.1 Introduction 67
3.2.2.2 Material and methods 68
3.2.2.3 Results 69
3.2.2.4 Discussion 69
3.2.2.5 Summary 71
3.2.3 Additional data: genetic correlation of growth and sex tendency over time 72

**3.3 Modelling the mid-term evolution of growth and sex ratio in farmed sea bass populations under selection for growth 74**
3.3.1 Why model the evolution of sex ratio under selection for growth ? 74
3.3.2 A stochastic simulation model 75
3.3.3 Parameters tested 76

3.3.4 Results and discussion 77

*4 General Discussion 79*

**4.1 Summary of the main results 79**

**4.2 A perspective for sea bass domestication and breeding programmes 82**

4.2.1 A proof of concept of the use of marker-based parentage assignment 82

4.2.2 Selection for increased body weight 86

4.2.3 Genotype by environment interactions for growth: which consequences? 91

4.2.4 Practical consequences of genetic variation for sex ratio 93

4.2.5 How to move towards monosex female sea bass populations? 96

**4.3 An evolutionary perspective on the genetic variation of sex ratio in sea bass, and its relations with growth 101**

4.3.1 Polygenic sex determination: a very peculiar system ? 101

4.3.2 The adaptive significance of polygenic sex determination (in sea bass) 105

4.3.3 Sex determination and sex differentiation? 109

**4.4 Conclusion 112**

*5 Bibliography 115*

# 1 General introduction

## 1.1 Aquaculture: a fast-growing animal production deserving optimisation

In historic times, the sea has always been considered as an inexhaustible source of high quality food for humanity. It is only very recently that it became more and more evident that this was not the case. When FAO records on capture fisheries started in 1951, 70 % of the world fisheries were considered undeveloped, and less than 10% were fully exploited, while in 1999 the picture was quite the opposite: 50% of the fisheries were fully exploited, and more than 40% were overfished or collapsed (Froese and Kesner-Reyes, 2002). This can also be seen in the production curve, where growth of fisheries production has stopped and is even declining since the 80's despite a continuous increase in fishing effort (FAO, 2009). Nowadays, although propositions are done by scientists to help better manage capture fisheries (e.g. Pauly *et al.*, 2002), nobody seriously expects that world capture fisheries production will ever produce more than they did in the 1980's, and some authors argue that capture fisheries could even completely collapse by the middle of the 21th century (Worm *et al.*, 2006).

Facing this rise and fall of capture fisheries, the demand for seafood has continuously increased, and indeed the total world production of fish[1] has never stopped growing since 1950 (FAO, 2009). Where does the difference come from? It comes from aquaculture, which is the fastest growing animal production in the world for more than 20 years (Figure 1-1). Aquaculture became more important than sheep and goats in the 1990s, and is now catching up with bovine meat and eggs production. When compared with capture fisheries, aquaculture now provides *ca.* 50% of the human consumption of fish (FAO, 2009). Thus, all the increase in fish production at the world level since the 1980's comes from aquaculture. This has to be mitigated by the fact that the bulk of aquaculture production comes from Asia, with 60-70% of the production originating from just China. Consequently, the world production trend may not be the same in all countries, and especially in Europe, the growth of the production is much slower. Still, in addition to significant inland productions like common carp (*Cyprinus carpio*) in Central Europe or rainbow trout (*Oncorhynchus mykiss*) all over Europe, there are some success stories. These are mainly marine aquaculture, - another difference with the global situation where freshwater aquaculture dominates. These successful European species are Atlantic salmon (*Salmo salar*) in Northern Europe, European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) in the Mediterranean area. The

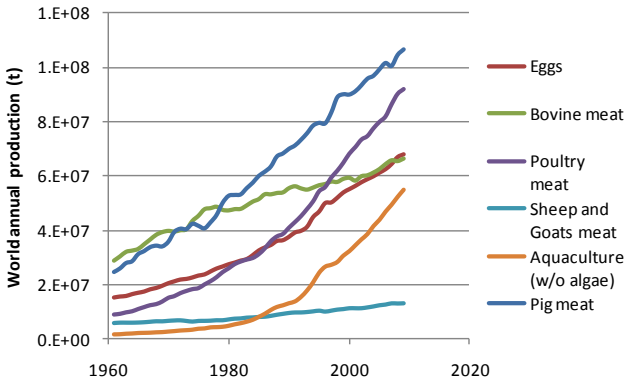


**Figure 1-1**: World production of farmed animal products (except milk) since 1960 (FAOSTAT data)

[1] in this section, the generic term "fish" represents finfish, mollusks and crustaceans.

methods to rear those last two species were developed only in the mid 80's, and since then the production of each of them has grown to more than 130,000t/year in 2008 (FEAP, 2008).

This very fast growth of aquaculture inevitably raises questions about its sustainability. Important concerns were raised about the use of fish meal and fish oil, originating from small pelagic fisheries (especially in Peru and Chile) in the aquaculture of carnivorous fishes. Some estimations of the global efficiency of the system led to the conclusion that aquaculture was not producing more fish, but indeed increased the pressure on wild fish stocks (Naylor *et al.*, 2000). However, the composition of fish feeds is evolving quickly with inclusion of more and more oils and proteins from plant origin, and provided this trend continues, feed source is not seen as a major threat to the sustainability of aquaculture production anymore (Tacon and Metian, 2008; Naylor *et al.*, 2009).

Selective breeding has been shown to be a major driver in the improvement of production efficiency in terrestrial species. In broiler chickens for example, it has been shown that selective breeding was responsible for more than 80% of the increase in growth rate observed between 1957 and 2001 (Havenstein *et al.*, 2003b). The same experiment also revealed an improvement in feed conversion ratio (1.62 in the selected strain *vs.* 2.14 in the control strain) and in meat yield (breast yield at 71 days: 21.3% in the selected strain, *vs.* 11.0% in the control strain - Havenstein *et al.*, 2003a). Such important productivity improvements have also been seen in almost all terrestrial livestock species (reviewed in Van Der Steen *et al.*, 2005), with a correlated improvement in production efficiency allowing a reduced production of greenhouse gases per ton of animal product (Hume *et al.*, 2011). Therefore, it can be reasonably forecast that genetic improvement also has potential to dramatically increase the productivity and efficiency of aquaculture production. This is of crucial importance as aquaculture will play an ever-increasing role in the production of aquatic products at the world level.

## 1.2 Starting from the wild: domestication and selective breeding in fish

A major difference between terrestrial livestock and aquaculture species is their domestication status. In terrestrial livestock, the bulk of the production (>90% in volume[2]) is based on four species, pig (*Sus scrofa domesticus*), chicken (*Gallus domesticus*), cattle (*Bos taurus*) and sheep (*Ovis aries*). All those species are long domesticated, between 10000 and 1500 years before present (reviewed in Mignon-Grasteau *et al.*, 2005). It should be noted that domestication involves an evolutionary process by which animal populations become adapted to man and the environment he provides, in addition to environment induced ontogenic changes at the level of the animal itself (Price, 1984). For this reason, only species which went through many generations of captive breeding can be considered domesticated.

In the case of fish, the two species considered domesticated are the common carp and the goldfish *Carassius auratus* (Balon, 2004), both domesticated at the end of the Middle Ages. The real possibility to domesticate other species arose with the discovery of artificial fertilization of salmonids in the 17th century (Coste, 1853). However, in many important species for aquaculture, the life cycle has been completely closed (by controlled reproduction of captive-born fishes) only very recently. In salmon for example, this occurred in the 1970's, thus leading to *ca.* 10 generations of captive breeding until 2010. In the European sea bass and the gilthead sea bream, the techniques for captive reproduction and efficient larval rearing were only established in the mid-1980's, but this does not ensure a domestication of those species, as many hatcheries still use wild broodstock. Moreover,

[2] Based on FAOSTAT data 2009, http://faostat.fao.org/site/569/default.aspx#ancor

even hatcheries which conduct breeding programmes on these species were only between 3 and 5 generations from the wild in 2009 (Chavanne, pers.comm.).

Domestication of fish is expected to increase the adaptation of fish lines to the farming environment, producing bolder animals, with increased motivation for food, losing anti-predator and reproductive behaviour (reviewed in Ruzzante, 1994; Gross, 1998; Vandeputte and Prunet, 2002). As one of the main determinants of domestication is "natural selection" for the captive environments, it can be foreseen that domestication may act very rapidly in fish species, where fertility is very high (up to $10^6$ eggs/kg female weight in many species), as the intensity of this natural selection may be very high (Doyle, 1983; Doyle *et al.*, 1995). Thus, domesticating fish is a first step towards farmed fish lines which has proven very efficient: when comparing wild and domesticated rainbow trout, after a few tens of generations of captive breeding, the difference in growth rate between the offspring of domesticated and wild trout lines is spectacular (from *ca.* x2 to x27 for body weight at a given age - Devlin *et al.*, 2001; Morkramer *et al.*, 1985). In Coho salmon *Oncorhynchus kisutch*, domestication has also been shown to increase growth rate after four generations, although to a lesser extent (Hershberger *et al.*, 1990).

The next step of genetic improvement following domestication is selective breeding for one or several traits of interest. Indeed, as we indicated before that in most fish species domestication is very recent, most of the time, unlike what happened in livestock, selective breeding starts as soon as the life cycle is closed. The reason for this is that the theory of breeding and the gains it can generate is well known, which was not the case for cattle 10,000 years ago! The first trait to be selected is always growth rate, which has the advantage to be easily measured and to give a visible result. The result is important genetic gains in growth rate in many species, in the range of 9-20% per generation as reviewed by Gjedrem and Thodesen (2005). More recent results are in the same range: 21.5% per generation in brown trout (Chevassus *et al.*, 2004), 7.1-18.7% in Nile tilapia *Oreochromis niloticus* (Khaw *et al.*, 2008; Thodesen *et al.*, 2011) and 10.2-13.9% in Coho salmon (Neira *et al.*, 2006).

These successful achievements are the consequence of 1) the relatively high heritability of body weight in fish (mostly between 0.20 and 0.50 - see review by Gjedrem and Olesen, 2005), 2) the high coefficient of variation of body weight (20-35% vs. 7-10% in land animals - Gjedrem, 1998) and 3) the possible use of high selection intensities, due to the high fertility and small individual size of fish.

Heritability has been estimated for a number of other traits like reproduction traits, processing yields, body shape, fat content, disease resistance,... and in most cases significant genetic variation can be identified, allowing the development of breeding programmes on virtually any trait (see Gjedrem and Olesen, 2005, for a general review, Quillet *et al.*, 2007 and Odegard *et al.*, 2011 for reviews focused on disease resistance traits).

Altogether, we can conclude that like in terrestrial livestock, and probably even more owing to the high possible selection intensities, domestication and selection have the potential to be major contributors to the development of efficient aquaculture industries throughout the world.

## 1.3 Selective breeding in fish: accessing the pedigree?

An important point, in any optimized selective breeding programme, is the capability to keep track of the pedigree. The knowledge of the pedigree has three main interests:

- allowing a better management of inbreeding, as with a known pedigree inbreeding can be calculated and constrained through optimized matings;
- permitting the estimation of heritability and genetic correlations through the evaluation of the within and between-family variance components for the trait(s) of interest, a strategy much more efficient than realized heritability, which is limited to one trait and requires a selected and a control line;
- setting up more efficient breeding programmes using family information as a means to improve the precision of the selection index.

In fish, knowledge of the pedigree is complicated by the fact that hatchlings are very small in size (from a few tens of micrograms to 150 mg) and cannot be physically tagged. There are three ways to solve the issue of using pedigrees in fish breeding programmes: (i) not use them (which is the case with mass or individual selection), (ii) use separate rearing of progenies until they reach a size where they can be tagged (usually *ca.* 20g mean body weight) or (iii) use genotyping of polymorphic markers to assess the parentage of individuals.

### 1.3.1 Not using the pedigree: individual selection

Using individual selection can yield interesting gains owing to the high selection intensity possible, and remains a choice of interest for selecting traits that can be measured directly on live breeding candidates. This method produced positive results selecting for body weight in channel catfish *Ictalurus punctatus* (Dunham and Smitherman, 1983), gilthead sea bream (Knibb *et al.*, 1998), Nile tilapia (Basiao and Doyle, 1999), brown trout (Chevassus *et al.*, 2004) and common carp *Cyprinus carpio* (Vandeputte *et al.*, 2008). However, this was not always the case, and unsuccessful trials in common carp (Moav and Wohlfarth, 1976) and Nile tilapia (Teichert-Coddington and Smitherman, 1988; Huang and Liao, 1990) initially led some to think that selection was not operating in fish (Gjedrem, 2012). It was also the rationale to develop the “Prosper” method, an optimized individual selection method for growth that proposes to control non genetic maternal effects and competition effects (Chevassus *et al.*, 2004) and has been the basis of the development of many breeding programmes in France (Haffray *et al.*, 2004; Vandeputte *et al.*, 2009a).

Other traits have also been successfully selected for by mass selection, like body shape in common carp (Ankorion *et al.*, 1992) and muscle fat content, estimated with a Distell Fat-meter, in rainbow trout (Quillet *et al.*, 2005).

Individual selection has the advantage that it is the easiest and cheapest to implement of selection methods, making it particularly suitable for small or medium companies. However, it also suffers from serious drawbacks. The first one is that this method is likely to generate important rates of inbreeding if not properly managed (Gjerde *et al.*, 1996; Dupont-Nivet *et al.*, 2006). Second, it cannot be used on traits that cannot be recorded (directly or indirectly) on the live breeding candidates. In addition, the genetic parameters of the traits selected remain generally unknown. Realized heritability for the selected trait can be estimated if a control line is maintained in parallel, but this is a rough estimate of the true heritability, and most of all the genetic parameters of other potentially

interesting traits (and the genetic correlations among those) will remain unknown unless specific selection experiments for these traits are set up. Finally, selection might be more effective, especially with low heritability traits, if family information can be used (Falconer and Mackay, 1996). This will be especially true when selection applies to a combination of several traits - and it is a normal fate for breeding programmes to incorporate more traits over time.

### 1.3.2 Separate rearing of the families

Family-based selection with separate rearing of progenies is the method which has been used and developed in the first "modern" breeding programmes for fish in the 1970's-1980's in Norway (Gjedrem, 2010, 2012) and in North America (*e.g.* Hershberger *et al.*, 1990). Typically, in such breeding programmes, each male is mated with 2-3 females in a hierarchical system, then progenies are reared separately until tagging (100-400 separate rearing units needed). After tagging[3] at ***ca.*** 20g (almost 1 year in Atlantic salmon - Gjerde *et al.*, 1994), some breeding candidates remain on the breeding site, while other tagged fish from the different families are sent to on-farm growing tests or to challenge testing for diseases (Gjedrem, 2010). This type of breeding programme was then extended to other species, with famous programmes like the GIFT (Genetically Improved Farm Tilapia) in the Philippines (Eknath and Acosta, 1998), the programme for the improvement of rohu *Labeo rohita* in India (Gjerde *et al.*, 2005) or several programmes for the Pacific white shrimp *Penaeus vannamei* (e.g. Gitterle *et al.*, 2005).

Knowledge of the multi-generational pedigree allows the use of optimal methods, like BLUP (Best Linear Unbiased Prediction) for the prediction of breeding values. Undoubtedly, such breeding programmes have generated the bulk of the genetic gain in the major genetically improved species of world aquaculture like Nile Tilapia, Atlantic salmon and Pacific white shrimp (Neira, 2010; Rye *et al.*, 2010). While they are very convenient to include traits recorded on sibs in challenge tests (including farm ongrowing data), the initial rearing phases are done in conditions that differ a lot from industry standards, owing to the necessity to have all families reared separately in small volume tanks or hapas. A fish that starts its life at a few milligrams (or tens of milligrams in the case of salmonids) has already increased its body weight by a factor of 200 to 2000 when it reaches 20g, while the way to commercial weight only implies a further multiplication by a factor of 20 to 200.

Therefore, common environment effects (= "tank effects") are expected to be large, and indeed they may be so when measured: 10-30% of the phenotypic variance for body weight in common carp (Ninh *et al.*, 2011), a "substantial" amount in rohu carp (Gjerde *et al.*, 2005), from 2 to 20% in Atlantic Salmon (Gjerde *et al.*, 1994), from 3 to 12% in Atlantic cod *Gadus morhua* (Gjerde *et al.*, 2004; Tosh *et al.*, 2010), 14-17% in rainbow trout (Henryon *et al.*, 2002). In some cases however, it appears that tank effects are contained to limited level (0 to 9% of phenotypic variance in rainbow trout- Elvingson and Johansson, 1993). High tank effects are problematic as they may bias the estimated family values, and then the estimated breeding value of individuals. In addition to this, the need to minimize family (and then rearing units) number tends to promote hierarchical mating designs, as for a given effective population size (needed to avoid inbreeding) they imply the production of less families than most factorial mating designs. However, hierarchical designs perform less than factorial designs both for estimation of genetic parameters (Vandeputte *et al.*, 2001) and conservation of

[3] Initially, tagging was performed by freeze branding, abaltion of fins or use of external tags (Gjedrem, 2010. Today, the majority of fish is tagged by injection of RFID glass tags (called PIT-tags) which provide reliable individual tagging at a modest cost (1-2 € per – reusable- tag)

genetic variability (Dupont-Nivet *et al.*, 2006). A last problem of using separate rearing of families is the initial cost of building the infrastructure. While the benefit to cost ratio of fish selective breeding is so high that at the industry level this initial cost should have no significant impact on the profitability of a breeding programme (Ponzoni *et al.*, 2008), this initial investment can clearly be a constraint for a small-medium company to engage in a breeding programme.

### 1.3.3 *A posteriori* parentage assignment with genetic markers

The last solution to access pedigree information is the use of genetic markers. This had been thought of a long time ago in fishes (Brody *et al.*, 1976; Moav *et al.*, 1976; Brody *et al.*, 1980), but at that time the available genetic markers (allozymes) did not exhibit enough variability to resolve parentage in more than a few families and involved highly invasive (even lethal) sampling. The idea had a second life when microsatellite markers became available, as those markers have a much higher genetic variability, and sampling is limited to a small piece of fin kept in ethanol at ambient temperature. Then, using either exclusion of incompatible parent pairs (Dodds *et al.*, 1996) or maximum likelihood approaches (SanCristobal and Chevalet, 1997), a new possibility to trace family relationships arose. The first small scale trials were done in salmon and cod (Doyle *et al.*, 1995; Doyle and Herbinger, 1995; Herbinger, 1995), and it soon became evident theoretically that large crosses with several tens of parents could be dealt with (Estoup *et al.*, 1998; Norris *et al.*, 2000; Villanueva *et al.*, 2002). The first large scale trials were done in rainbow trout and Atlantic salmon (Fishback *et al.*, 2002; Norris and Cunningham, 2004), with single assignment rates higher than 90%.

Several assignment softwares have been developed, some more focused on wildlife (CERVUS: Marshall *et al.*, 1998; PARENTE: Cercueil *et al.*, 2002; PAPA: Duchesne *et al.*, 2002), on forest trees (FAMOZ: Gerber *et al.*, 2003) or on aquaculture species (PROBMAX: Danzmann, 1997; VITASSIGN: Vandeputte *et al.*, 2006; FAP: Taggart, 2007). Differences between assignment results can appear in complex situations, especially with likelihood-based softwares in which more hypotheses are needed than with simple exclusion (Herlin *et al.*, 2007). The main drawback of exclusion-based softwares is their sensitivity to genotyping errors, which generates "impossible" genotypes and then unassigned offspring. This proportion of unassigned offspring can reach high levels even with modest genotyping error rates (Vandeputte *et al.*, 2006), but this problem can be easily solved by accepting a limited number of allelic mismatches (1 to 2 in general) in the evaluation of an offspring-sire-dam triplet (Vandeputte *et al.*, 2006; Christie, 2010). In this way, practical assignment rates higher than 90% can be obtained most of the time (Vandeputte *et al.*, 2011).

Compared to separate rearing systems, the major advantage of *a posteriori* parentage assignment is that all fish can be reared as a single batch from the beginning, thus completely eliminating the confusion of tank and family effects. This may be particularly important in species with small eggs (carps, marine fishes), where larval mortalities can be high (50 to 90%) and strongly interact with recorded growth rates at the larval and post-larval stages through rearing density effects. The possibility to have all families in a single batch also allows the use of industry rearing structures from the first stages, thus permitting a more realistic evaluation of the breeding values of the families, from a farmer's point of view. The third advantage of this method is that it allows the use of any type of mating design, including factorial designs which are the most informative and the best ones to keep genetic variability during the selection process (Dupont-Nivet *et al.*, 2006). Finally, from a practical point of view, genotyping is only an operating cost, and no initial investment is needed to use this methodology in practice, making it quite cost-effective in many cases (*e.g.* Ninh *et al.*, 2011).

Moreover, it allows a transition from individual selection to family selection just by genotyping the breeding candidates in a pre-existing individual selection programme, as has been done in the French fish breeding industry (Haffray *et al.*, 2004; Vandeputte *et al.*, 2009b).

The main drawback of using parentage assignment is the cost of individual fish data - linked to the cost of genotyping. In separate rearing, the primary limiting factor is the number of families, and increasing the number of fish per family just increases feeding, handling and tagging costs. In a parentage assignment system, in addition to feeding, handling and tagging, each extra fish will also incur a genotyping cost, which can be substantial (from 4 to 15 € /fish - see review in Ninh *et al.*, 2011). This can be a problem to record data on challenged sibs (e.g. field growth tests, disease challenge tests, processing traits) where several tens of fishes per family are usually needed. An additional problem is that due to differential survival of families and sampling error, uneven numbers of fish per family will be obtained. This is seen by some as a major problem (Gjerde, 2005), as for a given number of families, disequilibrium in family size limits the number of families which can be used to estimate breeding values with reasonable precision. However, this could probably be at least partially solved by increasing the number of families produced, which can be done at little cost in such programmes. Theoretical optimisation has been done for estimation of genetic parameters using such technology (Vandeputte *et al.*, 2001; Dupont-Nivet *et al.*, 2002) and is on the way for the set up of breeding programmes (Sonesson, 2005; Sonesson *et al.*, 2011). Additionally, breeding programmes using genotyping of progenies are implemented in 15 out of 37 European breeding programmes (Aquabreeding, 2009), showing their scope for practical application.

## 1.4 The genetics of European sea bass

At the time the present research was conceived, very little was known about the genetics of European sea bass. This is understandable as efficient rearing procedures allowing industry development only dated back a few years. Genetic studies first concentrated on the population genetics of the species, then a few trials attempted to describe genetic variability for some traits of interest.

### 1.4.1 Population genetics of sea bass

The study of population genetics in sea bass started with enzymatic markers, which showed some level of genetic variation between individual stocks (both natural and farmed - e.g. Martinez *et al.*, 1991) , and a general picture with a strong differentiation between Atlantic and Mediterranean populations (Allegrucci *et al.*, 1997) and some level of genetic differentiation within the Mediterranean sea (Allegrucci *et al.*, 1997) and locally in the North Atlantic (Child, 1992). The development of microsatellite markers (Garcia De Leon *et al.*, 1995) allowed wider and more precise studies, which nevertheless ended up with the same conclusions: there is a strong differentiation between Mediterranean and Atlantic populations, the limit being the Alboran sea in South-eastern Spain (Naciri *et al.*, 1999). In the Mediterranean, there is a clear differentiation between East and West Mediterranean, while the populations seem very homogeneous in the Western part, and much more differentiated in the Eastern part (Bahri-Sfar *et al.*, 2000; Castilho and Ciftci, 2005). Some samples collected in the wild in the Eastern part also show similarities to Western Mediterranean samples, and could be escapees from fish farms, which are very numerous in Greece and may use stocks from hatcheries using West-Mediterranean broodstock (Bahri-Sfar *et al.*, 2005).

### 1.4.2 Genetic variation for quantitative traits

Evaluation of populations were done for a West Mediterranean population, marine and lagoon populations from Egypt and crosses thereof (Gorshkov *et al.*, 2004), showing small though significant differences of specific growth rate, condition factor and survival, with no evidence of heterosis in the crosses. However, the lack of replication in some of the experiments, and the small numbers of broodstock used, together with the use of mass spawnings, make these results rather uncertain. First estimates of heritability for growth traits were obtained from a 9-10 males *3 females full factorial mating (Saillant *et al.*, 2006) where families were identified by genotyping. The overall heritability of body weight was 0.29±0.13, and was higher when the fish were raised at low temperature (0.50±0.19) or low density (0.60±0.22). Heritability of body weight tended to increase with age, and genetic correlations between body weights at different ages (average 0.70, range 0.61-0.85) was considered to be high enough to use early growth as a predictor for later growth, thus allowing early (and consequently less expensive) selection. Although these estimates had limited precision, as the number of parents used to generate the families was low, they constituted an incentive to go forward, as genetic variation seemed to be high enough to perform efficient breeding programmes in this species.

## 1.5 Sex ratio in the sea bass: a difficult trait to deal with

In most vertebrates, sex is genetically determined (Genotypic Sex Determination or GSD), in most cases by sex chromosomes, yielding stable 50:50 primary sex ratios. In the sea bass, it was soon discovered that farmed populations comprised a high proportion of males (75-95%, Piferrer *et al.*, 2005). This is seen as problematic, first because females grow faster than males (ca. 25% higher body weight at 1 year, Saillant *et al.*, 2001b; Gorshkov *et al.*, 2003) and mature one year later, thus making them more suitable for farming. A second problem, thinking of developing breeding programmes, is that both males and females are needed, and heavily biased sex ratios induce a lower selection intensity on the least present sex, which cannot be fully compensated by the higher selection intensity on the other sex.

In rainbow trout or Nile tilapia, two major farmed fish species, the better performance of one sex has led to the development of monosex technologies. In rainbow trout, female (with XX sex chromosomes) juveniles are sex-reversed as "neomales" using methyltestosterone, and these XX neomales, when mated with normal XX females, only produce XX (female) offspring, which is the sex of interest in this species (Breton *et al.*, 1996). In Nile tilapia, YY supermales can be obtained by mating feminized XY males with normal males (Mair *et al.*, 1997). These supermales, when mated with normal XX females, produce near to 100% male offspring. Sex-reversal with steroids was also tried in sea bass, but the sex ratios of the offspring of the sex-reversed parents was not compatible with a simple chromosomal sex determination system (Blazquez *et al.*, 1999). Uniparental reproduction (gynogenesis) also ruled out the possibility that females could bear XX sex chromosomes, as gynogenetic progenies had the same sex ratio as bi-parental diploid controls (Felip *et al.*, 2002; Peruzzi *et al.*, 2004).

In parallel, several studies investigated the effect of environmental factors on sex determination in sea bass, with a major interest on temperature. Indeed, temperature sex determination (TSD) is the most frequent case of environmental sex determination (ESD), and is rather common in reptiles, amphibians and fishes (see review in Kraak and Pen, 2002). In sea bass, it appears that high

temperatures (>17°C) during early development (before 60 days post fertilization -dpf) promote the appearance of increased numbers of males in the populations.

This general interpretation, proposed by Navarro-Martin *et al.* (2009b), fits well with most of the published data (Pavlidis *et al.*, 2000; Koumoundouros *et al.*, 2002; Saillant *et al.*, 2002; Mylonas *et al.*, 2005). However, some results are not explained by this interpretation. After a warm early rearing (20-24°C), Blazquez *et al.*, 1998 exposed groups of young sea bass to high (25°C) or low (15°C) temperatures from 57 to 137 dpf, which resulted in 0% females in the low temperature group *vs.* up to 27% females in the high temperature group (13% on average). Similarly, fish reared at 13°C from hatching to 346 dpf had only 11% females, *vs.* 32% in groups reared at 20°C (Saillant *et al.*, 2002). Moreover, variability of sex ratios in different batches (from different families or origins) exposed to the same temperature profiles remains very high (*e.g.* 21.7 to 90.0% females in four batches reared 64 days below 17°C, Navarro-Martin *et al.*, 2009b). Then, although the general tendency that cold early rearing would favour females seems quite established, its practical use remains unreliable, and the possibility that later cold temperatures may act in the opposite way cannot be excluded. Clearly, the TSD of sea bass is not as clearly defined as what is seen in some turtles, where a 2°C increase in nest temperature changes the sex ratio of the progeny from 100% male to 100% female (Bull *et al.*, 1982b). Environmental variables other than temperature, like rearing density (Saillant *et al.*, 2003c), salinity (Saillant *et al.*, 2003b) or photoperiod (Blazquez *et al.*, 1998) were tested in sea bass but did not induce changes in sex ratios. Surprisingly, although many investigations were conducted to identify the environmental causes of the sex ratio disequilibrium in farmed populations, no studies provided convincing evidence about the natural sex ratio of sea bass in the wild, which was implicitly thought to be even, but without formal proof thereof.

Finally, it was demonstrated that family or strain effects could have an important impact on the sex ratio of sea bass reared in the same environmental conditions (Saillant *et al.*, 2002; Gorshkov *et al.*, 2003; Gorshkov *et al.*, 2004). This was however achieved with a limited number of parents tested (2x2 factorial in Gorshkov *et al.*, 2003, 9x3 factorial in Saillant et al, 2002), which prevented the quantification of this variability in sex ratio. Interestingly, significant sire*temperature interactions were also demonstrated (Saillant *et al.*, 2002), although they were mainly explained by one sire which gave similar offspring sex ratios at both high and low temperatures, while the other 8 sire progenies had much less females at low temperature. Then, it clearly appears that the sex determination system in sea bass is not simple, and seems to be a complex mixture of genetic and environmental influences, with possible interactions inbetween. This is clearly not the best starting point for obtaining stable - and even monosex female- sex ratios for sea bass farming.

## 1.6 A two-steps approach for studying genetic variation and its application to sea bass culture

In order to study the genetic variation of growth and sex ratio traits in sea bass, we followed a two-steps approach: first the between-family variation of traits was estimated in a partial factorial mating design, and second selective breeding for growth was undertaken in order to evaluate the realized selection response. We evaluated both the direct response on the trait selected (growth) and the correlated response on sex ratio. This two-steps approach was expected to give accurate and robust results, and to provide the basis for setting up efficient breeding programmes in sea bass. To implement this approach, we made a number of important scientific and technical choices.

The first choice was to focus on the Atlantic sea bass population. This choice was governed by the availability of a large number of wild-caught Atlantic broodstock in our partner farm (Panittica Pugliese, Torre Canne di Fasano, Italy). Although some hatchery-reared broodstock might have been available, we chose this option of using wild caught fish as it was the guarantee to access a large genetic variability. Indeed, it is well known that, due to the general use of mass spawnings with uncontrolled participation of the broodstock present, it is not unusual to see low effective population sizes and consequently low genetic variability in hatchery-reared marine fish ( see Perez-Enriquez *et al.*, 1999 in red sea bream or Chatziplis *et al.*, 2007 in sea bass). Moreover, as many hatcheries (although not all of them) still use wild broodstock for their sea bass juveniles production, this was expected to be kind of a representative starting point for a selective breeding programme. Studying only fish from one base population was a limitation in the sense that a significant part of the genetic variation for production traits may lie between populations, as was seen for example in the Atlantic salmon (Refstie and Steine, 1978), brown trout (Chevassus *et al.*, 1992), common carp (Wohlfarth, 1993) or Nile tilapia (Bentsen *et al.*, 1998). However, the comparison of wild populations was scheduled for a later phase and has now been done, although not published yet.

The second important choice was to use genotyping of microsatellites to access the family structure of the experimental population chosen. This was indeed the only possible choice, as we did not have access to a family larval rearing unit that would have allowed separate rearing of independently produced families. Small-scale (in terms of family number) trials in the sea bass had proven the feasibility of this parentage assignment approach (Saillant *et al.*, 2002), and larger scale uses of this technology has already been done in other fish species (Fishback *et al.*, 2002; Norris and Cunningham, 2004; Vandeputte *et al.*, 2004). A decisive advantage of this approach was the ability to use industry larval rearing protocols, directly in the fish farm, allowing the fish to express their performance in a rearing environment representative of the production sector. Another decisive advantage was the certainty, through the use of a "common garden" experiment, to avoid any confusion of family effects with common environment (tank) effects. Finally, as the number of families produced is not a limiting factor with this approach, it also gave us the opportunity to choose any type of mating design to produce the experimental families.

This is where the third choice came in. Although in practice, all sea bass hatcheries use mass spawnings for their production of juveniles, we chose to use artificial fertilization. The main reason for this is that mass spawnings may give very unbalanced family representations, with only a small proportion of the present broodstock effectively participating to the reproduction. For example, in a mass spawning in with 58 female and 45 male sea bass parents, a single female contributed more than 95% of the progeny, with only 26 males participating, one of which sired *ca.* 50% of the progeny

(Chatziplis *et al.*, 2007). This kind of outcome is clearly not optimal at all for estimating genetic parameters. The ability to get relatively balanced family sizes by artificial fertilization and common garden rearing had been demonstrated earlier (Saillant *et al.*, 2002) on a small size mating design, and upscaling it to a larger size, although challenging, seemed feasible. Moreover, artificial fertilization allows the set up of factorial mating designs, which are the most informative for the estimation of genetic parameters, as not only of additive genetic variance, but also of non genetic maternal effects and dominance variance can be estimated. This was thought to be essential as maternal effects can be important in fish, as repeatedly demonstrated in salmonids (Aulstad *et al.*, 1972; Gall, 1974; Chevassus, 1976; Mckay *et al.*, 1986b; Blanc, 2002; Vandeputte *et al.*, 2002b). For dominance variance, very little data were available, but in salmonids the dominance component can represent up to 22% of the phenotypic variance (Rye and Mao, 1998; Pante *et al.*, 2002; Gallardo *et al.*, 2010), while most of the time it remained difficult to estimate. This difficulty was certainly at least partly due to the quite general use of nested designs in which dominance is confounded with the full-sib family effect, while it is not the case in factorial designs (Becker, 1984). A preliminary simulation study showed that common garden factorial designs with a few tens of sires tested were likely to give good estimates of additive and dominance variance (Vandeputte *et al.*, 2001).

A last important point to deal with was the possible existence of genotype by environment interactions for the traits of interest. Sea bass juveniles are typically produced in hatcheries which send their products for ongrowing in a variety of different structures (cages, ponds, tanks, raceways) with variable environmental conditions (temperature, salinity). As breeding programmes will be located in hatcheries, it is therefore extremely important to verify that the high performing families in one site will also perform well in another site. Therefore, we decided to conduct a multi-site estimation of genetic parameters, in order to quantify genotype by environment interactions through the estimation of genetic correlations between rearing sites for the same recorded trait. Studies of within population genotype by environment interactions (GxE) in fish have not been very numerous, and in most cases conclude that GxE interactions exist but not to a level requiring the building of environment-specific breeding programmes (e.g. Sylven *et al.*, 1991; Kause *et al.*, 2003; Kolstad *et al.*, 2006; Khaw *et al.*, 2012). However, in some rare cases, GxE interaction can be quite high with genetic correlations between environments below 0.5, like in Nile tilapia between freshwater and brackish water (Luan *et al.*, 2008). Under the general hypothesis that GxE interactions should not be very high, we decided to use very different rearing systems, in order to maximize them if present. The general characteristics of the systems chosen are shown in Table 2-1 (p.17). As too many parameters differed between the sites, we did not expect to be able to infer the origin of eventual GxE interactions, so the partner fish farms were left free to raise the fish with their usual practices (notably in terms of feed and density).

The second step of our approach was to estimate response to selection. To this end, we compared the performance of the offspring of fish either selected or not selected for growth. This estimation was done after only one generation of selection, and furthermore only comparing sires in a paternal testing design, as females start spawning only at three years of age while all males spermiate at two years. Moreover, as sex ratios in the first generation were highly male biased (18.2% females), it was easy to apply a high selection intensity (p=5%) on males. The high selection differential generated partially compensated the fact that a paternal testing design only allows the measurement of half of the additive genetic divergence between the lines tested. Knowing that the observed divergence between lines might be limited, we used a common garden strategy also for the estimation of

selection response, with all genotypes mixed as early as incubation. This avoided confounding any environmental effect with genetic groups, and increased the statistical power to detect even modest differences. However, it is well known that in fish, there is a magnification of genetic differences in communal rearing (Moav and Wohlfarth, 1974; Blanc and Poisson, 2003), so we also set up triplicated separate tanks of the groups tested to try and estimate this magnification effect in case it would operate in sea bass.

For the genetic groups tested, we compared sires mass selected for body length (5% selection pressure at commercial weight) to a control group of sires issued from the same parents, but which had an average length at the same age. Two more genetic groups were added to have a more complete description of the selection response. First, we also used a group of wild sires, in order to see if the control group had a modified breeding value linked to domestication selection, which is thought to operate quickly when starting from a wild fish stock (Doyle *et al.*, 1995; Araki *et al.*, 2007; Christie *et al.*, 2012). Second, we used a second selected population of sires, which originated from the same wild parents as our selected and control fish, but which had been selected for growth in an commercial breeding programme in our partner farm, Panittica Pugliese, using a method derived from the Prosper individual selection scheme designed by Chevassus *et al.* (2004) on brown trout. In the case of sea bass, the control of maternal effects recommended in Prosper could only be done by using spawns produced on the same day, but not by mixing progenies of females with similar egg sizes, as there was no proof of the existence of maternal effects in sea bass, nor that they would be linked to differences in egg size. The main feature of this commercial breeding programme was the use of repeated growth challenges, recommended in Prosper to avoid the establishment of competition through behavioural hierarchies, and to assess fish on their “real” genetic potential for growth rate. Instead of selecting fish only on body length at commercial size, like we did in our mass selected sires, they were selected based on three successive growth challenges at different ages, two of which were based on body length, and one on body weight. The final selection pressure was the same, with 5% of the fish selected.

Then, this selection response experiment allowed the comparison of two lines selected for growth, a massal (M) and a Prosper (P) line, with an unselected control line (which we called D for domesticated), and the base Wild population (W). Thus, we expected to be able to estimate the effects of selection and domestication on growth, as well as the correlated response on other traits (especially sex ratio, but also quality traits which are not reported in the present thesis). The influence of competition on those traits could be estimated by comparing the results in separated and mixed rearing groups.

The following chapters are a series of papers retracing the different steps in our approach:

**Chapter 2** presents the estimates of genetic variation for growth and sex ratio within an offspring group of sea bass issued from wild parents from an Atlantic population, reared in four different locations. This was conducted in the frame of the European project Heritabolum (Q5CR-2002-71720), funded by the EC and the private farms, Panittica Pugliese (Italy), Viveiro Villanova (Portugal) and Ardag (Israel).

The first paper (section 2.1) deals with heritability and genotype by environment estimates for body size and body shape traits: "Heritabilities and GxE interactions for growth in the European sea bass (*Dicentrarchus labrax* L.) using a marker-based pedigree", by Mathilde Dupont-Nivet, Marc

Vandeputte, Alain Vergnet, Olivier Merdy, Pierrick Haffray, Hervé Chavanne, and Béatrice Chatain, was published in 2008 in *Aquaculture* (volume 275: 81-87)

The second paper (section 2.2) came from a re-analysis of the same dataset, evaluating growth rates rather than body size and weight. We had focused the first paper on weight, as this is the commercial trait. However fish could only be tagged at 35g mean weight and were slaughtered at 400g, so that their final weight was indeed quite influenced by the tagging weight and finally not that much representative of GxE interactions, as all pre-tagging growth had been done in one site. This paper, "Genotype by environment interactions for growth in European sea bass (*Dicentrarchus labrax*) are large when growth rate rather than weight is considered" by Mathilde Dupont-Nivet, Bilge Karahan-Nomm , Alain Vergnet, Olivier Merdy, Pierrick Haffray, Hervé Chavanne, Béatrice Chatain and Marc Vandeputte, was published in 2010 in *Aquaculture* (volume 306: 365-368).

In section 2.3, I briefly present additional data about the genetic correlations of body weight and growth rate at different ages and in different sites, which were too many to include in the published papers but will be used in the general discussion to predict the outcome of various selection strategies to increase growth.

The third paper (section 2.4) describes the between families variation in sex ratio and its co-variation with body weight, and tests several hypotheses about the underlying genetic determinism of sex ratio. There is no genotype by environment component in this paper, as sex was fully determined at tagging, and was therefore not likely to be influenced by the different ongrowing conditions. It is titled "A polygenic hypothesis for sex determination in the European sea bass" by Marc Vandeputte, Mathilde Dupont-Nivet, Hervé Chavanne and Béatrice Chatain and was published in 2007 in *Genetics* (volume 176: 1049-1057). Complementary (unpublished) information about the threshold model for sex determination and some genetic and environmental correlations between size and sex ratio at different ages is added at the end of section 2.4.

**Chapter 3** is about the response to domestication and selection for increased body size, in terms of growth and sex ratio. The related experiments were done in the frame of the European project Competus, funded by the farms Ardag (Israel), Ecloserie Marine de Gravelines (France), Les Poissons du Soleil (France), Tinamenor SA (Spain), Viveiro Vilanova (Portugal) and the European Union (COOP-CT-2005-017633).

Direct selection response in terms of growth is studied in section 3.1, where we compare the offspring of wild founders, domesticated (first generation hatchery bred) and selected (2 populations, one mass selected for growth in Ifremer and the other in Panittica Pugliese, with a different protocol) sea bass populations. The paper, "Response to domestication and selection for growth in the European sea bass (*Dicentrarchus labrax*) in separate and mixed tanks" by Marc Vandeputte, Mathilde Dupont-Nivet, Pierrick Haffray, Hervé Chavanne, Silvia Cenadelli, Katia Parati, Marie-Odile Vidal, Alain Vergnet and Béatrice Chatain was published in 2009 in *Aquaculture* (volume 286: 20-27).

Correlated responses in terms of sex ratio to domestication and selective breeding for body size is the subject of section 3.2., where we first present evidence on the sex ratio in natural populations of sea bass. As pointed out before, this essential information was lacking in the numerous studies about the causes of sex ratio variations in farmed sea bass, and we felt it was a necessary starting point to

examine the possible environmental and genetic influences implied. We achieved this by exploiting the fisheries literature, where data about sea bass growth in natural population indeed comprised the sex of the recorded fish, although sex ratio was not their primary interest. This piece of work, "Are sex ratios in wild European sea bass (*Dicentrarchus labrax*) populations biased ?", by Marc Vandeputte, Edwige Quillet and Béatrice Chatain, was published in 2012 in *Aquatic Living Resources* (volume 25: 77-81).

Then, we tested our predictions formulated in section 2.3 that frequency-dependent selection should increase the proportion of females in the offspring of domesticated sea bass, while selection for growth should result in a further increase in the proportion of females. This paper, "Impact of domestication and artificial selection for growth on population sex ratios in the European sea bass (*Dicentrarchus labrax* L.)" by Marc Vandeputte, Mathilde Dupont-Nivet, Hervé Chavanne, Alain Vergnet, Edwige Quillet and Béatrice Chatain has been submitted. Additional data about genetic correlations of body size and sex ratio, estimated in the selection response experiment, are added at the end of section 3.2.

In section 3.3, I present a stochastic simulation model aiming at predicting the mid-term evolution of sex ratio (and growth) in selected populations of sea bass. The evolution of sex ratio in selected populations cannot be predicted simply by the breeder's equation, as sex ratio is not only submitted to direct or indirect selection (when growth is the trait selected), but also to frequency-dependent selection as soon as there is an uneven sex ratio in the population, which is indeed the general case in farmed sea bass. This part of the work was not published in a peer-reviewed journal, but the results of the simulations were used in several communications in French and international workshops and symposia.

**Chapter 4** is the general discussion of the thesis, with a double focus: after a summary of the main findings in section 4.1, I first examine the consequences of our findings on the design of breeding programmes for the sea bass, taking into account both growth and sex ratio (section 4.2). Then, in section 4.3, I more deeply discuss the evolutionary aspects of a polygenic sex determination system where sex tendency and growth are genetically linked, and try to infer new hypotheses that can be useful for improved farming of sea bass (which remains the primary focus of this thesis) through sex ratio control.

For the sake of homogeneity, I used the final version of the published manuscripts rather than the offprints, in order to put them in a homogeneous format. Some "additional data" sections were added after published manuscripts when needed for a better general interpretation of data. All bibliographic references were merged in a single list at the end of the manuscript, to avoid repetitions and save space.

