
Response to domestication and selection for growth in the European sea bass (*Dicentrarchus labrax*) in separate and mixed tanks

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Abstract:

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 (69 g mean weight) to day 611 post-fertilization (390 g 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).

Keywords: European sea bass; *Dicentrarchus labrax*; Selective breeding; Growth; Selection response; Realized heritability; Parentage assignment; Microsatellites; Competition; Nephrocalcinosis

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 (Chatziplis et al., 2007; Dupont-Nivet et al., 2008; Saillant et al., 2002; Saillant et al., 2006; Vandeputte et al., 2007). 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 (Herlin et al., 2007; Perez-Enriquez et al., 1999), 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 (Dupont-Nivet et al., 2008; Saillant et al., 2006). 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.

2. Materials and methods

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 m³ tank until 504 dpf (101g mean weight) then randomly separated in two 5 m³ tanks. At 594 dpf (202g mean weight), they were again separated at random in five 5 m³ 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 program 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 program 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 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.

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).

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 m³ 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.

2.4. Parentage assignment

The 1,200 seabass 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 MgCl₂, 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-labelled 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).

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 , μ 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 , μ 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_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 , μ 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 logarithmic 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. Results

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.

3.2. Selection response in separate tanks

The separate tanks results are reported in Table 1 and figure 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 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 \pm 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 - err), as well as faster growing between 268 and 368 days.

3.3. Selection response in mixed tanks

The ANOVA results for mixed tanks are reported in Table 2 and figure 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. 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.

4. Discussion

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.

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 1). 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 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 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 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 CO₂ concentrations (Fivelstad et al., 2003). This is likely to be caused by higher density, especially in a recirculated system with oxygenation where CO₂ stripping is not always efficient enough (Summerfelt et al., 2000).

Moreover, 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.

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.

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 be 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.

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Tables

Table 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.

Trait	Age (dpf)	Significance of model effects					Selection line effect			Selection level effect		
		Tank	Sex	Nephro	Mouth	Spine	<u>F</u> value	<u>df</u>	Sig. level	<u>F</u> value	<u>df</u>	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

Table 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.

Trait	Age (dpf)	Significance of model effects							Selection line effect		
		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

Figures

Figure 1: Evolution of Least-square means (\pm Standard Error) for body length, body weight and DGC (Daily Growth Coefficient) from 268 to 611 days post-fertilization in four selection lines of seabass (\triangle =Wild, \circ =Domesticated, \blacksquare =Mass selected for length, \blacklozenge =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). *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

Figure 1

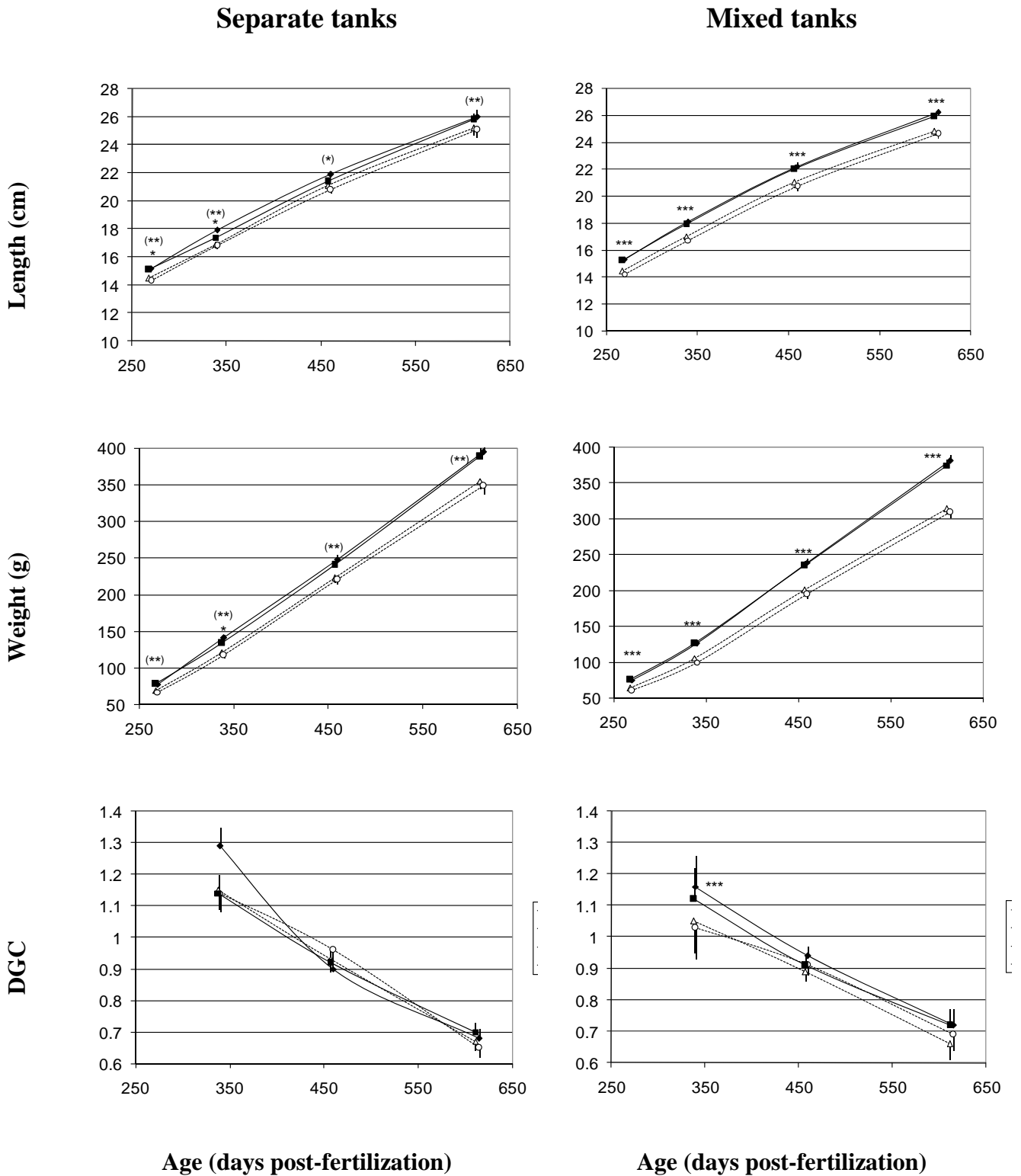


Figure 2: Mean weight superiority of selected over unselected sea bass ($[\text{mean weight of selected groups}/\text{mean weight of unselected groups}]-1$) in separate and mixed tanks from 287 to 611 days post-fertilization, plotted together with the mean rearing density of each period. Selected groups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

Figure 2

