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Version of Record: https://www.sciencedirect.com/science/article/pii/S2352513422001284
Manuscript_31e50eb6e9202e023c38ee29447f0318

Potential for genomic selection on feed efficiency in gilthead sea bream (*Sparus aurata*) based on individual feed conversion ratio, carcass and lipid traits

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Abstract

Genetic improvement of feed efficiency is key to improve the economic and environmental sustainability of fish farming. However, it requires individual phenotypes of feed efficiency which are difficult to obtain when fish are reared in groups, in tanks or cages. Here, we applied and validated on sea bream a method to evaluate individual feed efficiency based on individual rearing of fish in aquariums under restricted feeding. We also investigated the correlation between carcass and lipid traits with individual feed efficiency. We collected individual phenotypes of feed efficiency in aquariums on 538 sea breams (average weight = 54.50 g). Based on these individual phenotypes, fish (average weight = 174.6 g) were reared in groups of divergent phenotypes (high or low feed efficiency) during a period of 63 days in which the feed efficiency of each group was estimated. At the end of this group experiment, fish were harvested and yield traits were measured. All 538 fish, their parents as well as 794 sibs reared in cages in a production environment, were genotyped on a 57k SNP array to estimate genomic heritability and correlations between traits. We showed that feed efficiency was heritable and a GWAS analysis highlighted the polygenic architecture of feed efficiency. We also showed that feed efficiency was genetically correlated to viscera yield indicating that the most efficient fish had less viscera than the least efficient ones. Finally, over the 63 days period in groups, we confirmed that the groups composed of the efficient fish in aquariums were more efficient in group rearing than the groups composed of the least efficient fish in aquariums. Altogether, these results support that measuring individual feed efficiency in aquariums under restricted feeding may be used as a reliable phenotyping method to genetically improve feed efficiency, despite the bias intrinsically linked to individual rearing.

Keywords: fine phenotyping, selective breeding, aquaculture, genomic, restricted feeding, individual feed efficiency

Introduction

Improvement of the feed conversion ratio (FCR = Feed intake/bodyweight gain), which quantifies the ability of an animal to convert feed intake into biomass, is an important step in achieving sustainability in the aquaculture industry (Besson et al., 2016). In fish farming, the cost of feed ranges from 30 to 70% of the total production cost (Doupé and Lymbery, 2004; Kolstad et al., 2004). A decrease in the amount of feed needed per ton of fish produced would, therefore, be essential in enhancing economic sustainability. An example for Atlantic salmon is given by Kolstad et al. (2004), who state that an improvement in feed efficiency (FE) of 2-5% would save 8-20 million euros on feed costs in the production of salmon in Norway (considering 600 000 tons of feed consumed per year). From an environmental perspective, the improvement of FCR increases the proportion of nutrients converted into fish tissues, which reduces the load of nutrients to the environment at similar amount of whole fish produced. The production of feed is also a main contributor to the environmental impacts caused by fish production when analysed within a Life Cycle Analysis framework (Aubin et al., 2009). Therefore, improving feed efficiency would reduce the amount of feed needed per ton of fish produced, thus reducing the total environmental impacts per ton of fish produced, either on site with reduced eutrophication, or at a global scale with less resources consumed (Besson et al., 2017, 2016).

Genetic improvement and breeding programs have shown their capacity to improve feed efficiency in livestock production (Knap and Kause, 2018; Willems et al., 2013). However, to be fully efficient, this option requires collecting individual data to estimate genetic parameters of the trait and, later on, to estimate breeding values to select the most efficient fish among selection candidates. The problem is that individual data of feed intake are difficult to obtain for fish living in large groups and in water. To solve this issue, we recently developed a method based on the rearing of several hundred fish in individual aquariums under restricted feeding (Besson et al., 2019). Indeed, studies on rabbits (Drouilhet et al., 2016, 2013) and pigs (Nguyen and McPhee, 2005) showed that selecting for faster growing animals under restricted feeding was an efficient method to improve feed efficiency in the next generations. With this method, applied on European sea bass (*Dicentrarchus labrax*), we reported that individual FCR and growth (measured as Daily Growth Coefficient - DGC) obtained in aquariums had a genetic basis (h^2 = 0.47 and 0.76 respectively, Besson et al., 2019). Furthermore, we showed that groups of fish consisting of the best fish based on their individual FCR in aquarium (low FCR) were more efficient than groups of fish composed of the worst fish (high FCR) phenotyped in aquarium. These results suggest that selecting for fish based on their individual FCR measured under restricted feeding as proposed as in rabbit or pig would be possible, and would generate an improvement of FCR in fish reared in groups, which is the standard in the production environment.