# 2 Genetic variation as revealed by between-family variation in common garden experiments

## 2.1 Genetic variation for body size

This section has been published in 2008 in Aquaculture 275 (81-87) as:

"Heritabilities and GxE interactions for growth in the European sea bass (*Dicentrarchus labrax* L.) using a marker-based pedigree" by Mathilde Dupont-Nivet[a,*], Marc Vandeputte[b], Alain Vergnet[b], Olivier Merdy[a], Pierrick Haffray[c], Hervé Chavanne[d], Béatrice Chatain[b]

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### 2.1.1 Introduction

Domestication of sea bass *Dicentrarchus labrax* began in the 1980's and some breeding programmes already exist for the species (Italy, Greece, France …). However this is still the very beginning and some hatcheries still use wild broodstock. As in any animal production, breeding programmes are expected to provide important increases in productivity. However, to optimise breeding programmes, reliable estimates of genetic parameters in a wide range of rearing systems are needed. Some heritability estimates for growth traits exist for marine fish, but mainly for species other than sea bass, for example turbot *Scophthalmus maximus* (Gjerde *et al.*, 1997), Atlantic cod *Gadus morhua* (Gjerde *et al.*, 2004; Kolstad *et al.*, 2006), black bream *Acanthopagrus butcheri* (Doupe and Lymbery, 2005) and gilthead seabream *Sparus aurata* (Knibb *et al.*, 1997). Concerning sea bass, heritability estimates were published by Saillant *et al.* (2006), but based on a small design (3 dams X 10 sires).

One major constraint for estimating genetic parameters in fish is the inability to tag hatchlings, and consequently the need to separately rear the families until tagging size. This limits the number of families that can be used, and may bias family means by tank effects, thus biasing (full-sibs designs) or reducing the precision of (half-sibs designs) the estimated genetic parameters. An interesting alternative to separate rearing of families is the use of mixed rearing of progenies with a posteriori reconstruction of pedigrees using highly variable markers such as microsatellites. This was first proposed more than ten years ago (Herbinger, 1995; Estoup *et al.*, 1998) but it is only recently that mating designs of a size permitting reliable estimations of genetic parameters are being used (Norris and Cunningham, 2004; Vandeputte *et al.*, 2004). The major benefits of this methodology are the absence of between families environmental effects, and the possibility to use factorial designs which allow precise estimation of additive, maternal and dominance effects (Vandeputte *et al.*, 2001). Most of the previously cited heritability estimates in marine fish were obtained using the separate rearing method.

Sea bass farming takes place in very different system managements, and thus genetic and environment correlations of the same trait in different environments are also needed to set up optimised breeding programs. Indeed, it is a key point to know if a genetically improved strain in one environment would express superiority in other environments. This issue has been seldom studied in marine fish and never in sea bass except by Saillant *et al.* (2006) but with a small design.

The present work reports results from a large scale experiment involving 253 full sib families, communally reared, originating from 33 males and 23 females and distributed to four contrasted environments (France, Portugal, Italy and Israel). In this paper, we focus on growth traits (weight, length and condition factor) which are traits of high economical interest. The large design allows precise estimates of maternal and additive genetics effects as well as correlations between traits and genetic correlations between growth traits in each environment (genotype environment interactions)

### 2.1.2 Material and methods

#### *2.1.2.1 Animals*

The parents of the studied animals were wild fish of Atlantic origin collected by Panittica Pugliese (Italy) on the Northern coast of Brittany (France). Sperm was collected before the crossing and cryopreserved in 250 ml straws according to the method described in Fauvel *et al.* (1998). Further reproduction operations took place at Panittica Pugliese farm. Eggs were obtained by manual stripping following hormonal induction of ovulation. For all parents a fin clip was collected and kept in 90 % ethanol for DNA analyses and parentage assignment.

253 full-sib families from 33 males and 23 females were produced according to a partly factorial mating design. Crosses were conducted with three sets of different parents: 11 males X 9 females, 11 males X 7 females and 11 males X 7 females. Within each set a full factorial crossing was accomplished. All crosses were made by individual fertilization of identical volumes of eggs, and five minutes after fertilisation, eggs from the same female were mixed for further incubation.

Eggs were incubated (one female per incubator) for 48 hours after which two milliliters of floating eggs from each female were sampled and mixed to constitute a single batch of eggs that hatched in a 0.5 m3 incubator four days after the fertilization. They were all kept in the same tank for larval rearing until day 64. After which, they were transferred to a concrete raceway until they reached day 130. During larval rearing, the temperature gradually increased from 13 (at hatching) to 18°C (at day 15) after which it stabilised at the latter temperature. Fish were fed on artemia for 40 days, then weaned on dry food (Nippay, Hendrix). Food was first distributed manually to satiation, then, starting from day 66, one or two automatic feeders were used. During the pregrowing phase, the water temperature and salinity varied from 14.2 to 19.3°C and from 19.5 to 37.5 ‰, respectively.

At 134 days post hatch (about 4g), a random sample of 16000 fish was sent to Ifremer station in Palavas (France) and pregrown in a 5 m3 tank in a recirculating system (10-30% renewal/day, 18°C, 34 ‰ average salinity). At 156 days, the batch of fish was split at random into four 5 m3 tanks to lower the density. At 238 days, their weight was higher than scheduled, so the temperature was lowered to 14°C (0.5°C per day).

At 370 days, fish had reached a mean weight of 35 g and 7000 were randomly selected, individually PIT-tagged and fin-clipped (kept in 90% ethanol for further DNA analyses). Four batches of 1750 fish

each (on average, 6.9 per fullsib family) were constituted and each one was kept in a 5m3 tank prior to distribution to four different farms.

The four farms were chosen for their varying growing or rearing conditions. Main rearing conditions are reported in Table 2-1. One batch was kept at the Ifremer station in France (Farm A) in tanks where the density was maintained below 30 kg/m3. The temperature was raised progressively from 14°C and maintained throughout the whole experiment at 20-22°C.

**Table 2-1:** Growing conditions in the four rearing sites**.**

| | Rearing period (days) | Rearing system | Temperature (°C) | Volume ($m^3$) | Rearing density ($kg/m^3$) |
|---|---|---|---|---|---|
| Farm A | 420-714 | semi-closed recirculation system | 20-22 | 5 (x4) | < 30 |
| Farm B | 423-795 | concrete tank with borehole water | 19-20 | 12 | < 46 |
| Farm C | 420-873 | semi-intensive estuarine | 9-25 | 400 | < 2 |
| Farm D | 513-734* | floating cage in tropical waters | 22-27 | 216 | < 4 |

*: fish of farm D were reared in farm B during the period 423-510 days post hatching.

A second batch arrived at 423 days at Panittica Pugliese in Italy (Farm B) where it was reared in a 12 m3 concrete raceway supplied with 19°C (constant temperature) borehole water. A third one arrived at Viveiro Villanova in Portugal (Farm C) at 420 days and was first reared in an 8m3 tank. Then, they were transferred to a semi-intensive estuarine pond at 588 days. The last batch of fish was reared from day 513 in a 216 m3 sea-cage in tropical conditions at Ardag in Israel (Farm D). This batch was kept in the farm B from day 423 to day 510, due to transportation problems.

#### *2.1.2.2 Data collection.*

In each farm, fish were measured at commercial size (average 400 g), varying from 338 g (farm B) to 487 g (farm D). Number of fish measured, mean weight and age are reported in Table 2. Fish were starved 3 days prior to harvest. On harvest day, all fish were euthanized in an excess dose of 2-phenoxyethanol (0.6 ml.l-1) or eugenol (0.1 ml.l-1, farm B). In farm A, the fish were not euthanized but only anaesthetized (0.3 ml.l-1 phenoxyethanol).

Each fish was weighed (to the nearest 0.1g) and its length measured (to the nearest 1 mm) and its tag read to determine and record its parentage. The condition factor (*K*) was calculated. In farms B, C and D, internal deformities were scored after opening each fish. In farm A, fish were then reared until 1 kg and were slaughtered at this stage. Internal deformities were then noted at this later stage. Sex was determined by examination of the gonads.

#### *2.1.2.3 Parentage assignment*

Parentage assignment was performed by Landcatch Natural Selection (Scotland) using six microsatellite markers organised in a single PCR multiplex.

Assignments were redrawn using software (written by Landcatch) for pedigree analysis. The software uses two separate algorithms for pedigree assignment: a Bayesian probabilistic calculation computes the most likely parents; and a simple text matching algorithm compares parental and offspring

genotypes at each locus sequentially and excludes mismatches in turn. The two sets of results were then compared. There was almost perfect concordance between the two sets of assignments.

#### *2.1.2.4 Statistical analyses*

One major problem for data analysis was the high occurrence of spinal deformities (mainly lordosis). In most cases, even when accounted for by a fixed effect, deformities introduced uncontrolled variation in the models, and we preferred to work only on normal fish, as the increase of precision brought by the use of exclusively normal fish overcame or at least compensated the loss in precision due to the lower number of fish used when eliminating the deformed ones from analyses. An exception was done for farm A where the number of normal fish was so low that some slightly deformed fish were also used in the analysis. Occurrence of deformities and our method for accounting for them will be presented further in the Results section.

To determine the potential significant fixed effects, data were first analysed using proc GLM of the SAS ® System. Tank effect was not significant ($P > 0.1$) for all traits but sex effect was kept in further models ($P < 0.05$). A farm effect ($P<0.05$) was also included when all data were analyzed together. Interactions were not significant. A model with the deformity as a fixed effect was also tested to make decisions about including or not deformed fish in the analysis (see Results section).

Heritabilities, non genetic maternal effect and dominance were first analyzed for all data using Asreml (Gilmour *et al.*, 2002). An animal model with dominance and maternal effect (model 1) or without dominance effect (model 2) or without dominance nor maternal effect (model 3) was used.

$$Y = X\beta + Z_1u + Z_2m + Z_3fs + e \qquad \text{(model 1)}$$

where $Y$ is the vector of observations, $\beta$ is the vector of fixed effects (overall mean, sex and farm), $u$ is the vector of random additive genetic effects, $m$ is the vector of random maternal effects, $fs$ is the random vector of fullsib family effect (*i.e*. accounts for dominance) and $e$ is the vector of random residual effects. $X$, $Z_1$, $Z_2$ and $Z_3$ are known incidence matrices. Dominance effect was very low (see Results section) and was removed for further analyses. Then genetic parameters were also estimated for each site using model 2 (without dominance effect) without farm effect.

Genetic by environment interactions (GxE interactions) were estimated through genetic correlations between the trait of interest in environment 1 and the same trait in environment 2, considered as two different traits in the analysis. GxE interactions is the difference between 1 and the genetic correlation, and the closer the genetic correlation is to 1, the smaller is the interaction.

### 2.1.3 Results

Parental origin could be traced for 99.2% of the fish. The full-sibs family sizes were variable (from 0 to 66), but only 37 families (15%) had less than half the number of expected offspring and only one family had zero offspring.

The number of fish remaining at the final slaughtering and their age are given in Table 2-2. The survival during the ongrowing phase was satisfactory in all sites, ranging from 67 to 95%. Sex ratio was similar in all sites, ranging from 17 to 19.4% of females. Weight of females was 24% higher.

**Table 2-2:** Number, age and mean weight of measured animals in each site.

| | Age (days) | Number | Mean weight (g) | Proportion of deformed fish (%) | Survival rate (%) |
|---|---|---|---|---|---|
| Palavas (A) | 714 | 1473 | 398 | 83 | 84.2 |
| Panittica (B) | 795 | 1651 | 338 | 60 | 94.8 |
| Vila Nova (C) | 873 | 1177 | 358 | 55 | 67.3 |
| Ardag (D) | 734 | 1667 | 487 | 58 | 95.7 |

The growth rate was different among sites, as expected, and the differences between sites were largely due to temperature differences. The proportion of deformed fish (from the scoring of internal deformities) reached 83, 60, 55 and 58% in farm A, B, C  D, respectively. The main type of deformity was lordosis often associated with scoliosis, while a few fusions and cyphosis were also observed.

Estimates of heritabilities of weight, length and *K* in farm C are presented in Table 2-3 for all fish, normal + mildly deformed fish or normal fish only. A correction by introducing a fixed effect of deformity was also tested. Results are presented only for farm C but the conclusions were the same for other farms. Deformities seem to have almost no effect on the estimation of the heritability of weight. However, one can see that the precision of the estimation is not better when using the full data set, compared to the normal fish only, despite the 2 to 2.5 fold increase of the number of fish. This is probably due to a decrease of precision in the estimation of additive genetic values when deformed fish are integrated in the analysis. Deformities have an important effect on estimations for length and even more for *K*. This was expected, as deformities have an obvious impact on length and thus on the length-weight relationship. Despite this high impact of deformities, the correction with a fixed effect was not really efficient in most cases: heritability estimates remain lower with correction than when considering only normal fish. Thus, we have chosen to use only normal fish in the rest of our analysis.

**Table 2-3:** Effects of deformities on heritability estimates ($h^2$) and their standard errors (s.e.) at harvest size,  for farm C (Vila Nova, Portugal), depending on the groups of fish kept for analysis and on the models used : with (corrected data) or without ( raw data) a fixed effect accounting for the occurrence of deformities.

| Trait | Fish kept for analysis | Sample size | $h^2$ for raw data (± s.e.) | $h^2$ for corrected data (± s.e.) |
|---|---|---|---|---|
| Weight | All fish | 1151 | 0.62 ± 0.06 | 0.62 ± 0.06 |
| Weight | Normal + mildly deformed | 789 | 0.65 ± 0.07 | 0.65 ± 0.07 |
| Weight | Normal | 523 | 0.64 ± 0.07 | - |
| Length | All fish | 1151 | 0.54 ± 0.06 | 0.58 ± 0.07 |
| Length | Normal + mildly deformed | 789 | 0.64 ± 0.07 | 0.66 ± 0.07 |
| Length | Normal | 523 | 0.70 ± 0.08 | - |
| *K* | All fish | 1151 | 0.19 ± 0.04 | 0.23 ± 0.04 |
| *K* | Normal + mildly deformed | 789 | 0.40 ± 0.06 | 0.49 ± 0.07 |
| *K* | Normal | 523 | 0.53 ± 0.07 | - |

This considerably reduces the size of the available datasets: from 1177-1675 to 250-648 animals. For farm A, there were only 250 normal animals and we considered that this number was too low relative to the number of families. Thus we have added mildly deformed fish to reach a sample size of 610, for the sake of models stability. Moreover, as the deformities were scored at a later age in farm A, it is quite likely that fish scored as mildly deformed there could have been scored as normal if they had been slaughtered at 400g like in the other sites. In Table 2-5, sample size and means of the reduced data set are given for each trait.

**Table 2-5:** Sample size and means (± standard deviations) of growth traits in the reduced data sets used for estimation of heritability and genetic correlations at commercial size, in four different sites (A: Palavas, France; B: Panittica, Italy; C: Vila Nova, Portugal; D: Ardag, Israel)

| | Number | Weight (g) | Length (cm) | K |
|---|---|---|---|---|
| Farm A | 610 | 415.4 ± 119.2 | 25.9 ± 2.2 | 2.33 ± 0.24 |
| Farm B | 648 | 336.6 ± 94.3 | 25.4 ± 2.4 | 1.99 ± 0.16 |
| Farm C | 491 | 358.5 ± 71.7 | 26.4 ± 1.7 | 1.93 ± 0.13 |
| Farm D | 629 | 516.6 ± 139.4 | 28.2 ± 2.9 | 2.25 ± 0.32 |

**Table 2-4:** Estimates (± Standard Error) of heritabilities ($h^2$) and maternal effects ($m^2$) for growth traits at commercial size using model 1 (dominance and maternal effect), model 2 (without dominance) or model 3 (without dominance nor maternal effect) for all data. The relative explanatory powers of models are accounted for by the differences in -2Log-Likelihood between both models.

| Trait | Model | $h^2$ ± S.E | $m^2$ ± S.E | $d^2$ ± S.E | - 2 Log L |
|---|---|---|---|---|---|
| **Weight** | Model 1 | 0.34 ± 0.09 | 0.06 ± 0.05 | 0.01 ± 0.01 | 23952.0 |
| | Model 2 | 0.35 ± 0.09 | 0.06 ± 0.05 | - | 23953.8 |
| | Model 3 | 0.46 ± 0.08 | - | - | 23955.4 |
| **Length** | Model 1 | 0.24 ± 0.07 | 0.10 ± 0.05 | 0.02 ± 0.01 | 17208.14 |
| | Model 2 | 0.25 ± 0.06 | 0.10 ± 0.05 | - | 17212.84 |
| | Model 3 | 0.43 ± 0.07 | - | - | 17217.46 |
| ***K*** | Model 1 | 0.34 ± 0.09 | 0.01 ± 0.04 | 0.00 ± 0.01 | 16366.22 |
| | Model 2 | 0.34 ± 0.09 | 0.01 ± 0.04 | - | 16366.22 |
| | Model 3 | 0.36 ± 0.06 | - | - | 16366.32 |

Estimations of heritabilities for all farms together and according to models 1, 2 and 3 are given in Table 2-4. For all three traits, dominance effect is clearly non significant and can be removed from the model. According to differences of –2LogL between models, maternal effect is not significant for the three traits. However for weight and especially for length, maternal effect is not negligible and even (for length) at border of significance if we consider S.E. Moreover, if maternal effect is removed, heritability estimates are highly increased.

**Table 2-6:** Estimates (± Standard Error) of heritabilities ($h^2$) and maternal effects ($m^2$) for growth traits at commercial size using model 2 with maternal effect and no dominance effect, in four different sites (A: Palavas, France; B: Panittica, Italy; C: Vila Nova, Portugal; D: Ardag, Israel).

| Trait | Farm | $h^2$ ± S.E | $m^2$ ± S.E. |
|---|---|---|---|
| **Weight** | A | 0.40 ± 0.14 | 0.07 ± 0.07 |
| | B | 0.44 ± 0.14 | 0.04 ± 0.07 |
| | C | 0.39 ± 0.14 | 0.13 ± 0.08 |
| | D | 0.38 ± 0.14 | 0.08 ± 0.07 |
| **Length** | A | 0.41 ± 0.15 | 0.07 ± 0.07 |
| | B | 0.33 ± 0.12 | 0.09 ± 0.07 |
| | C | 0.34 ± 0.13 | 0.19 ± 0.09 |
| | D | 0.27 ± 0.11 | 0.10 ± 0.06 |
| **K** | A | 0.46 ± 0.15 | 0.05 ± 0.07 |
| | B | 0.45 ± 0.15 | 0.04 ± 0.07 |
| | C | 0.51 ± 0.18 | 0.03 ± 0.08 |
| | D | 0.26 ± 0.11 | 0.03 ± 0.05 |

**Table 2-7:** Phenotypic (above diagonal) and genetic correlations (± S.E. below diagonal) between weight, length and K at commercial size in sea bass for all farms, and in each of the four farms (A: Palavas, France; B: Panittica, Italy; C: Vila Nova, Portugal; D: Ardag, Israel)

| | Weight | Length | K |
|---|---|---|---|
| **All farms** | | | |
| Weight | | 0.87 | 0.47 |
| Length | 0.95 ± 0.02 | | 0.08 |
| K | 0.27 ± 0.15 | -0.05 ± 0.16 | |
| **Farm A** | | | |
| Weight | | 0.91 | 0.39 |
| Length | 0.91 ± 0.01 | | 0.01 |
| K | 0.34 ± 0.11 | -0.07 ± 0.12 | |
| **Farm B** | | | |
| Weight | | 0.95 | 0.01 |
| Length | 0.96 ± 0.01 | | 0.03 |
| K | 0.23 ± 0.11 | -0.05 ± 0.12 | |
| **Farm C** | | | |
| Weight | | 0.88 | 0.13 |
| Length | 0.94 ± 0.01 | | -0.32 |
| K | 0.02 ± 0.12 | -0.32 ± 0.11 | |
| **Farm D** | | | |
| Weight | | 0.93 | 0.44 |
| Length | 0.95 ± 0.01 | | 0.15 |
| K | 0.35 ± 0.11 | 0.05 ± 0.13 | |

Heritabilities estimated in each site using model 2 are reported in Table 2-6. Heritabilities were all medium to high. They are little higher in farm C, but in this latter farm the CV of weight within sex was lower (18.9% versus 24-26% in other sites). For length and K, heritability seems lower in farm D.

Phenotypic correlations and genetic correlations estimated with model 2 are gathered in Table 2-7 for all farms and for each farm. Correlation between weight and length is always very high (> 0.9). Genetic correlations between weight or length and K can change widely from one site to another.

Genetic correlations for weight between different farms are summarized in Table 2-8. They are especially high (thus very small interaction) between farm A, B and D. They are lower between farm C and other farms (<0.9), especially farm D (0.70) which suggests higher genotype-environment interactions.

**Table 2-8:** Estimations of genetic correlations for weight at commercial size measured in different environments (A: Palavas, France; B: Panittica, Italy; C: Vila Nova, Portugal; D: Ardag, Israel)

| | Farm A | Farm B | Farm C |
|---|---|---|---|
| **Farm B** | 0.99 ± 0.05 | | |
| **Farm C** | 0.84 ± 0.08 | 0.88 ± 0.07 | |
| **Farm D** | 0.97 ± 0.03 | 0.96 ± 0.04 | 0.70 ± 0.10 |

### 2.1.4 Discussion

#### *2.1.4.1 Deformities*

The cause of the deformities was apparently not to be sought during larval rearing, as the fish that were kept by farm B for its breeding programme, which were produced from the same parents on the same day and reared in the same conditions, did not suffer (at least externally) from such deformities. The most probable cause is the rearing conditions in farm A, prior to tagging. Indeed, the small fish that arrived from farm B (134 days, 3.6 g mean weight) were reared in 5m3 circular tanks, where a strong circular water current induced tank self-cleaning. However, it is known that the intensive swimming provoked by such water current is not suitable for this size of fish that have not completed their bone calcification (Chatain, 1994).

As the fish were chosen at random to constitute the different farm batches, we can make the hypothesis that the rate of deformities was initially the same in all batches. The differences observed at slaughter then should come from environmental effects of the rearing systems, allowing the fish to recover or not, or at least worsening or not the initial deformities. The much higher proportion of deformed fish in farm A is probably also accounted for by the bigger size (1 kg) at which the scoring was done. We cannot exclude a bias on estimates of genetic parameters even with removal of deformed fish. However this should lead to a decrease of estimates unless heritability of deformities is very high which is not the case ($h^2$ = 0.16-0.29 on the underlying scale).

#### *2.1.4.2 Maternal effect*

With our results, it is still difficult to conclude on maternal effects in growth of sea bass. Statistics show that they are not significant. Considering the small egg size in sea bass, the absence of maternal effects in large fish is not surprising. Similar results were found by Saillant *et al.* (2006). More generally maternal effects have been often described in different marine fish but only on early life history traits such as larvae weight or yolk-sac volume [see for example, Bang *et al.*, 2006 in Atlantic herring, Saillant *et al.*, 2001a in sea bass]. For later stages, to our knowledge, maternal effects in marine fish are not very well documented. In Atlantic cod, Gjerde *et al.*( 2004) found 0.03 to 0.12 as an estimation of common full-sibs effect for body weight at 25 g. This effect contains maternal effect but also tank effects and dominance, and maternal effect was thus probably low in this experiment. Doupe and Lymbery, 2005 in black bream found that maternal effect decrease gradually with age from 9.4 % (75 days old, 0.6 g) to 1.8% (180 days old, 17.2 g). In salmonid fish for which egg size is much larger and maternal effects are high in early stages, they are also known to decrease with age (for example, McKay *et al.*, 1986b; Crandell and Gall, 1993).

However, in our experiment, a systematic increase of heritability is observed when maternal effect is removed. This could be due to the introduction in the model of a non significant - and thus difficult to estimate - maternal effect than to a real maternal effect. But, the higher is the estimate of maternal effect, the higher is the increase of heritability estimate. Maternal effects are probably at the border of significance in our dataset and thus we cannot reject their existence. It is not impossible that egg quality can be the origin of a small but real maternal effect. We finally choose a conservative attitude, and kept the maternal effect in further models. Since it leads to lower heritabilities, we prefer this choice which leaves room for more genetic progress than expected. However introduction of maternal effect in the model mainly affects heritability estimates: estimations of genetic

correlations between traits are not changed and genotype by environment interactions little affected.

#### 2.1.4.3 Heritability estimates

Our results show moderate to high heritabilities. These results are in the range of those obtained by Saillant *et al.*, 2006. These authors published the first heritability estimates in sea bass, however our paper gives much more reliable estimates, obtained in different rearing conditions, with a large number of families and a design which prevents biases by dominance, maternal or other common environment effects. In other marine fish, medium to high heritabilities have also been found: 0.45-0.70 for body weight of turbot (Gjerde *et al.*, 1997), but probably overestimated because of the mating design (sires nested within dams)), 0.29 ± 0.27 to 0.52 ± 0.26 for body weight at 25 g for Atlantic cod (Gjerde *et al.*, 2004) and 0.51 ± 0.10 for body weight at two years in Atlantic cod (Kolstad *et al.*, 2006). However in black bream with a small design (five dams mated to six sires), Doupe and Lymbery, 2005) found moderate heritability of growth traits in juvenile stages.

Therefore, comparing to classically selected fish species, like salmonids, the heritability of growth seems a little larger in sea bass. Indeed, in salmonids, estimates generally range between 0.2 and 0.4 (for a review, see Gjerde, 1986). The fact that sea bass is not domesticated (here, all parents were caught from the wild) could be one explanation.

These heritability values are promising for genetic progress, at least in the short term. As an example, for weight, the expected genetic gain for a mass selection with a pressure of 5% should range between 16 and 25% of the mean per generation. But this has to be confirmed by selection experiments as many examples in litterature show unsuccessful selection experiments in fish (for example, Moav and Wohlfarth, 1976; Hulata *et al.*, 1986; Huang and Liao, 1990; Gjedrem, 1998, for a review).

#### 2.1.4.4 Genotype by environment interactions

Low interaction between farm B and D is not surprising knowing the long common life of both batches. The highest interactions are seen between Farm C and other farms. It is plausible that the semi-intensive nature of the Farm C rearing system, together with its low temperature in winter, leads to different rankings of the families, compared with the other sites which are all warmer and more intensive (the warmest being Farm A and Farm D).

For sea bass aquaculture, these results show that in most cases there would be similar response on weight if fish selected in one site would be reared in another site, except with highly divergent systems. This leaves open both the possibility to undertake a single breeding programme and the possibility to set up site-specific breeding programmes. Another possibility would be a single breeding programme with multisite testing and site-specific multiplication according to the best ranking families in each site. The choice is open for each farm, according to its characteristics and objectives. We must also underline that in the present work, all fish are reared in a common environment before being sent to the various rearing sites (farms), thus limiting the possible GxE interaction effects to the only on-growing period.

The genotype-environment interactions obtained here are much lower than those already reported in sea bass by Saillant *et al.*(2006). In Saillant *et al.*(2006), each experimental group was exposed to the different environmental conditions from fertilization time, thus extending the interaction action from the early larval stages. Therefore, it should be really interesting to test genotype environment interactions in the early stage in a larger design. Moreover, the significantly lower precision of estimates reported in the latter experiment reduces the accuracy of results.

In fish, genotype-environment interactions for growth traits have been studied mainly in salmonids, catfish, carp and tilapia species. They have been estimated through reaction norms or, as in this paper, through genetic correlations of a trait measured in different environments and considered as different traits, or through selection response in different environments. Most papers indeed studied GxE interactions through reaction norms: different strains or genotypes reared in different environments. Significant genotype-environment (environments were generally characterised by different temperature/photoperiod/nutritional environment/density) for growth was found in many fish species: carp (Wohlfarth *et al.*, 1983), catfish (Dunham *et al.*, 1990), tilapia (Romana-Eguia and Doyle, 1992), Rainbow trout (Iwamoto, 1986) and in marine fish: turbot (Imsland *et al.*, 2000), Atlantic halibut (Jonassen *et al.*, 2000) and Atlantic cod (Imsland *et al.*, 2005). Papers estimating genetic correlations between the same trait in different environments are less numerous and results highly variable. In rainbow trout, Sylven *et al.* (1991) found genetic correlations ranging from 0.58 to 0.86 between slaughter weight in freshwater (Sweden), brackish water (Sweden) and salt water (Norway), *i.e.* higher interactions than in our experiment. Still, in rainbow trout, Bagley *et al.*, 1994 found genetic correlations ranging from 0.32 to 0.9 for different stocking densities. Again in rainbow trout, Palti *et al.* (2006) found similar family rankings reared under a classical fishmeal diet or a gluten-based diet. In Atlantic salmon, Hanke *et al.* (1989) found similar family rankings for different photoperiods while Stefansson *et al.* ( 1990) found significant family x photoperiod interactions. In tilapia, Ponzoni *et al.* (2005) show small interactions for weight of selected fish (GIFT strain) in cages or ponds and concluded that 'selection response was being achieved in both environments and that there was not enough evidence to justify the conduct of separate genetic improvement programmes.

Generally speaking, GxE interactions depend on the traits, populations and environments studied and are still difficult to predict. It is however a key point when setting up a breeding programme in species with wide range of environments and large geographical area like in sea bass.

#### *2.1.4.5 Correlations between growth traits*

As the genetic correlation is high and heritabilities of both traits are also similar, selection on weight or length should yield the same results on weight. However, because of the correlations with K, selecting on weight or on length is not equivalent.

In farms A, B and D, the genetic correlation between length and K is close to zero, so selection on length would have no impact on K, but the positive genetic correlation between weight and K would lead to the selection of "fatter" fish if weight was used as a selection criterion. In this case, selection on length should be preferred. On the opposite, in farm C, the genetic correlation between weight and K is close to zero so that selecting on weight would have no impact on the global shape of the fish. However, the genetic correlation between length and K is negative, so selection on length would lead to leaner, though heavier, fish. This kind of fish is generally appreciated as it looks more like a

wild fish, and finally selection on length will probably be preferred again. In other sites, a specific selection on K would be necessary to obtain leaner fish.

### 2.1.5 Summary

**253 full-sib families from 33 males and 23 females of European sea bass were produced in a partly factorial mating design. All fish were reared in the same tank during 14 months, then 7000 of them were dispatched in four farms to different locations (France, Israel, Italy, Portugal) representing a wide variety of environmental conditions. Around 400 g mean weight, 1177 to 1667 fish in each site were weighed and length was measured. Condition factor (*K*) was calculated. Pedigrees were redrawn a posteriori using microsatellites markers: parental origin could be retraced for 99.2 % of fish. Due to a high incidence of deformities, the useful sample size was reduced to 491-670 fish per site. Maternal effects were small. Using a simple animal model, heritability of weight ranged from 0.38±0.14 to 0.44±0.14 in the different sites. Length was highly correlated to weight, with similar heritabilities. GxE interaction, estimated through genetic correlations of weight across the different environments ranged from 0.70±0.10 to 0.99±0.05. Genetic correlations between weight or length and K were not similar in the different sites.**

## 2.2 Genetic variation for growth rate

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### 2.2.1 Introduction

European sea bass is a major aquaculture species in the Mediterranean region and in the southern part of the north east Atlantic Ocean (Portugal, Canary Islands). Its domestication began in the 1980s, and while some breeding programmes are starting (France, Greece, Israel), some hatcheries still use wild broodstock. Breeding programmes are expected to provide important increases in productivity, as in all fish species (Gjedrem and Thodesen, 2005), especially because heritabilities of growth traits range from medium to high in this species (Saillant *et al.*, 2006; Dupont-Nivet *et al.*, 2008). High selection response (+23-42 % per generation) for weight at commercial harvest size was obtained in an individual selection experiment in a recirculating system (Vandeputte *et al.*, 2009b). However, hatcheries can provide fingerlings to a wide range of fish farms with very different culture conditions, thus efficient selective breeding for growth requires the knowledge of any GxE interactions. They are efficiently approached by calculating genetic correlations using a common family structure under different environmental conditions, and considering the traits at each site as separate traits. We previously published estimates of GxE interactions for weight at commercial size (Dupont-Nivet *et al.*, 2008), and showed that they were small in most cases (genetic correlation $r_A$ between sites >0.84), while it was moderate ($r_A$=0.70) between the two extreme sites in terms of rearing systems, especially regarding temperature. Fish in the experiment reported in this previous paper were tagged at a mean weight of 35g and harvested around 400g, and this relatively late tagging leaves the possibility that final weight performance was significantly influenced by weight at tagging (where all fish were in the same environment), thus reducing the possibilities to see GxE interactions. Thus, using the same dataset, we report in the present paper an additional analysis: GxE interactions for growth rate expressed as Daily Growth Coefficient (DGC).

### 2.2.2 Material and methods

#### *2.2.2.1 Animals*

Details regarding the production of experimental animals were given in Dupont-Nivet *et al.* (2008). Briefly, 253 full-sib families from 33 males and 23 females were produced according to a partly factorial mating design, and all families were reared as a single batch, starting at 48h post-fertilization. They were all kept in the same tank (Panittica Pugliese, Torre Canne di Fasano, Italy) until they reached 134 days post-fertilization (dpf), where a random sample of 16,000 fish was sent to the Ifremer station in Palavas (France) and pre-grown in a 5 $m^3$ tank. At 156 dpf, the batch of fish

was split at random into four 5 $m^3$ tanks to lower stocking density. At 370 dpf, fish were 35 g (mean weight) and 7000 were randomly selected, individually PIT-tagged and fin-clipped (fin clips were kept in 90% ethanol for further DNA analyses). Four batches of 1750 fish each were distributed to four different farms.

**Table 2-9** Growing conditions in the four rearing sites.

| | Rearing period (days) | Rearing system | Temperature (°C) | Volume ($m^3$) | Rearing density ($kg/m^3$) |
|---|---|---|---|---|---|
| **Farm A France** | 420-714 | semi-closed recirculation system | 20-22 | 5 (x4) | < 30 |
| **Farm B Italy** | 423-795 | concrete tank with well water | 19-20 | 12 | < 46 |
| **Farm C Portugal** | 420-873 | semi-intensive estuarine | 9-25 | 400 | < 2 |
| **Farm D Israel** | 513-734* | floating cage in tropical waters | 22-27 | 216 | < 4 |

*: fish of farm D were reared in farm B during the period 423-510 days post hatching.

The four farms were chosen for their varying growing or rearing conditions (Table 2-9), including a recirculating system (Palavas, France, Site A), a concrete raceway with well water (Torre Canne di Fasano, Italy, site B), semi-intensive estuarine earthen ponds (Vila Nova de Milfontes, Portugal, site C) and tropical seawater cages (Eilat, Israel, site D). These farms differed in many factors other than rearing system, such as water temperature (mean and variation), fish density, feed composition, feeding practices, associated pathogens and water quality. All these factors, and others not identified, may have contributed to GxE interactions. It must be noted that due to logistical problems, the batch of fish for site D remained at site B from 423 to 510 dpf, and they stayed at site D from 513 to 734 dpf. Each site used its own rearing procedures and feeds, the only restriction being that the fish had to be kept as one batch and should not at any time be sorted.

### *2.2.2.2 Data collection*

At each farm, fish were measured at commercial size (average 400 g), varying from 338 g (farm B) to 487 g (farm D). Number of fish measured, mean weight, age and DGC (defined below) are reported in Table 2-10. Each fish was measured (weight, length) and individually identified with its tag. At farms B, C and D, internal deformities (defined below) were scored after opening each fish. At farm A, fish were put back in the tanks after measurements, reared until 1 kg and were slaughtered at this stage. Internal deformities then were noted at this later stage. Sex was determined by examination of the gonads. Parentage assignment was performed by Landcatch Natural Selection (Scotland) using six microsatellite markers organised in a single PCR multiplex. The assignments were recovered with a home-made program (see details in Dupont-Nivet *et al.*, 2008). Parentage assignment yielded 99.2% unique assignments.

**Table 2-10 :** Number, age, mean weight, mean daily growth coefficient (DGC), proportion of deformed fish and survival rate at each site.

| | Age (days) | Number (N) | Mean weight (g) | Mean DGC | Proportion of deformed fish (%) | Survival rate (%) |
|---|---|---|---|---|---|---|
| **Farm A France** | 714 | 1473 | 398 | 1.18 | 83 | 84.2 |
| **Farm B Italy** | 795 | 1651 | 338 | 0.86 | 60 | 94.8 |
| **Farm C Portugal** | 873 | 1177 | 358 | 0.76 | 55 | 67.3 |
| **Farm D Israel** | 734 | 1667 | 487 | 1.25 | 58 | 95.7 |

#### 2.2.2.3 Statistical analyses

To account for the growth rate of the fish in the different sites, we used the daily growth coefficient [DGC = 100 × (final individual weight$^{1/3}$ – initial individual weight$^{1/3}$)/days], which was chosen because it is much more independent of initial body weight than weight gain and specific growth rate (Cho, 1992), and its use for estimating growth rate in aquaculture is therefore recommended (Bureau *et al.*, 2000). We also analysed the weight at tagging and the final weight (ie body weight at commercial size) of fish at the different sites. To test the potential significance of fixed effects on DGC, initial body weight (IBW) and final body weight (FBW) data were first analysed using proc GLM of the SAS ® System. Tank (prior to tagging) and sex were significant effects ($P$ <0.05) for all traits. Deformities (coded 1 for deformed fish, 0 for undeformed) were significant for FBW ($P$ <0.05) but not for other traits. A deformity effect was then kept as a fixed effect in the analysis model.

A very high proportion of the fish suffered from spinal deformities (65% of all examined fish had one or more kinds of deformities), mostly lordosis and scoliosis (43% and 30%, respectively). These probably were generated by forced swimming due to inappropriate hydrodynamics in the 5m3 tanks in Palavas in the early phases of the experiment (from 3 to 35 g mean weight - Bardon *et al.*, 2009). Because, in our previous paper (Dupont-Nivet *et al.*, 2008), where we analyzed body weight, length and condition factor, as these traits, especially length and condition factor were affected by deformities, we chose to work on a reduced dataset in order to avoid potential effects of imperfect correction by a fixed effect. As seen before, there was no impact of deformities on DGC and IBW, and only moderate impact on FBW. Heritability and genetic correlations involving FBW were similar when including or not deformed fish, but standard errors of genetic correlations were larger when deformed fish were removed. Then, for this paper, we used the full dataset, including data from deformed and undeformed fish, in order to increase the precision and relevance of estimates.

Heritabilities and non-genetic maternal effect were first analyzed for all data using VCE6 (Groeneveld *et al.*, 2008). A multi-trait animal model with maternal effect (model 1 shown below) or without maternal effect (model 2) was used:

$$Y = X\beta + Z_1u + Z_2m + e \qquad \text{(model 1)}$$

where $Y$ is the vector of observations, $\beta$ is the vector of fixed effects (overall mean, initial tank, sex, deformity – for final weight only –, site when data from all sites are treated as a single trait), $u$ is the vector of random additive genetic effects, $m$ is the vector of random maternal effects, and $e$ is the vector of random residual effects. $X$, $Z_1$, and $Z_2$ are known incidence matrices.

Genotype-by-environment (GxE) interactions were estimated through genetic correlations between the trait of interest in environment 1 and the same trait in environment 2, considered as two different traits in the analysis, using model 1. The correlation of residuals between sites was zero, as one individual is present at only one site. GxE interaction is measured by the difference between 1 and the genetic correlation; thus, the closer the genetic correlation to 1, the smaller the GE interaction.

### 2.2.3 Results and discussion

The estimated heritability and maternal effects of DGC, IBW and FBW at each site and across all sites are given in Table 2-11. Maternal effects were not significant, but not taking them into account caused inflated heritability estimates, so they were kept for further analyses. Heritability of initial body weight was very high (0.61±0.14), while the values were more moderate for final body weight (0.29-0.45 among the different sites) and for DGC (0.16-0.34). Interestingly, the heritability estimate across sites for DGC (0.12±0.04) was lower than the estimates at individual sites. So, even if heritability of growth rate within site is relatively high, family re-rankings between sites may cause both smaller between-family variance and higher within-family variance when all sites are considered, lowering heritability across all sites.

**Table 2-11 :** Heritability ($h^2$) and maternal effects ($m^2$) estimates (± S.E.) for daily growth coefficient (DGC), initial body weight (IBW) and final body weight (FBW) at four sites and across all sites. Heritability estimates are given for models with (model 1) and without (model 2) maternal effects. Estimates for IBW are given only across all sites, as all fish were reared in a single site (site A) at that stage.

| Trait | Parameter | Site A (France) | Site B (Italy) | Site C (Portugal) | Site D (Israël) | All Sites |
|---|---|---|---|---|---|---|
| **DGC** | $h^2$ model 1 | 0.19±0.07 | 0.32±0.07 | 0.34±0.10 | 0.16±0.06 | 0.12±0.04 |
| | $m^2$ model 1 | 0.03±0.03 | 0.00±0.00 | 0.06±0.06 | 0.04±0.03 | 0.03±0.02 |
| | $h^2$ model 2 | 0.25±0.06 | 0.32±0.06 | 0.45±0.08 | 0.23±0.05 | 0.17±0.03 |
| **FBW** | $h^2$ model 1 | 0.44±0.12 | 0.45±0.12 | 0.44±0.11 | 0.29±0.09 | 0.28±0.07 |
| | $m^2$ model 1 | 0.06±0.06 | 0.03±0.06 | 0.09±0.07 | 0.10±0.06 | 0.04±0.04 |
| | $h^2$ model 2 | 0.54±0.09 | 0.51±0.08 | 0.62±0.09 | 0.48±0.08 | 0.36±0.06 |
| **IBW** | $h^2$ model 1 | - | - | - | - | 0.61±0.14 |
| | $m^2$ model 1 | - | - | - | - | 0.05±0.07 |
| | $h^2$ model 2 | - | - | - | - | 0.70±0.09 |

Genetic correlations of DGC and FBW between sites are given in Table 2-12. Results for FBW were a little different, but consistent with results on the reduced dataset (without data from deformed fish) (Dupont-Nivet *et al.*, 2008). Genetic correlations of FBW were high (>0.80), revealing little GxE interaction, except between sites C and D (0.75). A very high value (0.93) was reached between site B and site D, where fish shared a longer common rearing period in site B, as noted above. In contrast, genetic correlations of DGCs between sites were much lower: although sites B and D were still close to each other ($r_A$=0.78), the other values ranged from 0.21 to 0.61. As the standard errors were moderate (0.08 to 0.16), the genetic correlations clearly differ from 1, and then reveal significant GxE interactions. Existence of GxE interactions is further confirmed by the observation that the value of heritability across sites for DGC was lower than the individual values estimated within site, as noted above.

**Table 2-12 :** Genetic correlations between sites and heritabilities within site (bold, on the diagonal) for Daily Growth Coefficient (DGC) and final body weight (FBW). Estimates ± S.E.

| trait | | site A (France) | site B (Italy) | site C (Portugal) | site D (Israël) |
|---|---|---|---|---|---|
| **DGC** | site A | **0.19±0.04** | 0.39±0.12 | 0.21±0.14 | 0.34±0.16 |
| | site B | | **0.26±0.05** | 0.61±0.10 | 0.78±0.08 |
| | site C | | | **0.35±0.06** | 0.42±0.14 |
| | site D | | | | **0.16±0.04** |
| **FBW** | site A | **0.42±0.09** | 0.81±0.06 | 0.81±0.06 | 0.86±0.06 |
| | site B | | **0.40±0.08** | 0.81±0.06 | 0.93±0.04 |
| | site C | | | **0.44±0.08** | 0.75±0.09 |
| | site D | | | | **0.29±0.07** |

Compared to our previous results, this genetic analysis of DGC between sites reveals a higher level of GxE interactions than previously expected based on commercial weight. We primarily focused on commercial weight, as it is the most important trait that generates income to the farmers. However, growth rate is also important, because in many cases the on-grower buys its fingerlings from a hatchery or a breeding company, fast growth rate during on-growing is of major interest as it

shortens the production cycle. Also, a faster growth rate is expected to generate improved feed conversion ratio (Thodesen *et al.*, 1999; Kause *et al.*, 2006; Quinton *et al.*, 2007), and thus lower production costs.

The GxE interactions estimated in this paper for DGC are similar to those observed by Saillant *et al.* (2006) on log (weight) in sea bass, who observed genetic correlations mostly in the 0.40-0.50 range. The major difference is that the environments tested by Saillant *et al.* (2006) were generated by manipulation of rearing parameters within the same facility (temperature, density), while the environments we tested were distinct, constrasting on-growing environments. Also, in Saillant *et al.* (2006), the range of conditions were applied since the early phases of rearing, making final weight a surrogate estimation of growth rate. In the present experiment, because our fish were tagged and separated between the environments relatively late, at 35g mean weight, it allowed the initial common rearing phase to have more impact on the final weight observed. This is reinforced by the very high heritability of weight at 35 g (0.61), which effectively makes divergences in size-at-tagging have a strong genetic component. Thus, these differences will still be present, at least partly, at commercial size, even if DGCs of the different families rank differently across the different rearing sites.

In fish, genotype-environment interactions for growth traits have been studied in many species, but mostly as responses of geographic strains to contrasting environments. Studies at the family level investigating genetic correlations between environments are few, but quite consistently show GxE interactions, apart for Chinook salmon (*Oncorhynchus tshawytscha*) where no family re-rankings were apparent (Winkelman and Peterson, 1994). Genetic correlations between environments ranged from 0.58 to 0.86 for body weight of rainbow trout across different systems and salinities up to 2-3 kg, starting from 1 year-old fish (Sylven *et al.*, 1991). In the same species, different densities from start-of-feeding yielded genetic correlations in the range 0.32 - 0.90 for body weight (Bagley *et al.*, 1994). For sea bream, genetic correlations for body weight at commercial size (360-480g) between cage and intensive tank rearing systems were 0.70±0.10, with fish tagged and separated at 4.8g mean weight (Navarro *et al.*, 2009). These results, together with those of Saillant *et al.* (2006) and our present results, suggest that high genetic correlations (i.e. low GxE interactions) for body weight across different environments are more an exception than a rule, especially when fish are reared separately for the largest part of their growth period.

Because in most cases, marine fishes (including sea bass) are sold as fingerlings at a small size (<10g), some practices should be adapted to avoid GxE interactions in order to allow expression of the potential of growth-selected fish. These practices should include rearing selected fish in conditions resembling those of production environment. Our data do not allow us to test specific hypotheses about the major factors (temperature mean and variation, density, feed composition and feeding practices, associated pathogens) generating GxE interactions, as we cannot tease at causes of the genetic correlations observed, except for the high correlation (0.78) between sites B and D, which can be explained by the long common life. Another unanswered question is the temporal stability of such interactions; *i.e.* would the respective families rank the same in different years at the same sites, or would there be genotype*year interactions within site? Further research is needed to understand the determinants of GxE interactions in sea bass before developing breeding programmes adapted to the different type of rearing systems (cage, ponds, closed water system, raceway) used across the industry.

### 2.2.4 Summary

**Two hundred fifty three full-sib families from 33 males and 23 females of European sea bass were produced in a partly factorial mating design. All fish were reared in the same tank for 14 months until reaching mean weight of 35 g, then 7000 of them were individually tagged and weighed, and dispatched to four farms in different locations (France, Israel, Italy and Portugal) representing a wide variety of environmental conditions. Around mean weight of 400 g, 1177 to 1667 fish at each site were weighed. Daily growth coefficient (DGC) was calculated. Pedigrees were successfully redrawn for 99.2% of fish using microsatellite markers. Genetic correlations between sites were high for body weight (>0.80 in all cases but one, *i.e.* five cases over six), but only moderate for DGC (0.21-0.61), with one exception. This indicates significant GxE interactions for growth rate, which were not revealed when studying body weight due to shared common environment of the fish prior to separation to the different rearing environments.**

## 2.3 Additional data: genetic correlations between initial weight, slaughter weight and growth rate

As we showed in section 2.2 that the heritability of initial body weight at tagging was very high (0.61), it could be of interest to perform selection at that stage to have high selection gains. However, as initial growth was done in one environment (recirculated systems at Ifremer Palavas), and there are high GxE interactions for growth rate between sites, it is interesting to know the genetic correlations of initial weight with slaughter weight (as well as growth rate) in the different sites.

To this end, we used the same dataset as in section 2.2 with multi-trait animal models where Final body weight (FBW) as well as DGC in each site were considered as different traits, while initial body weight (IBW) was considered as the same trait for all sites (as all fish were reared in Palavas until the measurement of IBW). Fixed effects were overall mean, initial tank, sex and deformity, and a maternal random effect was added. two different models, one for FBW and one for DGC were fitted with VCE6.0 (Groeneveld *et al.*, 2008). A third model, where FBW and DGC in each site were considered as different traits, was fitted with the same software. Due to lack of convergence, maternal effects were omitted in this model.

**Table 2-13:** Heritabilities of Final body weight (FBW) and DGC in four rearing sites and genetic correlations with initial body weight (IBW).

| | $h^2$ of FBW ± s.e. | $r_A$ IBW-FBW | $h^2$ of DGC ± s.e. | $r_A$ IBW-DGC |
|---|---|---|---|---|
| **Israel** | 0.38±0.06 | 0.83±0.04 | 0.17±0.04 | -0.16±0.16 |
| **France** | 0.55±0.07 | 0.94±0.02 | 0.20±0.04 | 0.54±0.11 |
| **Italy** | 0.46±0.05 | 0.85±0.03 | 0.26±0.05 | 0.00±0.14 |
| **Portugal** | 0.54±0.09 | 0.86±0.03 | 0.35±0.06 | -0.31±0.11 |

**Table 2-14:** Genetic correlations (± s.e.) of FBW in a site with DGC in the same (diagonal) or another (upper triangle) site.

| | Israel | France | Italy | Portugal |
|---|---|---|---|---|
| **Israel** | 0.70±0.04 | 0.66±0.08 | 0.55±0.05 | 0.21±0.05 |
| **France** | | 0.80±0.03 | 0.22±0.07 | 0.02±0.09 |
| **Italy** | | | 0.72±0.04 | 0.36±0.07 |
| **Portugal** | | | | 0.47±0.07 |

The heritabilities and genetic correlations of IBW with FBW and DGC are given in Table 2-13.

The heritability of IBW was 0.64±0.05 in both multi-trait models. The genetic correlation of IBW with FBW was high (>0.8) in all sites, and maximal in France (0.94). DGC from tagging to slaughter was positively genetically correlated with IBW in France ($r_A$=0.54), was not significantly genetically correlated with IBW in Italy and Israel, and was negatively correlated with IBW ($r_A$= -0.31) in Portugal.

The high genetic correlations between IBW and FBW show that the variation in FBW is largely a consequence of the genetic variation in IBW. When DGC is considered, only France, which is the site where the fish were reared prior to tagging, shows a positive genetic correlation of DGC with FBW (Table 2-13). Under the hypothesis that all fish start at the same size at hatching, IBW is a surrogate for growth rate until 370 dpf. The conclusions that can be drawn from the present data are that

1) genetic variation is higher for early growth rate ($h^2$=0.64 for IBW) than for late growth rate ($h^2$=0.38-0.55 for FBW and 0.17-0.35 for DGC),

2) late growth rate is poorly genetically correlated with early growth rate,

3) the high heritability of FBW is largely a consequence of the high heritability of IBW

4) selection for fast growth until 1 year of age can have a null or even negative impact on growth rate in a different environment.

The genetic correlations of FBW with DGC within and across sites are shown in Table 2-14. They imply that selection for FBW should yield relatively high response on DGC in the same site ($r_A$=0.70-0.80), except for Portugal, where the genetic correlation between both is only 0.47. This is most likely because of the negative genetic correlation between IBW and DGC in this site, which should mechanically reduce the correlation between FBW (which integrates IBW and DGC) and DGC. Genetic correlations between FBW in one site and DGC in another site vary from 0.66 between France and Israel to 0.02 between France and Portugal, showing that selection for harvest weight done in the recirculated systems of Palavas would be totally inefficient in increasing growth rate in the semi-intensive ponds of Portugal.

In fish, the consensus is that within population GxE interaction is rather limited (e.g. Kause *et al.*, 2003; Khaw *et al.*, 2012; Kolstad *et al.*, 2006; Sylven *et al.*, 1991). In this study, we find it very high, with even a negative genetic correlation, although in our case it is more than GxE, as the interaction is an interaction between genotype and a combination of age and environment.

A possibility that might increase this genotype by environment interaction in our case is the impact of sexual maturation on growth. In sea bass, males generally mature at 2 years of age (some precociously at one year) and females mature at 3 years, and maturation occurs in winter/spring. It has been shown that the maturation status had an important effect on the growth of sea bass (Begtashi *et al.*, 2004; Saillant *et al.*, 2001b), and maturation is also known, in rainbow trout, to perturb the estimation of genetic parameters (Dupont-Nivet *et al.*, 2010a). In the present study, until one year of age, fish were reared with a long photoperiod (16L:8D) aimed at limiting sexual maturation (Begtashi *et al.*, 2004). Fish were measured for IBW then dispatched to the ongrowing sites in February 2003, in the middle of the reproduction season. As they were under long photoperiod until that time, probably none or a very limited amount of fish (and only males) had matured at that time. FBW was measured in Palavas in January 2004, in Israel in February 2004, in Italy in April 2004 and in Portugal in June 2004. All fish were in natural photoperiod except in Palavas, where they were kept under a long photoperiod. Thus, it is likely that almost all males matured in Italy, Israel and Portugal, while only a limited proportion matured in Palavas – and this could be a good explanation for the fact that genetic correlation between IBW and DGC is significantly positive only in Palavas.

Nevertheless, apart from what we showed in section 2.2, the lowest genetic correlation estimates evidenced before in fish between two environments were 0.45 for body weight (meaning it might be less on growth rate) in Nile tilapia between freshwater and brackish water (Luan *et al.*, 2008), and in the same species 0.08-0.43 between intensive cage culture and pond environments (Bentsen *et al.*, 2012). The low estimates obtained in sea bass – and their possible causes - will have to be taken into account for the design of breeding programmes.

## 2.4 Genetic variation for sex ratio

### 2.4.1 A polygenic hypothesis for sex determination

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"A Polygenic Hypothesis for Sex Determination in the European Sea Bass *Dicentrarchus labrax*" by Marc Vandeputte*,†, Mathilde Dupont-Nivet*, Hervé Chavanne‡ and Béatrice Chatain†

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#### *2.4.1.1 Introduction*

In gonochoric species with genetically determined sex, a one to one sex ratio is known to be optimal in an infinite population of diploid individuals with random mating and Mendelian segregation (Fisher, 1930; Charnov, 1975). The observation of skewed sex ratios may imply, among others, non-Mendelian segregation like in drosophila (Vaz and Carvalho, 2004), non-random mating (Hamilton, 1967) or environmental sex determination (ESD - Bull, 1985). In the latter case, the sex of an individual is not fixed at conception, but is influenced by environmental conditions during its early life. ESD is expected to be favored when the offspring lives in patchy environments, which may confer advantages to being male or female, and neither the offspring nor the parent have control and/or predictive ability on the type of patch the offspring will live in (Charnov and Bull, 1977). Temperature (e.g. Bull and Vogt, 1979; Baroiller and D'Cotta, 2001) seems to be the main environmental factor implied, but density (Ellenby, 1954) and social status (Francis and Barlow, 1993) have been shown to be possible sex-determining environmental factors. In species with ESD, in many cases there is also a genetic variation (Bull *et al.*, 1982a; Conover and Heins, 1987a; Janzen, 1992; Baroiller and D'Cotta, 2001), which in some cases has been described as polygenic (Bull *et al.*, 1982a; Janzen, 1992). Polygenic sex determination, however, is considered to be evolutionary unstable (Rice, 1986) and its maintenance is still poorly understood. It is thought by some authors to be the ancestral type of sex determinism in fish (Kirpichnikov, 1981), but organisms where it is accepted that sex has a polygenic component are indeed very few: the parasitic wasp *Nasonia vitripennis* (Orzack and Gladstone, 1994), the turtles *Graptemys ouachitensis* (Bull *et al.*, 1982a) and *Chelydra serpentina* (Janzen, 1992), and probably the swordtail *Xiphophorus helleri* (Kosswig, 1964).

The European sea bass (*Dicentrarchus labrax*) is a gonochoristic teleost fish distributed in the North-Eastern Atlantic, the Mediterranean and the Black Sea (Pickett and Pawson, 1994). They live in shallow, coastal waters, estuaries, lagoons and harbours, moving to deeper waters (up to 100 m deep) as they grow. Although they can live in waters under 5°C, their seek temperatures above 10°C, and even 15°C in their first year (Kelley, 1988b). They spawn in open waters in late winter-early spring, depending on the latitude. The eggs hatch in 4-9 days, and the young fish move inshore in their first month, towards the warmest waters, especially in estuaries (Pickett and Pawson, 1994). Sex remains undifferentiated for a long period: differentiation occurs between 128 and 250 days post-fertilisation - dpf (Saillant *et al.*, 2003a). Records of sea bass sex ratio in wild populations are scarce. They show balanced sex ratios (Saillant *et al.*, 2003a), excess of males (Menu, pers.comm.) or excess of females (Arias, 1980), but as a whole do not contradict the hypothesis of balanced sex

ratios in the wild. The sea bass is an important species in Mediterranean aquaculture, and it appears that in all aquaculture populations, sex ratios are strongly biased towards males (75 to 95%, e.g. Blazquez *et al.*, 1998; Saillant *et al.*, 2002; Saillant *et al.*, 2003a), which is a problem for farmers as males mature earlier and grow less than females. Temperature has been shown to have a major effect on sex determination in sea bass (Blazquez *et al.*, 1998; Pavlidis *et al.*, 2000; Saillant *et al.*, 2002; Mylonas *et al.*, 2005). The effect of temperature is not fully understood, as two studies show an increased proportion of males with cold temperature (15°C: Blazquez *et al.*, 1998, 13°C: Saillant *et al.*, 2002) while the other two show an increased proportion of females at 13 and 15°C (Pavlidis *et al.*, 2000; Mylonas *et al.*, 2005). The current hypothesis is that low temperatures early in development (before 100 dpf) may favor female sex differentiation, but that long-lasting low temperatures, through a negative effect on growth, may preclude female differentiation and result in an increased proportion of males (Piferrer *et al.*, 2005). Thus, the excess of males observed in culture would be due to the use of temperatures higher than in nature, for productivity reasons. From the genetic point of view, in addition to the environmental effect on sex, simple female homogamety can be excluded, as the sex ratios of normal diploid and gynogenetic offspring are equivalent (Felip *et al.*, 2002; Peruzzi *et al.*, 2004). The sex ratio of the offspring from masculinized females is not female biased and would rule out both XX-XY (female homogamety) and ZW-ZZ (male homogamety) systems (Blazquez *et al.*, 1999). In this latter study however, the possible male bias induced by high rearing temperatures (22.5°C), and the impossibility to ascertain the genetic sex of the sex-reversed parents makes the demonstration a little weak. Therefore, male homogamety with environmentally male-biased sex ratios would still be a possibility. Additionally, parental influence on the sex ratio of progenies has also been demonstrated, however with very limited experimental settings (Saillant *et al.*, 2002; Gorshkov *et al.*, 2003), showing that there is a genetic component of progeny sex ratio. Although it is clear that the sex of sea bass is determined both by genetic factors and by the environment (mostly temperature), the sex determination system of this species remains basically unknown (Piferrer *et al.*, 2005).

In this study, our aim was to describe the genetic component influencing sex ratio in sea bass, using a large number of families in classical aquaculture conditions, and to determine which genetic models could describe it best. We described sex using a threshold model with an underlying variable (sex tendency), as this type of model integrates both genetic and environmental effects, which are known to influence sex ratio in the sea bass.

#### *2.4.1.2 Material and methods*

**A Partly Factorial Mating Design:** The brood fish used were from a group of 33 males and 51 females of wild Atlantic origin, collected in 2000 on the French coasts of Brittany. Each brood fish was individually tagged and fin clipped for DNA extraction. The sperm of males was cryopreserved in 250 µl straws (Fauvel *et al.*, 1998). In January 2001, 51 females were injected with 10 µg/kg LHRH (SIGMA, D-TRP6-LHRH), and eggs were stripped 72 hours later. Twenty-three females gave a sufficient quantity of good quality oocytes. From these spawns, we produced a mating design combining 33 males and 23 females in 3 full factorial sets of 11♂ x 9♀, 11♂ x 7♀ and 11♂ x 7♀, for a total of 253 families. All full-sib families were fertilized individually, then eggs were grouped by female for incubation (48 hours at 13°C), after which 2 ml of viable eggs per female (approx. 2.000 eggs/female) were collected to create one batch containing all families. Standard rearing conditions were used, with temperature gradually increasing from 13 to 18°C in the first 64 days. Temperature was then kept at 18°C until 238 days (mean length of fish 117 mm, mean weight 23.6 g), then

lowered to 14°C in order to slow down growth until the time scheduled for tagging. Although late low temperatures are suspected to masculinize the progenies (Piferrer *et al.*, 2005), this does not apply at 238 days, as it was shown before that lowering temperature from 20 to 13°C at 149 days (mean length 81 mm) had no impact on the sex ratio (Saillant *et al.*, 2002).

**Recording of Traits and Parentage Assignment:** At 370 days, the fish had reached a mean weight of 35 g. Seven thousand of them were randomly selected, on which individual weight and length were measured. Each fish was individually tagged and fin clipped for DNA extraction. The fish were then sent to four different sites (1.750/site), where they were reared until approx. 400 g mean weight. This rearing in different sites was designed for estimating genetic parameters and genotype-environment interactions for growth and quality traits, in parallel of the present study. Still, it was not expected to have any impact on the sex ratio, as the differentiation period is over well before 370 days. At 400 g, the remaining fish (5.988) were slaughtered and sex was recorded by visual observation of the gonads after dissection, and 5.960 of those had an identifiable sex phenotype. In all sites, the difference between males and females was straightforward (female gonads were orange, and male gonads pink/white), and only 28 fish in total could not be determined with certainty. Parentage assignment was done by Landcatch Natural Selection (Alloa, UK) using 6 microsatellite markers on both parents and offspring. Out of the 5.960 offspring with a sex phenotype, 5.896 (98.9%) could be assigned to a single parental pair.

**Statistical Methods:** Sire 23 (set 3) gave only 3 offspring, probably due to bad sperm quality. It was removed from the analysis, as it created a major disequilibrium in the data, thus reducing the number of families studied from 253 to 246. Then, the base data set comprised 5.893 offspring from 246 families. Apart from sire 23, 245 of 246 possible families had offspring. No offspring were found in the sire 9 x dam 2 family (set 1), probably due to a bad quality straw of cryopreserved sperm, as both male 9 and female 2 gave satisfactory results in all other crosses. In order to avoid computational problems, these missing data were replaced by simulated data corresponding to the expected numbers of male and female offspring in this family (19 males and 3 females, which are the average numbers of males and females per family produced by male 9 and female 2)

In a first analysis, the number of females was calculated in each paternal or maternal half-sib family, and compared to the expected number of females with a uniform proportion corresponding to the observed proportion of females in the whole sample, using chi-square tests, to test for the existence of significant genetic variation in progeny sex ratio. In a second step, the family sex ratios in each of the three full-factorial sets was analyzed by logistic regression using SAS® proc Logistic, where the proportion of females was explained by a sire and dam effect. The model fit was tested using the Hosmer and Lemeshow test (Hosmer and Lemeshow, 1989). In a third step, sex was considered as a threshold trait with a polygenic basis (Bulmer and Bull, 1982). Sex was analyzed using a single trait model including additive random effects for sire and dam, and a residual error. Restricted maximum likelihood (REML) estimates of variance components for the random effects in the model were obtained on the underlying liability scale using the ASREML software (Gilmour *et al.*, 2002). Both sire and dam heritabilities were estimated, using standard formulae (Becker, 1984). Genetic correlations between sex and growth were estimated with a trivariate (sex, weight and length at 370 days) animal model, with sex coded on the observed scale (0 or 1), using the VCE5.0 software (Kovac and Groeneveld, 2003). This model included an animal additive genetic effect for all traits, and for length and weight, a fixed effect of sex, which was necessary due to sex dimorphism on length and weight

(females are larger than males). We used the observed scale since it produces unbiased genetic correlations as long as the threshold trait does not have both low heritability and low incidence (Olausson and Ronningen, 1975). We also estimated the heritability of sex dimorphism for growth using classic formulae of (co)variance for a difference (Chapuis *et al.*, 1996).

Then, we used the estimates generated to simulate samples of 5.893 fish from 23 dams and 32 males in three sets of 9x11, 7x10 and 7x11, using a heritability of sex of 0.62 on the underlying scale, and a mean proportion of females of 18.3%. We also generated simulated samples of the same size using a threshold model where the underlying sex tendency *m* would be determined, instead of polygenes, by one or two bi-allelic loci with effect size *f*, such that genotype *aa* had a genetic effect of *–f* on *m*, genotype *Aa* had a genetic effect of 0 and genotype *AA* had a genetic effect of *+f*. The allelic frequencies were 0.5 for each allele, except for the one locus case where allelic frequencies 0.2, 0.4, 0.5, 0.6 and 0.8 were tested. The effect size *f* was tuned so that the proportion of genetic variance over phenotypic variance was 0.62, and a random residual effect representing 38% of the total phenotypic variance was added. This model is an extension to skewed population sex ratios of the two-factor model with environmental variance (Bull, 1983). Purely genetic threshold models were also tested, either polygenic with $h^2=1$, or with one to five bi-allelic loci, but no residual environmental variance. For each model, we generated 10.000 samples, and compared the simulated distribution of full and half-sib family sex ratios with the observed one, to test for the coherence of the model with the observed dataset.

### 2.4.1.3 Results

The overall proportion of females in the population was 18.3%, and there were no sex ratio differences between fish from each of the four growing sites (17.4%-19.4%, $\chi^2$=4.62, 3 d.f., $P$>0.20). Family sex ratios are given in Appendix 2-1 (p.43). Comparison of observed values to expected values under the hypothesis of equal sex ratio in all families shows a strong inequality of contributions between half-sib families ($\chi^2$=350.6, 31 d.f., $P$<0.0001 for paternal half-sib families, $\chi^2$=327.6, 22 d.f., $P$<0.0001 for maternal half-sib families). Proportions of females ranged from 4.7% to 46.3% in paternal half-sib families, and from 0.5% to 40.3% in maternal half-sib families. The logistic regression analysis showed that both sire and dam had a highly significant effect on the progeny sex ratio ($P$<0.0001), and that this model without interaction was enough to explain the observed dataset, the Hosmer and Lemeshow chi-square tests being far from significance ($\chi^2$=6.04, 8 d.f., $P$>0.6 in set 1, $\chi^2$=1.94, 8 d.f., $P$>0.9 in set 2, $\chi^2$=6.04, 8 d.f., $P$>0.6 in set 3). The estimated heritability of sex on the underlying scale was 0.52 ± 0.13 (sire heritability), 0.72 ± 0.20 (dam heritability), or 0.62 ± 0.12 (sire+dam heritability). The maternal effect ratio (non genetic maternal variance/phenotypic variance) that would explain the difference between sire and dam heritability is $m^2$=0.05 ± 0.06, which is clearly non significant. The heritability of growth sex dimorphism was low (0.15 for weight and 0.09 for length at 370 days). The estimated genetic correlation was 0.50 ± 0.09 between sex and weight, and 0.48 ± 0.09 between sex and length. Some graphical illustration of this can be seen on Figure 2-1, which shows that apart from the fixed effect of sex on weight (females being on average 40.8% heavier than males at 370 days), larger fish tended to be found in the families with the highest proportion of females. The distribution of proportions of females among the 55 half-sib families scored (32 paternal half-sib families, 23 maternal half-sib families) is plotted on Figure 2-2, and compared with simulated distributions in the same experimental setting. Detailed information about models fit can be found in Table 2-15. Additive polygenes with $h^2$=0.62 could explain the data with excellent fit. For a single locus with environmental variance, the best fit was observed with allelic frequencies of 0.6 for the male-orienting allele and 0.4 for the female-orienting allele, but this could not account for the observed half-sib data.

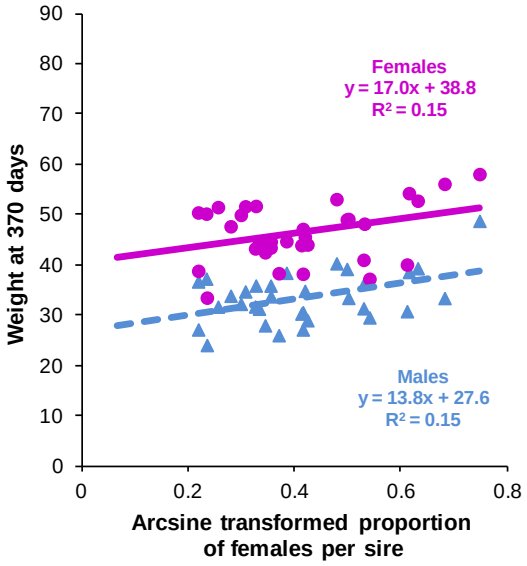


**Figure 2-1:** Relationship between arcsine transformed proportion of females in sire half-sib families of European sea bass and mean weight at 370 days of male and female offspring in the same families.

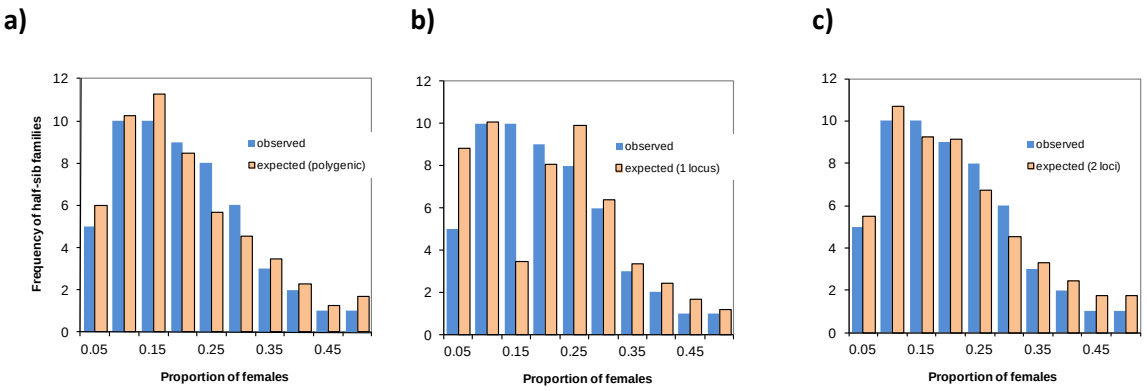


**Figure 2-2:** Observed frequencies of females in 55 half-sib families of European sea bass, and expected frequencies under a threshold model with 18.3% females in the offspring and 38% environmental variance, where the genetic component of the underlying variable is: a) polygenic; b) 1 bi-allelic locus with p=0.6 for the masculinizing allele, q=0.4 for the feminizing allele; c) 2 bi-allelic loci with p=q=0.5.

On the contrary, a two-locus system with environmental variance could fit both half-sib and full-sib observed data. No purely genetic model (without environmental variance) could explain the observed data, whatever the number of loci implied (from monogenic to polygenic).

**Table 2-15:** Comparison of family sex ratio distributions observed in the present study with simulated distributions from various sex-determination models.

| | Half-sib families sex ratio distribution d.f.=6 | | Full-sib families sex ratio distribution d.f.=11 | |
|---|---|---|---|---|
| Model | $\chi^2$ | P-value | $\chi^2$ | P-value |
| Polygenic, $h^2$=0.62 | 2.07 | >0.9 | 10.9 | >0.4 |
| 1 locus with envir. variance, p=0.2 [a] | 58.1 | <0.001 | 171 | <0.001 |
| " , p=0.4 | 59.6 | <0.001 | 18.4 | >0.05 |
| " , p=0.5 | 22.9 | <0.001 | 6.2 | >0.8 |
| " , p=0.6 | 14.8 | <0.05 | 10.2 | >0.5 |
| " , p=0.8 | 18.2 | <0.01 | 28.1 | <0.01 |
| 2 loci with envir. variance, p=0.5 | 1.42 | >0.9 | 12.6 | >0.3 |
| 1 locus without envir. Variance | 383 | <0.001 | 865 | <0.001 |
| 2 loci without envir. Variance | 23.9 | <0.001 | 139 | <0.001 |
| 3 loci without envir. Variance | 12.8 | <0.05 | 63.3 | <0.001 |
| 5 loci without envir. Variance | 6.33 | >0.4 | 38.2 | <0.001 |
| Polygenic without envir. variance ($h^2$=1) | 8.04 | >0.2 | 40.1 | <0.001 |

*P-value <0.05 shows incompatibility between simulated and observed data, p= frequency of the male-orienting allele*

#### *2.4.1.4 Discussion*

**The genetic influence on sex in sea bass:** Our results are fully in accordance with a polygenic model, as described by Bulmer and Bull (1982), where the sex of an offspring is determined by the fact that an underlying sex tendency (determined both by polygenes and environmental effects) is greater or less than a threshold value. In our experiment, both sires and dams have an effect on the sex ratio of their progenies, and the effects are similar in size, pleading for an additive genetic effect on sex ratio. The heritability is high ($h^2_{s+d}$=0.62 ± 0.12), and clearly different from zero. It may not be biased by non additive genetic factors, as the mating design is factorial and all fish were in the same environment at the time of sex determination. Moreover, we verified that each dam ($\chi^2$=73, 66 d.f., $P$>0.2) and each sire ($\chi^2$=104, 93 d.f., $P$>0.2) contributed the same proportion of offspring in all sites, and that the same sex ratios were observed in all sites, so even unexpected late actions of the environment on the sex ratio would not bias the between-family data. The "classical" vertebrate chromosomal sex determination model is expected to give 50% males and 50% females, and clearly cannot explain the data obtained, even with eventual sex-reversal of genetic females to males due to temperature. This holds as long as sex reversal is homogeneous among families (i.e. not genetically determined). If sex reversal was not homogeneous among families, this would imply the existence of a secondary genetic component of sex ratio, in addition to the chromosomal system. Purely environmental sex determination (ESD) can also be excluded, as it is not supposed to yield any between families differences in sex ratios. This was already suggested by previous results (Blazquez *et al.*, 1999; Saillant *et al.*, 2002; Gorshkov *et al.*, 2003; Peruzzi *et al.*, 2004). As it is clear that environment can influence sex ratio in sea bass (Piferrer *et al.*, 2005), the genetic component we evidence can be either considered as a genetic variation of the primary sex ratio, or as a genetic sensitivity to the environmental effects. As we tested only one environmental condition however, we cannot distinguish between both. The observed occurrence of intra-testicular oocytes in many (up to 62%) young sea bass males (Gorshkov *et al.*, 1999; Saillant *et al.*, 2003a) could be in favor of a polygenic primary sex ratio, with sex reversal occurring as a consequence of environmental effects. However, the existence of genetic by environment interactions, which would favor the second hypothesis, has already been evidenced (Saillant *et al.*, 2002). Nevertheless, we show that whatever its true nature, there is a genetic effect which leads to a continuous distribution of family sex ratios in this species, at least in one (masculinizing) environmental condition. We also show that, in addition to the global effect of temperature, which certainly skewed sex ratio towards males in the present experiment, it is necessary to include environmental variance within this global environment to explain the observed distribution of sex ratio, meaning that a purely genetic model where individual sex would be uniformly influenced by the environment could not explain our data. Even with environmental variance allowed, a two-factor system can be excluded, but a four-factor system (two bi-allelic loci) can explain the observed data. Similarly, in the apple snail, it was concluded that a continuous variation in family sex ratios was most likely due to at least four sex factors (Yusa, 2007). Unlike what was seen in the silverside fish *Menidia menidia* (Conover and Heins, 1987b), we could not observe a multimodal distribution of family sex ratios, which was considered an indication of the existence of only a few sex factors in this species. Indeed, as pointed out by Bull (1983), it is extremely difficult to ascertain the polygenic nature of a sex determining system, when compared to a system with only a few factors and some environmental variance. Nevertheless, both systems are expected to behave in very similar manner, and may be described by the Bulmer and Bull threshold model, provided sex factors have individually weak effects (Bull, 1983). Finally, we showed there was considerable genetic variation for sex ratio in a given environment, as half-sib family sex ratios range from 0.5% to 46.3%

of females (the proportions range from 0 to 75% in full-sib families, but their small size – on average 24 individuals – makes it less significant). We can compare this range of variation in female proportions to that produced by temperature treatments: 0-27% (Blazquez *et al.*, 1998), 18-66% (Koumoundouros *et al.*, 2002), 24-74% (Pavlidis *et al.*, 2000) and 11-32% (Saillant *et al.*, 2002). This shows that in this species, the genetic and environmental components of sex determinism are of comparable magnitude, which confirms there are no fundamental barriers between ESD and GSD, as proposed by Bull (1983).

**The genetic relationship between size and sex:** An interesting feature of our results is the relatively strong genetic correlation between sex and size ($r_A$=0.50 ± 0.09), which means that some of the genes acting on sex determination and growth are the same, or at least are strongly linked in our sample. This is in accordance with previous experimental evidence showing that females are larger at the time of sex differentiation (Blazquez *et al.*, 1999; Saillant *et al.*, 2001b). Still, we have shown that once corrected for this sex dimorphism, there was still a size advantage in families with high proportions of females (Figure 2-1). This strengthens a lot the connection that was established between growth and sex. Indeed, as sea bass is a group spawner, it is clear that the size of females should have a strong impact on their fitness, through their absolute fertility, whereas it may be less important for males. Therefore, the idea that a minimum size is needed at the time of sex differentiation to be able to differentiate as female is plausible, and strengthened by the fact that the size advantage of females is never as large as at the time of sex differentiation (+41% weight at 1 year, +20% at 2 years in the present study, +67% at 10 months and +25% from 2 years in Saillant *et al.*, 2001b). This type of determinism is observed in nematodes (Ellenby, 1954) and eels (Roncarati *et al.*, 1997), where high density (hence limited resources for growth) favors male differentiation. However, density had no effect on sex ratio in sea bass (Saillant *et al.*, 2003c), but in this latter case the densities and rearing conditions were chosen to avoid any impact on growth, in order not to confound effects of growth and density *per se*.

**Evolutionary consequences of polygenic sex in sea bass:** Our heritability estimates for sex ratio are high (0.62), as in turtles, where high estimates (in the range of 0.5-0.8) have also been found (Bull *et al.*, 1982a; Janzen, 1992). However, the estimates in turtles were likely inflated by maternal and/or dominance effects, due to the use of full-sib designs, which is not the case in our experiment. Moreover, in turtles, the impact of high heritability estimates was considerably lowered in natural conditions by the high variance between nests temperature, which, combined with the very narrow temperature range for complete sex change, reduces a lot the potential for selection on sensitivity to ESD (Bull *et al.*, 1982a). In our case, although temperature has a large effect on sex ratio, it cannot produce 100% female progenies, and there is a wide spectrum (13-25°C) having an impact on sex determination. Still, the temperature of the coastal waters during the first year of life of the sea bass may be quite variable, and thus reduce the effectiveness of natural selection on polygenic sex ratio in this species. As our experimental growing conditions are different from natural ones (fish are 13.5 cm at 1 year, *vs*. 7-10 cm in the wild in the Atlantic -Pickett & Pawson 1994), and with the suspected genotype-environment interactions for sex ratio in sea bass (Saillant *et al.*, 2002), variation which may be hidden in wild (low temperature) conditions may be expressed in experimental or farm (warm) conditions. This may be even accentuated by the growth-sex genetic relationship, as growth also has a high heritability in this experiment (0.54 ± 0.08 for weight), and is expected to have a higher heritability in fast-growing conditions than in slow-growing conditions.

Considering the high heritability of sex ratio observed in our conditions, we could calculate the effects of frequency-dependent selection on sex using the Bulmer and Bull (1982) model (Figure 2-3). It shows that equilibrium sex ratio should be reached in 7-8 generations. This shift towards females, which are more interesting for aquaculture, could even be accelerated through artificial selection of female-producing families. If artificial selection on growth is also practiced, as is the case in several hatcheries, the shift in sex ratios in aquaculture populations should be even faster, and may lead to predominantly female populations. As aquaculture of sea bass expands in the Mediterranean area, the impact of aquaculture escapees, with modified sex determinism, will have to be carefully evaluated, as sex ratio is doubtlessly a major determinant of fitness, and may therefore have an important impact on the fitness of natural populations of this species (Lynch and O'Hely, 2001).

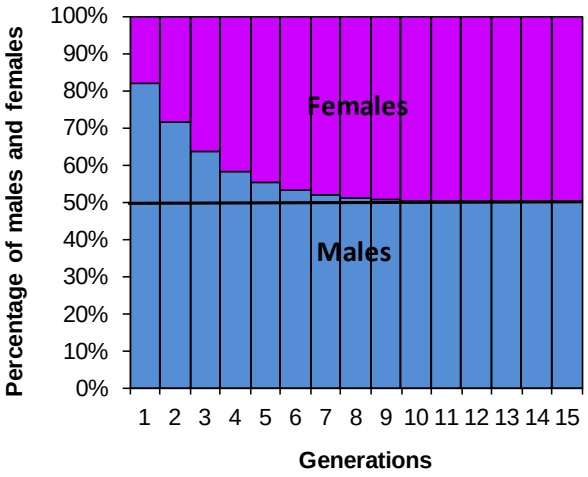


**Figure 2-3 :** Expected evolution of sea bass sex ratio along generations of random mating in constant environmental conditions, using the frequency-dependent model of Bulmer and Bull (1982), with heritability 0.62 and initial sex ratio 18.3% females (this study's estimates)

**Conclusion:** We have demonstrated that sex determinism in the sea bass is not monogenic, is sensitive to within-tank variations in the environment, and that the genetic component is essentially additive, is linked to the growth capacity of the fish, is of the same magnitude as the environmental component controlled by temperature, and can be precisely described using a polygenic threshold model with $h^2$=0.62 on the underlying scale.

Selective breeding experiments are under way to explore the effective response to sex ratio selection and the correlated sex ratio response to selection for growth. They will also provide material for QTL search in the coming years, hopefully allowing us by that time to determine more precisely if sex determinism in this species is effectively polygenic or only oligogenic.

**Appendix 2-1**. Numbers of males and females (M:F) in 246 European sea bass families, representing 5,893 fish from three full-factorial matings

A) Set 1: 11 sires x 9 dams

| Dams / Sires | D34 | D35 | D36 | D37 | D38 | D39 | D40 | D41 | D42 | Mean proportion of females | Total offspring number |
|---|---|---|---|---|---|---|---|---|---|---|---|
| S01 | 19:2 | 21:4 | 20:5 | 20:12 | 20:11 | 19:12 | 30:9 | 19:8 | 30:5 | 0.256 | 266 |
| S02 | 17:0 | 13:0 | 16:0 | 14:3 | 35:4 | 26:6 | 33:3 | 33:5 | 21:0 | 0.092 | 229 |
| S03 | 16:1 | 22:0 | 25:1 | 14:3 | 25:1 | 36:2 | 30:9 | 26:8 | 31:2 | 0.107 | 252 |
| S04 | 7:3 | 15:0 | 12:3 | 10:7 | 27:9 | 17:12 | 36:11 | 17:7 | 18:3 | 0.257 | 214 |
| S05 | 14:4 | 21:2 | 19:5 | 5:5 | 11:7 | 15:6 | 20:4 | 27:7 | 13:3 | 0.229 | 188 |
| S06 | 16:0 | 9:0 | 21:2 | 10:2 | 18:3 | 33:4 | 22:4 | 27:8 | 18:1 | 0.121 | 198 |
| S07 | 14:8 | 18:3 | 24:10 | 12:8 | 22:8 | 12:17 | 29:15 | 15:10 | 7:3 | 0.349 | 235 |
| S08 | 9:1 | 11:1 | 11:2 | 6:0 | 22:0 | 18:0 | 31:0 | 6:2 | 7:0 | 0.047 | 127 |
| S09 | 16:2 | [a] | 14:0 | 21:1 | 37:4 | 24:3 | 22:4 | 15:5 | 17:0 | 0.103 | 185 |
| S10 | 3:7 | 14:3 | 8:3 | 4:12 | 14:7 | 12:14 | 18:16 | 15:12 | 13:13 | 0.463 | 188 |
| S11 | 11:8 | 24:0 | 20:5 | 20:21 | 17:11 | 19:16 | 28:13 | 22:14 | 30:6 | 0.330 | 285 |
| Mean proportion of females | 0.202 | 0.072 | 0.159 | 0.352 | 0.208 | 0.285 | 0.227 | 0.279 | 0.149 | | |
| Total offspring number | 178 | 181 | 226 | 210 | 313 | 323 | 387 | 308 | 241 | | |

[a] no offspring observed in this family

B) Set 2: 11 sires x 7 dams

| Dams / Sires | D43 | D44 | D45 | D46 | D47 | D48 | D49 | Mean proportion of females | Total offspring number |
|---|---|---|---|---|---|---|---|---|---|
| S12 | 29:4 | 17:3 | 15:0 | 20:1 | 19:0 | 27:0 | 12:0 | 0.054 | 147 |
| S13 | 15:8 | 34:8 | 30:4 | 47:2 | 29:4 | 35:1 | 11:6 | 0.141 | 234 |
| S14 | 11:17 | 27:2 | 12:0 | 28:0 | 26:4 | 26:2 | 7:3 | 0.170 | 165 |
| S15 | 18:3 | 28:3 | 18:1 | 19:0 | 24:0 | 18:0 | 16:0 | 0.047 | 148 |
| S16 | 9:16 | 21:11 | 26:4 | 28:3 | 38:12 | 20:4 | 13:6 | 0.265 | 211 |
| S17 | 25:18 | 34:15 | 27:1 | 37:1 | 39:6 | 34:1 | 25:2 | 0.166 | 265 |
| S18 | 11:10 | 29:5 | 31:1 | 26:1 | 41:1 | 35:2 | 9:1 | 0.103 | 203 |
| S19 | 11:10 | 22:2 | 13:1 | 21:0 | 26:3 | 27:1 | 18:2 | 0.121 | 157 |
| S20 | 19:10 | 24:9 | 14:1 | 28:1 | 29:1 | 31:1 | 14:1 | 0.131 | 183 |
| S21 | 18:10 | 11:5 | 11:2 | 25:1 | 18:1 | 26:3 | 14:2 | 0.163 | 147 |
| S22 | 16:17 | 11:15 | 24:8 | 22:1 | 14:8 | 16:3 | 11:5 | 0.333 | 171 |
| Mean proportion of females | 0.403 | 0.232 | 0.094 | 0.035 | 0.117 | 0.058 | 0.157 | | |
| Total offspring number | 305 | 336 | 244 | 312 | 343 | 313 | 178 | | |

C) Set 3: 10 sires x 7 dams

| Dams / Sires | D50 | D51 | D52 | D53 | D54 | D55 | D56 | Mean proportion of females | Total offspring number |
|---|---|---|---|---|---|---|---|---|---|
| S24 | 3:1 | 4:0 | 5:0 | 5:0 | 5:1 | 7:0 | 13:2 | 0.087 | 46 |
| S25 | 13:3 | 24:9 | 12:2 | 29:0 | 24:9 | 18:1 | 43:20 | 0.213 | 207 |
| S26 | 34:9 | 9:5 | 10:1 | 32:0 | 12:2 | 11:0 | 15:7 | 0.163 | 147 |
| S27 | 22:4 | 17:6 | 9:0 | 27:0 | 22:2 | 6:0 | 21:4 | 0.114 | 140 |
| S28 | 44:4 | 19:2 | 8:0 | 23:0 | 24:4 | 9:0 | 18:2 | 0.076 | 157 |
| S29 | 13:8 | 9:9 | 4:3 | 10:1 | 6:4 | 5:1 | 6:9 | 0.398 | 88 |
| S30 | 37:10 | 21:9 | 17:2 | 33:0 | 38:7 | 16:0 | 20:7 | 0.161 | 217 |
| S31 | 33:6 | 20:9 | 14:1 | 25:0 | 18:11 | 14:1 | 19:15 | 0.231 | 186 |
| S32 | 29:3 | 31:4 | 16:0 | 18:0 | 28:2 | 6:0 | 30:0 | 0.054 | 167 |
| S33 | 26:0 | 27:3 | 9:0 | 12:0 | 22:1 | 11:0 | 24:5 | 0.064 | 140 |
| Mean proportion of females | 0.159 | 0.236 | 0.080 | 0.005 | 0.178 | 0.028 | 0.254 | | |
| Total offspring number | 302 | 237 | 113 | 215 | 242 | 106 | 280 | | |

#### *2.4.1.5 Summary*

**Polygenic sex determination, although suspected in several species, is thought to be evolutionary unstable, and has been proven in very few cases. In the European sea bass, temperature is known to influence the sex ratio. We set up a factorial mating, producing 5,893 individuals from 253 full-sib families, all reared in a single batch to avoid any between-families environmental effects. The proportion of females in the offspring was 18.3%, with a large variation between families. Interpreting sex as a threshold trait, the heritability estimate was 0.62 ± 0.12. The observed distribution of family sex ratios was in accordance with a polygenic model or with a four sex factors system with environmental variance, and could not be explained by any genetic model without environmental variance. We showed there was a positive genetic correlation between weight and sex ($r_A$= 0.50 ± 0.09), apart from the phenotypic sex dimorphism in favor of females. This supports the hypothesis that a minimum size is required for sea bass juveniles to differentiate as females. An evolution of sex ratio by frequency-dependent selection is expected during the domestication process of *D. labrax* populations, raising concern on the release of such fish in the wild.**

### 2.4.2 Supplemental information

#### *2.4.2.1 The threshold model for sex determination*

In chapter 2.4.1, we referred to a threshold model for polygenic sex determination which was introduced by Bulmer and Bull (1982). This model and its consequences are not necessarily intuitive, and due to the paucity of species with polygenic sex determination, it is indeed little used. Then, we will develop here a few aspects of this model to make it more familiar to the reader.