This method of phenotyping fish in individual aquariums is promising but it is extremely tedious. In Besson et al. (2019) the phenotyping of 588 sea bass involved a full-time position dedicated to the daily routine of counting and cleaning uneaten pellets for 200 individually housed fish over 6 months. Therefore, finding correlated traits to individual feed efficiency that are easier to measure could enhance or even replace (if the genetic correlation with FCR is high enough) the selection of feed efficiency via individual phenotyping in aquarium. Gilbert et al. (2017) showed that nine generations of divergent selection on Residual Feed Intake (RFI, another measure of feed efficiency) in pigs yielded a favourable correlated response on dressing percentage and higher lean meat content, while showing a reduction in backfat and viscera weight. Therefore, this means that selecting on RFI would in fact select for animals that allocate more resources towards lean tissue (i.e. muscle and/or bones) rather than to fat tissue. This is because deposition of lipids is less efficient in terms of energy used per unit of wet weight gain than the deposition of protein (Knap and Kause, 2018). Similarly, in broilers, genetic selection over half a century considerably increased carcass yield while at the same time reducing lipid content and improving feed efficiency (Havenstein et al., 2003a, 2003b). Hence, in many breeding programs, selection for feed efficiency is achieved by indirect selection of leanest animals, which can be done using non-invasive technology easier to implement than direct measurement of feed intake (Knap and Wang, 2012). This principle has also been demonstrated for rainbow trout by Kause et al. (2016) who showed that the most efficient fish had a lower lipid percentage in the muscle than less efficient fish. Therefore, Knap and Kause (2018) suggested to select fish against lipid deposition to improve feed efficiency such as in Janhunen et al. (2017). However, Besson et al. (2019) could not establish any correlation between individual feed efficiency and intramuscular fat percentage measured indirectly by microwaves (Distell Fish FatMeter) in sea bass. Still, we know that lipid deposition in different parts of the body are genetically different traits (Kause et al., 2006; Tobin et al., 2006). In the sea bass, visceral fat has a low phenotypic correlation (r_p=0.31), and no genetic correlation (r_g=-0.02 ± 0.27) with muscle fat (Saillant et al., 2009). In this species, visceral fat represents 66% of the total visceral weight and the percentage of viscera is highly phenotypically and genetically correlated to the percentage of visceral fat (r_p = 0.92 and r_g = 1.00, Saillant et al., 2009). A hypothesis therefore would be that selecting for lower visceral percentage could effectively improve feed efficiency because more resources would be directed towards muscle growth and because less lipids would be stored by the fish.

The gilthead sea bream (*Sparus aurata*) is the most important species of Mediterranean aquaculture, with 228.000 tonnes produced in 2018 (FAO, 2020). Feed efficiency is a key driver of profitability in this production, and several breeding programs are being operated in Europe, but none of them directly targets feed efficiency (Chavanne et al., 2016). Given the promising results recently obtained about selective breeding for individual feed efficiency in sea bass in aquariums (Besson et al., 2019), we also explore the feasibility of such selection in gilthead sea bream. Testing and validating