In a quantitative genetics framework, the sex of an animal can be determined by a threshold model with an underlying liability called sex tendency. Sex tendency ($t$) has a genetic and an environmental component, both normally and independently distributed. If the value of the sex tendency is below a fixed threshold, the animal becomes a male, while if it is over the threshold, it becomes a female. One thing that is important to consider is that in such a model, there is no hierarchy in environmental or genetic effects. Either one can modify sex tendency, and as a consequence make the animal cross the threshold that will make it male or female.

In a given group of individuals (population of family), the observed sex ratio allows the calculation of the average sex tendency of males and females in the group ($t_m$ and $t_f$, respectively – see Figure 2-4). In this thesis, we will use a fixed reference value, considering that a population with a mean sex tendency ($t_{mean}$) equal to zero would have an even sex ratio. We also arbitrarily consider that the phenotypic standard deviation of $t$ is unity, and that $t$ follows a normal distribution. Under these hypotheses, in a group with a proportion $P_f$ of females, the sex tendency parameters are calculated as follows, after adaptation from Bulmer and Bull (1982):

$$t_{mean} = \text{probit}(P_f) \qquad \text{(Equation 2-1)}$$

$$\boldsymbol{t_f = t_{mean} + \frac{\varphi(t_{mean})}{P_f}} \qquad \text{(Equation 2-2)}$$

$$t_m = t_{mean} - \frac{\varphi(t_{mean})}{1-P_f} \qquad \text{(Equation 2-3)}$$

With *probit* the inverse of the cumulative distribution of the standard normal distribution and $\varphi$ the probability density function of the standard normal distribution. $t_f$ is the average sex tendency of the females in the population, while $t_m$ is the average sex tendency of the males. Using the case of our G1 population, where $P_f$=0.182 as an example, we obtain the following values, plotted in Figure 2-4: $t_{mean}$=-0.91, $t_f$=0.54, $t_m$=-1.23.

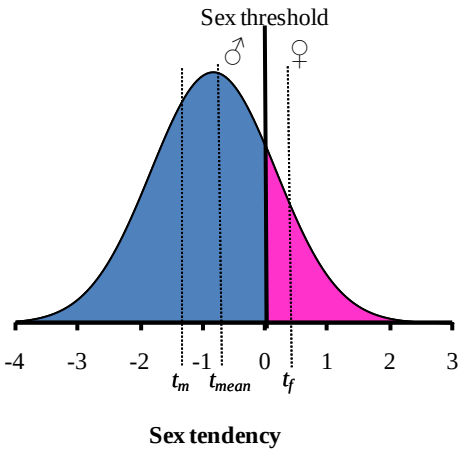


**Figure 2-4:** Threshold model for sex ratio in a population with a normally distributed sex tendency and 20% of females. $t_m$= mean sex tendency of males, $t_{mean}$= mean sex tendency in the population, $t_f$= mean sex tendency of females

We will see later on (section 4.2.4) how this can practically be used to predict the evolution of sex ratio and sex tendency in a domesticated population of sea bass.

#### 2.4.2.2 Genetic and environmental correlations of sex tendency and body length at different ages

We only reported before the heritability of sex tendency and its genetic correlation with growth measured by body weight or body length at 370 dpf. The same model also gave the heritability of body length at that stage: the raw value was 0.72±0.10, while the value with potential maternal effects removed (as suggested section 2.2) was 0.62±0.11. The environmental correlation of sex tendency with body weight ($r_E$=-0.06±0.04) and body length ($r_E$=-0.05±0.04) was also estimated. This value did not significantly differ from zero, however its interpretation is rather tricky as in the model concerned the phenotypic effect of sex on size is removed by a fixed effect. Then, as all fish are also reared in a common environment, it cannot be really expected that this environmental correlation would in any case depart from zero.

For further predictions of what would happen if fish were selected for increased growth, we also estimated the genetic correlation of body length and sex tendency at slaughter size (around 400 g mean weight), adding a site effect in the model for body length to account for the different mean lengths of fish at slaughter in the different sites. In this case, the genetic correlation of body length and sex tendency reduced to 0.33±0.12, while the environmental correlation was still close to zero at -0.04±0.04. All these values will be useful to simulate the effects of selection for growth on sex ratio, as will be done in section 4.3.

# 3 Effects of domestication and directional selection for body length

## 3.1 Selection response for growth

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### 3.1.1 Introduction

European sea bass (*Dicentrarchus labrax*) is a leading species of Mediterranean aquaculture, but a large proportion of the broodstock used today remains unselected, with many hatcheries using only wild brood fish. Sea bass culture would undoubtedly benefit from selective breeding for productivity traits. As in almost every species, the first trait for which selection is desired is growth. This is particularly critical for the sea bass, as its growth rate is slow: it is not exceptional to need 24 months from hatching to produce a commercial size (400g) sea bass. Still, these figures may vary largely, depending on rearing conditions, and especially the temperature regime. Selective breeding for growth has already proven effective in many species (see review in Gjedrem and Olesen, 2005), with gains in the range of 5-20% per generation.

The potential of sea bass for breeding has gained interest quite recently, thanks to the application of parentage assignment with microsatellite loci. They allow to identify families, and hence the quantitative variation among and within them, with fish reared in a single batch (Saillant *et al.*, 2002; Saillant *et al.*, 2006; Chatziplis *et al.*, 2007; Vandeputte *et al.*, 2007; Dupont-Nivet *et al.*, 2008;). The availability of this method was particularly critical for this species, as, like other marine species, it goes through a difficult period of larval rearing, where the environmental (i.e. non genetic) variation of growth and survival between tanks is particularly high. This makes the use of separate family rearing (the alternative method to recover family information) particularly challenging. The use of microsatellite theoretically allows the use of any kind of progeny, including progeny from mass spawnings. Still, like in other marine species (Perez-Enriquez *et al.*, 1999; Herlin *et al.*, 2007), only very few parents may be represented in a mass spawning of sea bass (Chatziplis *et al.*, 2007). Thus, for estimating accurate genetic parameters, another important point was the control of reproduction through artificial fertilization, allowing the use of particularly informative factorial designs (Vandeputte *et al.*, 2001). The combination of artificial fertilization and parentage assignment has allowed the estimation of genetic parameters for growth in the sea bass (Saillant *et al.*, 2006; Dupont-Nivet *et al.*, 2008). The estimates of heritability for commercial weight are medium to high (0.31-0.60), giving good prospects for genetic improvement of growth. Nevertheless, these estimates were obtained in mixed tanks with possibly competing families. The advantage of mixed tanks is to prevent from biases in $h^2$ estimates due to common environment effects, but all fish compete for

growth in the same environment and this might lead to the selection of the most aggressive fish, which do not necessarily have the best genetic potential for growth (Ruzzante and Doyle, 1991). Competition effects might also increase the difference between genetic groups (here families) with different growth potentials, as seen in common carp *Cyprinus carpio* (Moav and Wohlfarth, 1974) and rainbow trout *Oncorhynchus mykiss* (Blanc and Poisson, 2003), which would likely lead to different heritability estimates in separate family tanks and mixed families system.

In addition to the effect of selective breeding, domestication selection may occur when closing the cycle of sea bass, considering the fact that the starting point is wild fish. Domestication selection may be defined as the process by which a captive population becomes adapted to the rearing environment through genetic modification along generations. Domestication selection has been evidenced in many fish species (e.g. Fleming and Einum, 1997; Hershberger *et al.*, 1990), and seems to be very strong in the first generation in cod *Gadus morhua* (Doyle *et al.*, 1995). Domestication is very recent in marine fish species, but concerns a largely growing number of species (Duarte *et al.*, 2007).

In the present experiment, the growth of offspring from wild, domesticated (first generation) and selected (first generation of selection for growth) sea bass were compared both in mixed tanks and in separate tanks. The contrast between the selected lines and the domesticated line will be the response to selection for growth, whereas the difference between the domesticated and the wild line is expected to estimate domestication selection (*i.e.* "natural selection" in captivity during the first captive breeding cycle). The comparison of responses in mixed and separate tanks will give a first estimation the effect of competition on growth in sea bass.

### 3.1.2 Materials and methods

#### *3.1.2.1 Selection of sires*

The population in which the sires were selected originated from a partial factorial cross of 33 G0 sires and 23 G0 dams of wild European sea-bass, comprising 253 full-sib families (see Vandeputte *et al.*, 2007 for details). The mixed G1 families were reared as a single batch in a recirculated system, and at 370 days post-fertilization (dpf), 2228 fish were randomly selected, individually tagged with Passive Integrated Transponder glass tags, and a piece of fin was collected in ethanol for further parentage assignment. The fish were reared as a single batch in a 5 $m^3$ tank until 504 dpf (101g mean weight) then randomly separated in two 5 $m^3$ tanks. At 594 dpf (202g mean weight), they were again separated at random in five 5 $m^3$ tanks, where they were reared until 714 dpf (398g mean weight). At this age, each fish was individually measured for length and weight, and the 103 longest fish out of the 1953 remaining ones were sexed with a polypropylene endometrial suction curette (Pipelle de Cormier, Unimar, Neuilly-en-Thelle, France). When used for sexing fish, it is introduced in the genital duct until reaching the gonad where a small piece of genital material is biopsied. 60% of the fish in the population suffered from spine deformities, but only a very low proportion of fish were deformed in the longest ones. Out of these 103 longest fish, 31 undeformed putative males were identified ("massal" group, mass selected for growth), and separated to allow them to reach sexual maturation (under natural photoperiod and temperature). Additionally, 69 undeformed individuals were collected at random in the population and maintained in maturation conditions to constitute the control (domesticated) group. Among the remaining fish, 1473 were slaughtered and sexed, allowing to see that the female rate was 17.2% in the population, and to estimate the distribution of

length in the undeformed males in the population. Among the fish allowed to mature, 23 massal and 25 domesticated males gave sperm which was cryopreserved (Fauvel *et al.*, 1998). Seventeen massal males were selected among the 23 cryopreserved ones for their mean deviation to the population mean on length to be 2.07 phenotypic SD (equivalent to a 5% pressure in a normally distributed population) when related to the distribution of undeformed males in the population. Twenty domesticated males were chosen among the 25 cryopreserved ones for their mean phenotypic deviation from the mean for length to be –0.04 SD.

We also randomly chose 20 cryopreserved males from the 33 initial G0 population wild males to be used as wild control.

Finally, we had the opportunity to test males from the first generation of an industry breeding programme, ran by Panittica Pugliese (Torre Canne di Fasano, Italy). This programme started from the same larvae as our mass selected and domesticated populations, with 2 supplementary G0 females from the same origin (25 instead of 23). We do not have all the details on how the selection was performed, but this programme is an adaptation to the sea bass of the PROSPER selection scheme developed by INRA on brown trout *Salmo trutta fario* (Chevassus *et al.*, 2004). The eggs of the different females were initially grouped in homogeneous groups according to egg size. These groups were reared separately, and densities adjusted to make them reach the same mean size around 10 gram. Then, three repeated growth challenges were applied (one on weight, two on length) with the objective of a 5% selection pressure on males (identical to that of our massal group). The percentage of males in the population (75%) was assessed by slaughtering a sample of fish at the time of the first challenge, allowing the determination of the number of males to be kept in the end to reach the expected selection pressure. Nineteen cryopreserved sperms from PROSPER-like selected males were made available to us by Panittica Pugliese.

To sum up, all the sire lines compared in the present experiment originate from the same G0 wild base population: the Wild sires were a random sample from the G0 population males, the Domesticated sires a random sample of the males from the G1 population (derived from a 33 sires by 23 dams cross from G0 broodfish), the Massal sires were from the 5% longest sires at commercial (400g) size selected from the G1 population, and the Prosper sires were issued from the G1' population (same crossing where two more G0 females were added) and selected with three challenges on growth totalizing a 5% selection pressure.

#### *3.1.2.2 Constitution of the experimental progeny*

The matings and the rearing of the progenies were done in the Ifremer experimental facility of Palavas (France). In March 2005, 19 wild females were injected with 10 µg/kg LHRH (SIGMA, D-TRP6-LHRH), and eggs were stripped 72 hours later. Thirteen females gave a sufficient quantity of good quality oocytes. From these spawns, we produced a full factorial mating design using cryopreserved sperm from the 76 males previously chosen, *i.e.* 20 wild (W) males, 20 domesticated (D) males, 17 mass selected (M) males and 19 PROSPER-like selected (P) males. DNA samples were available for all parents. An equal volume (150 ml) of each of the 13 spawns used was mixed in a single egg pool, which was then used to produce 76 aliquots of eggs (25 ml each) that were each individually fertilized by the cryopreserved sperm from one male and activated by hatchery sea water. The eggs were grouped by type of male for incubation (48 hours at 13°C), and at that time, floating eggs were dispatched in 12 larval tanks of 500l each, i.e. 3 tanks per group (W, D, M, P) were each seeded with

46 ml (*ca.* 27.000) eggs. Additionally, 3 "mixed" tanks were seeded each with equal volumes of eggs from each group (11.5 ml W + 11.5 ml D + 11.5 ml M + 11.5 ml P = 46 ml/tank).

#### *3.1.2.3 Rearing conditions and phenotyping*

A standard rearing protocol was applied (Chatain, 1994) until day 90 post-fertilization. At that stage, the fish were counted in each tank, and 2,500 fish per tank were randomly chosen and transferred to 5 $m^3$ tanks, keeping the same 12 tanks structure (3W, 3D, 3M, 3P, 3 mixed). The measurements started at 268 days, when the fish had reached a mean weight of 65 g. In each separate tank, 120 fish were randomly chosen, individually tagged, their weight and length were recorded, and they were reintroduced in their tank, together with 680 randomly chosen untagged fish from the same group, so that the number was adjusted to 800 fish/tank. In each mixed tank, 400 fish were randomly chosen, on which individual weight and length were recorded. Each of them was individually tagged and fin-clipped for DNA extraction. The 400 fish were reintroduced in their tank with 400 randomly chosen untagged fish from the same tank to adjust the number to 800 fish per tank.

The tagged fish were individually measured for length and weight at days 338, 457 and 611. During all the rearing phases, the fish were fed *ad libitum* with a standard sea bass pellet (Le Gouessant, France). At day 457, the numbers were adjusted to 500 fish per tank removing randomly chosen untagged fish. At day 611, they were slaughtered, and sex, mouth deformities and spine deformities were recorded. Unexpected occurrence of nephrocalcinosis (whitish stones in the kidney) was noticed and recorded on all fish by visual inspection of the dissected kidney.

#### *3.1.2.4 Parentage assignment*

The 1,200 sea bass in the mixed tanks were assigned to their parents using microsatellite markers analysis. Eight markers were used, *Dla016*, *Dla020*, *Dla105*, *Dla116*, *Dla119*, *Lab13*, *Lab3* and *Dla022* (Chistiakov *et al.*, 2004; Ciftci *et al.*, 2002; Garcia De Leon *et al.*, 1995).

Genomic DNA was extracted using AB6100 (Applied Biosystems) with Nuc-Prep (Applied Biosystems) chemistry. Amplification was performed in a 20 µl polymerase chain reaction (PCR) mixture containing 25 ng of genomic DNA, 2.0 µl PCR buffer, 1.2 µl $MgCl_2$, 0.4 units Amplitaq Gold (Applied Biosystems), 1.25 mM dNTPs mix (Applied Biosystems) and 10pmol for each primer. The reverse primers were 5' end-labbelled with FAM, NED and VIC fluorochrome. The samples were amplified on a Thermal Cycler (Applied Biosystems 9600 Geneamp PCR System) according to the following protocol: 10min initial denaturation at 95°C (hot start) followed by 30 cycles of 1 min at 94°C, 30 s at 55°C,1 min at 72°C and extension at 72°C for 60 min. The polymorphism was screened in a capillary sequencer (Applied Biosystems 3100).

The parentage assignment was established with a new software (Galli, in prep.) using the exclusion method based on Mendelian rules of inheritance (Jones and Ardren, 2003) or calculating the likelihood of each potential parental pair (Duchesne *et al.*, 2002). Data were further tested with PROBMAX program (Danzmann, 1997).

#### 3.1.2.5 Statistical analyses

The individual data analyzed were weight (*W*) and length (*L*) as well as daily growth coefficient:

$$DGC_{1-2} = \frac{W_2^{1/3} - W_1^{1/3}}{date_2 - date_1} \times 100$$

The analysis of data in the separate tanks was done using SAS-Mixed using the following model:

$$Y_{ijklmno} = \mu + s_j + d_k + m_l + n_m + l_n + T_{i(n)} + \varepsilon_{ijklmno} \quad \text{[Model1]}$$

Where $Y_{ijklmno}$ is the performance of individual *o*, $\mu$ is the general mean, $s_j$ is the fixed effect of sex *j* (1=male, 2=female), $d_k$ is the fixed effect of spine deformity (0= normal, 1=deformed), $m_l$ is the fixed effect of mouth deformity (0=normal, 1=deformed), $n_m$ is the fixed effect of nephrocalcinosis (0=normal, 1=affected), $l_n$ is the fixed effect of selection line (W, D, M, P), $T_{i(n)}$ is the random effect of tank *i* nested within selection line *n*, and $\varepsilon_{ijklmno}$ is the random residual. First, a complete version of model 1 was used to determine the significant fixed effects. Following this, a reduced version, where non significant fixed effects were removed, was ran to assess the significance of the "selection line" effect and to estimate the least square means of the four selection lines, which were compared with a Tukey-Kramer test when the "selection line" effect was significant. The effect of selection line and the differences between least square means were tested using tank mean square as the error term (in fact an adjusted mean square provided by SAS using Satterthwaite's approximation for degrees of freedom), thus testing the fact that selection lines differ relative to the random tank effects (*i.e.* significantly different selection line means that the differences seen between the offspring of the tested sire groups are not due to tank effects). Here, no information was known about the parents of any particular offspring.

Another model was also used to describe the separate tanks data:

$$Y_{ijklmno} = \mu + s_j + d_k + m_l + n_m + b_n + T_{i(n)} + \varepsilon_{ijklmno} \quad \text{[Model2]}$$

Where $Y_{ijklmno}$ is the performance of individual *o*, $\mu$ is the general mean, $s_j$ is the fixed effect of sex *j* (1=male, 2=female), $d_k$ is the fixed effect of spine deformity (0= normal, 1=deformed), $m_l$ is the fixed effect of mouth deformity (0=normal, 1=deformed), $n_m$ is the fixed effect of nephrocalcinosis (0=normal, 1=affected), $b_{\underline{n}}$ is the fixed effect of selection level (*b*=1 for M and P, *b*=0 for W and D), $T_{i(n)}$ is the random effect of tank *i* nested within selection level *n*, and $\varepsilon_{ijklmno}$ is the random residual. Again, a reduced version with only the significant fixed effects was used to test the significance of the selection level effect. The difference between model 1 and model 2 is that model 2 considers only two levels of the selection effect, and compares selected fish with unselected fish, with 6 tank replicates for each level, giving more statistical power. This may seem a little artificial as it is clear that neither the M and P lines nor the D and W lines do originate from the same parents. However we did it considering that 1) they originate from the same base population 2) the M and P parents undergone the same selection pressure on growth, while the D and W parents were not submitted to any directional selection, so that a 'selection level' effect makes sense 3) it can be seen that in most cases the mean values of M and P or D and W are very similar.

The analysis of data in the mixed tanks was done using SAS-Mixed first using the following model:

$$Y_{ijklmnopq} = \mu + T_i + s_j + d_k + m_l + n_m + l_n + S_{o(n)} + D_p + \varepsilon_{ijklmnopq} \quad \text{[Model3]}$$

Where $Y_{ijklmnopq}$ is the performance of individual $q$, $\mu$ is the general mean, $T_i$ is the random effect of tank $i$ ($i$=1, 2, 3), $s_j$ is the fixed effect of sex $j$ (1=male, 2=female), $d_k$ is the fixed effect of spine deformity (0= normal, 1=deformed), $m_l$ is the fixed effect of mouth deformity (0=normal, 1=deformed), $n_m$ is the fixed effect of nephrocalcinosis (0=normal, 1=affected), $l_n$ is the fixed effect of selection line (W, D, M, P), $S_{o(n)}$ is the random effect of sire $o$ nested within selection line $n$, $D_p$ is the random effect of dam $p$, and $\varepsilon_{ijklmnopq}$ is the random residual. Prior to using model 3, we had tested a more complete model including random interaction terms between tank and selection line, tank and dam and tank and sire. As these interaction terms were never significant (Wald test for random effects, $P$>0.05), they were excluded from the model to obtain model 3. As before, the significance of the fixed effects was assessed with the full version of model 3, and then a reduced version omitting non-significant fixed effects was ran to test for the effect of selection line and to estimate the least square means of the four selection lines, which were compared with a Tukey-Kramer test when the "selection line" effect was significant .The effects of selection line and the differences between least-square means were tested using sire mean square as the error term (in fact an adjusted mean square provided by SAS using Satterthwaite's approximation for degrees of freedom, accounting for different numbers of offspring per sire). Thus, we tested the fact that selection lines differ relative to the sampling of sires (*i.e.* significantly different selection line means that the true genetic mean of the populations from which the sires were sampled differ).

In all models, residuals were checked for normality and homoscedasticity. Residuals for weights strongly departed from normality in models 1 and 2, which could be fixed by logartithmic transformation. However, the significance levels of all effects were the same in with or without logarithmic transformation. Therefore, we chose to present the results for weight with model 1 and 2 using data in original scale.

### 3.1.3 Results

#### *3.1.3.1 Parentage assignment*

Out of the 1,200 initial samples, 1,151 were assigned to a single parental pair (95.9%) with 1 mismatch tolerated, 32 were assigned two pairs (2.7%), 15 were not assigned to any pair (1.3%), and 2 had multi-allelic loci and were excluded. Then, 1,151 individuals could be used for further analysis in mixed tanks. Among those, 271 were from the W group (23.5%), 279 from the D group (24.2%), 357 from the M group (31.0%) and 244 from the P group (21.2%). This representation was not even ($\chi^2$=24.5, *d.f.*=3, $P$<0.001) but still provided enough individuals per group for correct evaluation of the response to selection.

### 3.1.3.2 Selection response in separate tanks

The separate tanks results are reported in Table 3-1 and Figure 3-1. The effect of selection line was significant for length at 268 days, where the D group was smaller than P, the other groups being in between. At 338 days, P was larger than both D and W, M being in between. Selection lines differed also for weight at day 338 with the same pattern as for length. For all other traits and periods, the effect was never significant. However, this is probably due to a limited number of tank replicates. When W and D were merged in an "Unselected" group, while M and P were merged in a "Selected" group (Model 2), the pattern was quite different: selected fish were larger than unselected ones at all times, both for length and weight ($P$<0.01 in most cases – see Figure 3-1). However, even with this new model, DGCs were never different ($P$>0.2) between selected and unselected fish. Tank effects were significant or close to significance on all traits at all periods. Sex effects were highly significant on weight and length at all ages (with females larger than males) but were never significant on DGC, showing a comparable growth rate of both females and males from day 268 to day 611. Nephrocalcinosis (recorded at day 611 – 6.2% incidence) had no impact on length at 268 days and weight at 268 and 338 days, but always had significant effects later on affected fish being smaller. Its effect on DGC was large at all periods, *e.g.* from 457 to 611 days, the DGC least-square mean of non-affected fish was 0.82 while that of affected fish was only 0.53. Mouth deformities (9.9% incidence) had an impact on weight at 611 days, and on DGC457-611. Their effect on growth was negative. The effect of spine deformities (3.4% incidence) was seen on weight, but not length, at all ages, as well as on DGC268-338. Surprisingly, the deformed fish were heavier than the undeformed ones (*e.g.* mean ± SE is 73.4±3.1 g for affected ones *vs.* 66.4±0.7 g for normal fish at day 268, and 372±16 g *vs.* 338±8 g at day 611), as well as faster growing between 268 and 368 days.

**Table 3-1:** Significance levels of model effects on growth in separate tanks for four selection lines (W, D, M, P) of sea bass. dpf= days post-fertilization. DGC= Daily growth coefficient. Nephro = nephrocalcinosis. Mouth, Spine = spine deformities. Significance levels for effects other than selection line and selection level are from Model 1. *F*-values, degrees of freedom and significance levels are from model 1 with non-significant fixed effects removed for selection lines, and from model 2 with non-significant fixed effects removed for selection level. *P*-values noted 0.05 mean 0.05 < *P* < 0.06 (not significant). Non-integer *dfs* appear due to the use of Satterthwaites's approximation.

| | Significance of model effects | | | | | | Selection line effect | | | Selection level effect | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Trait** | Age (dpf) | Tank | Sex | Nephro | Mouth | Spine | *F* value | *df* | Sig. level | *F* value | *df* | Sig. level |
| **Length** | 268 | 0.07 | <0.001 | 0.49 | 0.79 | 0.07 | 5.63 | 3, 8.0 | <0.05 | 19.3 | 1, 10.1 | <0.01 |
| | 338 | 0.07 | <0.001 | <0.05 | 0.67 | 0.05 | 6.90 | 3, 8.2 | <0.05 | 14.4 | 1, 10.1 | <0.01 |
| | 457 | <0.05 | <0.001 | <0.001 | 0.54 | 0.06 | 2.86 | 3, 8.1 | 0.11 | 7.03 | 1, 10.1 | <0.05 |
| | 611 | 0.06 | <0.001 | <0.001 | 0.10 | 0.07 | 3.46 | 3, 8.1 | 0.07 | 11.0 | 1, 10.1 | <0.01 |
| **Weight** | 268 | 0.05 | <0.001 | 0.71 | 0.45 | <0.05 | 3.81 | 3, 8.0 | 0.06 | 13.5 | 1, 10.1 | <0.01 |
| | 338 | 0.08 | <0.001 | 0.23 | 0.39 | <0.05 | 7.36 | 3, 8.2 | <0.05 | 19.5 | 1, 10.2 | <0.01 |
| | 457 | 0.06 | <0.001 | <0.001 | 0.22 | <0.01 | 3.32 | 3, 8.1 | 0.08 | 10.9 | 1, 10.1 | <0.01 |
| | 611 | 0.06 | <0.001 | <0.001 | <0.05 | <0.001 | 3.25 | 3, 8.1 | 0.08 | 11.6 | 1, 10.2 | <0.01 |
| **DGC** | 268-338 | <0.05 | 0.98 | <0.001 | 0.70 | <0.05 | 1.80 | 3, 8.0 | 0.22 | 1.36 | 1, 10.0 | 0.27 |
| | 338-457 | <0.05 | 0.21 | <0.001 | 0.35 | 0.10 | 0.36 | 3, 8.0 | 0.78 | 0.86 | 1, 10.0 | 0.37 |
| | 457-611 | <0.05 | 0.76 | <0.001 | <0.001 | 0.49 | 0.57 | 3, 8.1 | 0.65 | 1.28 | 1, 10.1 | 0.28 |

### *3.1.3.3 Selection response in mixed tanks*

The ANOVA results for mixed tanks are reported in Table 3-2 and Figure 3-1. The effect of selection line was always highly significant on length and weight where M and P outperformed D and W at all ages. Selection line also had an effect on DGC268-338 where P outperformed both unselected groups while M outperformed the D group but not the W group (*P*=0.08, still being close to significance). At later ages, no effect of selection line was seen on DGC, so most of the response was established before 338 dpf and even before 268 dpf. Here, tank effects were never significant.

**Table 3-2:** Significance of model effects on growth in mixed tanks for four selection lines (W, D, M, P) of sea bass. dpf= days post-fertilization. DGC= Daily growth coefficient. Nephro = nephrocalcinosis. Mouth, Spine = spine deformities. Significance levels for effects other than selection line and selection level are from Model 3. The sire effect is nested within selection line. *F*-values, degrees of freedom and significance levels for selection lines are from model 3 with non-significant fixed effects removed. *P*-values noted 0.05 mean 0.05 < *P* < 0.06 (not significant). Non-integer dfs appear due to the use of Satterthwaites's approximation. NE: *P*-value not estimated as random covariance component estimated to be zero.

| | | | Significance of model effects | | | | | | Selection line effect | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Trait** | Age (dpf) | Tank | Sex | Nephro | Mouth | Spine | Sire | Dam | *F* value | *df* | Sig. level |
| **Length** | 268 | NE | <0.001 | 0.20 | 0.39 | 0.81 | <0.001 | <0.05 | 12.95 | 3, 67.7 | <0.001 |
| | 338 | 0.18 | <0.001 | 0.26 | 0.42 | 0.71 | <0.001 | <0.05 | 13.00 | 3, 69.4 | <0.001 |
| | 457 | 0.25 | <0.001 | 0.06 | 0.52 | 0.65 | <0.001 | <0.05 | 12.14 | 3, 66.1 | <0.001 |
| | 611 | 0.18 | <0.001 | <0.001 | 0.55 | 0.33 | <0.001 | <0.05 | 11.95 | 3, 65.5 | <0.001 |
| **Weight** | 268 | 0.22 | <0.001 | 0.33 | 0.22 | 0.54 | <0.001 | 0.05 | 9.32 | 3, 69.0 | <0.001 |
| | 338 | 0.18 | <0.001 | 0.27 | 0.33 | 0.53 | <0.001 | 0.07 | 11.02 | 3, 70.2 | <0.001 |
| | 457 | 0.35 | <0.001 | 0.08 | 0.49 | 0.50 | <0.001 | 0.06 | 10.43 | 3, 67.8 | <0.001 |
| | 611 | 0.17 | <0.001 | <0.001 | 0.49 | 0.24 | <0.001 | 0.05 | 11.01 | 3, 68.5 | <0.001 |
| **DGC** | 268-338 | 0.16 | 0.77 | 0.28 | 0.17 | 0.92 | <0.001 | 0.13 | 8.75 | 3, 65.2 | <0.001 |
| | 338-457 | 0.17 | <0.05 | <0.001 | 0.88 | 0.09 | <0.01 | <0.05 | 2.12 | 3, 60.1 | 0.11 |
| | 457-611 | 0.17 | 0.16 | <0.001 | <0.05 | 0.17 | <0.01 | 0.06 | 2.46 | 3, 61.4 | 0.07 |

The effect of sex was always significant, except on DGC 268-338 and DGC 457-611. Still, female sex had a positive effect both on length and weight but had a negative effect on DGC338-457 (0.903±0.013 for females, 0.930±0.013 for males). Nephrocalcinosis (3.9% incidence) again, had a negative effect on DGC but this effect started only on DGC338-457. Its effect on weight and length was noticed only on day 611. In mixed tanks, no effect of mouth deformity (12% incidence) was seen except on DGC457-611 (negative effect), and no effect of spine deformity (5.6% incidence) was seen at any time.

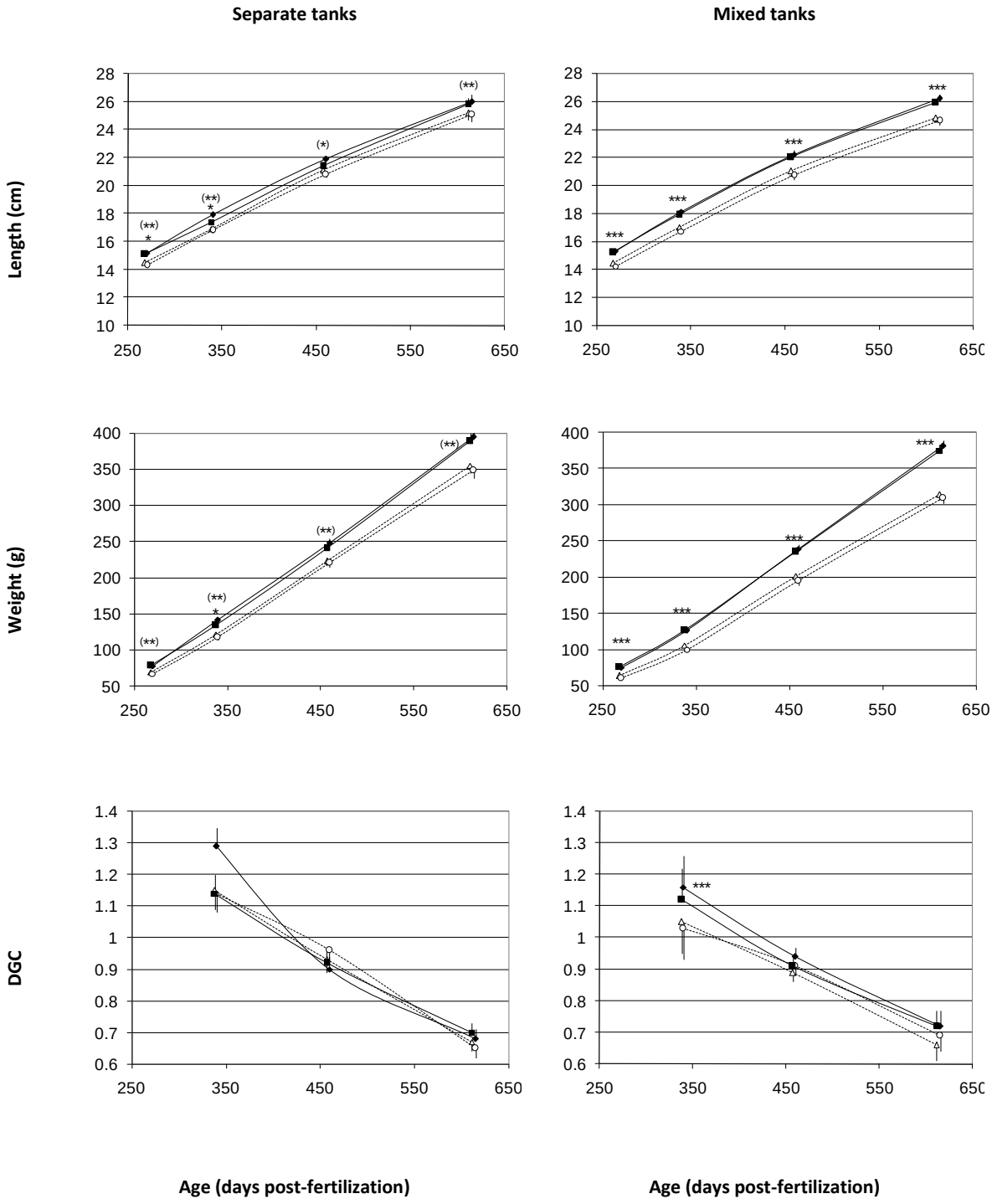


**Figure 3-1:** Evolution of Least-square means (± Standard Error) for body length, body weight and DGC (Daily Growth Coefficient) from 268 to 611 days post-fertilization in four selection lines of sea bass (△=Wild, ○=Domesticated, ■=Mass selected for length, ◆=PROSPER-like selected for length – see text for more details), reared in triplicates either separately (left column) or in mixed tanks (right column). Asterisks denote significance levels for the effect of selection lines (Model 1 for separate tanks, Model 3 for mixed tanks). Asterisks between brackets in the "separate tanks" graphs denote significance levels of the Selected (M, P) *vs*. Unselected (W, D) effect (Model 2). *** $P<0.001$; ** $P<0.01$; * $P<0.05$.

### 3.1.4 Discussion

#### *3.1.4.1 Parentage assignment in mixed tanks*

The proportion of genotyped fish assigned to a single pair was 95.9% which is in the usual range of other parentage assignment studies in fish (in the 90-99% range, *e.g.* Fishback *et al.*, 2002; Norris and Cunningham, 2004; Vandeputte *et al.*, 2004; Wesmajervi *et al.*, 2006). This is still a good result considering that we used a large mating scheme (76 x 13 full factorial) with related sires which may generate lower assignment rates (as seen in carp, Vandeputte *et al.*, 2008). However, we had to increase the number of loci from an initially planned number of six to eight as the first run of assignments with 6 loci gave a proportion of uniquely assigned fish (88%) which was below our expectations. The proportion of unassigned fish was low (1.3%) as well as the number of fish for which one mismatch was needed to achieve unique assignment is also low (two fish in total). This is indicative of a low genotyping error rate (Vandeputte *et al.*, 2006). Moreover, the proportion of fish with multiple parental pairs assigned is low (2.7%), indicating a good assignment power. Therefore, the assignment results obtained using mismatches can be used with good confidence for the genetic analysis.

#### *3.1.4.2 Response to selection*

The response to selection was high, both in separate and mixed tanks. However, differences between lines in separate tanks were significant only at 268 and 338 days for length, and at 338 days for weight. This partly results from the model used which uses the between-tank random variation as the residual, with only 8 *dfs* on average for the residual. Still, when both selected lines are pooled as a "selected" group and both unselected lines as a "control" group, significant differences in weight and length are seen between both groups, showing the reality of selection response in separate tanks. We also have to point out that all of the response seen on length and weight in separate tanks was established at the first measurement, as no differences in DGC appear at any time. In mixed tanks, some increase in response is seen between days 268 and 338, but not after. Selection was done on length at 400 g mean weight in the M males with a selection differential of 2.11 phenotypic SD (SD) between the D and the M males. In the present response estimation experiment, the difference between the M and the D offspring at day 611 (390g) was 1.4 cm in mixed tanks (0.63 phenotypic SD) and 0.8 cm in separate tanks (0.36 phenotypic SD). As we are using a paternal testing system, the difference between the offspring groups is expected to be half the true genetic difference between the parental groups. Therefore, the full response estimate between M and D would have been 1.26 phenotypic SD in mixed tanks and 0.72 SD in separate tanks, yielding realized heritability estimates of 0.60 and 0.34 on length, respectively. This is in the range of what was expected from heritability estimates in Palavas based on covariance between relatives (0.41, Dupont-Nivet *et al.*, 2008). The corresponding full correlated response on weight at day 611 is 131.2 g in mixed tanks and 79.4 g in separate tanks, respectively representing 42% and 23% of the mean of the unselected (D) strain least square mean for weight. This value is clearly in the high range of observed response to selection in fish (10-30% per generation, see review by Gjedrem and Thodesen, 2005), and is promising for the future of sea bass selective breeding. It is worth noting that both selected groups, Massal and Prosper-like, give mostly the same results. Both represent mass selection processes keeping the 5% largest males, but in different farms, and with different methods (one single selection event at 400g for the Massal, three progressive eliminations for the Prosper-like). This shows that selection for growth should be effective in several conditions, as predicted by the low G x E interactions ($r_A$=0.99) that were formerly estimated for weight at commercial size between

Panittica and Palavas (Dupont-Nivet *et al.*, 2008). Prosper was initially designed to be more efficient than mass selection, based on the control of maternal effects linked to egg size and the control of competition through recurrent size challenges. Although Prosper showed its efficiency in brown trout (+21.5% weight/generation, Chevassus *et al.*, 2004), it has never been experimentally proven to be more efficient than mass selection. This is the first attempt to do so, and it is not conclusive. Among the possible reasons, the moderate level of maternal effects influencing growth in sea bass (estimated $m^2$=0.10 on length, although not significant) might make the maternal effects control procedure of Prosper useless. We have no precise clue on how the recurrent growth challenges were performed in the Panittica breeding programme. Still, our data show that in this case Prosper is as efficient as mass selection in sea bass.

Over time, selection response increased from day 268 to day 338, then decreased at days 457 and 611 (Figure 3-2). The response in mixed tanks at 268, 338, 457 and 611 days was higher than that in separate tanks by 45%, 43%, 71% and 67%, respectively. This raises three questions: 1) why is the response higher in mixed tanks , 2) why does it decrease and 3) why does it decrease more in separate tanks ?

The fact that the response is higher in mixed tanks is likely to be due to a competition effect, as was seen in communal testing of common carp strains (Moav and Wohlfarth, 1974) or rainbow trout families (Blanc and Poisson, 2003), and in the comparison of up and down-selected sea bream *Sparus aurata* (Knibb *et al.*, 1997). However, this competition does not seem to be active between days 268 and 338, as the difference in response between separate tanks and mixed tanks remains the same. Similarly, this difference remains stable between days 457 and 611, but on the contrary largely increases from day 338 to day 457. Therefore, although it seems plausible that competition explains part of the difference in response between mixed and separate tanks, it seems unlikely that it would explain its evolution from day 268 to 611, which is not regular at all.

It is interesting to see that the mean rearing density seems to be inversely correlated with selection response (Figure 3-2). In rainbow trout, density has been shown to interfere with genetic variance (Bagley *et al.*, 1994), and in sea bass, it has already been shown that heritability of growth is higher at low densities (0.60) than at high densities (0.31 – Saillant *et al.*, 2006). Conversely, it also has been shown in rainbow trout that density might increase phenotypic variation (Leary *et al.*, 1991). All this indicates that density may seriously interfere with the expression of genetic variation between genotypes. Therefore, it is plausible that the selection response observed in the later stages was limited by density, possibly through an effect on water quality. Moreover, as the number of fish in each tank was the same, density was highest in the separate tanks containing selected fish (which were

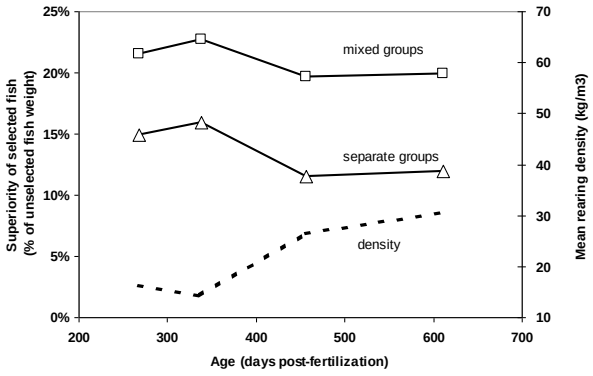


**Figure 3-2:** Mean weight superiority of selected over unselected sea bass ([mean weight of selected groups/mean weight of unselected groups]-1) in separate and mixed tanks from 287 to 611 days post-fertilization, plotted together with the mean rearing density of each period. Selected groups are Massal and Prosper-like, unselected groups are Wild and Domesticated

heavier). This could have limited their growth more than that of the unselected fish, and explain the decrease in response in separate tanks when density was high (days 457 and 611). In mixed tanks, as all fish are subjected to the same environment (density) conditions, the impact would have been the same for all genotypes. This could explain why selection response decreases more in separate tanks relative to mixed tanks.

A third level of explanation would imply the effect of nephrocalcinosis: in mixed tanks, the absence of response on DGC is concomitant with the appearance of the nephrocalcinosis effect. This incidence is not different among selection lines within mixed or separate tanks (Chi-square test, $P>0.05$) but is different between separate and mixed tanks (3.9% *vs.* 6.2%, $P<0.05$). This pathology may be induced by excessive $CO_2$ concentrations (Fivelstad *et al.*, 2003). This is likely to be caused by higher density, especially in a recirculated system with oxygenation where $CO_2$ stripping is not always efficient enough (Summerfelt *et al.*, 2000). Morevover, only fish which had kidneys showing evident signs of nephrocalcinosis were recorded as affected, but it is quite likely that some less affected fish were recorded as normal. It is therefore possible that a proportion of "normal" fish may have decreased late growth rates caused by nephrocalcinosis, thus decreasing the overall estimated growth rate of the population.

Over all, the simplest answers to our three questions would be: 1) selection response is higher in mixed tanks because it is amplified by competition , 2) it decreases over time because of density and nephrocalcinosis effects and 3) it decreases more in separate tanks because density affects more selected fish in separate tanks.

#### *3.1.4.3 Effect of domestication*

All performances were similar between offspring from wild males and from domesticated males. Therefore, no effect of domestication selection could be shown. This is in contrast with what was seen in coho salmon *Oncorhynchus kisutch*, where domestication selection was found to account for a significant part of the improvement in growth generated by the selective breeding process (Hershberger *et al.*, 1990). Of course, this could be due to the fact that our domesticated males are only in their first generation, which limits the potential for efficient domestication selection, as well as the expected effect size of domestication selection. Moreover, as the comparison was only done through the use of different males on the same females, the observed difference is only half the expected additive genetic difference between the W and D populations, and might be too small to be detected. Still, it was shown in cod that large differential mortalities occur in the first generation of captive breeding (Doyle *et al.*, 1995), leaving room for significant domestication selection, and it was also recently shown that just one generation of captive breeding could significantly impair the fitness of rainbow trout in the wild (Araki *et al.*, 2007). In sea bass, domestication will have to be studied on later generations of captive breeding, using traits other than growth (survival, reproduction, behaviour, stress response,…) which may be more subject to natural selection in the rearing process.

#### *3.1.4.4 Possible application of the results at commercial scale*

In this experiment, realized heritability is higher in mixed tanks. However, farmers will never grow one selected and one non-selected line in the same rearing unit, and therefore the realized heritability to keep for economical simulations of potential genetic progress is the one observed when the two selection lines were reared separately (0.34).

In addition, these results underline the high potential gains expected from the application of optimized mass selection as performed here (artificial fertilization for creating factorial mating designs, management of potential non genetic maternal effect induced by different success at hatching between dams). Such selective breeding protocols (with or without repeated successive grading) should create a significant improvement of growth, as observed experimentally in the first generation. This potential progress needs to be balanced by the fact that in the present experiment the realized heritability was only tested between selected and control sires. As sex ratio is generally skewed towards high proportions (85-95%) of males (Piferrer *et al.*, 2005), mass selection will not be equally efficient between sexes and a lower mean genetic progress should be observed as the selection pressure, and hence the response on females will be lower due to their lower number in the population of candidates.

### 3.1.5 Summary

**Selective breeding of European sea bass (*Dicentrarchus labrax*) receives a growing interest, as the estimated heritability of growth is medium to high. In this study, we compared the offspring of four groups of sea bass sires, mated with the same wild dams: wild (W), first generation of domestication (D), first generation of mass selection for length (M), first generation of PROSPER-like selection for length (P). The comparison was done both in replicated tanks (separate rearing) and in mixed tanks (mixed rearing) where sire origins were recovered by genotyping of eight microsatellite markers. Weight, length and growth rate were measured from day 238 post-fertilization (69g mean weight) to day 611 post fertilization (390g mean weight). Both in mixed and separate tanks, both selected groups (P, M) were larger than unselected groups (W, D). No difference was seen at any time between W and D, nor between M and P. The selection response estimate on weight was larger in mixed tanks when compared to separate tanks (+42% in mixed tanks, +23% in separate tanks at day 611), yielding realized heritability estimates of 0.60 and 0.34, respectively, and confirming the excellent potential of the species for growth improvement through selective breeding. Both selection response and the amplification effect between mixed and separate tanks decreased as rearing density increased. Our hypothesis is that selection response is magnified by competition in mixed tanks, while sub-optimal rearing conditions lower the observed selection response, more in separate tanks (where selected thus larger fish are at a higher density than unselected ones) than in mixed tanks (where all fish experience the same density effects).**

## 3.2 Selection response for sex ratio

### 3.2.1 A necessary baseline: sex ratios in wild sea bass populations

This section was published in 2012 in Aquatic Living Resources 25 (77-81) with the title:

"Are sex ratios in wild European sea bass (*Dicentrarchus labrax*) populations biased ?", by Marc Vandeputte[1,2,3] *, Edwige Quillet[1], Béatrice Chatain[2]

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#### *3.2.1.1 Introduction*

The European sea bass (*Dicentrarchus labrax*) is a fish species that has been domesticated in the 1980's for aquaculture. Its production rose steadily since then, and it has become one of the major species of Mediterranean aquaculture, together with gilthead sea bream (*Sparus aurata*). It has been repeatedly observed that sex ratios in farmed populations were strongly biased towards males (75 to 95% Piferrer *et al.*, 2005). This is not optimal for fish farming, as males may mature before commercial size, and then experience reduced growth. Moreover, at a similar age, females are larger than males (Saillant *et al.*, 2001b). Finally, as selective breeding of sea bass becomes a reality, with high potential gains in productivity (Vandeputte *et al.*, 2009b), a good balance between males and females in the populations is needed to allow efficient selection of both sexes.

There is a wide variation in sex determination systems in fish, where sex can be determined by environmental factors (mainly temperature), major genetic factors like sex chromosomes and minor genetic factors, or a combination of those (see Baroiller *et al.*, 2009 for a review). In the European sea bass, sex is not yet determined at hatching, and temperature has been shown to play a major role in its determination, although its effect is not yet fully understood. The current hypothesis is that high temperatures early in development (before 100 dpf) lead to decreased female rates (Piferrer *et al.*, 2005), probably through an inhibition of female differentiation (Navarro-Martin *et al.*, 2009b). However long-lasting low temperatures also produce an excess of males interpreted as caused by a low growth rate precluding female orientation (Navarro-Martin *et al.*, 2009b). Thus, the excess of males observed in culture would be due to the use of temperatures higher than in the wild. In addition to temperature effects, between-families variation of sex ratio shows that genotypic effects also exist (Saillant *et al.*, 2002; Vandeputte *et al.*, 2007), and the distribution of family sex ratios was shown to be compatible with a polygenic system, but not with a "classical" GSD system with sex chromosomes (Vandeputte *et al.*, 2007). This type of sex determination system has seldom been evidenced in Vertebrates (McGaugh and Janzen, 2011), and is believed to be evolutionarily unstable (Bulmer and Bull, 1982; Rice, 1986), as it should evolve in most cases towards a chromosomal system where sex is determined at conception. In some cases however, when the environment has differential effect on the fitness of both sexes (e.g., environment influences growth rate and females benefit more of a large size than males, as in *Menidia menidia* - Conover, 1984), the polygenic system may in some rare cases be maintained or alternatively evolve towards a system where sex is determined by environmental factors only (Bulmer and Bull, 1982).

The genetic component of sex ratio evidenced in culture conditions is a strong lever for natural selection to act and stabilize sex ratios at 1:1, as predicted by Fisher's theory of equal investment in both sexes (Fisher, 1930). The rationale is simple: if there is more of one sex than of the other, then each individual of the more abundant sex will produce less offspring (and hence have a lower fitness) than individuals of the less abundant sex, which will then be favoured by natural selection. If the sex of an individual is governed by a system where there is a genetic variance for sex tendency (the propensity of an individual to differentiate as male or female), then frequency-dependent selection should stabilize the population at an even sex ratio.

The environmental and genetic components of sea bass sex determination in farmed populations have been and remain subject to many investigations. However no reliable estimation of wild population sex ratios exists to date, although it is important to know if the excess of males is a characteristic of the species or is linked to farming conditions. The aim of the present study was to use published data from the fisheries literature to examine population sex ratios of the sea bass over its distribution range. When age class data were available, we also examined the possible variation of sex ratio between years, an indicator of environmental effects existing in the wild.

#### *3.2.1.2 Material and methods*

##### 3.2.1.2.1 Data sets

We used data from nine publications, covering the major part of the natural range of the species, with data from the Atlantic Ocean (Ireland, UK, Spain), the West of the Mediterranean Sea (France, Algeria), and the East of the Mediterranean Sea (Egypt, Turkey). These samples encompass the major populations (Atlantic, East and West Mediterranean) identified by population genetics (Naciri *et al.*, 1999; Bahri-Sfar *et al.*, 2000). In those papers, sex ratio was not the parameter studied, but the sex of the fish was recorded, creating a valuable data base for our purpose.

##### 3.2.1.2.2 Statistical methods

Observed numbers of males and females in each population were compared to the expected numbers under an even sex ratio hypothesis with a chi-square test. A significant difference indicated that the observed sex ratio departed from the expected 1:1. Such tests were also performed on population sub-samples comprising only younger fish (<30 cm body length) or older fish (>40cm body length). Body size limits were chosen as a surrogate for age, which was available in three populations only, but remain imperfect as their relation to age may be influenced by sex and water temperature.

#### *3.2.1.3 Results*

##### 3.2.1.3.1 Population sex ratios

Nine publications concerning thirteen population samples were examined (Table 3-3), with altogether 4889 wild sea bass sexed. The gross proportion of females across all population samples was 59.6%, which is higher than an expected 50% ($\chi^2$=180, 1 *df*, *P*<0.001). Some variation of sex ratios was seen between population samples (50.0 to 73.6% of females), and twelve samples out of thirteen had an excess of females, although it was significant in eight samples only. It should be noted that eight of the nine samples from the north Atlantic had a significant excess of females, while this was the case for only two of the five South Atlantic / Mediterranean samples.

When considering only 1314 young fish (<30cm), the proportion of females was 52.0%, not different from 50% ($\chi^2$=2.05, 1 *df*, *P*=0.15). None of the population samples, taken individually, did significantly depart from 1:1 (*P*>0.05, with observed sex ratios varying from 49.1% to 57.0%). So, the primary sex ratio can be considered to be even. On the contrary, the largest fish were mostly females (69.5% in 1811 fish > 40cm, *P*<0.001). In this case, eight of the 10 population samples with available data significantly departed from 1:1 (observed sex ratios 58.2% to 95.6%), while two did not due to small sample size, although observed sex ratios were high (61 and 67% females, see Table 3-3)

#### 3.2.1.3.2 Age class/age group sex ratios

In the study on Irish bass, age-class sex ratios were available, and three "good" brood years with a high contribution to the population were identified (Kennedy and Fitzmaurice, 1972). Among the 126 fish (<4.5 kg, as all fish >4.5 kg were females) sampled from 13 year classes, 82 (65%) were born in those three years, and among those 65.9% were female *vs* 45.5% in the other 10 year classes, a significant difference ($\chi^2$=4.92, 1 *df*, *P*=0.03).

Age group sex ratios were available in the Egypt study (Wassef and El Emary, 1989), but no "good " brood years could be identified there, as the number of fish per age group was an essentially monotonous decreasing function of age. Of the 11 age groups with more than 10 fish (age groups X to XV were merged to obtain sufficient numbers of fish), six were balanced (46 to 61% females, $P(\chi^2)$>0.05), three had an excess of females (88% in age group VII, *P*<0.001, 100% in age groups IX and X to XV, *P*<0.01) and one was lacking females (26% in age group VI, *P*<0.01). In the Turkish population where age-group sex ratios were also available (Ergene, 1999), none of the six age groups with more than 10 fish significantly departed from the expected 50:50 sex ratio (*P*>0.05).

### *3.2.1.4 Discussion*

Our analysis of the sea bass fisheries literature allowed to show that wild sea bass populations taken as a whole exhibited a slight but significant excess of females (59.4% females on average), and thus differed from the cultured populations where a large excess of males (75 to 95%) is the rule (Piferrer *et al.*, 2005). The first question that comes is the representativeness of samplings, as sex ratios can vary between locations and times of the year (Pawson and Pickett, 1996).

Most of the studies were done with repeated sampling all along the year, and sometimes over several years, thus eliminating the "time of the year" bias. Capture methods were diverse and included commercial fisheries (Arias, 1980; Pawson and Pickett, 1996; Wassef and El Emary, 1989), research vessels surveys (Pawson and Pickett, 1996), rod and line (Kelley, 1988a; Kennedy and Fitzmaurice, 1972; Pawson and Pickett, 1996), spear fishing (Barnabé, 1973; Kara, 1997), gill netting (Kara, 1997; Ergene, 1999) and fyke netting (Ergene, 1999). The homogeneity of the observed results would however rule out a large effect of capture method on the sex ratio. One more important point is that most of the captures were done in coastal or lagoon areas, and that offshore catches were little represented. Kelley (1988) suggested that large males might be more numerous in offshore areas - therefore one cannot exclude that at least some of the distortion of sex ratios in favour of females would be due to insufficient sampling in offshore areas.

**Table 3-3**: Numbers and proportions of male and female European sea bass sampled in 9 locations. Ref = reference; Nm = number of males; Nf = number of females. Chi-square ($\chi^2$) test for $H_0$: equal proportions of males and females (significant results are highlighted with italics). NA: not available.

| | | All fish | | | | Fish < 30 cm total length | | | | Fish > 40 cm total length | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Area of capture** | Ref[c] | *Nm* | *Nf* | Female ratio (%) | *P<χ²* | *Nm* | *Nf* | Female ratio (%) | *P<χ²* | *Nm* | *Nf* | Female ratio (%) | *P<χ²* |
| **Ireland** | 1 | 52[a] | 91 | *63.6* | *0.001* | NA | NA | NA | NA | NA | NA | NA | NA |
| **Yealm & Blackwater, UK** | 2 | 30 | 30 | 50.0 | 1.00 | 30 | 30 | 50.0 | 1.00 | NA | NA | NA | NA |
| **Northern UK** | 3 | 169 | 309 | *64.6* | *<0.001* | 43 | 53 | 55.2 | 0.31 | 72 | 175 | *70.9* | *<0.001* |
| **Central UK** | 3 | 241 | 339 | *58.4* | *<0.001* | 81 | 85 | 51.2 | 0.76 | 85 | 185 | *68.5* | *<0.001* |
| **Southern UK** | 3 | 256 | 320 | *55.6* | *0.008* | 51 | 60 | 54.1 | 0.39 | 151 | 210 | *58.2* | *0.002* |
| **Western UK** | 4 | 259[b] | 428[b] | *62.3* | *<0.001* | 81[b] | 85[b] | 56.0 | 0.30 | 116[b] | 258[b] | *69.0* | *<0.001* |
| **Southern UK** | 4 | 96[b] | 256[b] | *72.7* | *<0.001* | 15[b] | 19[b] | 55.9 | 0.49 | 50[b] | 194[b] | *79.5* | *<0.001* |
| **South-eastern UK** | 4 | 34[b] | 95[b] | *73.6* | *<0.001* | NA | NA | NA | NA | 34 | 95 | *73.6* | *<0.001* |
| **Cadiz, Spain** | 5 | 170 | 189 | *52.6* | 0.31 | NA | NA | NA | NA | NA | NA | NA | NA |
| **Sète, Gulf of Lion, France** | 6 | 74 | 136 | *64.8* | *<0.001* | 44 | 43 | 49.4 | 0.92 | 17 | 56 | *76.7* | *<0.001* |
| **Gulf of Annaba, Algeria** | 7 | 227 | 300 | *56.9* | *0.001* | 74 | 98 | 57.0 | 0.07 | 17 | 27 | 61.4 | 0.13 |
| **Goksu Delta, Turkey** | 8 | 136 | 158 | 53.7 | 0.20 | 88 | 87 | 49.7 | 0.94 | 8 | 16 | 66.7 | 0.1025 |
| **Alexandria, Egypt** | 9 | 232 | 262 | 53.0 | 0.18 | 172 | 166 | 49.1 | 0.74 | 2 | 43 | *95.6* | *<0.001* |
| **TOTAL** | | 1976 | 2913 | *59.6* | *<0.001* | 631 | 683 | 52.0 | 0.15 | 552 | 1259 | *69.5* | <0.001 |

[a] *data from 1967 and 1968 captures (Table 7, p 579)*

[b] *Numbers of males and females from Kelley (1988) were estimated from the total numbers recorded and the proportions of males in each size class deduced from figure 10 of the article. In this case also, fork length was used instead of total length to classify fish.*

[c] *References: (1) Kennedy and Fitzmaurice, 1972 (2) Pawson et al., 2000 (3) Pawson and Pickett, 1996 (4) Kelley, 1988a (5) Arias, 1980 (6) Barnabé, 1973 (7) Kara, 1997 (8) Ergene, 1999 (9) Wassef and El Emary, 1989.*

Females tended to grow faster than males in some cases (Kelley, 1988a; Pawson and Pickett, 1996), but also sometimes had the same size at age (Wassef and El Emary, 1989; Ergene, 1999). In general however, wild sea bass females tend to be slightly larger than males of the same age class (reviewed by Pickett and Pawson, 1994). The observed predominance of females in large fish could then partly be due to a faster growth of females, but this effect is expected to be limited as the body length of males and females of the same age differ by less than 5% on average (average of 5 populations reviewed by Pickett and Pawson, 1994). Another possible bias is that using size limits as a surrogate for age implies that the age limits of the size categories will differ among populations, depending on the growth conditions encountered. However when age-group data are available, it is also apparent that there are usually more females in older fish (Kennedy and Fitzmaurice, 1972; Wassef and El Emary, 1989), which has been attributed to a higher longevity of females (Kelley, 1988a).

Finally, the sex ratio of the younger fish (<30 cm) was balanced between males and females, and then conformed to Fisher's theory. More females were present in older fish presumably because 1) males are shorter-lived than females and 2) biased sampling may occur if large males tend to live more offshore, as discussed before. This observation of unbiased sex ratio in the wild shows that the excess of males observed in cultured populations is not linked to an intrinsic characteristic of the species to show male biased sex ratios. Previous experimental evidence showed that temperatures higher than 17°C in the larval rearing phase, which are typically used by the industry, have a male biasing effect (reviews by Piferrer *et al.*, 2005; Navarro-Martin *et al.*, 2009b). It can be then reasonably postulated that the excess of males in farmed populations is actually linked to the environmental conditions during larval rearing.

Additional information was obtained from Irish data, in which 'good' brood years were correlated with warm summers or springs, and contributed a high proportion of the catches (Kennedy and Fitzmaurice, 1972). It appeared that in those good brood years, sex ratio was biased towards females. The 1959 year class, which was specially numerous, had 67.2% females. The same year class was also found to be very abundant in the UK by Kelley (1988), who also found a predominance of females (71.2%). One of the major issues to explain the abundance of females in good brood years in these Irish and UK data is to know whether the fish in the good years are 1) issued from local spawns or 2) originating from Biscay and migrating North, as hypothesized by Kelley (1988). In the first case, the source of the higher female ratio would be linked to local environmental conditions, while in the second case it could also include genetic effects if the fish come from a different population. The general picture of sea bass population genetics is a genetic homogeneity of populations within the Atlantic (Naciri *et al.*, 1999; Fritsch *et al.*, 2007), although some local differences may occur. Then, even if fish were massively migrating it seems quite unlikely that genetic differences would impact on sex ratios. This is further supported by the remarkable homogeneity found in the present study among population level sex ratios in young fish. Therefore, the environmental cause for excess of females in some years seems more plausible than the genetic one. It has to be noted that in the Mediterranean, we could not identify "good" years (specially abundant year classes) in the published data from Egypt (Wassef and El Emary, 1989) and Turkey (Ergene, 1999), though variation in age-group sex ratios was evidenced in Egypt. In this latter case however, three cases of unbalanced age group sex ratios reflected excess of females in old age groups (>VIII), which can be explained by the higher longevity of females, as seen before. Still, the very low female ratio in age group VI (26.4%) cannot be explained by differential longevity between the sexes and should is likely to be the result of environmental variation between years.

There is little doubt that the variation in sex ratio between age classes, when it cannot be explained by differences in longevity or a sampling bias between sexes, is of environmental origin. However, the available data do not allow the determination of the time at which the differences appear and of the mechanism involved. Still, as temperature in early life has been shown to influence sex ratios, and temperature is highly variable between years, the fact that variation in natural temperature may also induce variations in sex ratio seems plausible. The European sea bass has a polygenic sex determination system, with an estimated heritability of 0.62±0.12 for sex tendency (Vandeputte *et al.*, 2007). In such a system, when environmental variation induces different sex ratios in different years, evolution should drive the system towards chromosomal sex determination, or environmental sex determination if environment variations have different effects on the fitness of males and females (Charnov and Bull, 1977; Bulmer and Bull, 1982) As chromosomal sex determination has been excluded (Blazquez *et al.*, 1999; Vandeputte *et al.*, 2007), as well as purely environmental sex determination (Vandeputte *et al.*, 2007), the only possibility remaining is polygenic (or at least oligogenic) sex determination, but this is expected to be only a transient state, as in most conditions it is evolutionary unstable (Bulmer and Bull, 1982; Rice, 1986; Hatcher and Tofts, 1995). As environmental conditions are expected to be quite variable in the Atlantic, the West Mediterranean Sea and the East Mediterranean Sea, it is likely that this system combining genetic variation and environmental influences on sex ratios could have reached different equilibrium states in the main wild sea bass populations, as has been observed in the Atlantic silverside *Menidia menidia*, where the relative influence of genetics and temperature on sex ratios differs along a latitudinal gradient (Lagomarsino and Conover, 1993), or in the lizard *Niveoscincus ocellatus* in which highland populations show genotypic sex determination and lowland populations show temperature-dependent sex determination (Pen *et al.*, 2010). Therefore, exploring the between population variation in sex determination patterns might be of great help to better control sex ratios in sea bass aquaculture, and to further increase our knowledge of the evolution of sex-determining mechanisms.

#### *3.2.1.5 Summary:*

**Sex ratios in farmed European sea bass are highly biased towards males (75 to 95%), which is problematic for aquaculture. In this mini-review, we re-analyse fisheries literature data about sex ratios in wild sea bass from 13 population samples, representing altogether 4889 individuals covering the major part of the distribution range of the species. We find that as a whole, the sex ratio of wild populations is biased towards females (59.4% females, $P<0.001$), but that the sex ratio of the younger fish (<30 cm total length) is balanced (52.0% females, $P=0.15$), while the sex ratio of the older fish is heavily biased towards females (69.5% females, $P<0.01$). Possible causes of these differences (differential longevity, biased sampling) are discussed. When age-group sex ratios are available (three population samples out of 13), significant variation between age groups appears, part of which is most likely of environmental origin. This study shows that the excess of males in culture is not a characteristic of the species, but rather a consequence of the environments used in culture, interacting with a complex system where both environmental and genetic influences govern sex determination in sea bass.**

### 3.2.2 Sex ratio changes in domesticated and selected populations

This part of the work was submitted as:

"Domestication and artificial selection for growth induce sex ratio shifts in the European sea bass (*Dicentrarchus labrax* L.)" by Marc Vandeputte[1,2,3 *], Mathilde Dupont-Nivet[1], Hervé Chavanne [4,5],Alain Vergnet [2], Pierrick Haffray[6], Edwige Quillet[1], Béatrice Chatain[2]

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**corresponding author*

#### *3.2.2.1 Introduction*

The evolution of sex ratios is a major subject in evolutionary biology, as it has important implications on fitness and allows testing of explanatory models with a relatively easily assessed phenotype, numbers of males and females (West and Herre, 2002). In gonochoristic species, two main types of sex determination have been described, genotypic (GSD) and environmental (ESD) sex determination. It has been proposed that these two categories were the extremes of a continuum (Kraak and de Looze, 1993; Kraak and Pen, 2002; Sarre *et al.*, 2004), with GSD possibly subdivided between major and minor genetic factors (Baroiller *et al.*, 2009). In fish, both types of sex determination are present, with ESD being in most cases studied temperature-dependent sex determination (TSD).

The European sea bass is a gonochoristic fish distributed in the North-Eastern Atlantic, the Mediterranean and the Black Sea (Pickett and Pawson, 1994). It is also a major species in Mediterranean aquaculture, and in farmed populations, 75 to 95% of the fish are males (Piferrer *et al.*, 2005).

In this species, sex remains undifferentiated for a long period (Saillant *et al.*, 2003a): differentiation occurs between 128 and 250 days post-fertilisation (dpf). Temperature has been shown to play a major role in sex determination of sea bass, but its effect is not fully understood. The current hypothesis is that high temperatures early in development (before 100 dpf) lead to decreased female rates (Piferrer *et al.*, 2005), probably through an inhibition of female differentiation (Navarro-Martin *et al.*, 2009b). However long-lasting low temperatures also produce an excess of males interpreted as caused by a low growth rate precluding female orientation (Blazquez *et al.*, 1998; Saillant *et al.*, 2002; Navarro-Martin *et al.*, 2009b). In wild populations, the primary sex ratio is 50:50, although some variation between year-classes, probably due to the environment, has been observed (Vandeputte *et al.*, 2012). Thus, the excess of males observed in culture would mostly be due to the use of temperatures higher than in the wild early in development. In addition to the temperature effects, between-families variation of sex ratio shows that genetic effects (Saillant *et al.*, 2002; Vandeputte *et al.*, 2007) and genotype by environment interactions (Saillant *et al.*, 2002) also exist. The distribution of family sex ratios was shown to be compatible with a polygenic system, or at least with an oligogenic system with a minimum of four sex factors and additional environmental variance, whereas pure ESD or pure GSD could be excluded (Vandeputte *et al.*, 2007).

The aim of the present study was to demonstrate experimentally that, as predicted in our previous study (Vandeputte *et al.*, 2007), sex ratio should evolve in farmed populations of sea bass as a result of frequency-dependent selection and selection for growth. For this, we compared the sex ratios of the offspring of growth-selected males, unselected males born in captivity and wild-born males.

#### *3.2.2.2 Material and methods*

##### 3.2.2.2.1 G0 and G1 base populations for experimental selection

The constitution of the first generation population (G1) was described in details in a previous paper (Vandeputte *et al.*, 2007). Briefly, 253 families were produced by artificial fertilization according to a partly factorial mating design involving 33 G0 sires and 23 G0 dams of wild Atlantic origin. All families were reared as a single batch in a common garden experiment since fertilization. Rearing temperature increased from 13 to 18°C in the first 64 days. Temperature was then kept at 18°C until 238 days post fertilization (dpf), an age at which changes in temperature have no impact on progeny sex ratio any more (Saillant *et al.*, 2002). Fish were individually tagged at 370 dpf, their pedigree was recovered by the genotyping of 6 microsatellite markers, and their sex was determined later on by visual observation of the gonads after dissection. The proportion of females among the offspring was 18.2%, indicating that the G1 environment was masculinising as expected.

Among the tagged G1 fish, 17 males were selected for high body length at 714 dpf, representing a 5% selection pressure ("Selected" or S group, on average +2.07 phenotypic standard deviations ($\sigma_P$) over the mean for body length), 20 unselected males were chosen to be representative of the average length of the population ("Domesticated" or D group, born in captivity but with average growth capacity, on average -0.04 $\sigma_P$ for body length). We also randomly chose 20 cryopreserved males from the 33 initial G0 population wild males to be used as wild control parents (W group). More details on the selection procedures are available in a previous paper (Vandeputte *et al.*, 2009b).

##### 3.2.2.2.2 Response to selection

The matings and the rearing of the G2 progenies were carried out at the Ifremer experimental facility of Palavas (France) and were detailed elsewhere (Vandeputte *et al.*, 2009b). The spawns of 13 wild females (West Mediterranean origin) were used to produce a full factorial mating design using cryopreserved sperm from the 57 males previously chosen, *i.e.* 20 wild (W) males, 20 domesticated (D) males and 17 selected (S) males (see before). Progenies from the factorial mating were reared according to different designs: 1) as a single batch with all progenies (W, D, S offspring) mixed since fertilisation in 3 replicated tanks 2) as triplicated separate batches in nine tanks (3 tanks for each of the W, D, S groups). Temperature increased from 16 to 20°C in the first 55 days, then sharply increased to 24°C, and progressively decreased to 20°C from 110 to 268 dpf. At 268 dpf, 962 fish from the mixed batches were individually tagged, and 859 survived until they were sexed at 611 dpf. Among those, 826 (96.1%) could be unambiguously assigned to their parents and genetic origin (W, D, S), using eight microsatellite markers, *Dla016*, *Dla020*, *Dla105*, *Dla116*, *Dla119*, *Lab13*, *Lab3* and *Dla022* (Garcia De Leon *et al.*, 1995; Ciftci *et al.*, 2002; Chistiakov *et al.*, 2004) and the assignment software VITASSIGN (Vandeputte *et al.*, 2006). In parallel, 970 fish from the 9 separate batches (84-114 per batch) were sexed at 611 dpf.

##### 3.2.2.2.3 Statistical methods

Sex ratios were compared among the three G2 offspring groups (W, D, S) in order to assess the effects of domestication, selection for growth and type of rearing (separate tanks *vs.* mixed tanks) on

progeny sex ratio. A logistic regression on proportion of females in the offspring was performed, first with a model including the effect of sire line (W, D, S), the effect of rearing type (mixed, separate), the interaction between sire line and rearing type, and a block effect, corresponding to three recirculation systems with 5 tanks each used for rearing the fish from 78 to 611 dpf (Model 1). After removal of the non significant effects, the effects in the model were only sire line and rearing type, and this model (Model2) was used to estimate the significance of contrasts for offspring group and rearing type.

#### 3.2.2.3 Results

The average proportion of females in unselected G1 fish was 18.2% (*N*=5915). The average sex ratio of G2 progenies was 46.6% females on a total of 1796 fish. Logistic regression (model 1) indicated that line (W, D, S) and rearing type (mixed, separate) had a highly significant effect on sex ratio (*P*<0.001), while neither line*rearing type interaction nor block were significant (*P*>0.4 and *P*>0.9, respectively). These last two factors were removed in model 2, which was then used to estimate the contrasts between lines, as reported in Figure 3-3. Additionally, it can be noted that model 2 did not display overdispersion (*P*>0.8), nor lack of fit (Hosmer and Lemeshov test, *P*>0.7).

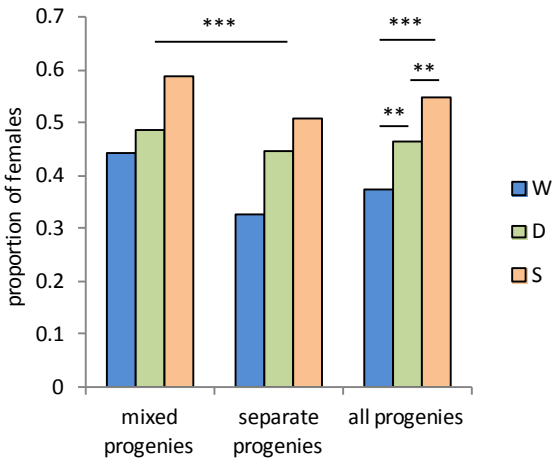


**Figure 3-3:** Proportion of females in G2 progenies of sea bass from different lines (Wild, Domesticated, Selected) reared in mixed tanks or in separate tanks. ** *P*<0.01; ***P*<0.001

Figure 3-3 shows that the overall proportion of females was higher in mixed rearing (51.5%) when compared to separate rearing (42.5%), and that the three lines had different sex ratios. As line*rearing interaction was not significant, line sex ratios were calculated on the whole dataset, and were 37.5% females for the progeny of Wild sires, 46.4% for Domesticated and 55.0% for Selected fish, which were all significantly different from each other (*P*<0.01).

#### 3.2.2.4 Discussion

From the study of between-family variation for sex ratio and body size in G1 (Vandeputte *et al.*, 2007), we hypothesized that sex ratios in farmed populations of sea bass should evolve towards more females as a result of frequency-dependent selection in domesticated populations. The present selection experiment clearly showed that sex ratio was modified by simple domestication (W *vs.* D), as an adaptive response to a new environment producing male-biased sex ratios. As predicted by Fisher's theory and its adaptation to the case of polygenic sex determination (Bulmer and Bull, 1982; Fisher, 1930), frequency-dependent selection tends to increase the proportion of females in the offspring of males born and reared in a masculinising environment. Our second hypothesis, based on a positive genetic correlation between body size and sex tendency (Vandeputte *et al.*, 2007), was that selection for fast growth should further increase the proportion of females in the farmed populations. In the present experiment, we demonstrated that fast-growing males selected in G1 produced more females in their G2 offspring than domesticated males did. It should be noted that S offspring were significantly larger than D offspring, which in turn had a size similar to that of W

offspring (Vandeputte *et al.*, 2009b). Thus, the effect of domestication on sex ratio was not due to an indirect effect of growth, but to frequency dependent selection, while the effect of selection for growth on sex ratio could be seen as correlated effect of fast growth rate.

The mean proportions of females in the offspring of wild sires in G1 (18.2%) and G2 (37.5%) were quite different, although both male-biased. We expected that the thermal profile in G2 would produce more females, through the stimulation of growth by high temperatures towards the end of the sex determination window. This is what we observed, but we must honestly state that the use of the same thermic profile in other experiments also sometimes gave very low sex ratios (*ca.* 10% females). Nevertheless the difference in mean sex ratio between separate and mixed rearing shows that environment effects on sex ratio (including temperature) are not fully understood yet. In the present experiment, it happened that the 'rearing type' effect was confounded with a 'larval rearing room' effect during the first 77 days. Both rooms had the same set point for temperature, but some very limited variation happened (S.D. for the difference in daily temperature between both rooms= 0.7°C) and sex ratios were substantially modified (32.7 *vs.* 44.1% females in the G2 offspring of wild sires reared separately or mixed, respectively). Then, the temperature profile can still be one reason for the shift in sex ratio between G1 and G2, but other environmental effects such as water quality or social effects, which may be different in mixed and separate groups could be invoked. A second possible reason is that the G1 fish were from Atlantic parents, but were crossed with Mediterranean females to produce the G2 - as we did not manage to have ready to spawn Atlantic females. Previous experience with this Mediterranean wild population did not yield remarkably high female ratios (usually 10 to 30% females), so a large additive genetic effect is unlikely. Dominance (heterosis) effect could be an explanation, considering the fact that Mediterranean and Atlantic populations show genetic divergence (Naciri *et al.*, 1999). Then, different sex factors may develop under different environmental conditions, as seen in Atlantic silversides *Menidia menidia* (Lagomarsino and Conover, 1993), and combine to yield an increased proportion of females in hybrids between populations. This possibility is supported by additional unpublished results with Atlantic and Mediterranean sea bass hybrids showing suggestive heterosis on sex ratio in a highly masculinizing environment (10.7% females in hybrids *vs*. 9.1% in Atlantic and 6.7% in Mediterranean so 7.8% on average in pure lines, 2886 fish sexed, $\chi^2$=7.32, 1 *d.f, P*<0.01). Anyhow, if the global shift in sex tendency in G2 is caused by a genetic effect of the Mediterranenan population (additive or heterosis), it should apply to all groups which are all Atlantic x Mediterranean hybrids, all males being derived from the same Atlantic base population, and should therefore not bias the relative values of sex ratio between G2 offspring groups (W, D and S).

Polygenic sex determination has not often been evidenced in vertebrates (ten species reviewed in McGaugh and Janzen, 2011), where GSD with sex chromosomes and to a lesser extent ESD are by far more represented. However, in fish, there are several cases of species with sex chromosomes (thus GSD) where minor genetic factors, sometimes influenced by the environment, allow modifications of the sex ratio (see Baroiller *et al.*, 2009 for a review). Then, the idea that the ancestral sex determination system (in fish) would be polygenic (Kirpichnikov, 1981) remains an interesting hypothesis. The condition for a high genetic variance in sex ratio, like the one observed in sea bass, to exist in a polygenic system is a variable level (between years or locations) of environmental effects on sex tendency (Bulmer and Bull, 1982). This condition is plausible in the case of sea bass (coastal water temperatures are highly variable), but such a pattern also favours invasion of the population by major sex factors (Bulmer and Bull, 1982). If there is a differential response in fitness between the

two sexes for variations of the quality of the environment, the system is supposed to evolve towards "true" ESD, meaning sex ratio being essentially determined by the environment, with an abrupt change in sex ratio around a threshold environment value, as seen in many reptiles (Charnov and Bull, 1977; ; Bulmer and Bull, 1982; Bull, 1983). This is not the case in sea bass, as proven by the difficulties to precisely assess the effect of temperature on sex ratio (Piferrer *et al.*, 2005; Navarro-Martin *et al.*, 2009b). When there is both temporal variability of environment and sex-specific fitness effects, the evolution towards ESD or genic (meaning GSD with major sex factors) sex determination should depend on the relative values of environmental effects on sex ratio and fitness (Bulmer and Bull, 1982; Pen *et al.*, 2010). For a specific case where a major sex factor is closely linked to a gene with sex-specific fitness effects, the prediction is that the major sex factor should in general invade the population (Rice, 1986). If we consider that body size has a positive effect on female fitness in sea bass, as seen in the Atlantic silverside (Conover, 1984), a major sex factor in a region with growth promoting genes should then invade the population. However, it has been shown that growth was highly heritable in sea bass, but also subject to important genotype by environment interactions (Dupont-Nivet *et al.*, 2010b), meaning that the genotypes favouring growth rate are not the same in different environments. In this case, the linkage of a major sex factor with growth would not be permanent, possibly preventing a fast increase in its frequency in the population. Finally, genotype by environment interactions were also evidenced for sex ratio, suggesting variable sensitivity to temperature of different genotypes (Saillant *et al.*, 2002), and then again the possibility that genes with a direct effect on sex tendency would not be the same in all environments. This could be part of the subtle equilibrium allowing the maintenance of polygenic (or at least plurigenic) variation for sex tendency in sea bass.

We have shown that population sex ratio could evolve rapidly in captive sea bass populations, due to a high genetic variance, and to an association with growth. The variation was expressed in captive breeding conditions, and the relative changes may be different in wild populations experiencing different environmental conditions, or having different genetic backgrounds. Previous reports on Atlantic silverside, another fish where environmental and genetic factors influence sex ratio, showed that populations from different latitudes may have different main sex determination systems (Lagomarsino and Conover, 1993). Our work was done on the Atlantic population of the sea bass, but it would be highly valuable to study the genetic variation of sex ratio also in the Mediterranean population, in order to see if the balance between genotypic and environmental sex determination is similar or not. This could also have applied outcomes, as both populations are used for developing selective breeding programmes in aquaculture.

#### *3.2.2.5 Summary*

**In cultured populations of the European sea bass *Dicentrarchus labrax*, sex ratios are usually highly male-biased. It has been showed previously that there is a high between family variance in sex ratio, with a positive genetic correlation between growth and percentage of females. Through experimental selection, we showed that there were more females (46.4%) in the offspring of captive-bred males (1st generation domestication) than in the offspring of wild males (37.5% females) when reared in the same environment . A further shift in sex ratio was observed in the offspring of males selected for fast growth rate (55.0% females). The genetic architecture of sex ratio, with high genetic variation and a linkage with growth rate probably has an adaptive value for the sea bass, and implies that farmed populations sex ratios will quickly be modified by domestication and selective breeding of the species, with important outcomes for aquaculture.**

### 3.2.3 Additional data: genetic correlation of growth and sex tendency over time

In section 3.2.2, we evaluated the effect of selection on body length and of domestication on offspring population sex ratios. We could especially do this in mixed populations thanks to the use of genotyping of microsatellites to recover parentage. Genotyping of microsatellites not only gave us the information on the group of sires each offspring originated from, information that we used in section 3.2.2), but also the family information, so which sire and dam each offspring had as parents, and this for 826 sexed offspring. For each of these fish, we also had measured body length at 238, 338 and 457 dpf. In addition to these data, we measured the body length of 583 larvae at 10 dpf and of 637 juveniles at 90 dpf. All those fish were also genotyped, and 10 dpf larvae (*n*=560) as well as 90 dpf juveniles (*n*=612) could be assigned to their parents. We did not have access to the sex of these juveniles, however the pedigree allowed to link their performance to that of the 826 sexed fish.

To this end, we used a multi-trait animal model where the traits were body length at 10, 90, 238, 338 and 457 dpf and the binary sex trait. Using binary traits on the observed scale to estimate genetic correlations between the liability variable and continuous traits is expected to give unbiased results (Mercer and Hill, 1984; Olausson and Ronningen, 1975). The fixed effect in the model were larval rearing tank and sire type (W, D, M). The model was run with VCE6 (Groeneveld *et al.*, 2008).

Genetic correlations between body length and sex tendency (Figure 3-4) were not stable at all over time, and peaked at 90 dpf ($r_A$=0.77±0.16), showing that early growth seems to have a major link with sex tendency, while late growth would be less relevant.

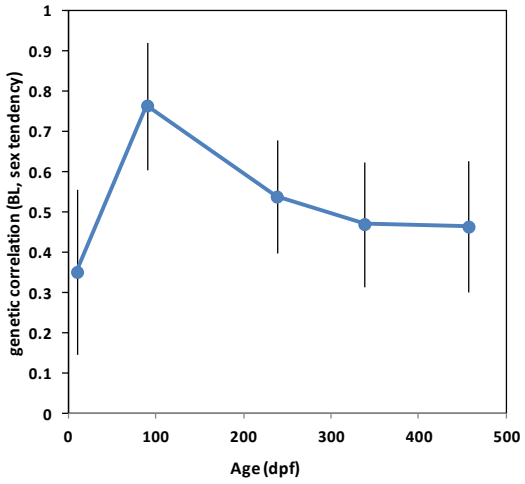


**Figure 3-4:** Evolution over time of the genetic correlation between body length and sex tendency in sea bass. Error bars account for standard errors of estimates.

Due to the limited sample size and the special data structure however (only 826 fish with a sex phenotype), the standard errors of the genetic correlations estimated in this section were rather high. Another limitation is that, although sex has a phenotypic effect on body size which already exists at 84 dpf (Saillant *et al.*, 2003c), this was not included in the model, because sex information was only available for the fish measured from 238 dpf, and we wanted to treat body length data at all ages in a similar way. Not taking into account the fixed effect of sex on body length would be likely to inflate the genetic correlations estimated, because the average body length is expected to be larger in female-rich families, solely by the phenotypic effect of the larger size of females . Conversely, we chose to include a sire type effect in the model, and as the sire types have both different sex tendencies (section 3.2.1) and different body sizes (section 3.1), it can be expected that removing this with a sire type fixed effect is likely to decrease the genetic correlation. Not including the sire type effect would be possible but then the distribution of sire additive genetic values could not be considered to be random and normal, potentially causing estimation biases.

It has been shown before that sex dimorphism for body size is already present at 84 dpf, long before the differentiation of gonads (Saillant *et al.*, 2003c). As all fish initially start at the same size (constrained by egg size in the same pool of females), and there is genetic little correlation of sex tendency with size at 10 dpf (start feeding - see Figure 3-4) it is then likely that growth rate is strongly linked to sex tendency somewhere between 10 and 90 dpf. Whether growth rate acts on sex tendency or sex acts on growth rate at that stage remains unsolved, although we can note that this time window is well before the differentiation of the gonads, which starts around 150 dpf (or 80 mm standard length - Saillant *et al.*, 2003a; Piferrer *et al.*, 2005) although some differences in the expression profile of *cyp450a* can be seen as early as 120 dpf (Blazquez *et al.*, 2009). In any case, no sign of differentiation has ever been seen before 120 dpf, so the idea that growth rate may influence sex orientation remains plausible.

## 3.3 Modelling the mid-term evolution of growth and sex ratio in farmed sea bass populations under selection for growth

This part of the work has not been published in a peer-reviewed journal, but various outcomes of the simulation program have been used in several poster presentations in national and international conferences, among which:

Vandeputte, M., Dupont-Nivet, M., Chavanne, H., Haffray, P., Chatain, B., 2007. La sélection sur le sexe chez le bar : comment produire des populations riches en femelles ? Journées Recherche Filière Piscicole, Paris, 3-4 juillet 2007.

Vandeputte, M., Dupont-Nivet, M., Haffray, P., Chavanne, H., Vergnet, A., Quillet, E., Chatain, B., 2011. Modification of population sex ratios by domestication and artificial selection in the European sea bass (*Dicentrarchus labrax* L.). Colloque de la Société Française de Génétique "Genetics, Epigenetics and Evolution of Sex Chromosomes", Paris, 9-10 June 2011.

Vandeputte, M., Dupont-Nivet, M., Haffray, P., Chavanne, H., Vergnet, A., Quillet, E., Chatain, B., 2011. Can we obtain monosex sea bass populations through selective breeding ? 62nd EAAP Annual Meeting, Stavanger, Norway - 29 August - 1 September 2011.

### 3.3.1 Why model the evolution of sex ratio under selection for growth ?

European sea bass is an aquaculture species in which sex ratios of farmed populations are generally strongly biased towards males (75-95%, Piferrer *et al.*, 2005). These skewed sex ratios are a characteristic of farmed populations, as wild European sea bass quite generally exhibit balanced sex ratios (Vandeputte *et al.*, 2012). Several experiments have shown that temperature during early development is a key factor influencing sex determination in this species (reviewed in Navarro-Martin *et al.*, 2009b), and it seems very likely that the temperature profile during larval rearing, possibly interacting with other unknown environmental factors, is the key reason for these sex ratio biases. Nevertheless, it has been demonstrated that sea bass sex ratio also has a genetic component in both Mediterranean (Saillant *et al.*, 2002) and Atlantic (Vandeputte *et al.*, 2007) populations of sea bass. In the Atlantic population, sex ratio can be modelled as a threshold trait with an underlying sex tendency, the heritability of which was estimated to be 0.62±0.12 on the liability scale. Interestingly, it has also been shown, in the same Atlantic population, that there was a positive genetic correlation between body weight at one year and sex tendency ($r_A$= 0.50±0.09), as well as between body length and sex tendency ($r_A$= 0.48±0.09, Vandeputte *et al.*, 2007). Based on these results, we hypothesize that

1) frequency-dependent selection in closed farmed populations of sea bass should induce a shift in farmed population sex ratios, which would be expected to reach a balanced value in 7 to 8 generations of domestication (Vandeputte *et al.*, 2007), and

2) selection for growth should speed up the modification of sex ratio towards females.

These two predictions have been empirically verified by a selective breeding and domestication experiment (see section 3.2.2) where the offspring of wild sea bass males had a sex ratio of 37.5% females, while the offspring of first generation domesticated males were 46.4% females and the offspring of fish mass selected for body length were 55.0% females.