111 this method on sea bream would allow to confirm its interest to be more broadly applied in the
112 aquaculture sector, as it would increase the genericity of this approach. Therefore, the aim of the
113 present study was to evaluate the genetic basis of individual feed efficiency in gilthead sea bream, and
114 to investigate the relationships between individual feed efficiency and carcass and adipose traits in this
115 species.
116
117 To do so, we applied to gilthead sea bream the same method developed by Besson et al. (2019)
118 on Europeans sea bass to phenotype fish for their individual feed efficiency in individual aquaria. The
119 animals were genotyped on a 60k SNP array. Then, we could estimate genetic parameters, identify
120 potential quantitative trait loci (QTL) and assess the efficiency of genomic selection compared to
121 pedigree-based selection for feed efficiency, when only few animals can be phenotyped. After
122 collecting individual phenotypes of feed efficiency, we verified that these individual estimations were
123 well linked to group feed efficiency. At the end of the experiment, the animals were finally harvested
124 to estimate carcass yield and to estimate genetic correlations with feed efficiency. Finally, to estimates
125 genetic correlations with the growth and yield performance of fish reared in commercial conditions in
126 sea cages, a sib group was reared in a sea cage in Greece and harvested at commercial weight.

Material and methods

Ethics statement

The experiment was evaluated by the Ethical Committee n° 036 and authorized by the French Ministry of Higher Education, Research and Innovation (Authorization number APAFIS#12550-2015071718471859v9). All experimental procedures were conducted following the guidelines for animal experimentation established by Directive 2010-63-EU of the European Union and the corresponding French legislation.

Animals, maintenance and summary of trials

The sea bream originated from a partial full factorial mating design performed on the selected line of Les Fermes Marine du Soleil breeding company (La Brée-les-Bains, France). The general protocol to produce the families was similar that reported by Aslam et al. (2018). Artificial mating was performed within a day in September 2018, using 61 sires and 28 dams. Eggs from each dam were fertilized by 10 to 11 different sires and each the semen of each sire fertilized oocytes from 4 to 5 dam. Maternal half-sib families were incubated separately between dams. After hatching, an equivalent number of larvae per dam were transferred in a single tank and reared in common environmental conditions. At 91 days post-hatching, about 800 randomly selected sea bream (average body weight= 2.7 g) were tagged with Passive Integrated Transponders (PIT-tag) and individual fin sampling was performed for further DNA extraction, genotyping and parentage assignment. The fish were transferred at 108 dph to Ifremer in Palavas-les-Flots (France) and randomly split in 2 tanks of 1.5 m^3 in a recirculation system with natural salinity water kept at 20 ^{o}C. They were first individually weighed at 136 dph (BW_136, mean weight = 17.21 g) and then at 161 dph (BW_161, mean weight = 27.65 g). At 161 dph, we started the individual feed efficiency experiment in aquariums (details in "Evaluation of individual feed efficiency"). Then, at 323 dph, we started the group feed efficiency experiment (details in "Evaluation of group feed efficiency"). Finally, at 420 dph, fish were euthanized to measure production and yield traits (details in "Harvest traits"). The timeline of the experiment and a summary of traits measured are given in figure 1. For the entire experiment, we used a sea bream feed made by Sparos LDA (Olhão, Portugal). The composition of the feed is given in Appendix 1. The experimental feed was formulated to fulfil the sea bream nutritional requirements while minimizing the use of fish meal and fish oil in the diet.