As it can be foreseen that selective breeding will develop in this species, and that the first trait selected for in breeding programmes is almost always growth, it is of great interest to try and predict the impact of selection for increased body size on population sex ratios. This cannot be done in a straightforward manner, as due to the genetic correlation of body size and sex tendency, selection for body size is expected to increase sex tendency, and this will be accelerated by frequency

dependent selection as long as the proportion of females is lower than 50% in the population. Conversely, if the proportion of females in the population reaches values beyond 50%, frequency-dependent selection will counteract the effect of selection for increased body weight towards an increased sex tendency (and hence more females in the population). We can empirically predict that the population will reach an equilibrium point, beyond 50% of females, at which both selection pressures will neutralize themselves.

In this part of the work, we will examine by stochastic simulation the fate of a sea bass population submitted to selection for increased body size, in order to predict the rate and level of sex ratio shifts, and to estimate the equilibrium sex ratio reached in different situations.

### 3.3.2 A stochastic simulation model

We considered a population of sea bass selected for increased body length. Additive genetic and phenotypic values for body length, sex tendency and phenotypic sex in the population were simulated as described below.

A base population of wild broodstock was simulated. The additive genetic values of each fish for body length (*Al*) and sex tendency (*At*) were drawn from a binormal distribution N(0, $\Sigma_A$) and the environmental values of each fish for body length (*El*) and sex tendency (*Et*) were drawn from a binormal distribution N(0, $\Sigma_E$), where $\Sigma_A$ and $\Sigma_E$ were two variance-covariance matrices:

$$\Sigma_A = \begin{pmatrix} \sigma_{Al}^2 & r_A\sigma_{Al}\sigma_{At} \\ r_A\sigma_{Al}\sigma_{At} & \sigma_{At}^2 \end{pmatrix} \text{ and } \Sigma_E = \begin{pmatrix} 1-\sigma_{Al}^2 & r_E\sqrt{(1-\sigma_{Al}^2)(1-\sigma_{At}^2)} \\ r_E\sqrt{(1-\sigma_{Al}^2)(1-\sigma_{At}^2)} & 1-\sigma_{At}^2 \end{pmatrix}$$

Where $\sigma_{Al}$ is the additive standard deviation of body length, $\sigma_{At}$ is the additive standard deviation of sex tendency, $r_A$ is the genetic correlation between body length and sex tendency and $r_E$ is the environmental correlation between body length and sex tendency. Under a simple additive genetic model the environmental variance $\sigma_E$=1-$\sigma_A$, hence the values in matrix $\Sigma_E$.

The phenotypic values of the wild broodstock for body length and sex tendency were calculated as $P_l = \mu_l + A_l + E_l$ and $P_t = A_t + E_t$ . When $P_t$ was positive (= exceeding a zero threshold), the phenotypic sex of a given wild broodstock was set as female, while it was set as male if $P_t$ was negative. Thus, on average half of the wild broodstock was male and half was female, as observed in natural populations (Vandeputte *et al.*, 2012).

The first generation of unselected hatchery fish was generated using randomly drawn male and female wild broodstock. The phenotypic values for offspring *i* of sire *s* and dam *d* were generated with $P_{li} = \mu_l + \frac{1}{2}A_{ls} + \frac{1}{2}A_{ld} + \delta_{Ali} + E_{li}$ and $P_{ti} = probit(p_f) + \frac{1}{2}A_{ts} + \frac{1}{2}A_{td} + \delta_{Ati} + E_{ti}$ where $A_{ls}$ is the additive value for body length of sire *s*, $A_{ld}$ is the additive value for body length of dam *d*, $\delta_{Ali}$ is the mendelian sampling term of offspring *I* for body length and $E_{li}$ is the environmental value of offspring *I* for body length. Similar notations were used for the terms relating to sex tendency, and probit($p_f$), with probit the inverse of the cumulative distribution of the standard normal distribution evaluated at $p_f$, the average proportion of females in the hatchery conditions observed with offspring from wild broodstock. This probit term accounted for the environmental displacement of the mean sex tendency in hatchery conditions, as explained in section 2.4.2.1. This environmental term was kept constant over generations, under the hypothesis that rearing conditions (and their impact on

sex tendency) do not change over time. $\delta_{Ali}$ and $\delta_{Ati}$ were drawn from a binormal distribution N(0,½$\Sigma_A$), while $E_{li}$ and $E_{ti}$ were drawn from a binormal distribution N(0, $\Sigma_E$). As before, the phenotypic sex of offspring *i* was male or female depending on whether $P_{ti}$ was negative or positive, respectively. However, due to the environmental effect on sex tendency accounted for by probit($p_f$), this resulted in a population of offspring where the expected proportion of females was $p_f$ and the expected proportion of males was (1-$p_f$).

The individual selection process for body length was modelled as follows: the number of male ($N_m$) and female ($N_f$) broodstock to be selected was fixed at each generation, as well as the size of the population of candidates ($N_o$, number of offspring from the previous generation). This resulted in a global proportion selected of $p_s$=($N_m$+$N_f$)/$N_o$. Within each sex, the candidates were ranked according to their phenotypic body length. The $N_m$ largest males and the $N_f$ largest females were selected and mated at random in a full factorial design. Then, the genotypic and phenotypic values of each offspring for body length and sex tendency were generated from the additive genetic values of their parents as explained previously. Simulations were run for 15 generations, with 30 replicate populations. The mean and standard errors of sex tendency were calculated from the 30 replicate values at each generation

### 3.3.3 Parameters tested

The size of the simulated population of candidates was 1000 individuals at each generation. The total number of parents selected was calculated as a function of the proportion selected chosen, *i.e.* 10 parents for $p$=0.01, 50 parents for $p$=0.05, 200 parents for $p$=0.20, 500 parents for $p$=0.50. The number of male and female parents chosen was always equal to half the total number of parents.

The basic genetic parameters used were a 0.62 heritability for sex tendency, a 0.62 heritability of body length (the conservative value for BL at tagging with potential maternal effect removed - see section 2.4.2.2) and a 0.48 genetic correlation between both. The coefficient of variation of body length was set to 0.10, the average value observed, and the phenotypic variance of sex tendency was arbitrarily set to 1. The environmental correlation between body length and sex tendency was set to zero, as it never significantly differed from this value (section 2.4.2.2).

In a first simulation, we considered a proportion selected on body length equal to 0.05, and compared different initial proportions of females in the G0 hatchery population, in order to reflect the influence of the level of masculinisation induced by hatchery conditions. Initial proportions of females tested were 0.05, 0.182 (the one observed in section 2.4.1) and 0.50.

In a second simulation, we investigated the effect of the proportion selected for body length. The values tested were 0.01, 0.05, 0.20 and 0.50, with an initial proportion of females set at 0.182.

Then, we investigated the effect of different values for the heritability of body length (0.3 to 0.7), and the effect of different levels of genetic correlation (0.3 to 0.8) with constant heritabilities of body length (0.62) and sex tendency (0.62).

### 3.3.4 Results and discussion

The first set of simulations (Figure 3-5) showed that selecting the 5% largest fish for body length should quickly result in an increase in the proportion of females in the population, which then reaches an equilibrium value. Interestingly, the masculinising effect of the hatchery environment, reflected by the proportion of females in generation zero (unselected offspring of wild-caught parents), had no impact on the equilibrium proportion of females, which was 81.6% with the parameters chosen. The masculinising effect also had no impact on the time for reaching the equilibrium value, which was approximately 8 generations.

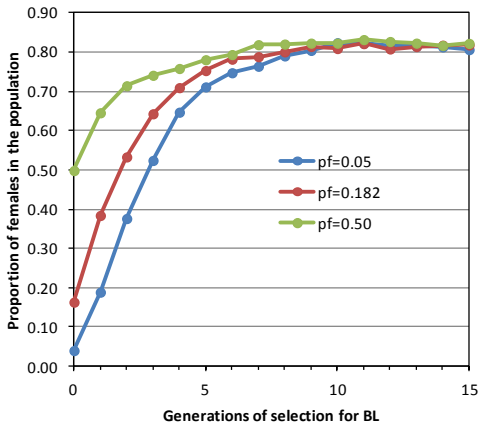


**Figure 3-5:** Simulated proportion of females over generations of selection for body length for different proportions of females ($p_f$) in the unselected population.

However, it has to be noted that although the equilibrium sex ratio was the same whatever the masculinising effect of the rearing environment, the result in terms of genetic component of the sex tendency is different. The reason for this is the following: as the equilibrium sex ratio is always the same, we can derive from equation 1 in section 2.4.2.1 that the mean population sex tendency is the same at the end. However, in generation zero, before the selection process starts, proportions of females largely differ. As the base population parents are always drawn from a wild population with an even sex ratio, their mean genetic component for sex tendency is the same, and then the difference comes from the environment. The mean effect of the environment in generation zero can be estimated by the mean sex tendency of the population at that time, namely $t$=-1.64 for $p_f$=0.05, $t$=-0.91 for $p_f$=0.182 and $t$=0 for $p_f$=0.5. If we remove this environmental component from the equilibrium sex tendency ($t$=0.90 with 81.6% females), we end up with quite different levels of sex tendency: $t$=2.55 for $p_f$=0.05, $t$=1.81 for $p_f$=0.182 and $t$=0.90 for $p_f$=0.5, which means that if offspring from the three different populations were put in the same environment, this would result in very different sex ratios.

The second set of simulations investigated the influence of the selection intensity for body length (Figure 3-6). It clearly appeared that selection intensity had an impact on the level of the equilibrium value. In the range tested the equilibrium value was proportional to the standardized selection intensity $i$ (Falconer and Mackay, 1996) on body length. This should however not be the case for very high selection intensities as there cannot be more than 100% females in the population! Here again, the plateau was reached at the same time (8-9 generations) whatever the selection intensity. In the next set of simulations, we showed that the heritability of body length had an impact

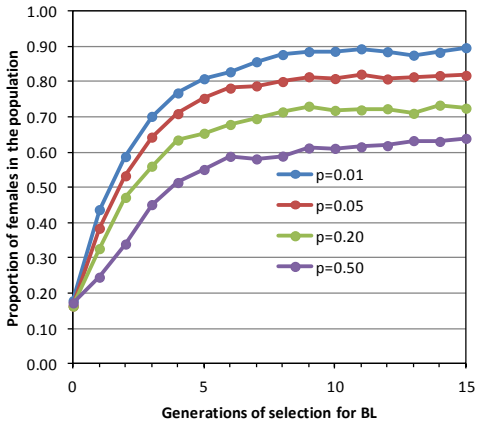


**Figure 3-6:** Simulated proportion of females over generations of selection for body length for different proportions selected ($p$).

on the level of the plateau, but this impact was rather limited for the range of values tested (73.5% females for $h^2_{BL}$=0.30, 83.5% for $h^2_{BL}$=0.70 - Figure 3-7). The impact of genetic correlation was higher, with values of the plateau at 70.3% females for $r_A$=0.30, 81.5% for $r_A$=0.48 and 94.8% for $r_A$=0.80 (Figure 3-8).

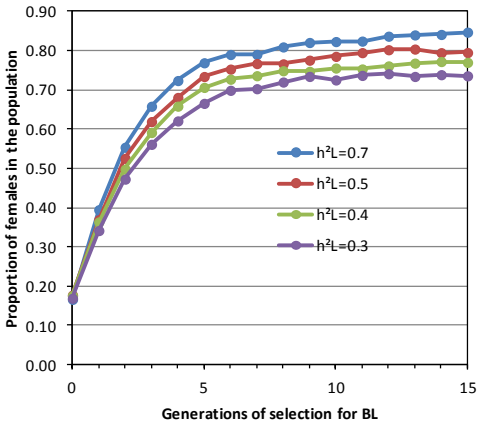


**Figure 3-7:** Simulated proportion of females over generations of selection for body length for with different heritabilities for BL.

We did not try to simulate different values for the heritability of sex tendency, as in the experiment where we had a good design to estimate it (section 2.4.1), we had only one estimate for the heritability of sex tendency, as the sex of individuals does not vary over time, contrary to length for which we had two genetic correlation values with sex tendency (0.48 for length at 1 year and 0.33 for length at slaughter - section 2.4.2.2). We also showed in a more limited design (section 3.2.3) that an even higher genetic correlation (0.76) was possible when considering length at 90 dpf.

We already showed before that without selection, the domestication process of sea bass should lead to balanced sex ratios in 7-8 generations by frequency-dependent selection (section 2.4.1). Here we show that selection for growth, which is the most commonly applied in domesticated stocks, should further increase the proportion of females in farmed populations. However, this is true in the population tested (Atlantic base population) and could be different, as heritabilities and genetic correlations may differ, in other (Mediterranean) populations of sea bass. Another important potential limitation is that genetic correlations may not be stable in variable environments (Simons and Roff, 1996) or after some generations of selection, although in this case their sign is expected to remain constant (Leroi *et al.*, 1994).

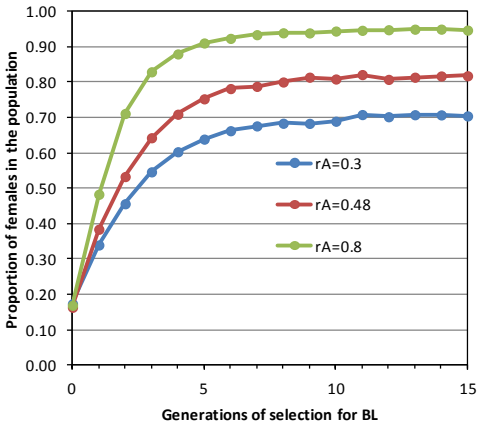


**Figure 3-8:** Simulated proportion of females over generations of selection for body length for with different genetic correlations between BL and sex tendency.

# 4 General Discussion

## 4.1 Summary of the main results

The first important technical results of the present research were that the production of large numbers of reasonably balanced families using artificial fertilization was feasible in sea bass, and that pedigree tracing with microsatellites was reliable, with single assignment rates exceeding 95%. These results open the possibility to use such techniques in sea bass breeding programmes, with evident potential in terms of conservation of the genetic variability and of use of family information to improve the estimation of breeding values.

The analysis of covariance between relatives showed that the heritability of body weight and body length is reasonably high ($h^2$= 0.27-0.44), and in general moderately affected by non genetic maternal effects ($m^2$= 0.04-0.19). Dominance variation for weight and length is low and can be reasonably ignored ($d^2 \leq$ 0.02). Together with the high level of phenotypic variation observed for these growth traits, this gives good perspectives to increase harvest weight, either by direct selection on weight or by indirect selection on length as both traits were found to be highly genetically correlated ($r_A$=0.91-0.96). When growth rate rather than body weight was considered, heritability estimates were still reasonable though lower (0.16-0.34 within sites).

These good prospects were confirmed by comparing the offspring of first generation selected and unselected (domesticated) sires with that of wild sires. Two lines of selected fish showed a selection response of 23% body weight when compared to wild or unselected control. The two lines of selected fish both represented the top 5% males in terms of body weight and body length, but with two selection strategies: mass selection for length at 398g mean weight in the Ifremer experimental station, and an industry breeding programme (Panittica) with three successive cullings on weight (1) and length (2) at different ages. The offspring of the two selected lines had the same growth performance over the whole period studied, showing no superiority of the Prosper method in this case - although the sampling variance inherent to selection does not allow us to conclude on this absence of differences. Similarly, no difference in growth could be observed between the offspring of domesticated and wild males - with the same limitation.

Competition was shown to amplify differences between genetic groups, as the selection response when all four genotypes were mixed in the same tanks reached 42%, compared to the 23% observed in separate tanks. Realized heritability was 0.34 when selection response was evaluated in separate tanks, in agreement with the previous estimates from covariance between relatives. When selection response was estimated from mixed tanks, the realized heritability was estimated to be 0.60, a clearly over-estimated figure including the competition effect – this then has to be taken into account when designing experiments for comparing genetic groups of sea bass. Nevertheless, this selection response experiment, although done in a single environment, showed that very fast gains in growth could be achieved in sea bass by simple individual selection.

The GxE interaction component of the study was double faced. We approached GxE interactions through the estimation of genetic correlations between the four farm sites: tropical sea cages in Israel, intensive recirculated system in France, raceways with well water in Italy and semi-intensive estuarine ponds in Portugal. When we studied the genetic correlations of harvest body weight (*ca*. 400g) between sites, we found they were rather high: 0.84-0.99, with one exception of a genetic

correlation at 0.70 between Israel and Portugal, the extreme sites in terms of rearing temperature. The logical conclusion from this finding was that GxE interactions were not an important constraint for the development of sea bass breeding programmes.

However, in this study, the fish were tagged at *ca*. 1 year of age and a mean weight of 35g, thus limiting the possibility for GxE interaction to have a very high impact on harvest weight. When we studied growth rate instead of body weight, the picture was completely different: genetic correlations between sites were low to moderate (0.21-0.61) with one exception ($r_A$=0.78) between Israel and Italy, where a long common life due to transportation problems could explain the (relatively) high level of the genetic correlation. When choosing growth rate rather than body weight as the target trait, the GxE interaction is then expected to be high – which is also revealed by the fact that the heritability of growth rate across sites is much lower (0.12) than the average of within site heritabilities (0.25). Still, we can mention that we did not observe any negative genetic correlation between sites, meaning that selection for growth in a given site is not expected to generate adverse effects in any other site. However, a negative genetic correlation appeared between initial weight (before 370 dpf) in Palavas and growth rate in Portugal ($r_A$=-0.31±0.11), showing that selection for early growth should be considered with caution, especially when the ongrowing environment differs from the selection environment.

Through the analysis of the fisheries literature related to sea bass, we could show that, as predicted by Fisher's theory, the sex ratio in wild sea bass populations, either from the Atlantic or from the Mediterranean, was 1:1 in young adults. Females were dominating in older fish, probably due to a higher longevity. This indirectly showed that the excess of males in farmed sea bass was neither an intrinsic characteristic of the species, nor linked to the genetic background of the farmed sea bass lines, and thus was actually linked to the farming environment, as hypothesized before on the basis of environmental manipulations in the early rearing phases (as reviewed by Navarro-Martin *et al.*, 2009b). Moreover, anecdotal evidence in some cohorts indicated that cohort sex ratios could also be male or female-biased in the wild, most likely due to specific environmental conditions in some (rare) brood years. This indicates that mimicking natural conditions should allow the production of balanced sex ratios in farmed sea bass populations, but also that the manipulation of the environment could lead to an excess of males (farmers know that...) or of females (which has not been reliably established until now).

The average sex ratio in the first (covariance between relatives) experiment was 18.2% females, well in line with usual sex ratios in production. Between family variation for sex ratio was very high, with proportions of females ranging from 4.7 to 46.3% in paternal half-sib families and from 0.5 to 40.3% in maternal half-sib families. Full-sib families had 0 to 75% females, but their small size (21 fish on average) limits the significance of this last observation. Still, we could observe from logistic regression that sex ratios were mostly additive.

We demonstrated that the distribution of sex ratios among families was not compatible with a purely genetic sex determination system (existence of environmental variance was a necessity, whatever the number of loci implied). Even with environmental variance, at least two bi-allelic loci were necessary to explain the distribution observed, while polygenic sex determination was also plausible.

When sex ratio was described as a threshold trait with an underlying polygenic sex tendency, the heritability of sex tendency on the underlying scale was 0.62±0.12, and interestingly it was

significantly positively correlated with body weight ($r_A$= 0.50±0.09). We concluded from these observations that closing a sea bass population in farming conditions should lead to balanced sex ratios in 7-8 generations through frequency-dependent selection. Due to the positive genetic correlation with growth, selection for growth would be expected to further increase sex-tendency, and fasten the reach of a balanced sex ratio – and even induce female-dominant populations in later generations.

These hypotheses were tested in the selection response experiment where we compared sex ratios in the offspring of wild, first generation domesticated and first generation mass selected for growth sea bass males. Although the average sex ratio was higher in this experiment, probably due to environment conditions and/or an heterosis effect[4], the proportion of females was significantly different between all groups, being 37.5%, 46.4% and 55.0% in the offspring of wild, domesticated and selected males, respectively. This qualitatively confirmed the predictions done from the analysis of sex ratios in the families of the first experiment, that domesticated fish should have more females, and that this should be further increased by selection for growth. In the same experiment, we could show that the genetic correlation between body length and sex tendency seemed to be maximal at the early juvenile stage (90 dpf) where it could reach 0.77±0.16.

As indirect selection of sex tendency through selection for growth involves interactions between frequency-dependent selection and indirect selection, its outcome cannot be easily predicted in quantitative terms. To this end, I developed a tool in Excel-VBA to perform stochastic simulations of the evolution of growth and sex tendency under selection for growth. This allowed me to demonstrate that selecting the 5% longest fish should result in an increase in the female ratio in the population, reaching a plateau around 80% of females in 8-9 generations. The phenotypic level of the sex ratio plateau is positively influenced by the genetic correlation between body length and sex tendency, the selection intensity on body length and the heritability of body length, but not by the environmental level of masculinisation linked to the hatchery environment.

We will now examine more precisely the consequences of these findings for 1) developing selective breeding programmes for the aquaculture of sea bass and 2) increasing our understanding of the very peculiar sex determination system in this species.

[4] as the males, from Atlantic origin, were mated to Mediterranean females which were the only ones available the year the experiment was performed

## 4.2 A perspective for sea bass domestication and breeding programmes

### 4.2.1 A proof of concept of the use of marker-based parentage assignment

Fish breeding programmes have historically been based on separate rearing of the progenies (for family based breeding programmes) or on individual selection, not using family information. The first option may be very efficient but costly in terms of structures and labour, while the second one may be easier to implement, but limited in terms of precision and most of all in terms of control and optimization of the selection process: the only heritability that can be known ( and only *a posteriori*, if a control line has been kept) is realized heritability of the one trait selected for, and there is a total ignorance of the efficiency or not of the methods used to conserve genetic variability and avoid inbreeding. In addition, selecting for lethal traits is difficult if not impossible in individual selection schemes, and selection on an index combining several traits cannot be optimized when genetic parameters are not known.

In the present work, we have, for the first time in sea bass, used a mating design which is not too far from what a commercial sea bass breeding programme could be, using genotyping of microsatellites to recover parentage. The previous trials using molecular pedigrees with this species had been done on very small family numbers (9 sires*3 dams, Saillant *et al.*, 2006). In other species, larger mating designs had been used (Table 4-1).

**Table 4-1 :** Examples of parentage assignment with microsatellites in farmed fish, for different species, type of mating designs (FF: full factorial, IF: incomplete factorial, NE: nested, MS: mass spawning) with percentage of unambiguous assignment and estimated effective population size, based on two calculations, $N_{e1}$ which takes into account the sex ratio of broodstock and $N_{e2}$ which also incorporates the real numbers of participating broodstock and the disequilibrium in family sizes.

| Species | mating (type) | # full sib families[a] | #markers | % unique assignments | $N_{e1}$[b] | $N_{e2}$[c] | Reference |
|---|---|---|---|---|---|---|---|
| **O.mykiss** | 48 ♂ x 4♀ (FF) | 192 | 10 | 90 % | 14.7 | 14.6 | Chevassus *et al.*, 2002 |
| ***O.mykiss*** | 2 ♂ x 36♀ (FF) | 96 | 14 | 91-95% | 7.6 | 7.4 | Fishback *et al.*, 2002 |
| ***S.salar*** | 78 ♂ x 149♀ (NE) | 149 | 8 | 95.8% | 204.8 | ? | Norris and Cunningham, 2004 |
| ***C.carpio*** | 24 ♂ x 10♀ (FF) | 240 | 7-8 | 95.3% | 28.2 | 22.3 | Vandeputte et al, 2004 |
| ***C.carpio*** | 147 ♂ x 8♀ (FF) | 1176 | 6-11 | 75.7% | 30.3 | 29.7 | Kocour *et al.*, 2007 |
| ***C.carpio*** | 58 ♂ x 58♀ (IF) | 135 (113) | 7 | 96.8% | ? | ? | Ninh *et al.*, 2011 |
| ***H.molitrix*** | 36 ♂ x 36♀ (IF) | 144 | 10 | 96.4% | 72.0 | 60.6 | Gheyas *et al.*, 2009 |
| ***D.labrax*** | 41 ♂ x 8♀ (FF) | 328 (261) | 5-6 | 99.5% | 26.8 | 18.0 | Grima *et al.*, 2010a |
| ***D.labrax*** | 33 ♂ x 23♀ (IF) | 253 (252) | 6 | 99.2% | 54.2 | 52.0 | This study (chap.2) |
| ***D. labrax*** | 76 ♂ x 13♀ (FF) | 988 | 8 | 95.9% | 44.4 | 29.8 | This study (chap.3) |
| ***G.morhua*** | 24 ♂ x 26♀ (MS) | 101 | 4 | 98.6% | 49.9 | ? | Bekkevold *et al.*, 2002 |
| ***G. morhua*** | 30 ♂ x 70♀ (MS) | (523) | 5 | 91.2% | 84.0 | ? | Wesmajervi *et al.*, 2006 |
| ***P.major*** | 250 ♂+♀ (MS) | (87) | 4 | 73.5% | 84.6 | 63.7 | Perez-Enriquez *et al.*, 1999 |
| ***D. labrax*** | 45 ♂ x 58♀ (MS) | | 6 | 59.9% | 101.4 | 3.4 | Chatziplis *et al.*, 2007 |
| ***P.herzensteini*** | 26 ♂ x 35♀ (MS) | | 5 | 92.2% | 59.7 | 15.3 | Kim *et al.*, 2007 |

[a]theoretical number, observed number between brackets

[b]estimated by $4N_mN_f/(N_m+N_f)$, $N_m$=number of male parents, $N_f$=number of female parents

[c]estimated by $4N_{em}N_{ef}/(N_{em}+N_{ef})$,with $N_{em}=(N_mk_m-1)/(k_m-1+V_m/k_m)$ and $N_{ef}=(N_fk_f-1)/(k_f-1+V_f/k_f)$, where $k_m$ ($k_f$) is the average number of offspring per male (female) parent and $V_m$ ($V_f$) is the variance of the number of offspring per male (female) parent (Kimura and Crow, 1963).

In our results as well as in the literature (Table 4-1), it appears that high assignment rates (95%) can be achieved in most cases. In a few cases however, low assignment rates have been achieved. The first reason of this is the insufficient assignment power of the marker set used, which is often

developed with the aim to minimize the number of markers for a given projected assignment power. This can lead to disappointing results, where the observed power can be well below the calculated one (Jerry *et al.*, 2004; Dong *et al.*, 2006; Slabbert *et al.*, 2009; Vandeputte *et al.*, 2011). Population-specific problems like the occurrence of null alleles (Hedgecock *et al.*, 2004), or the use of related parents which share more alleles than unrelated parents (Matson *et al.*, 2008; Villanueva *et al.*, 2002) can also decrease the assignment power. Genotyping errors may also be a problem, but are easily dealt with both by exclusion-based (Christie, 2010; Vandeputte *et al.*, 2011) and maximum-likelihoods based (SanCristobal and Chevalet, 1997) assignment software as long as they are not too many. Last, but not least, missing parents, due to loss of samples over time or delayed collection are an avoidable but potentially serious problem, as exemplified in Chatziplis *et al.* (2007) where the 59.9% single assignment rate comes together with 36.9% unassigned offspring, probably largely due to the fact that 15 parents out of 103 had missing DNA samples.

Apart from parentage assignment success, the ability to have a good representation of families is essential for setting up a breeding programme as it has an impact on the conservation of genetic variability from one generation to another, and also on the value of family information. In the early days of the technique, this was seen as a major drawback of molecular pedigrees when compared to separate rearing of families (Gjerde, 2005).

One way to have a synthetic view of the equilibrium of family sizes is the calculation of effective population size, using a formula that includes the effect of variance in family size ($N_{e2}$ in Table 4-1). As effective population size is extremely sensitive to variance in family size, the comparison with an estimation of $N_e$ which does not include variance in family size ($N_{e1}$ in Table 4-1) is an efficient way to account for the impact of this imbalance on the conservation of genetic variance in a breeding programme. In our results, the difference between the two $N_e$ estimates was -4.1% in our first experiment and -33% in the second one. The main difference between the two was that in experiment 1 the spawn of each females was split to be individually fertilized with several males and incubated separately, while in experiment 2 the eggs of the 8 females were pooled in equal quantities before they were separated in aliquots for fertilization by the individual males. When looking at other experiments summarized in Table 4-1, it appears that the experiment by Grima *et al.*, 2010a, which also used pooling of eggs before fertilization, also exhibited a 33% reduction in $N_e$ when variance of family size is accounted for. Other studies with separate incubation of females in salmonids also show very minor reductions in $N_e$ (Chevassus *et al.*, 2002; Fishback *et al.*, 2002). The source of such variations is probably linked to differential fertilization rates between females, which can be compensated when eggs are mixed by equal volume once unfertilized eggs have been eliminated - which isnot possible when eggs are pooled before fertilization. However, this is not a general case, as in common carp one experiment with pooling of eggs prior to fertilization gave a significant $N_e$ reduction (-21% in Vandeputte *et al.*, 2004) while another did not (-2.0% in Kocour *et al.*, 2007).

When comparing these figures obtained by artificial fertilization under a controlled mating scheme to figures obtained in mass spawnings, it anyway appears that even mixing of spawns prior to fertilization is better than the disequilibrium incurred when collecting mass-spawned eggs ($N_e$ reductions of -24.8% in red sea bream, Perez-Enriquez *et al.*, 1999; -74.4% in brown sole, Kim *et al.*, 2007; and even -96.6% in European sea bass, Chatziplis *et al.*, 2007 - see Table 4-1). All in all, it appears that combining artificial fertilization with parentage assignment seems an efficient way to

provide a usable family structure for fish breeding. If it is anticipated that the number of well-represented families is to some extent below the expected number, this can easily be corrected by producing more families, as the number of families is not constrained by the number of rearing structures, as is the case when separate rearing is used. For a given offspring sample size, precision will always be lower with unequal family sizes, but using more families may also have a positive impact on the genetic gain through higher between family selection intensity, so the final result is uncertain and will require further modelling to take appropriate decisions. Increasing the number of families ultimately will also decrease the assignment power of the marker set used, but this effect is expected to be very small if the assignment power is high enough. Using the formulae of Villanueva *et al.* (2002), it can be estimated that a set of 10 loci with 5 equally frequent alleles would have a combined exclusion probability of 0.9999996177, resulting in a theoretical assignment rate of 99.6% in a 100*100 factorial mating, and the same marker set would still assign 98.5% of a progeny in a 200*200 mating, with twice as many parents and four times more potential families. Thus, when the assignment power is high enough, the number of families (within a reasonable range) will have very little impact if any on the efficiency of parentage assignment.

A decisive advantage of parentage assignment is that the "common garden" methodology allows to avoid any bias in genetic parameters linked to tank effects common to full-sibs, which can be high in some cases in fish, as highlighted before (section 1.3.2, p.5). A potential drawback is that as fish are reared together from hatching, maternal effects linked to egg size or egg quality may give initial advantages to some offspring, which may then persist over time. In the present study, although they were not significant, maternal effects could be high at some times: $m^2$ was estimated to be 0.19±0.09 for body weight at slaughter in Portugal although the estimate for all data at slaughter was only 0.06±0.05 (section 0). Still, this seemed rather dataset dependent, as this was estimated with a reduced dataset (without deformed fish) while with the full data set the same estimates reduced to 0.09±0.07 and 0.04±0.04 (section 0). Nevertheless, when separate rearing is used, maternal effects are known to fade over time in salmonids (Gall, 1974; Mckay *et al.*, 1986a; Mckay *et al.*, 1986b; Blanc, 2002) while they may persist in mixed rearing (Blanc, 2002). In mixed rearing trials, maternal effects have been shown to be less than 0.04 in common carp (Vandeputte *et al.*, 2004; Ninh *et al.*, 2011) but more data is needed to draw a general conclusion on this subject.

Another advantage of parentage assignment is that it allows, without investment in rearing structures, to obtain precise estimates of genetic parameters (heritabilities, genetic correlations), as exemplified in Chapter 2. Moreover, these genetic parameters can be obtained in industry conditions, as the fish are reared in a single batch that can be reared as any production batch (with the exception that it should not be sorted by body size to avoid underestimation of heritability - Blonk *et al.*, 2010). As there is no investment in structures, there is no need to start designing a breeding programme before the genetic parameters are known, and it is quite commonsense that the options for the design of breeding programmes may to a large extent depend on the estimates of genetic parameters (*e.g.*, using family information is highly valuable when heritability is small, but may be much less relevant when it is moderate to high - Falconer and Mackay, 1996). Once the genetic parameters are known, all options (mass selection - without family information, family based selection with separate rearing or genotyping) remain open to practically establish a breeding programme, as they are not constrained by the initial methodology chosen.

Experimentally, genotyping of progenies can also be extremely useful to compare the performances of several genotypes (in our case wild, domesticated and selected - see Chapter 3) in a common environment. In fish, common environment or "tank effect" can be very high, and this may require a high number of replicate tanks to achieve enough experimental power in comparing various genotypes (e.g. Vandeputte *et al.*, 2002a). Then, communal testing, where all genotypes are physically tagged and mixed in the same rearing structure has long been advocated as a way to avoid common environment effect and to increase statistical power to detect differences between genotypes (Wohlfarth and Moav, 1985). However, "classical" communal testing is flawed by the necessity to separately rear fish before they can be physically tagged (at least 3 g body weight - Navarro *et al.*, 2006, most of the time 10-20g). The differences in growth established during this initial separate rearing phase, where genetic and environmental effects cannot easily be disentangled except with replication of rearing structures, may or may not impact growth in the communal rearing phase. In common carp, the multiply nursed samples method has been developed in Israel to overcome this problem, but it remains difficult to set up in practice (Wohlfarth and Moav, 1972), as it involves creating artificial environmental divergences in an homogeneous genetic group during the separate rearing phase, and then mixing these "multiply nursed samples" with the tested groups to regress gains on initial environmental differences . Solving this question is quite straightforward when the pedigree is recovered by genotyping, as all fish can be mixed from the first stages (hatching, or even egg stage).

However, as shown in Chapter 3, whatever the method chosen, the remaining problem, is that in communal rearing, competition may magnify differences in size between groups with different genetic potential for growth. This was also shown in other species such as rainbow trout (Blanc and Poisson, 2003), gilthead sea bream (Knibb *et al.*, 1997) and common carp (Moav and Wohlfarth, 1974).

The main drawback of parentage assignment with markers remains its cost (4-15€per individual fish), and a precise assessment of its economic value will be needed in each situation. Such evaluations have seldom been published, but when they have, they showed that parentage assignment compared well to separate rearing on the economic side (Ninh *et al.*, 2011). It is expected that Single Nucleotide Polymorphisms (SNP) genotyping would permit genotyping large samples of fish at a moderate cost in the future. The advantage of SNPs is their adaptability to high throughput automated genotyping methods and their low genotyping error rate, but more SNPs that microsatellites are needed to provide efficient parentage assignment (Hauser *et al.*, 2011). However, in early 2012, there are still no routinely implemented SNP genotyping methods that outperform the cost/benefit ratio of microsatellite-based parentage assignment.

Our results show that genotyping of progenies is an efficient tool to estimate genetic parameters and to conduct response to selection experiments in sea bass - although for this last use estimates of differences between genotypes may be biased upwards. Moreover, we showed that the technique was practical even in relatively large mating designs ($N_e$≈50), which could be subsets of a real size breeding programme for the species with $N_e$=100-200 as classically recommended to keep inbreeding to a level not impacting population fitness (Meuwissen and Woolliams, 1994).

### 4.2.2 Selection for increased body weight

Body weight has always been and remains the main trait selected for in fish breeding programmes (Gjedrem and Thodesen, 2005). However, as breeding programmes develop on a given species, the tendency is to decrease the part of body weight in the selection index, to the benefit of quality and fitness (disease resistance) traits. Examples can be seen at http://aquagen.no for the breeding goals of Aquagen in Atlantic salmon and in Vandeputte *et al.* (2009a) for the French rainbow trout breeding system. However, the benefits of selection for body weight are not as straightforward as it seems, as faster growing fish will also eat more - so the increased output is not for free!

One of the "objective" reasons to select for growth is the supposed correlated increase in feed efficiency, as has been observed in land animals where fast growers tend to direct a higher proportion of their energy budget to growth rather than to maintenance (*e.g.* Arthur *et al.*, 2001 in young Charolais cattle). When simulating the economic gains of selection for growth in fish, it appears that a major parameter influencing profitability is the correlated response in feed efficiency (Ponzoni *et al.*, 2007; Ponzoni *et al.*, 2008). However though some results highlight a positive impact of selection for growth on feed efficiency in fish (Thodesen *et al.*, 1999; Kause *et al.*, 2006; Quinton *et al.*, 2007), it is not always the case (Sanchez *et al.*, 2001; Ogata *et al.*, 2002; Mambrini *et al.*, 2004). Then, the interest of selecting for body weight if no improvement in feed efficiency would occur would be questionable.

Another way to see the question would be to consider that body weight is an easily recorded trait, which quite generally has a moderate to high heritability (0.20 to 0.50 in most cases, see review in Gjedrem and Olesen, 2005) and a high phenotypic variance, making it suitable for individual selection with high, visible genetic gains (10-30% per generation, reviewed by Gjedrem and Thodesen, 2005). In a given species then, it can be considered that selective breeding first has to prove its efficiency to farmers through increased weight gains (as was done in the Atlantic salmon in Norway, Gjedrem, 2010), while more directly profitable - but more difficult to select- traits (disease resistance, quality, processing yields) will only be selected later on, as seen before. Whatever the real benefits of selection for growth, breeding programmes are now in their early generations in sea bass, and growth rate clearly remains an important breeding goal for the sea bass breeding companies.

Our results show that the heritability of growth traits in sea bass is moderate to high, with many variations depending on the trait, age of the fish, ongrowing site and statistical model to analyze data: the highest estimate we obtained was $h^2$=0.70±0.09 for tagging weight (at 1 year, 35g mean weight) in a model without maternal effects, while the lowest was $h^2$=0.12±0.04 for growth rate (DGC) across all sites in a model with maternal effect (section 0). For designing a breeding programme, it is then clear that choices regarding the estimates used will have important consequences on the estimation of potential gains.

First of all, we have to choose between heritability estimates obtained from models with or without maternal effects. As we saw that maternal effects were never significant, it would be tempting to use estimates of heritability obtained without maternal effects, which are higher. However, if maternal effects are existent but small (less than 0.05) they could noticeably bias heritability estimates, although not statistically significant. Therefore, we will rather use conservative estimates obtained from models integrating maternal effects. When maternal effects are large, technical ways to overcome them by separately rearing the offspring from different female groups with similar egg size

have been proposed in trout, in the PROSPER methodology for individual selection (Chevassus *et al.*, 2004, Haffray *et al.*, in press). However, using such a method is beneficial only if maternal effects are relatively large ($m^2$>0.10), which is obviously not the case here, and moreover requires a measurable predictor of maternal effects. Such a predictor is available in salmonids, as egg size has been shown to explain most of the maternal effects, at least in the early stages (Blanc, 2002; Vandeputte *et al.*, 2002b), while in sea bass we have no idea of a predictor which could be egg size but maybe also any parameter linked to egg quality. It is then neither advisable nor efficient to take into account maternal effects in a sea bass breeding programme, apart from using conservative heritability estimates obtained with maternal effects included in the model. To increase growth, there are basically three traits that can be chosen: body weight, body length, and growth rate (as $DGC = (FBW^{1/3} - IBW^{1/3})/t$). Each of them has its pros and cons:

- Body weight, and especially body weight at harvest, is the trait chosen in many breeding programmes, as the weight of fish sold is directly linked to the economic turnover of the farms. When reviewing the aquaculture genetics literature, it is by far the most studied trait, and the most straightforward to use. One of its disadvantages is that it is generally positively genetically correlated with the condition coefficient *K* - and this is the case in sea bass with genetic correlations in the range 0.23-0.35, except in one site - thus leading to a correlated increase in *K* with the increase in body weight. As in many cases consumers prefer thin "wild-like" shape, this can be seen as a negative consequence of selection for body weight.

- Body length can be used as an indirect predictor of body weight, as the genetic correlation between both traits is very high (0.91 to 0.96), so it can be almost as efficient as direct selection for increasing body weight, but with a null to negative genetic correlation with *K* (-0.32 to +0.05 in our data), thus selecting thinner fish. We have proven the efficiency of the first generation of selection on body length in section 3.1, with a conservative estimate of the gain (in separate tanks) of 23% for one generation of selection with a proportion selected of 5% on body length. Other selection experiments have shown the ability to efficiently select for increased body weight using body length as a predictor: +21.5% in weight per generation in four generations of brown trout, with a correlated decrease in *K* (-9.6%, Chevassus *et al.*, 2004). The advantage of using body length is also that it is extremely practical and fast to perform individual selection of fish above a given threshold, using a simple ruler.

- Growth rate, measured as DGC, does not seem to be a direct economic trait, however it also has its interests. By construction, growth rate is linked to final body weight, so increasing growth rate will doubtlessly increase final body weight. If we consider an integrated system where selection and ongrowing are done by the same company, harvest weight or growth rate from hatching to slaughter describe the same phenomenon, and seem to be of equal interest. However, in fish farming, breeding companies may be only hatcheries whose commercial product is juveniles, which are usually sold to ongrowing farms at 2-20 g mean weight in marine fish. For the buyer, it makes a big difference if the gain in growth is done before or after the moment when he buys the fish. For him, the important parameter will be the growth rate from fingerling to harvest size, which may be poorly predicted by final body weight, or even worse by fingerling weight at the hatchery stage (section 2.3). In this case, if the objective of a hatchery is to satisfy its customers, growth rate during the ongrowing

phase will be the trait to improve. This does not fully hold for hatcheries selling selected eggs and larvae, for which selection for final body weight may be appropriate, as the whole of the growth cycle is performed in the customer's installations.

As in most cases any of those growth traits have a moderate to high heritability ($h^2$>0.25), if growth is the only trait considered, the most economical solution for selection will be individual selection, as family information will bring in only limited additional precision, but a much higher cost due to genotyping and individual tagging of the fish, while tagging will not be necessary for individual selection on harvest weight or length. Individual selection for DGC however implies using repeated growth measurements on individual fish, thus making individual tagging of selection candidates necessary.

For individual tagging, the use of RFID glass tags is technically operational, as demonstrated in our experiments and other ones with other species (*e.g.* Navarro *et al.*, 2006 in sea bream). In practical farm conditions however, as tagged fish are not externally distinguishable from untagged fish, it might be difficult to use if there is a risk of mixing with fish that have to be sold for human consumption. Using tagged fish implies that selection candidates are reared separately from production fish, which differs from strategies where it was suggested that selection could be done on normal production batches (see the "walk-back" selection concept by Doyle and Herbinger, 1995).

In Table 4-2, we give estimates of selection response for growth traits in one site, using genetic parameters estimated in the Ifremer Palavas experimental facility from our heritability estimation experiment (chapter 0). We calculated the direct selection response using the breeder's equation $\Delta G = ih^2\sigma_P$ (Falconer and Mackay, 1996) for individual selection, where $\Delta G$ is the genetic gain per generation, $i$ is the standardised selection intensity ($i$=1.75 for 10% selected, 2.06 for 5% selected and 2.66 for 1% selected), $h^2$ is the heritability of the trait and $\sigma_P$ is the phenotypic standard deviation of the trait. When trait #1 (e.g. body length) was selected to obtain indirect response on trait #2 (e.g. slaughter weight), the following equation was used: $\Delta G_2 = i\sqrt{h^2_1 h^2_2} r_{A(1,2)}\sigma_{P2}$ (Falconer and Mackay, 1996), where $r_{A(1,2)}$ is the genetic correlation between trait 1 and trait 2. In all cases, $\Delta G$ was standardized by the mean of the trait and expressed as percent gain per generation.

**Table 4-2:** Estimated selection response (% of the mean per generation) for slaughter weight and DGC from 370 dpf to slaughter in sea bass in Ifremer Palavas conditions, for different traits and proportions selected.

| Selected trait | proportion selected | selection response slaughter weight | Selection response DGC |
|---|---|---|---|
| **BW at 370 dpf** | 10% | 32% | 3.7% |
| | 5% | 37% | 4.4% |
| | 1% | 48% | 5.7% |
| **BL at 370 dpf** | 10% | 30% | 3.6% |
| | 5% | 36% | 4.2% |
| | 1% | 46% | 5.5% |
| **BW at slaughter** | 10% | 29% | 4.7% |
| | 5% | 34% | 5.5% |
| | 1% | 44% | 7.1% |
| **BL at slaughter** | 10% | 23% | 3.8% |
| | 5% | 27% | 4.5% |
| | 1% | 35% | 5.8% |
| **DGC 370dpf-slaughter** | 10% | 15% | 3.9% |
| | 5% | 18% | 4.5% |
| | 1% | 23% | 5.9% |

Estimated selection responses for slaughter weight are high in general, and the best selection criterion appears to be BW at 370 dpf, meaning that due to its high heritability (0.61), it is more efficient than direct selection on slaughter weight. Even BL at 370

dpf (with the above mentioned advantage to also select thinner fish) is more efficient than direct selection on slaughter weight. The worst criterion is DGC, which yields approximately half the gain on BW compared to other selection criteria - and this is linked to its rather low heritability (0.19). In addition, we can see that the response in BW at slaughter when BL at slaughter is used as a selection criteria is estimated at 27%, while we observed 23% in our selection response experiment which was done in this way (Chapter 3) - thus confirming the reliability of these estimates.

If the objective is to increase DGC, the best trait to select for is by far slaughter weight. Direct selection on DGC or indirect selection for BL at slaughter or BL and BW at 370 dpf are all roughly equivalent in terms of gain. It should be noted that estimated gains in DGC are much smaller (3-7% per generation) than gains in body weight (15-48% per generation), due to both its low heritability and its relatively low coefficient of variation (11%, *vs.* 37% for BW at slaughter).

In our selection response experiment, the DGC of selected (M,P) and unselected (W,D) fish differed in the period 268-338 dpf (1.14±0.02 for selected vs. 1.04±0.02 for unselected, $P<0.01$) but were the same (0.780±0.018 for both groups, $P=0.97$) in the period 338-611 dpf, confirming the lower response on DGC after the first year, although selection was performed at 714 dpf in this experiment. This shows that the important weight gain we observed was mostly a consequence of early growth, although in this case negative interactions with rearing density could also partly explain the lack of response for late growth rate (section 3.1.4.2).

From a technical point of view, our results also raise a question about the relevance of DGC to study growth rate in sea bass. DGC has been developed in salmonids based on the observation that under optimal growing conditions, the cubic root of body weight was a linear function of time in rainbow trout (Iwama and Tautz, 1981; Bureau *et al.*, 2000). The consequence is that $DGC = (FBW^{1/3} - IBW^{1/3})/t$ is constant over time $t$ (and by extension independent of fish size). In sea bass however, we can see that DGC decreases with time in our selection response experiment (Figure 3-1, p56). Taylor series expansion of the calculation of weight gain as a function of DGC, time and IBW shows that variations in weight gains should be proportional to variations in DGC if DGC is constant over time. Therefore, it should be expected that the CV of DGC would be the same as the CV of weight gain (or of FBW if we consider the growth period from hatching to slaughter). The observation we do that the CV of DGC is approximately one third of the CV of body weight is therefore not expected. It could be the consequence of the decrease of DGC over time (or with body size), which would tend to mechanically underestimate growth rate in large fish while overestimating it in small fish. This reduced variability could then explain to some extent the parallel limitation of heritability estimates of DGC (0.24 on average), which are lower than estimates of heritability of BW (0.39 on average) in our data. Such difference is not seen in rainbow trout, where heritability of thermal growth coefficient (TGC, a temperature-adjusted DGC) between 43 and 440g mean weight is more similar to that of BW (0.46 *vs.* 0.61, Le Boucher *et al.*, 2011). It may be necessary to develop new formulae for DGC in sea bass, as has been done in rainbow trout where it appeared that the exponents applied to weight were rather different depending on the mean weight of the fish, although stable inside relatively large intervals ($BW^{0.21}$ from 0.2 to 20g, $BW^{0.33}$ from 20 to 500 g, $BW^{0.97}$ for BW>500g, Dumas *et al.*, 2007).

The simplest trait to select for then remains BW at slaughter, but BW or BL at 370 dpf may also be considered as they would decrease the cost of selection by avoiding the rearing of a large quantity of

selection candidates until slaughter. Precise economic evaluation has to be done, but rearing 20000 fish up from zero to 35g (700 kg biomass) to apply a 1% selection pressure on BL at 370 dpf would give the same gain in DGC and a higher gain on BW than rearing 4000 fish from 0 to 400g (1600 kg biomass) to apply a 5% selection pressure on BW at slaughter.

These calculations are valid within one site, but an important question remaining is the definition of a strategy when the selection environment differs from the production environment, as we saw that GxE interactions could be high in some cases.

### 4.2.3 Genotype by environment interactions for growth: which consequences?

In our genetic parameters estimation experiment, GxE interactions were limited for BW at slaughter ($r_A$ between different sites in the range 0.70-0.97), but rather high for DGC ($r_A$=0.21-0.78). When genetic correlations were estimated between initial growth (BW at 370 dpf) and DGC in different sites, negative correlations even appeared (-0.32 with Portugal). As sea bass hatcheries (and breeding programmes) are rather concentrated in a few places (Aquabreeding, 2009), it is important to figure out whether the genetic gains obtained could profit the whole ongrowing industry, and to propose ways to optimize them.

We will first estimate the selection response for a 5% selection pressure on body weight in one environment on final body weight in another environment, as well as on DGC in another environment. The correlated effects of selection for DGC or initial body weight (IBW) on DGC and BW will also be examined. All these estimates are presented in Table 4-3 below

**Table 4-3:** Predicted selection response(in % of the initial mean per generation) for final body weight (FBW) or DGC in one environment (columns) when initial body weight (IBW), FBW or DGC are selected for in another environment (rows).

**a)** Selection on FBW-response on FBW

| | FBW Israel | FBW Palavas | FBW Italy | FBW Portugal |
|---|---|---|---|---|
| **FBW Israel** | 16% | 17% | 17% | 11% |
| **FBW Palavas** | 16% | 24% | 18% | 14% |
| **FBW Italy** | 17% | 19% | 22% | 14% |
| **FBW Portugal** | 15% | 20% | 18% | 18% |

**b)** Selection on DGC-response on FBW

| | FBW Israel | FBW Palavas | FBW Italy | FBW Portugal |
|---|---|---|---|---|
| **DGC Israel** | 8.2% | 2.6% | 7.9% | 4.3% |
| **DGC Palavas** | 8.5% | 12.8% | 11.0% | 8.2% |
| **DGC Italy** | 8.4% | 4.1% | 12.6% | 5.8% |
| **DGC Portugal** | 3.7% | 0.4% | 7.3% | 7.5% |

**c)** Selection on FBW-response on DGC

| | DGC Israel | DGC Palavas | DGC Italy | DGC Portugal |
|---|---|---|---|---|
| **FBW Israel** | 4.0% | 3.7% | 4.2% | 1.2% |
| **FBW Palavas** | 1.2% | 5.4% | 2.0% | 0.1% |
| **FBW Italy** | 3.8% | 4.9% | 6.3% | 2.4% |
| **FBW Portugal** | 2.8% | 4.9% | 3.9% | 3.3% |

**d)** Selection on DGC-response on DGC

| | DGC Israel | DGC Palavas | DGC Italy | DGC Portugal |
|---|---|---|---|---|
| **DGC Israel** | 4.2% | 1.4% | 4.3% | 1.8% |
| **DGC Palavas** | 1.6% | 4.5% | 2.3% | 1.0% |
| **DGC Italy** | 4.2% | 2.1% | 7.0% | 3.3% |
| **DGC Portugal** | 2.6% | 1.3% | 5.0% | 6.2% |

**e)** Selection on IBW-response on FBW

| | FBW Israel | FBW Palavas | FBW Italy | FBW Portugal |
|---|---|---|---|---|
| **IBW(Palavas)** | 19.1% | 27.0% | 22.7% | 17.8% |

**f)** Selection on IBW-response on DGC

| | DGC Israel | DGC Palavas | DGC Italy | DGC Portugal |
|---|---|---|---|---|
| **IBW(Palavas)** | -1.3% | 4.4% | 0.0% | -2.5% |

Again, selection on FBW or IBW seems the most efficient to increase FBW. Selection response for FBW can decrease by a maximum of 42% when compared to selection response in the selection environment (14% for response in Portugal when selected in Palavas, *vs.* 24% for response in Palavas - Table 4-3a), but it is also striking to see that the predicted response on FBW when selecting for IBW in Palavas (Table 4-3e) is better than or equivalent to direct selection for FBW in the production environment (Table 4-3a). This may be indicative of the fact that due to late tagging at 35g mean weight, FBW is to a large extent a consequence of IBW, as hypothesized before.

Selection on DGC always leads to limited responses in DGC (1.0-7.0% per generation, Table 4-3d), and in all cases but one selection response on the selection site is better than response on an alternative

site. The same is observed when selection on DGC is used to obtain gains in FBW (Table 4-3b). In this case, although response can be close to zero (FBW reponse in Portugal to DGC selection in Palavas), it is always positive. This is not the case anymore when IBW (in Palavas) is used as a criterion to improve DGC. In this case, response is positive only in Palavas, while it is zero in Italy and negative in Israel and Portugal).

As discussed before, there are good reasons to think that DGC may not be an appropriate measurement of individual growth rate in sea bass, which may then affect its genetic and phenotypic variability estimates. Nevertheless, it has the advantage to measure growth rate on the ongrowing site, and to allow its study by removing at least part of the initial history of the fish. Genotype by environment interactions seem to be rather large and they could be even larger if fish had been dispatched at an earlier age. Had we tagged and dispatched fish at 3-4 g mean weight (a situation which better represents hatchery practices), they would have had the opportunity to multiply their body weight by 100 in the production environment, instead of 10 in the present experiment. In this case, it can be foreseen that GxE interactions could have been even larger. Conversely, we also have to take into account that we purposely chose highly divergent ongrowing environments, in order to maximize GxE interactions, as we anticipated that they would be limited based on the existing aquaculture literature. For further progress on this subject, it might be appropriate to set up a typology of rearing conditions in sea bass (temperature, water quality, structures, management) in order to be able to test sea bass families in environment really representative of the major rearing systems, and evaluate GxE interactions in these conditions.

We can conclude that in sea bass, genotype by environment interactions are large, and have to be taken into account in breeding programmes if some of the production environments strongly differ from the selection environment. The way to take into account GxE interactions can be at the extreme to implement separate breeding programmes for different environments, but this also implies a multiplication of the costs. An alternative way is to evaluate fish in several environments, and select based on an index aimed at maximizing gains in all environments. This is what has been done in the GIFT selection programme in Nile Tilapia, where families were split in several test environments to evaluate their growth performance, as some genetic correlations between environments (especially between cage and pond environment) were very low (0 to 0.4, Eknath and Acosta, 1998). In the Norwegian Atlantic salmon breeding programme, families were also sent to several test stations representative of different test environments. Interestingly in this case, GxE interactions for growth rate were small, and the main purpose was to take into account GxE interactions for age at first maturation, which were rather important (Gjedrem, 2010). It is important to note that these strategies which imply sending and recording family samples imply the use of family information, so an extra cost compared to individual selection, which was the option we first considered due the relatively high level of heritability for BW. If individual selection is to be used, then it has to be done in an environment as close as possible to the possible ongrowing environments.

Nevertheless, decisions concerning the need to implement one or several breeding programmes depend on complex economic calculations. In an estimation of the effects of GxE interactions on BW, survival and feed intake in common carp in Vietnam, it was concluded that a breeding programme was industry-wise profitable even with genetic correlation of traits between environments equal to 0.5 (Ponzoni *et al.*, 2008). However, in this case (but not when $r_A$ was 0.7 or more), it was more profitable to run two breeding programmes instead of one. One limitation is that such calculations

are done at the whole industry level. In Europe, breeding programmes are commercial operations which are paid for by private companies and must be profitable. Then optimal structures and decision-making processes are probably quite different.

### 4.2.4 Practical consequences of genetic variation for sex ratio

One peculiarity of sea bass is that the sex ratio of farmed populations is usually highly biased towards males, influenced by early rearing temperature (review by Navarro-Martin *et al.*, 2009b), but genetically variable and subject to frequency-dependent selection (sections 2.4 and 3.2.2). In addition, females are known to be larger than males of the same age, at least in farming conditions (Saillant *et al.*, 2001b). This body of observations will have important practical consequences for setting up breeding programmes in sea bass.

The mechanism of frequency-dependent selection will drive sex ratios towards 50-50 in all cases where direct selection on sex ratio or a correlated trait (growth) is not applied. In section 2.4.1, we used the polygenic threshold model to predict the evolution of sex ratio in farmed populations of sea bass by frequency-dependent selection. The model for frequency-dependent selection can very easily be derived from the equations presented in section 2.4.2.1 (p. 46). As both males and females are needed to produce offspring, the average sex tendency of the parents in a population of mean sex tendency $t_0$ will be:

$$(t_f + t_m)/2 = t_0 + \varphi(t_0)\frac{1-2P_f}{2P_f(1-P_f)}$$

(Equation 4-1)

and this will automatically create a selection differential between the mean of the parents and the mean of the population they are sampled from ($t_0$) equal to:

$$\Delta_S = \varphi(t_0)\frac{1-2P_f}{2P_f(1-P_f)} \qquad \text{(Equation 4-2)}$$

In a bisexual population, $0 < P_f < 1$, and $P_f(1-P_f)$ is always strictly positive. As $\varphi(t_0)$ is also always

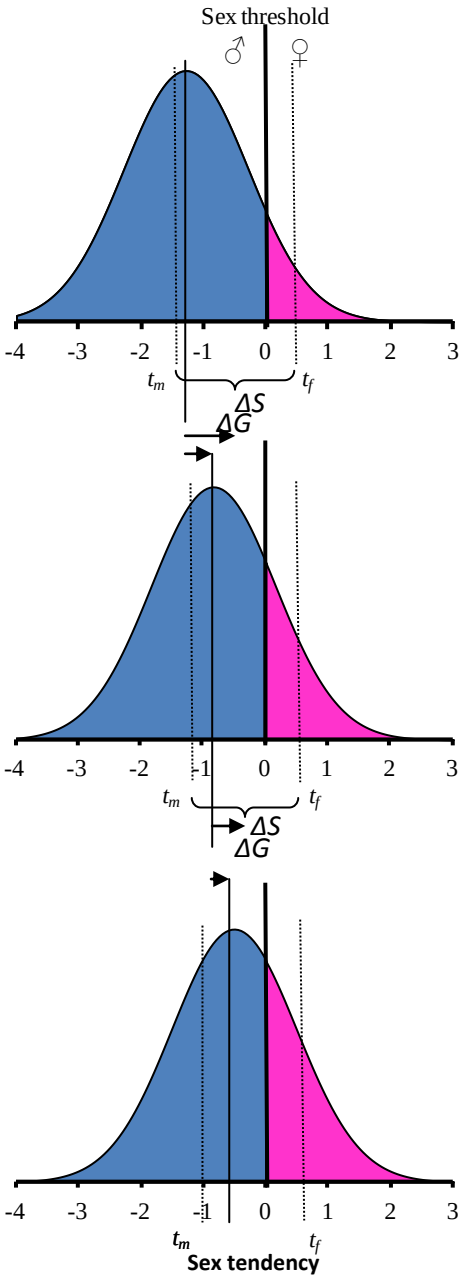


**Figure 4-1 :** Frequency-dependent selection in three successive generations of sea bass, starting with 10% females. $t_m$= mean sex tendency of males, $t_f$ = mean sex tendency of females. The selection differential $\Delta S$ is the difference between $(t_m+t_f)/2$ an the mean sex tendency of the population. $\Delta G=h^2\Delta S$ is the genetic gain in sex tendency at each generation.

strictly positive, the sign of the selection differential will depend on the sign of 1-2$P_f$, and then be positive when $P_f$<0.5, negative when $P_f$>0.5 and zero when $P_f$=0.5. The response to selection will be $\Delta G = h^2 \Delta S$, and thus selection will always tend to move the population mean towards an even sex ratio, as shown in Figure 4-1.

In case of selection for growth, the correlated response on sex tendency may increase the proportion of females over 50%, up to 70-90% (section 3.3).

Concerning the sensitivity to rearing temperature, we can consider sex ratios as rather unpredictable. This is seen in practice, and confirmed by experimental data where replicates of temperature treatments highly vary in terms of observed sex ratio. In the experiments of Navarro-Martin *et al.* ( 2009b), for example, the treatment with 64 dpf below 17°C led to female proportions varying between 21.7 and 90.0% among four replicate batches.

Sex ratio will then change due to frequency-dependent selection and eventually selection for growth, but also vary among batches of fish due to environmental variability. One consequence of this is that a sea bass breeder will never be able to rely on equal numbers of males and females in the population. As long as the population of breeding candidates is large enough, this should not lead to difficulties in obtaining sufficient numbers of males and females to propagate the next generation. However, one consequence is that selection intensity on any trait will not be similar in males and females, as the number of selection candidates of each sex will not be the same. Then, selection intensity will be the mean of selection intensity on males and females, but not the usual estimate of selection intensity estimated by the global proportion selected. The ratio of this mean selection intensity ($i_m$+$i_f$)/2 to the global selection intensity $i_t$, under the constraint that an equal number of males and females are kept as broodstock at each generation, is plotted in Figure 4-2. The ratio equals 1 when the proportion of females is 0.5, and decreases when it departs from 0.5. The decrease is larger for low selection intensities, but in most cases remains below 20% for values of $P_f$ between 10 and 90%, and below 10% for values of Pf between 20 and 80%. Then, the efficiency of selection for growth (or any other trait) will be somewhat lower in the beginning of the selection process, when populations of sea bass are mostly male, and increase as frequency-dependent selection for sex ratio proceeds. If selection for growth increases proportion of females beyond 50% as a result of correlated selection response on sex tendency (section 3.3), the efficiency of selection for growth and other traits will again decrease to a certain amount.

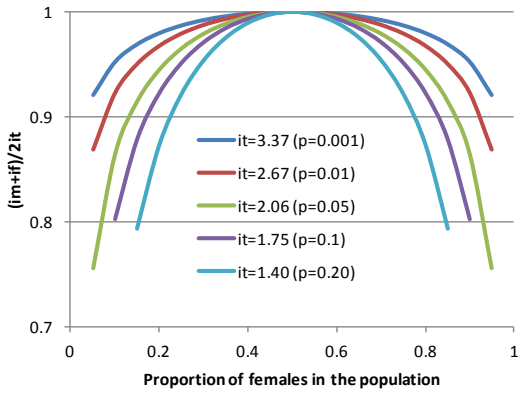


**Figure 4-2:** Ratio of the average selection intensity in males and females ($i_m$+$i_f$)/2 to the global selection intensity $i_t$ as a function of proportion of females in the population of breeding candidates. *p* is the global proportion of candidates selected as broodstock for the next generation.

A last important point to consider is sex dimorphism for body size, with females larger than males (Saillant *et al.*, 2001b). Male and female sea bass cannot be externally distinguished before maturity, and it seems reasonable to select for growth in non-maturing conditions, as maturation may interfere with growth and then lower the heritability of growth traits, as seen in the rainbow trout

(Dupont-Nivet *et al.*, 2010a). However, the uncertainty of sex ratio, linked to the poor repeatability of environmental control with temperature and to the evolution towards females due to selection will lead to very different proportions of males and females in the top growers of a population. A practical demonstration of that can be seen in Figure 4-3. In the Italian batch of our genetic parameters estimation experiment, there was a total of 17% females. When we simulate the phenotypic selection of the 10% largest fish on this population, we end up with 35% females and 65% males in the selected fish (Figure 4-3 a). In the massal group (separate rearing) of our response to selection experiment, we reached 50% females (section 3.2.2). In this case, if we simulate the selection of the 10% largest fish, the proportion of females in the selected batch reaches 90% (Figure 4-3 b). It is not difficult to figure out that the 10 % largest fish in a population with 80% females (which should happen after 7-8 generations of selection for growth, see 3.3) may comprise very few males if any.

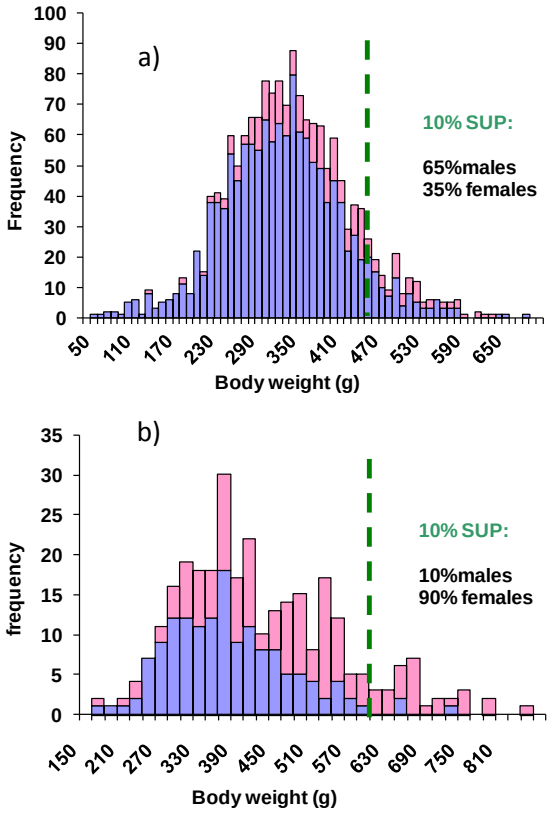


**Figure 4-3:** Proportions of males (blue) and females (pink) in size classes of two sea bass populations: a) Italian batch of our genetic parameters estimation experiment (17% females) and b) massal group of our response to selection experiment (50% females)

The solution to this problem is not straightforward. The first option is to find a way to distinguish males and females before maturation. Differences in external shape have been demonstrated to exist between males and females (Coban *et al.*, 2011). However, translation of such differences in diagnostic tools have failed in some cases (Saillant, pers. comm) while in other cases the use of geometric morphometrics seems to give promising results (Costa, pers. comm.). Differential expression of several genes between males and females has also been established (Blazquez and Piferrer, 2004; Blazquez *et al.*, 2008; Blazquez *et al.*, 2009; Deloffre *et al.*, 2009; Navarro-Martin *et al.*, 2009c; Blazquez *et al.*, 2011), but these molecular techniques are more directed towards sexing (and understanding the sex determination cascade) during or before the early stages of differentiation, and anyway require the sacrifice of sexed animals as brain or gonad samples are the base material of these studies. Until today then, only lethal measurements allow a reliable discrimination of male and female sea bass, through dissection and squash of gonads (Menu *et al.*, 2005). A promising method to sex fish is ultrasound tomography, which has been successfully used in many species (reviewed in Newman *et al.*, 2008), although in striped bass *Morone saxatilis,* a species taxonomically and morphologically close to European sea bass, only adult (age V) male and female could be successfully sexed (Blythe *et al.*, 1994) with high accuracy, while juveniles could not. In European sea bass, preliminary sexing trials with that method were not successful at 150g mean weight (Saillant, pers comm.).

Another possible option to sex fish would be to try to identify a few major sex QTLs, that would allow a prediction of the sex tendency of an animal, and thus of its likelihood to be male or female. If combined with a parentage assignment suite, the use of such markers could be done at a minor marginal cost. However, for the time being, this remains purely speculative as we do not know how many markers would be needed to reliably predict the sex tendency of an individual. Moreover, such markers would not take into account the micro-environmental variation in sex tendency, which is rather important (approximately half of the phenotypic variance).

Until early sexing methods become available, one practical solution to consider is to raise the fish in conditions where they can mature (natural photoperiod and temperature) and sex males by stripping of sperm in the reproduction season. In natural photoperiod, a certain proportion of the males can precociously mature at 1 year (5.3% in Rodriguez *et al.*, 2001, 21.9% in Begtashi *et al.*, 2004, 67% in Navarro-Martin *et al.*, 2009a), while virtually all males mature at 2 years (Rodriguez *et al.*, 2001). As it seems clear that precocious males are larger than later maturing males around one year of age (Begtashi *et al.*, 2004), it can be foreseen that letting maturation proceed probably would perturb the expression of growth, and possibly its heritability, as seen in rainbow trout (Dupont-Nivet *et al.*, 2010a). Moreover, precocious maturation appears at one year of age (100-150 g mean weight), well before commercial size, thus preventing accurate selection of fish on the trait of interest (growth at harvest size). A good alternative is probably to rear fish under controlled photoperiod with long or even continuous days in the first year (thus avoiding precocious maturation - Begtashi *et al.*, 2004), while coming back to natural photoperiod in the second year to allow maturation. In this way, growth should only be marginally perturbed by maturation, and if fish are individually tagged, it would be possible to know which ones are males at 2 years of age by stripping, and then select them on the basis of their growth performance earlier on. In any case however, this strategy has a cost as it requires individual tagging of fish, repeated recording of growth and keeping of all selection candidates alive until their second year, in rearing conditions where artificial lighting is possible. One additional question could be the relevance of selecting fish on their growth rate before maturation, while presently sea bass populations are mostly male and sold at 18-24 months of age, so in maturing or pre-maturing conditions. However, if we anticipate that selection for growth works well, as well as sex ratio control methods, future sea bass could well be mostly female and sold well before sexual maturation – and in this case selection on immature body weight would be the good option.

A second option to rationalize selection for growth could be the use of hormonal treatments to produce separate batches of males and females, which can be done using different hormones (Blazquez *et al.*, 1995; Blazquez *et al.*, 1999; Chatain *et al.*, 1999; Blazquez *et al.*, 2001; Navarro-Martin *et al.*, 2009a). This would allow full control of the number of male and female breeding candidates, and thus of the selection intensity in each sex. However, this may have other consequences and will be studied in the next section about selection for sex ratio.

### 4.2.5 How to move towards monosex female sea bass populations?

In fish, the sex phenotype is usually rather plastic, and can be influenced by hormonal treatments at an early age. As sex dimorphism for growth and age at puberty exists in many species, it is quite commonplace in aquaculture to manipulate phenotypic sex in order to obtain monosex populations of the most "advantageous" sex (reviewed in Devlin and Nagahama, 2002). In several species, sex determinism is a simple chromosomal system, as in salmonids and common carp where it is a mammalian-type system with sex chromosomes, XX for females and XY for males. Hormonal

inversion of XX rainbow trout produces XX "neomales", which produce male gametes (spermatozoa) carrying only the X chromosome. When mated with normal XX females, they produce only XX progeny, which is 100% female. This type of treatment is routinely used for rainbow trout production, which now widely uses those monosex female populations, which are of great interest due to the delayed maturation of fish (2-3 years instead of 1-2 years for males - Breton *et al.*, 1996). In common carp, such a system can also be applied, but is little used as monosex female populations have little advantages over bisexual populations (Gomelsky *et al.*, 1995; Cherfas *et al.*, 1996; Kocour *et al.*, 2003). This method of using neomales is of interest because it produces genetically monosex populations, without the need to use hormones to modify the sex on production batches directed to human consumption. In other species, hormonal inversion of sex may be used on production batches, for example in the Nile tilapia where use of methyltestosterone to produce all-male populations is quite commonplace (*e.g.* Phelps, 2001). However, such use of hormones, is today forbidden by EU regulations (Directive 96/22/EU) and suffers from a very negative image from the public. Then, genetic control of sex is viewed as highly preferable to direct sex inversion by steroid hormones.

In the case of sea bass, the size advantage of females (+20-30% at harvest, Saillant *et al.*, 2001b) as well as their delayed puberty compared to males makes them the "advantageous" sex, although their advantage in growth rate is clear in early stages (+67% in weight at 10 months of age), but less in the later stages, where the growth rate of females significantly exceeds that of males only during short periods (Saillant *et al.*, 2001b). Sex reversal with androgens to obtain "neomales" of sea bass has been tested earlier on (Blazquez *et al.*, 1995; Chatain *et al.*, 1999), but mating of neomales with normal females failed to produce 100% female offspring , as only 5 to 50% females were found in different families (Blazquez *et al.*, 1999). In light of our findings on the polygenic nature of sex determination in sea bass, such a result could be expected.

We showed that simple domestication should result in an even sex ratio in farmed sea bass populations, and that complementing it with selection for growth should result in 70-90% females after 8-9 generations (section 3.3). As we showed that the genetic correlation between growth and sex tendency has a strong impact on the sex ratio response and is highest in young fish (section 3.2.3), putting selection pressure on the younger stages (90 dpf) could help reaching the highest proportion of females (90% if the genetic correlation is 0.8, section 3.3.4). However, note that our previous results on selection for growth tend to suggest that for growth itself, early selection may well be suboptimal. Anyway, sex ratio values of 70-90% females, although interesting, do not correspond to monosex populations. Then, are there strategies that would lead to a monosex or quasi-monosex female status in farmed sea bass populations?

The first possibility to exploit is the fact that the sex ratio equilibrium reached by selection for growth does not depend on the environmental masculinisation level of the selection environment (section 3.3.4). The reason for this is that this equilibrium is reached when the selection against sex tendency by frequency-dependent selection compensates the indirect selection for sex tendency induced by selection for growth. If heritabilities and genetic correlations are constant, as well as the overall proportion selected for growth, the intensity of frequency-dependent selection against sex tendency only depends on the frequency of males and females in the population, and this is the same for indirect selection for sex tendency *via* growth (as the frequency of males and females also impacts on the intensity of selection for growth, as shown in section 4.2.4). However, we showed in section 3.3.4

that although the equilibrium was always the same in terms of sex ratio, it was very different in terms of sex tendency when the masculinising effect of hatchery environment differs: the equilibrium values of sex tendency we calculated (p.77) were $t$=2.55 for $P_{f0}$=0.05 (an initial proportion of females of 0.05 when wild parents are used), $t$=1.81 for $P_{f0}$=0.182 (the proportion observed in our genetic parameters estimation experiment) and $t$=0.90 for $P_{f0}$=0.5 (a case where we would be able to control the environment so that offspring of wild fish would have a balanced sex ratio). If we now consider that we have a temperature protocol for production (not selection) that would give even sex ratios in offspring of wild fish (*e.g.* the G90 treatment in Navarro-Martin *et al.*, 2009b), this means that such a treatment brings no environmental bias to sex tendency. Then, the proportion of females from the offspring of the selected animals can be calculated as $P_f=\Phi(t)$, with $\Phi(t)$ the cumulative distribution function of the normal distribution. This results in 99.5% females for $P_{f0}$=0.05, 96.5% females for $P_{f0}$=0.182 and 81.6% females for $P_{f0}$=0.5.

This highlights the fact that it is highly beneficial to use strong masculinising conditions in the selection environment, while feminizing or at least "neutral" conditions should be used for the production environment. The steps of progress in sex tendency using such an approach are described in Figure 4-4. Under such conditions, almost monosex female populations should be reached in approximately 8 generations. Still, there is a limit to the sex tendency that can be reached with this method, which is linked to the impact of frequency-dependent selection, which sets the asymptotic sex tendency to a level depending on the masculinising effect of the hatchery environment. It could be most efficient provided a very highly masculinising environment was available, but as we said before the repeatability of temperature treatments is rather low, and there is no temperature protocol available that repeatedly gives less than 5% females in the offspring of wild fish.

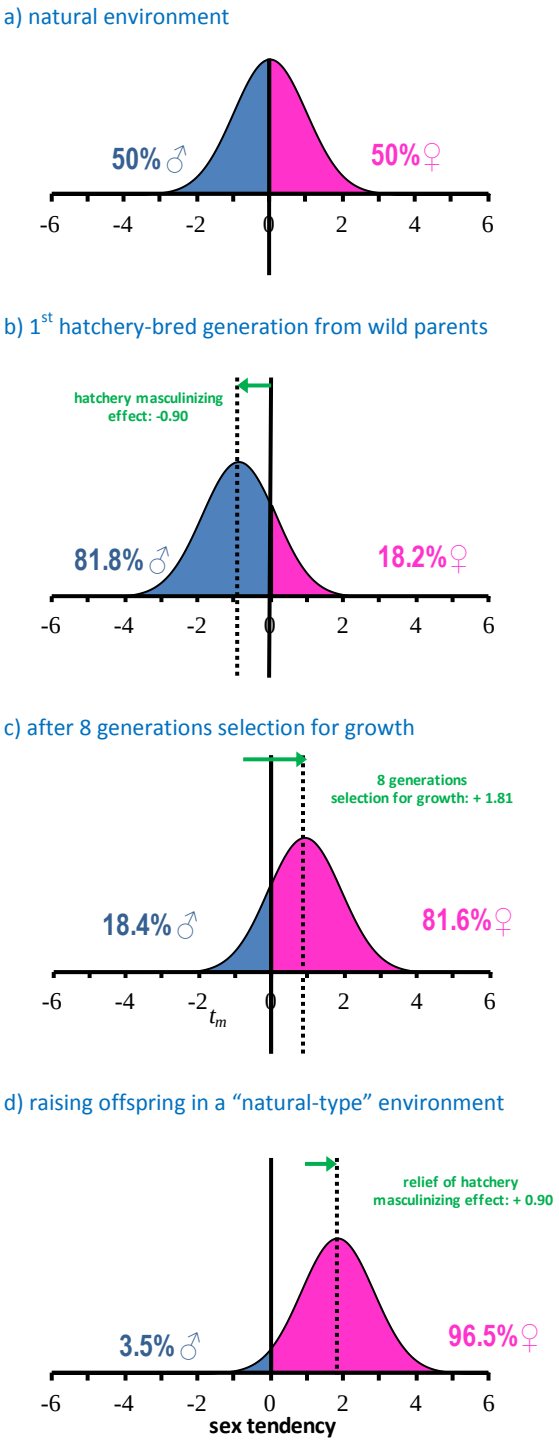


**Figure 4-4 :** Progress of sex tendency with selection for growth combined with manipulation of the masculinization level of the hatchery environment.

Beyond this suggested method, the possibility to control phenotypic sex by hormonal treatments opens another range of possibilities to obtain monosex female populations in sea bass. We saw

before that early trials using sea bass neomales failed to produce monosex progenies, and were therefore abandoned, except as a tool for studying the mechanisms of sex differentiation (*e.g.* Navarro-Martin *et al.*, 2009a). Nevertheless, methodologies of hormonal inversion in early feeding stages are available and allow the production of 100% male (Blazquez *et al.*, 1995; Chatain *et al.*, 1999; Blazquez *et al.*, 2001; Navarro-Martin *et al.*, 2009a) or 100% female (Navarro-Martin *et al.*, 2009a) fish batches. Use of 17α-methyltestosterone (MT) or 17α-methyldihydrotestosterone (MDHT) should be able to provide the strong (and maybe absolute) masculinisation tool that we cannot access through environmental manipulation – although their use has not yet been tested on strongly female-biased populations. Indeed, it is even more than that. Masculinising all of an offspring group, it would nullify frequency-dependent selection on males which prevents sex tendency of growth selected fish to increase indefinitely. In this case, it would be possible, by separating offspring in two equal batches, one put in "normal" masculinising conditions and one reverted to neomales, to reach almost 100% females in the "normal" batch and keep having 100% males in the neomales group. One condition for maximal efficiency would be that there is no genotype by masculinization treatment interaction for growth, *i.e.* that the family ranks for growth would be the same in the "normal" batch and in the neomales batch. To the best of my knowledge, such information is not available in any fish species and should be acquired to really evaluate this strategy.

An additional benefit of using neomales is that it would allow us to reach the monosex female status in the population even in the absence of genetic correlation between growth and sex tendency. We showed that the value of the genetic correlation is an important parameter determining the efficiency of growth mediated selection for sex tendency (section 3.3.4). We cannot take it for granted that this value is as high as we estimated it in any sea bass population. In the case of a population where there would be a null genetic correlation, selection for growth would have no impact on sex tendency, which would then rise to 50:50 as in the case of simple domestication. In this case, using neomales would make the sex tendency of the neomales population equal to the mean sex tendency of the population. If we go back to the corresponding equations (p 46), in generation *g*:

$t_{m(g)} = \text{probit}(P_{f(g)})$ in neomales and $t_{f(g)} = t_{m(g)} + \frac{\phi\,(t_{m(g)})}{P_{f(g)}}$ for the females in the "normal" batch , then in the next generation $t_{m(g+1)}=(t_{m(g)}+t_{f(g)})/2 = t_{m(g)} + \frac{\phi\,(t_{m(g)})}{2P_{f(g)}}$

As $\varphi(t_{m(g)})$ is always strictly positive, this shows that the sex tendency of the neomales (which is equal to that of the population as a whole) can only increase at each generation and ultimately reach a level where only females will be present in the "normal" group.

We can conclude that selection on sex ratio is possible in sea bass, with or without use of hormonal inversion of broodstock, to reach more than 90% females in production batches in 8 generations or so. Reaching 100% females is more complicated, and almost certainly requires the use of hormonal inversion of some broodstock to produce neomales. It must be noted that these projections require considering sex tendency as a simple threshold trait with additive genetic variance. It might not be that simple, as the trait selected might be a reaction norm to hatchery temperatures, as could be suggested by the existence of GxE interactions for sex ratio in an earlier study in sea bass (Saillant *et al.*, 2002). If this was the case, the selection scheme to be applied might have to be a little more complex, as successfully done in the Nile tilapia to select for temperature sensitivity of sex ratio, in

this case to obtain more males (Wessels and Hörstgen-Schwark, 2007; Wessels and Hörstgen-Schwark, 2011). Similar selection was also shown to be possible in rainbow trout (Magerhans *et al.*, 2009; Magerhans and Hörstgen-Schwark, 2010), a species usually considered as having a XX/XY chromosomal sex determination system, although possibly altered by minor sex factors (Quillet *et al.*, 2002). In the case of sea bass, if genetic variation of sex ratio was linked not to a simple additive threshold trait but to the expression of a reaction norm to a specific environment (hatchery temperature), our previous developments on selective breeding strategies would still be valid, except that the environmental manipulation to go back to "natural-type" environment might not be as efficient as supposed here.

## 4.3 An evolutionary perspective on the genetic variation of sex ratio in sea bass, and its relations with growth

In the previous section we saw many possibilities to exploit the genetic variation for growth and sex ratio in the sea bass. This genetic variation, which we revealed in the offspring of wild sea bass males and females, also has a meaning in the natural environment. This is especially true for sex determination, which exhibits a wide variety of systems in Teleost fishes (Mank *et al.*, 2006; Mank and Avise, 2009) while it is highly conserved in birds and mammals (Fridolfsson *et al.*, 1998; Potrzebowski *et al.*, 2008).

### 4.3.1 Polygenic sex determination: a very peculiar system ?

Sex determination systems observed in gonochoristic Vertebrates are male heterogamety (XX/XY) or female heterogamety (ZZ/ZW), polyfactorial (polygenic[5]) sex determination and environmental sex determination (ESD - Bull, 1983). The first three systems are usually qualified as GSD (genotypic sex determination). Interestingly, all of those mechanisms are observed in Teleost fishes (Mank *et al.*, 2006), the most common being male heterogamety. Reproductive modes other than gonochorism also appear (sequential or simultaneous hermaphroditism, unisexuality - reviewed by Mank *et al.*, 2006) - but those are not considered as sex determination models but rather as patterns of sexuality in the terminology defined by Bull (1983).

Polyfactorial sex determination was first suggested (Winge, 1932) to explain sex determination in the guppy, then developed as "polygenic sex determination" by Kosswig (1964), when studying the sex determination system of the swordtail *Xiphophorus helleri*. In these cases however, the hypothesis was that several genes had an effect on phenotypic sex, but that phenotypic sex was completely determined by the sum of several individually small gene effects. The effect of the environment was invoked only in the case where the sum of masculinizing gene effects equalled that of feminizing gene effects , to "avoid" the formation of hermaphrodites. However, this does not correspond to the usual definition of polygenic genetic variation used by quantitative geneticists, where individually small genetic and environmental effects add up to build the phenotype, resulting in genetic and environmental variance for the trait considered. Bulmer and Bull (1982) were the first to clearly formalise polygenic sex determination in this framework, considering that there is an underlying “sex tendency” phenotype, which has a polygenic determinism and which itself determines the observed phenotype (sex: male or female) depending on whether it is below or beyond a fixed threshold. It is important to realise that in this case, the usual classification of sex determination as GSD or ESD becomes meaningless, as any environmental or genotypic effect can equally affect the phenotype (sex tendency) and in the end put it below or beyond the threshold, and then determine the sex of the animal. We must remind that this is just a model, which may be viewed as artificial by physiologists and geneticists dealing with sex determination, as it does not target any "master gene" that would be the initial cause of orientation towards one sex or another. However, this model is

[5] Bull (1983) uses both terms, polyfactorial and polygenic. Polygenic is widely used in quantitative genetic theory to qualify traits which conform to the infinitesimal model, where the genotypic part of performance is determined by very high number of genes which all have a very small effect – and where the environment explains the non genetic part of the performance. Polyfactorial refers more to a limited number of sex factors (which are also often referred to as “minor sex factors”), not necessarily influenced by environment, although this is commonly the case . However, as polyfactorial systems with more than three factors will behave in the same way as real polygenic systems in most cases (Bull, 1983), and then have similar evolutionary consequences, the difference between both is more a question of degree than of principle.

very commonly used in quantitative genetics to describe the genetic variation of binary traits, with a high predictive ability (Falconer and Mackay, 1996; Lynch and Walsh, 1998).

Bull (1983) proposed three criteria to be able to demonstrate polyfactorial sex determination: (i) a large between-family sex ratio variance (ii) paternal and maternal effects on family sex ratio and (iii) a sex ratio response to selection. The last two conditions require the capacity to perform breeding experiments, which is not by far available in all species - and might be an explanation for the limited number of species with a proven polyfactorial sex determination. Sometimes, the first condition only is then considered, but systems with two sex factors and environmental variance (so not polyfactorial) can satisfy it and hence it cannot be considered a satisfying proof of polyfactorial sex determination (Bull, 1983). One additional difficulty raised by Bull is that all systems with more than three sex factors will generally satisfy the three conditions, making it very difficult to infer the real number of factors. In our study, the three conditions were demonstrated, although condition (iii) was only demonstrated through frequency-dependent selection and indirect selection of sex tendency by selection for growth. Anyway, other experimental evidence on a few families proves that direct selection on sex ratio is possible in sea bass (Ky *et al.*, 2006). Then, the conditions proposed by Bull are all satisfied by the sea bass, which makes it one of the rare Vertebrate species with polygenic sex determination.

Indeed, cases where sex determinism is considered polygenic are rare at least in Vertebrates. McGaugh and Janzen (2011) reviewed ten studies estimating the genetic variance of sex determination in Vertebrates with TSD, covering 8 species (three turtles *Graptemys ouachitensis, Chelydra serpentina* and *Chrysemis picta*, one lizard *Eublepharis macularius*, one crocodile *Alligator mississipiensis* and two fishes, the European sea bass - based on our results- and the Atlantic silverside *Menidia menidia*). In the reptiles considered here, there are temperatures at which only males or females are produced, often with a very narrow transition: 100% males below 28°C and 100% females beyond 30°C in *G. ouachitensis* and *C.picta* (Bull, 1980; Bull *et al.*, 1982a), 100% males from 24 to 26°C, 100% females below 20°C and beyond 30°C in *C.serpentina* (Bull, 1980; Janzen, 1992), 100% females at 31.5°C and below, 100% males from 32.5 to 33°C and again 100% females at 35°C in *A.mississipiensis* (Lang and Andrews, 1994), 100% females below 29°C and beyond 34°C in *E.macularius* (Rhen *et al.*, 2010). This shows that some levels of temperature can entirely determine sex, hence the TSD classification of these reptiles. In both fish species, the result is quite different: no temperature treatment yields 100% of either sex, neither in *M.menidia* (Conover and Heins, 1987a) nor in the sea bass (reviewed in Piferrer *et al.*, 2005, with the exception of one 100% male batch of only 30 fish in Blazquez *et al.*, 1998). Then, it is questionable to consider them as TSD species.

Conversely, there are cases where GSD species also show a certain level of polyfactorial sex determination. In Tilapias, the occurrence of major sex factors has been well demonstrated (e.g. Mair *et al.*, 1991; Cnaani *et al.*, 2008). However, some experiments showed a high between-family variance in sex ratio in *O. niloticus*, with contributions from both males and females, and a potential to respond to selection (Wohlfarth and Wedekind, 1991). Similar observations were done in the rainbow trout, a species known to have a XX/XY chromosomal sex determination system (Magerhans *et al.*, 2009; Magerhans and Hörstgen-Schwark, 2010). Those two experiments satisfy the three conditions put forward by Bull (1983) to ascertain polyfactorial sex determination. Interestingly, sex ratio variation in rainbow trout was revealed by a temperature treatment (30 days at 18°C instead of 12°C, starting at 42 dpf, Magerhans *et al.*, 2009), and temperature treatments had also been

previously shown to induce sex ratio shifts in another XX/XY salmonid, *Oncorhynchus nerka* (Azuma *et al.*, 2004). In rainbow trout, it has been separately shown that a minor sex factor could lead to masculinisation of XX females, probably in interaction with other loci and with the environment conditions (Quillet *et al.*, 2002). In Nile tilapia, where sex ratio shifts with temperature are well documented (reviewed by Baroiller *et al.*, 2009), it also appears that the propensity to produce more males by means of a temperature treatment can be selected for (Wessels and Hörstgen-Schwark, 2007; Wessels and Hörstgen-Schwark, 2011). In a third species, the turbot *Scophtalmus maximus*, sex determination mostly conforms with a ZZ/ZW system, but unusual sex ratios appear in some families, as well as an effect of early rearing temperature and a family*temperature interaction (Haffray *et al.*, 2009).

So, it seems that both TSD and GSD can be influenced by minor and/or polyfactorial genetic effects. But there, terminology becomes important: there is an unresolved debate about the definition of GSD and TSD, and the links between both. One view is that TSD and GSD are discrete processes (Valenzuela *et al.*, 2003), such that in GSD the sex of the zygote is determined at conception, while in TSD sex cannot be predicted by the zygotic genotype, and is permanently determined after fertilization by environmental factors. The other view is that GSD and TSD are the two ends of a continuum (Kraak and de Looze, 1993; Kraak and Pen, 2002; Sarre *et al.*, 2004).

Under the "discrete" hypothesis, coexistence of environmental and genetic effects is considered possible, but is generally considered as GSD with Environmental Effect (GSD-EE). The authors valuably argue that many of the temperature treatments which have been shown to modify sex ratios are outside the usual temperature range of the species during the period of sex determination. For the examples above, this is clearly true for the experiments with *O. nerka* and rainbow trout, where 18°C is out of the normal incubation range (8-10°C, reviewed in Ospina-Ãlvarez and Piferrer, 2008). In this case, as highlighted by Ospina-Ãlvarez and Piferrer, aromatase, an enzyme that produces estrogens essential for female sex differentiation in fish (Piferrer *et al.*, 1994), might be inhibited by temperature, as seen in fish and reptiles (Crews, 1994; Uchida *et al.*, 2004), potentially causing increases in number of males by impairing sex differentiation (downwards sex determination).

Another reason to reject TSD is the existence of heteromorphic sex chromosomes, which is considered as a proof of GSD, as in TSD *"there is little if any genetic difference between the sexes"* (Valenzuela *et al.*, 2003). This interpretation of the terms (no TSD when effective temperatures are ouside of the normal range or when heteromorphic sex chromosomes are present) has led to the re-classification of sex determination systems in fish previously considered as TSD (Ospina-Ãlvarez and Piferrer, 2008). Out of 27 species[6] initially considered as TSD, only 8 are considered as having true TSD, mostly based on these two criteria. Ironically, *Menidia* is considered as TSD in this study (Ospina-Ãlvarez and Piferrer, 2008), while it was considered as GSD in the initial paper proposing the dichotomous process (Valenzuela *et al.*, 2003). Ospina-Alvarez and Piferrer consider *Menidia* as TSD because 1) there is no evidence of heteromorphic sex chromosomes and 2) sex ratio changes with temperatures within the range of temperatures during development in the wild (RTD), while Valenzuela *et al.* considered that what changes is the proportion of GSD and TSD individuals within a population, stating *that "even in a species where sex is determined by the sum of polyfactorial genes*

[6] in this count, 33 *Apistogramma* species are taken as one species as they all have the same pattern

*plus a TSD gene, if a TSD gene puts the zygote above or below the necessary threshold, then that individual has TSD [so that...] at the individual level, the process is dichotomous*". This last sentence could equally be turned the other way round, stating that if a polyfactorial gene puts the zygote above or below the necessary threshold, then that individual is GSD. Anyway, such considerations at the individual level are of little interest to qualify populations and their evolution: what is important in my view is the fact that an individual becomes male or female based on the combination of genetic and environmental influences, without a notion of order, which may be difficult if not impossible to prove (especially if the proof has to be done at the level of each individual). Indeed, the GSD end of the dichotomic view can probably be at least partly explained as put forward by Mank and Avise (2009): *"In scientific thinking and research, there is a bias towards sex chromosomes, which is primarily due to the fact that most of the major animal models, including humans, possess them [...] this produces a misconception that sex chromosomes are the predominant mechanism by which sex is conferred in animals"*. For the TSD part of the dichotomic view, we could do the same observation based on the predominance of TSD (with narrow pivotal range) in reptiles. But in the case of fish, heteromorphic sex chromosomes have been observed in only 10% or so of the species surveyed (Devlin and Nagahama, 2002), although this percentage might rise to 50% in teleosts if homomorphic (more recent) sex chromosomes are taken into account (Mank and Avise, 2009). Symmetrically, TSD in fish does not involve as narrow pivotal ranges as in reptiles (10 to 15°C wide, reviewed in Ospina-Ãlvarez and Piferrer, 2008 , making it more difficult to see TSD as an all-or-none switch.

Then, I clearly favour the GSD-ESD continuum hypothesis, which in my view is clearly supported by the fact that different populations of the same species can have different sex determination systems: GSD in the Nova Scotia population of *Menidia menidia vs.* ESD (with genetic variation between families...) in more Southern populations (Lagomarsino and Conover, 1993), TSD in lowlands and GSD in highlands for the lizard *Niveoscincus ocellatus* (Pen *et al.*, 2010). Moreover, theoretical models show that a continuous state of equilibrium between heterogamety (the ultimate GSD model) and ESD exists if ESD is mediated by polyfactorial sex determination with environmental variance (Bull, 1983; Bull, 1981; Bulmer and Bull, 1982). Then, all the above-mentioned systems can in my view be considered as systems mixing genotypic and environmental influences on sex, with various states of equilibrium towards one end or another of the GSD-ESD continuum. In this framework, the proposition that the sex determination of fish can be described as a position in a triangle formed by major genetic factors, minor genetic factors and environmental influences, done by several authors (Baroiller *et al.*, 2009; Penman and Piferrer, 2008) would not be fully appropriate, as in reality we would have GSD and ESD, and in between modulation by minor (polyfactorial) genetic factors. We will come back to this later on when dealing with the evolution of sex-determining systems.

Coming back to the sea bass, we can state that it stands in the middle of the continuum, as genotypic and environmental effect grossly have the same variance, so explain the same proportion of the variation in sex tendency, at least in our hatchery conditions. Here, we have to consider that some of the temperatures used in hatcheries (24°C at some time in our selection response experiment) are higher than the range of temperatures during development in the wild (13-18°C, Ospina-Ãlvarez and Piferrer, 2008) and thus may induce sex ratio responses irrelevant to natural populations. Fortunately, the temperatures used in our genetic parameters experiment remain within this range, although they produce a male-biased sex ratio, so their relevance is less questionable. In addition, although the evidence remains anecdotal, we showed that biased sex ratios, probably linked to environmental influences, could happen in natural populations. Therefore, the fact that the sea bass

lies in the middle of the GSD-ESD continuum is probably not just an artefact generated by extreme experimental conditions. This makes it an interesting model to study the evolutionary implications of sex determination, considering that although "true" polygenic sex determination remains exceptional, implication of polygenes in sex determining systems in fish might be much more common than usually thought.

### 4.3.2 The adaptive significance of polygenic sex determination (in sea bass)

The literature about the evolution of polygenic sex determination is scarce, probably due to the fact that this system can be considered rare (at least "pure" polygenic sex determination) and raises less interest than the evolution of sex chromosomes or of TSD, as we just discussed. Nevertheless, when formalizing the threshold model with sex tendency, Bulmer and Bull (1982) performed a number of calculations and simulations to predict its possible evolution. The general outcome of these simulations is that the system is supposed to be evolutionarily unstable, which would explain its limited occurrence.

The first possibility is evolution towards sex chromosomes, with the following rationale: as soon as the sex ratios are not even in the populations, especially due to environmental effects[7], the variance of sex tendency among parents will be higher than the phenotypic variance of sex tendency in the population as a whole[8], which will then tend to increase phenotypic variance in the next generation. Such disruptive selection tends to increase genetic variance by encouraging heterozygosity, and makes the population respond more quickly to frequency-dependent selection, but also will make it susceptible to invasion by any mutant with major effect on sex determination (Bulmer and Bull, 1982). This mechanism will work both in the case where population sex ratios fluctuate between years, and in the case where they fluctuate among sub-populations exchanging gene flow.

The second possibility for a polygenic system to evolve conforms to the Charnov-Bull model (Charnov and Bull, 1977), which remains one of the most widely accepted models explaining the potential benefits of ESD, due to its robustness and to empirical evidence (Valenzuela, 2004). The conditions for the Charnov-Bull model to operate are 1° a patchy environment, with "good" and "bad" environments influencing individual fitness 2° a differential effect of the environment on the fitness of both sexes and 3° an inability of the parents to predict the quality of the environment. When there is a variance in environmental effects, and differential fitness effects of the environments in males and females, the system is shown to evolve towards a reduction in genetic variance (stabilizing selection), which means that the phenotypic variance in sex tendency will also be reduced, and hence the pivotal environmental range will be reduced, ultimately leading to abrupt changes in sex for small variations of the environment variable - a typical feature of "true" ESD (Bulmer and Bull, 1982). The magnitude of the environmental variance relative to the differential male/female fitness effects will influence the magnitude of the residual genetic variation, more environmental variance favouring more genetic variation, and hence more progressive transitions from one sex to the other around the pivotal environment value (Bull, 1983). Those more progressive transitions in TSD are observed in fish (Ospina-Ãlvarez and Piferrer, 2008) when compared to reptiles (Bull, 1980),

[7] as in an additive polygenic system, there is always environmental variance (as long as heritability is less than 1, which is the general case), and this implies that environment may have an effect on the phenotype (sex tendency, then sex ratio), as the phenotype is the sum of genotypic and environmental effects

[8] this is because the variance among parents will be the average of the variance among males and of the variance among females plus a quadratic term of the difference in average sex tendency between males and females. Except when $P_f$=0.5, this variance term is always higher than the phenotypic variance (Bulmer and Bull, 1982, p. 20)

suggesting higher environmental variance in fish – although reptiles are already thought to favour TSD because of the very high environmental variance in nest temperatures which would override canalization of GSD mechanisms (Georges *et al.*, 2010). Note that in this case "true" TSD is only the extreme of polygenic sex determination, and that the genetic variation in polygenes, although existing, cannot be seen out of the pivotal range. This does not prevent it to be very high within the pivotal range: in the map turtle *G. ouachitensis*, a clear TSD species, a (high) heritability of 0.82 for sex tendency has been estimated within the pivotal temperature range (Bull *et al.*, 1982a). However, as the pivotal range is very narrow, indeed in nature most of the nests are mostly male or mostly female, so that the genetic variance usable by selection is strongly reduced and could be as low as 0.06 (Bull *et al.*, 1982a). Similar results were obtained on the common snapping turtle *Chelydra serpentina* with heritability estimates of 0.34-0.76 for sex tendency within the pivotal range (Janzen, 1992).

Apart from evolving ESD or GSD from an initial polygenic system, it has also been shown that ESD could evolve from heterogametic GSD in a continuous manner provided that (i) environment can "revert" a proportion of the homogametic sex to the other sex, (ii) there is heritable variation in the ability of the homogametic sex to develop the sex phenotype of the heterogametic sex and (iii) the environment variable has male/female specific fitness effects (Bull, 1983; Bull, 1981).

Another theoretical model involving polygenic sex determination has been developed by Rice (1986). In Bulmer and Bull's model, a major sex factor is supposed to invade a population with polygenic sex determination if there is an environmentally induced fluctuation in sex ratio between years or connected populations. Rice proposed a model where such variation is not necessary, provided that the major sex factor Y has a pleiotropic positive effect on fitness or, more subtle, that it is linked to some extent to a gene having a positive effect on the fitness of the Y-induced sex.

Finally, a last theoretical model shows that even in the absence of selection and mutation (i.e. appearance of major sex factors), polygenic sex determination in a finite population is expected to evolve by genetic drift towards ESD, heterogamety or a system combining both, but cannot remain polygenic (Hatcher and Tofts, 1995). The conclusion of this study is that polygenic sex determination in intrinsically unstable – however cases where there would be a selective advantage of GSD or ESD are not studied, thus limiting the generality of the model proposed.

To summarize, GSD and ESD could well be the two ends of a continuum (Figure 4-5), as we could see that ESD and polygenic variation are intrinsically linked, ESD being possibly modelled as one extreme of polygenic sex determination, where genetic variation can be hidden by steep sex ratio responses to the environmental variable.

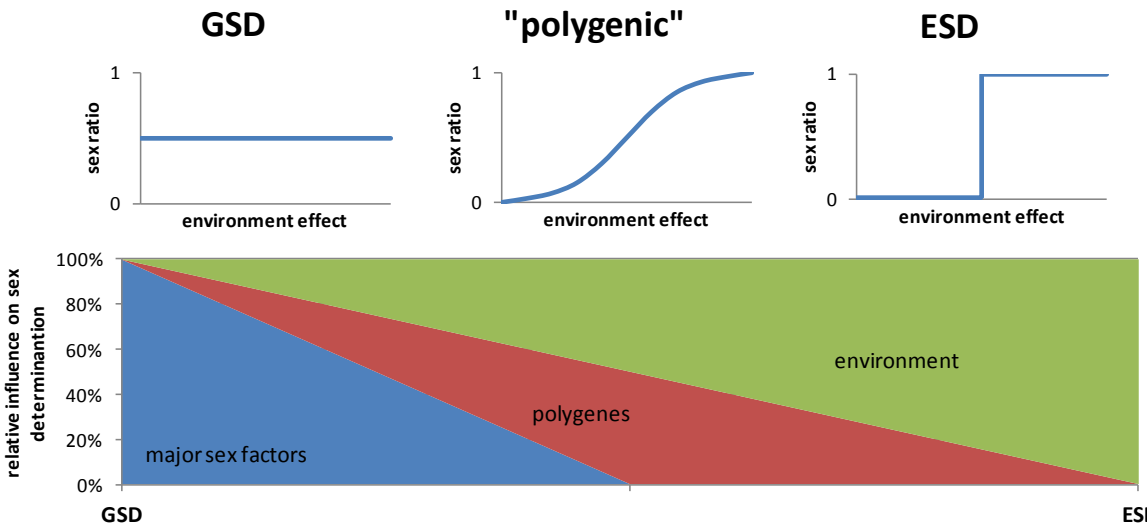


**Figure 4-5 :** Schematic representation of the GSD-ESD continuum. Top: response of sex ratio to changes in the environmental variable (adapted from Bull, 1983). Bottom: relative influence of major sex factors, polygenes (or minor sex factors) and environment on the determination of sex. Note that the decreasing effect of polygenes on the right end of the continuum is not necessarily due to a reduction in genetic variance, as shown in the map turtle (Bull *et al.*, 1982a).

Then, the GSD-EE model evoked earlier could in reality be a combination of major sex factors and a proportion of polygenic (including environmental) sex determination. When the temperature effect operates only outside of the natural temperature range during development (as seen in many cases by Ospina-Ãlvarez and Piferrer, 2008), this would be indicative of a good canalization of sex determination in nature, but still reveal the presence of cryptic genetic variation.

Evolutionary shifts along the continuum, according to the available theory, could be done in both ways, from GSD to ESD in case of a patchy environment with differential male-female fitness effects of the environmental variable (the Charnov-Bull model), and from ESD to polygenic or even GSD in case of environmental fluctuations affecting sex ratios among years or populations[9], the shift to GSD being speeded up by the appearance of major sex factors by mutation – and possible even in the absence of environmental fluctuations if the major factors are linked to sex-specific-fitness-related genes (the Rice model). Additionally, even in the absence of environmental fluctuations or sex-specific fitness effects, just genetic drift could evolve a polygenic system in both directions (the Hatcher and Tofts model).

However, one point has to be highlighted: GSD is expected to ultimately evolve towards heteromorphic sex chromosomes (Charlesworth *et al.*, 2005), and in this case going back to a system with polygenic/environmental sex determination might be difficult due to accumulation of deleterious mutations and sex-specific fitness genes on the chromosomes (Bull, 2008), and may need strong environmental cues to override the canalization of genetic sex determination and reveal potential hidden genetic variation (Georges *et al.*, 2010). These strong environmental cues are not expected to happen in mammals and birds where the environment of the embryo is highly stable, regulated by viviparity or brooding. Indeed, mammals and birds only exhibit GSD (with sex chromosomes), while TSD among vertebrates is restricted to poikilothermic clades, namely reptiles, amphibians and fishes. Phylogenetic analyses show both sex determination models (ESD and GSD) in

[9] Without sex-specific fitness effects in this case

fish (Mank *et al.*, 2006) and in reptiles (Janzen and Phillips, 2006), and the distribution of GSD and ESD within fishes and reptiles seems little linked with the phylogeny. Although there is no definitive phylogeny in fish and reptiles, this strongly supports the fact that the evolution of ESD and/or GSD independently arose several times (Mank and Avise, 2009). It is also noteworthy that in many fishes, even in the case of GSD, sex determining chromosomes have not evolved to heteromorphic sex chromosomes, remaining proto sex chromosomes (Peichel *et al.*, 2004; Charlesworth and Mank, 2010), so not at the ultimate state of GSD. I postulate that the genetic mechanism allowing these transitions is the polygenic variation, which should be attached to all environmental effects on sex determination.

Coming back to the sea bass, we have no direct evidence of a differential effect of temperature on the fitness of either sex in this species. In *Menidia*, which also exhibits (at least in southern populations) an influence of rearing temperature and a family variation of sex ratio, Conover (1984) showed that 1) early spawning (linked with low water temperatures) yielded larger fish, with better winter survival, and 2) a large size probably benefited more to females than to males). If we make the parallel with *Menidia*, we could hypothesize that early spawning gives a longer growing season, and that female sea bass benefit more from a larger size than males as hypothesized by Ghiselin (1969) for protandrous hermaphroditic fishes. In this case, it would be expected that cold temperatures early in development (indicative of earliness in the season) would induce the development of more females, which is precisely what is observed (Navarro-Martin *et al.*, 2009b).

However, additional predictions can be done if this was the case. An alternative and often complementary way organisms use to assess precocity in the season is photoperiod (Bromage *et al.*, 2001). In particular, photoperiod is often a major driver in the control of the spawning season in fish (and particularly in the sea bass - Carrillo *et al.*, 1989). It can then be anticipated that if starting development early in the season effectively favours females, early season photoperiods (so short or even photoperiods) should favour females. This could happen in two different ways, directly on the juveniles or through the genetic control of spawning date. For the first option, the effect of photoperiod on sex ratio in sea bass has been investigated by Blazquez *et al.* (1998). Unfortunately, this study was done with a late treatment (from 57 to 137 dpf) with an initial high rearing temperature during the first 57 days (20-24°C), a treatment now recognized to be highly masculinising (Navarro-Martin *et al.*, 2009b). Indeed, a very high proportion of males was observed, and no significant effect of photoperiod was seen, with 97% males in the short photoperiod treatment (9L:15D) and 93% in the long photoperiod treatment[10] (15L:9D). Therefore, adequate photoperiodic treatments with temperatures permitting the appearance of a significant proportion of females remain to be tested in sea bass. The second option, which relates to the genetic control of spawning date, also remains to be tested. There is no knowledge about the genetic variation of spawning date in sea bass, but this trait is known to present high genetic variability in fish species (e.g. $h^2$=0.60-0.86 in rainbow trout, Gall *et al.*, 1988), so it can be foreseen that genetic variability for this trait should also exist in sea bass[11], and it would be interesting to compare sex ratio proportions in the offspring or early and late breeders from a given sea bass population.

[10] Only 30 fish were sampled from each treatment, thus making the difference non significant.

[11] Genetic variation for spawning date is indirectly revealed by a 1-2 months difference between the spawning date of North Atlantic and West Mediterranean sea bass in the same housing conditions, Chatain, pers. comm.

The last interesting point is the relation between growth and sex, first with the phenotypic sex dimorphism in weight in favour of females, which is observed at all ages but particularly in the first year (+67% at 10 months Saillant *et al.*, 2001b). This can be considered an indirect proof that fast growth should benefit more females than males, as hypothesized before. Second, it is rather intriguing to see that the sex dimorphism for growth is already present at 84 dpf (Saillant *et al.*, 2003c), long before the differentiation of gonads, which starts around 150 dpf (Piferrer *et al.*, 2005; Saillant *et al.*, 2003a) although some differences in the expression profile of *cyp450a* can be seen as early as 120 dpf (Blazquez *et al.*, 2009). In addition to those phenotypic differences, we have also shown that body weight is highly genetically correlated with sex tendency, the highest correlation (0.77) being observed at 90 dpf. This can be interpreted in two different ways: either growth is beneficial to females, and then it is logical that female promoting genes are associated with growth-promoting genes[12], or fast growth (no matter whether mediated by genetics or by the environment) is an additional signal to the undifferentiated fish indicating the interest to differentiate as female. The high genetic correlation would tend to favour the first explanation, while the high sex dimorphism before any sign of sex differentiation appears would rather favour the second one. We presently have no means to choose between these two options or a combination of both.

### 4.3.3 Sex determination and sex differentiation?

In all the previous parts of this work, I did not mention any details about the mechanisms of sex differentiation in sea bass. The reason for this is rather simple: my objective is really to describe the genetic variation which determines the male or female orientation of a juvenile sea bass, and the cascade by which these determinants happen to allow the differentiation of males and females has indeed little relevance, except if we were able to identify (and then to understand the regulations of) the "master switch" which initiates the cascade.

However, this "master switch" concept is probably a mammalian-based concept, as in most eutherian mammals, sex is determined by a single gene located on the Y chromosome, *SRY* (Sinclair *et al.*, 1990). However, this master switch concept is not universally accepted even in mammals as it has been proposed that this simple monogenic determinism would in fact be a quantitative threshold mechanism (Mittwoch, 2006). In birds, there is also a master gene *DMRT1*, located on the Z chromosomes, although in this case the situation is also more complex as its effect is dosage-sensitive (Smith *et al.*, 2009). In fish, only in the medaka *Oryzias latipes* has a homologue of *DMRT1*, *DMY*, been identified (Kondo *et al.*, 2006; Matsuda *et al.*, 2002), and indeed there might well be a number of different "master genes" upstream of a highly conserved differentiation cascade (Volff *et al.*, 2007). For example, it has been shown that the sex determining locus maps to non homologous chromosomes in the closely related fish species threespine stickleback *Gasterosteus aculeatus* and ninespine stickleback *Pungitius pungitius* (Shapiro *et al.*, 2009). Another very recent finding is the master sex determining gene in salmonids, *SdY*, which is conserved but maps on different linkage groups in different species - and which is not a gene with a known function in the sex differentiation pathway (Yano *et al.*, 2012). Interestingly, the sex determination system in the zebrafish *Danio rerio*, which is influenced both by genetic factors and by the environment has long resisted identification (Siegfried and Nüsslein-Volhard, 2008; Traut and Winking, 2001), despite the availability of exceptional experimental animals (clones) and genomic resources. In this species, temperature

[12] as explained by Rice, 1986, mutations favouring female orientation will have a higher chance to invade the population if linked to a gene promoting female fitness through growth

effects during the sex differentiation phase (15-25 days post-hatching) have been shown to exist (Uchida *et al.*, 2004), as well as earlier effects during the incubation phase (Abozaid *et al.*, 2011). Recent research pointed out a ZZ-ZW system (Tong *et al.*, 2010), a polygenic system (Liew *et al.*, 2012) or a mixture of major genetic factors, minor genetic factors and environmental effects (Abozaid *et al.*, 2012) as possible sex determination systems in this species, thus pointing out the complexity of the situation.

An important thing that we can learn from the study of genes implied early in the cascade of differentiation is that the same genes may be used in species with different sex determination systems, but keep some of their "initial" characteristics. For example, it has been shown that *Wt1* was implied in the gonadogenesis of two turtle species, one with GSD and the other one with TSD. Interestingly, *Wt1* is highly regulated by temperature in the TSD species, and retains a relic thermosensitivity in the GSD species (Valenzuela, 2007). This type of expression control could be a way to explain how environmental variability may override the canalization of sex in GSD species, a necessity for ESD to be able to evolve from GSD (see before). Another very instructive experiment has been done with the worm *Caenorhabditis elegans*: using two mutant strains in which the mutations in sex determining genes induce thermal sensitivity of sex determination (Chandler *et al.*, 2009), an experimental evolution experiment was conducted over 50 generations. The results show that *"after 50 generations, evolved lines clearly recovered toward wild-type phenotypes. However, changes in transcript levels of key sex-determining genes in evolved lines cannot explain their partially (or in some cases, nearly completely) rescued phenotypes, implying that wild-type phenotypes can be restored independently of the transcriptional effects of these mutations.* [...This] *highlight*[s] *the microevolutionary flexibility of sex determination pathways and suggest*[s] *that compensatory adaptation to mutations can elicit novel and unpredictable evolutionary trajectories in these pathways, mirroring the phylogenetic diversity, and macroevolutionary dynamics of sex determination mechanisms"* (Chandler *et al.*, 2012). This highlights the fact that under TSD there need not be one master gene, and that other previously unidentified genes can do the job to restore the effects of a mutation. This is strongly in favour of the polygenic + environmental hypothesis that we put forward before as a mechanism permitting the evolution of sex determining systems.

If we come back to the sea bass, the "master switch" remains unknown. However, as in many fishes and reptiles, aromatase, the enzyme that converts androgen into oestrogens, seems to play an important role, rather upward in the sex determination/differentiation cascade. It has indirectly been shown during the thermolabile period that aromatase (*cyp19a1a*) expression was higher in future females, while 11βhydroxylase (*cyp11b*) expression was higher in future males, and this one month before the first signs of histological sex differentiation (Blazquez *et al.*, 2009). For aromatase, the mechanism probably involves methylation of the promoter, as it could be observed that the methylation levels of the promoter were twice higher in males than in females and that methylation decreases the expression of aromatase (Navarro-Martin *et al.*, 2011). Exposure to high temperature increased the *cyp19a* promoter methylation levels of females, indicating that temperature-induced masculinization involves DNA methylation-mediated control of aromatase gene expression (Navarro-Martin *et al.*, 2011). We saw before that cold temperatures could be a sign of earliness in the season, which, by analogy to *Menidia*, could be a reason to preferentially differentiate as females, which may benefit more from a higher growth. It seems that this temperature signal could then be mediated by the methylation of the aromatase promoter.

However, as we pointed out before, growth itself may also be an indicator of the advantage to differentiate as females. It could be observed that 89% of the young sea bass kept at a low temperature during the whole of the thermolabile period (13°C until 346 dpf – which causes very low growth) differentiated as males (Saillant *et al.*, 2002). However, following the previous results, it could be postulated that the methylation level of these fish should be low, thus favouring differentiation as females. Then, we consider likely that low temperature is a kind of "priming" allowing preferential differentiation as females in case later growth would be high enough. Then, a second level of regulation, dependent on growth, may be acting later on, eventually with a different mechanism. The "priming" by low temperature would allow the young sea bass to make the difference between the late season, where growth may be high due to high temperatures, but limited in time, and the early season, where fish will both profit from a longer time to grow and from fast growth when temperature increases later on. More experiments would be needed to test this hypothesis, but interestingly a two-levels mechanism acting at different levels of the cascade would be a seducing hypothesis to explain genotype by environment interactions on sex ratio observed when families of fish are reared at different temperatures in the sea bass by Saillant *et al.* (2002), but also in Nile tilapia by Baroiller *et al.* (1995) and in *Menidia* by Lagomarsino and Conover (1993).

One additional point relating to sex differentiation can help us better understand the process. It has been shown previously that a certain number of sea bass males exhibited intratesticular oocytes. This happens both in wild fish (Saillant *et al.*, 2003a) and in farmed fish (Saillant *et al.*, 2002; Saillant *et al.*, 2003a). Additionally, it appeared that among the 89% of males long-reared at low temperature we mentioned before, 63% had intratesticular oocytes, *vs.* only 36% in the less numerous males (68%) obtained at high temperature (20°C – Saillant *et al.*, 2002). The excess in cold-reared fish was interpreted as an indication of masculinization by environmental factors of "sensitive" fish, which implicitly are considered as sensitive "protofemales". It should be noted that the proportion of males in the high temperature group remained high (68%), which can be explained by a lack of "priming" by aromatase promoter methylation. However, the high percentage of males in the low temperature group requires an additional mechanism, as explained before. The idea that approximately half of the young sea bass are genetic females, which are masculinized by inadequate temperature treatments, has also been proposed by Navarro-Martin *et al.* (2009b). However, with the model of polygenic sex determination I defend, there need not be half of genetic females (and indeed the term "genetic females" has no meaning) – and it has been demonstrated elsewhere that some specific family crosses could exhibit a proportion of females as high as 82%, in environmental conditions where the average sex ratio was 38% females (Ky *et al.*, 2006).

Altogether, the mechanisms involved in sex differentiation in sea bass do not contradict our polygenic hypothesis, and observations done in other taxa suggest that there is room for such an hypothesis. However, environmentally influenced polygenic sex determination may rely on successive mechanisms, so as to maximize the chances for the largest fish to become females[13]. As pointed out by Valenzuela (2008), ESD implies a much longer time frame for the process of sex determination to take place when compared to pure GSD, where sex is fixed at conception.

[13] The fact that the sex dimorphism of weight in favor of females and/or the genetic correlation of growth with sex tendency is highest early in the first year, and specifically around the initiation of sex differentiation for the genetic correlation, strongly (although indirectly) supports this hypothesis.

## 4.4 Conclusion

We have demonstrated that the sea bass had a high potential for selective breeding, for the two major traits growth and sex ratio. Interestingly, both traits are linked making selection for growth also beneficial to improve the proportion of females in farmed populations. Then, there is a sound basis to start breeding programmes enhancing productivity in this species. Such breeding programmes have already started in France and Greece, and we can hope that positive results occur promptly. However, even for simple selection for growth, we pointed out that there was a need to better understand the connections between early growth and final growth rate, as well as the genetic by environment interactions which may act when fish are sent in rearing systems very different from the selection environment. So, even for a simple trait like growth, we cannot say that the story ends. One point that was uncovered and can quickly be solved is the provision of an adapted measurement of growth rate for sea bass, which would be independent of the age of the fish.

For sex ratio, we saw that the sea bass had a rather peculiar polygenic system - indeed probably peculiar only because the influences of environment and of genetics on the determination of sex are of the same magnitude, which prevents choosing between the usual dichotomic ESD and GSD systems. Although peculiar, this system can be efficiently modeled as a quantitative threshold trait. Developing the implications of such a model, we showed that combined with selection for growth and environmental or hormonal manipulation of the sex phenotype in the broodstock, it was indeed rather flexible and gave perspectives to obtain monosex female populations in a few generations. Monosex female populations would be expected to grow faster and mature later, so would be highly favored by sea bass farmers. Another benefit of monosex female populations would be, combined with triploid induction, to allow hatcheries to sell only sterile fish to ongrowers. Induced triploidy by means of temperature of pressure shocks a few minutes after fertilization is possible in many fish species (reviewed by Piferrer *et al.*, 2009), and especially in the sea bass (Peruzzi and Chatain, 2000). However, one frequent characteristic of triploids, which is verified in the sea bass, it that gonadal development is almost completely stopped in females while it may still be present in males (Peruzzi *et al.*, 2004). Therefore, monosex female populations would be perfect to obtain complete sterility through triploidy. The potential advantages of sterile fish are three: 1) quality is improved as no detrimental effects of maturation on flesh quality occur 2) in case of escapes in the natural waters, there is no possibility to have genetic interactions with wild populations and 3) for a breeding company, selling sterile fish is an efficient way to protect its investment. Nowadays, use of sea bass triploids is hampered by the lower growth of these fish (Peruzzi *et al.*, 2004), but also by the necessity to use artificial fertilization to produce triploids, as the temperature or pressure shock has to be applied a very precise timing following fertilization, which is not possible with mass spawning in tanks. Mass spawning is the usual way hatchery produce sea bass eggs, as it is less labour intensive and it provides an overall better productivity of female broodstock. More research is needed to achieve this, but the move towards farming of sterile fish is doubtlessly a major trend for the decades to come, mainly due to the pressure to avoid genetic impact of farmed fish on wild populations (Piferrer *et al.*, 2009).

Although monosex populations may in theory be obtained in a few generations, sea bass generations are rather long (2 years for the males, 3 years for the females), and it might be welcome to find ways to speed up the process. As we saw, the nature of polygenic sex determination makes it prone to

invasion by major sex factors, but it would also be likely that different sex factors would appear and be selected for in different populations (living in different environments). This paves the way to two options to speed up the establishment of monosex (or at least female dominant) populations: exploring the variability of wild populations, which may respond differently to the farming environment in terms of sex determination, and trying to identify potential major (or large) sex factors. QTLs for sex determination have already been found in Tilapia (Shirak *et al.*, 2006) and turbot (Martinez *et al.*, 2009), and the development of next generation sequencing now allows the identification and genotyping of thousands of Single Nucleotide Polymorphism (SNP) markers even in species where no sequence is available (Baird *et al.*, 2008; Willing *et al.*, 2011). The use of such technologies is clearly the way forward, for potential practical selective breeding applications but also for evolutionary biology, as it could help identify new sex determination factors - and thus answer more formally to the questions about the polygenic nature of sex determination in sea bass.

More than those two simple (but necessary) productive traits, growth and sex ratio, there is a need to develop other selection criteria for aquaculture species in general: genetic improvement has been shown to be a major driver in productivity gains in terrestrial livestock species, and it seems clear that aquaculture needs improved productivity and sustainability. The future of fish production clearly depends on the development of aquaculture, as fisheries worldwide are not expected to deliver more fish than they presently do, and could be at a serious risk to collapse (Worm *et al.*, 2006). Already today, aquaculture provides approximately half of the human consumption of fish worldwide. More sustainable breeding goals mean being able to produce more edible fish from less inputs, with a lower production of waste. Traits that can contribute to these sustainability goals are obviously feed efficiency, disease resistance, processing yields and adaptation to novel feed ingredients. The latter is important as the amount of fish meal and fish oil, which are essential ingredients of fish feeds, is limited - so they have to be substituted by other protein and lipid sources, mostly plant-based, although the culture of macro and micro algae could also provide interesting raw materials. Experiments have started to adapt carnivorous fish to plant-based ingredients by selective breeding, and show some room for improvement (Le Boucher *et al.*, 2011; Overturf *et al.*, 2011; Pierce *et al.*, 2008; Quinton *et al.*, 2007 in salmonids, Le Boucher *et al.*, 2010 in sea bass). Genetic variation for feed efficiency also exists, but remains difficult to exploit due to the difficulty to measure individual feed intake in group-reared fish. Indirect criteria are necessary here, and some have started to be developed, especially in the sea bass (Grima *et al.*, 2008; Grima *et al.*, 2010b; Grima *et al.*, 2010a). For disease resistance, genetic variation exists in many fish species, but has not yet been revealed for the major disease of the sea bass, Viral Nervous Necrosis (for which cod has a very high heritability of resistance Odegard *et al.*, 2010). Improving fillet yield by selection seems feasible in some species (Gjerde and Gjedrem, 1984; Haffray *et al.*, 2012; Kocour *et al.*, 2007; Rutten *et al.*, 2005), but is also difficult due to sometimes very small phenotypic variation (Powell *et al.*, 2008). In sea bass, fillet yield is not an issue yet as sea bass are mostly sold as round fish, but it will probably become important when growth rate will be sufficiently improved to make filleting economically feasible. Altogether, we can see that there is a wide array of criteria that could be used to increase the output of aquaculture production of edible fish through selective breeding, and it seems rather clear that these options have to be used to make aquaculture, as the future main provider of fish for human consumption, more sustainable.

However, will this be enough for future development to take place in a sustainable way? Until now, this is not evident at all, although this is not specific to aquaculture, this problem being the same for

many sectors of today's economy. As all animal production systems, aquaculture uses a wealth of primary (often fossil) energy to produce its outputs. Edible protein energy from aquaculture products represents only a limited fraction of the industrial energy consumed in the whole production cycle, as can be accounted for by Life Cycle Analysis (Tyedmers and Pelletier, 2007) : 1.4 to 13% for intensive aquaculture, 13 to 100% for extensive aquaculture, to be compared to 8% on average for fisheries and 1.8 to 25% for various terrestrial livestock production. Although the relative positions of the different systems can certainly be debated, and energy consumption is not the only relevant sustainability indicator (greenhouse gases potential, eutrophication potential, land use are other important categories, see *e.g.* Aubin and Van der Werf, 2009), these values clearly indicate that non renewable energy consumption of aquaculture remains important. The fact that the best converter of intensive aquaculture (13% industrial energy converted to protein energy) is rainbow trout, which is probably the most domesticated species for intensive aquaculture, highlights the potential benefits of domestication and selective breeding of fish to improve the productivity of inputs. This improvement can be done and has to be done, but will it allow the production of fish to feed an increasing population with the same, or even a lower input level? This would be needed to reach a level of decoupling of outputs and inputs satisfying the challenges of climate change and limited resources at the world level, as advocated by Jackson (2009). Efficiency enhancement of the fish alone cannot be the solution, and we will certainly also have to look for other production systems (other feed sources, extensive or multi-trophic systems) to increase resource use efficiency to a higher level (essentially using solar energy through primary production). This will also be an exciting challenge for fish geneticists, as the mass productivity of extensive systems is bound by primary production, making the use of "clever" breeding objectives (increasing conversion efficiency of inputs into edible fish flesh) a necessity. In the meantime, anticipating the change may be difficult, as for the moments we see no sign of extensification of aquaculture production – rather the opposite indeed, as we are still in the oil-doped economy!

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# Résumé

**Etude des variations génétiques de la croissance et du sex ratio chez le bar à l'aide de pedigrees moléculaires**

Le bar (*Dicentrarchus labrax*) est une espèce majeure de l'aquaculture méditerranéenne, dont la production est passée de presque rien en 1985 à plus de 100 000 tonnes annuelles aujourd'hui. Dans un grand nombre de cas, des géniteurs sauvages sont encore utilisés pour produire des juvéniles chez cette espèce, et l'on constate une forte prédominance des mâles, aux performances zootechniques inférieures, dans les populations d'élevage. Le but du présent travail de recherche était tout d'abord de quantifier les variations génétiques de la croissance et du sex ratio entre familles de bar produites par fécondation artificielle et élevées en commun, en utilisant le génotypage de locus microsatellites pour reconstruire les pedigrees des animaux mesurés. Dans un second temps, nous avons également étudié la réponse en termes de croissance et de sex ratio à une sélection expérimentale sur la croissance en longueur.

Nous avons tout d'abord pu montrer que la technique expérimentale choisie (fécondation artificielle, élevage en commun et reconstruction des pedigrees par génotypage) était efficace et susceptible d'être appliquée non seulement en expérimentation, mais aussi pour la mise en place de programmes de sélection chez le bar.

La croissance chez le bar montre une héritabilité élevée pour le poids à taille commerciale de 400g environ ($h^2$=0.38-0.44), mais plus modeste pour le taux de croissance de 35 à 400g (0.16-0.34), montrant l'importance de la croissance précoce, très héritable ($h^2$=0.61) dans la construction de la performance à taille commerciale. Par ailleurs, la croissance du bar n'est pas significativement influencée par des effets maternels non génétiques ou de dominance. Nous avons estimé les interactions génotype-milieu pour la croissance entre des sites de grossissement très différents, et si ces interactions se sont révélées modestes pour le poids à taille commerciale ($r_A$=0.70-0.99 entre sites), elles étaient beaucoup plus fortes pour le taux de croissance ($r_A$=0.21-0.61 entre sites). Bien que nous ayons à dessein choisi des environnements très différents pour ce test, ceci souligne l'importance de conduire les programmes de sélection dans un environnement proche de l'environnement d'élevage.

Nous avons montré que le sex-ratio des populations naturelles de bar ne différait pas de 50-50 en moyenne, mais que certaines classes d'âge pouvaient avoir un sex-ratio biaisé, vraisemblablement du fait d'effets environnementaux. En élevage, les sex-ratios sont variables entre familles et influencés à la fois par le père et par la mère. Aucun modèle purement génétique ne permet d'expliquer les distributions observées, qui peuvent être décrites soit par un modèle ayant au moins deux loci bialléliques et une variance micro-environnementale, soit par un modèle polygénique à seuil ($h^2$=0.62 pour la tendance sexuelle sur l'échelle sous-jacente). Avec ce dernier modèle, on note une corrélation génétique positive ($r_A$=0.50) entre tendance sexuelle et croissance. Ceci permet de prédire que la domestication devrait permettre un rééquilibrage du sex-ratio vers 50-50, la sélection croissance biaisant le sex-ratio vers plus de femelles. C'est ce que nous observons ensuite dans notre expérience de réponse à la sélection pour la croissance. Cette même expérience nous permet de confirmer le potentiel de l'espèce pour une amélioration génétique de la croissance, avec un gain de 23% en première génération.

Le modèle polygénique (ou à tout le moins polyfactoriel) de déterminisme du sexe est *a priori* rare chez les Vertébrés. Après avoir développé son utilisation possible pour obtenir à terme des populations de bars d'élevage monosexes femelles, le modèle polygénique est replacé dans la théorie du déterminisme du sexe chez les Vertébrés ectothermes, où il semble pouvoir être considéré comme beaucoup plus répandu qu'on ne le considère classiquement. Il pourrait être un moyen permettant aux espèces et aux populations de se déplacer le long du continuum ESD-GSD (déterminisme environnemental ou génétique du sexe).



**Mots-clés:** Aquaculture, génétique, sélection, sex ratio, croissance, *Dicentrarchus labrax*

## Summary

**Genetic variation of growth and sex ratio in the European sea bass (*Dicentrarchus labrax* L.) as revealed by molecular pedigrees**

The European sea bass (*Dicentrarchus labrax*) is a major species of Mediterranean aquaculture, the production of which rose from almost nothing in 1985 to more than 100.000 tonnes annually at present. In many cases, wild-caught broodstock is still used to produce juveniles for aquaculture, and farmed population are predominantly male – which unfortunately perform less than females. The aim of the present research was first to quantify the genetic variation of growth and sex ratio between families of sea bass produced by artificial fertilization and reared in a "common garden" approach, using the genotyping of microsatellite markers to reconstruct the pedigrees. In a second phase, we also studied the response in terms of growth and sex ratio to an experimental selection applied on body length.

We first could show that the experimental technique chosen (artificial fertilization, common garden rearing and pedigree reconstruction by genotyping) was efficient and could be applicable not only to conduct experiments but also to set up breeding programmes in sea bass.

Growth is a heritable trait in sea bass, with a high heritability for body weight at commercial size ($h^2$=0.38-0.44 around 400 g mean weight), but a lower value for growth rate from 35 to 400g (0.16-0.34), showing the importance of the highly heritable ($h^2$=0.61) early growth in the building of the performance at commercial size. Additionally, we showed that sea bass growth was not significantly impacted by dominance or non genetic maternal effects. We estimated genotype by environment interactions for growth between highly divergent ongrowing sites, showing that although interactions were moderate for body weight at commercial size ($r_A$= 0.70-0.99 between sites), they were much higher for growth rate ($r_A$=0.21-0.61 between sites). Although we purposely chose very divergent ongrowing environments, this highlights the importance of conducting breeding programs in environments resembling the production environment.

We showed that the sex ratio of natural populations in the wild did not differ from 50-50 on average, although some age classes could have a biased sex ratio, probably due to environmental effects. In a farmed population, sex ratios were shown to differ between families and to be equally influenced by the sire and the dam. No purely genetic model could account for the distributions observed, which could fit either to a model with a minimum of two bi-allelic loci plus micro-environmental variance, or to a polygenic threshold model with $h^2$=0.62 for sex tendency on the underlying scale. This last model also revealed a positive genetic correlation ($r_A$=0.50) between sex tendency and growth. This allowed us to predict that domestication should tend towards a balancing of the sex ratio at 50-50, while selection for faster growth should bias population sex ratios towards females. This is precisely what we observed later on in our selection response experiment, which also confirmed the potential of the species to be selected for faster growth, with a 23% gain in body weight in the first generation.

The polygenic (or at least polyfactorial) model of sex determination is considered rare in Vertebrates. After developing its possible use to tend towards monosex female farmed populations of sea bass, we assessed its position in the theory of sex determination in ectotherm Vertebrates, where it seems that it could well be more frequent as initially thought. Polygenic sex determination could be a means for species and populations to move along the ESD-GSD continuum (Environmental or Genetic Sex Determination).



**Keywords:** Aquaculture, genetics, selective breeding, sex ratio, growth, *Dicentrarchus labrax*