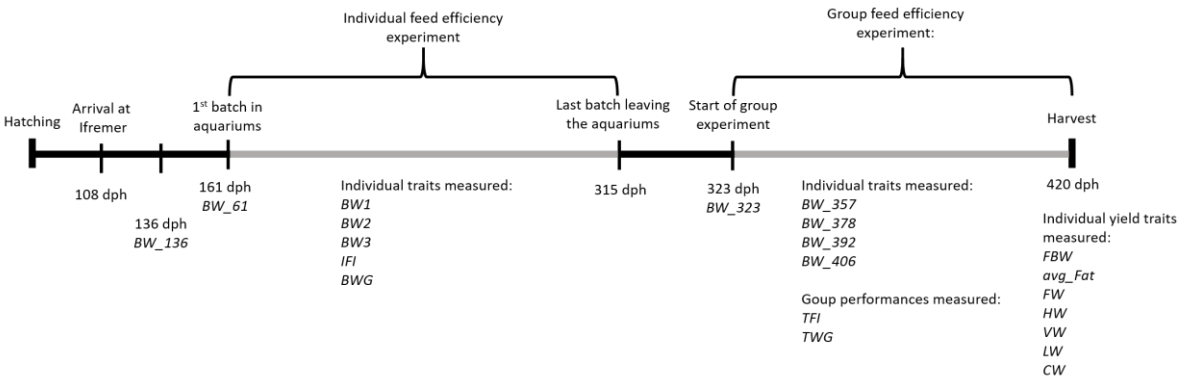


Figure 1. Timeline of the experiment. Time is express in days post hatching (dph). BW refers to body weights, IFI is the individual feed intake, BWG is the individual body weight gain, TFI is the feed intake of tanks, TWG is the weight gain of tanks. FBW = final body weight, avg_Fat = average muscle fat content using Distell Fatmeter, FW = fillet weight, HW = head weight, VW = viscera weight (except liver), LW = liver weight, CW = carcass weight.

In addition, a group of 1530 sibs was transferred to a 150 m^3 cage in Greece at 128 dph. These fish were fed a standard feed (SMART from Irida S.A.). They were reared until 432 dph, when they were harvested, and individual fin sampling was performed for further molecular-based parentage assignment.

Phenotyping individual feed efficiency

For the measurement of individual feed efficiency two hundred 10 L aquariums were used in a recirculation system where natural salinity sea water was kept at a temperature of 20 °C. Before the start of individual rearing, at 161 dph, the length and weight of all sea bream were measured. At 161 dph, a first batch of 150 fish was randomly split in the aquariums. After 14 days of acclimation in isolation, the fish were weighed again in a "go, no go" step. Fish that lost weight during this acclimation period were removed from the aquariums and returned to the 1.5 m^3 communal tanks, considering they were not adapted to the individual evaluation system. The fish that gained weight during the acclimation period were kept in aquariums for two more periods of 14 days each. In total, a "successful" fish stayed 42 days in aquarium and was weighed four times (Figure 2). To reach the maximum capacity of the facility (200 fish), new batches of fish were introduced in the aquariums every two weeks to replace the fish that did not pass the "go, no go" biometry or the ones that completed the 42 days trial. The phenotyping of the first batch of fish started at the age of 161 dph (mean weight = 38.41 g for this 129 first fish) while the last batch started at 273 dph (mean weight = 61.99 g). Before and after the trial in aquariums, the fish were kept in the 1.5 m^3 communal tanks.

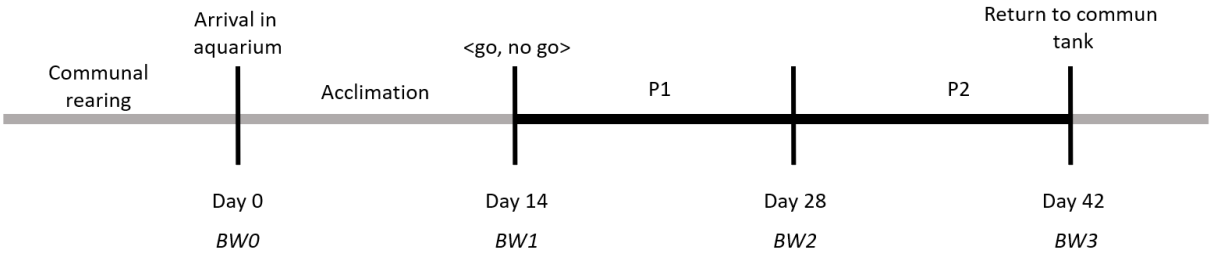


Figure 2. Timeline of the individual feed efficiency experiment. BW refers to body weights.

Individually reared sea breams were fed once in the morning with an automatic feeder. Each day, 3 mm feed pellets were delivered at a rate of 1.54% (corresponding to 70% of a standard feeding rate) of an individual's body weight (fish body weight range: 9.4 - 114.9g), re-evaluated at the beginning of each 14 days period. Fish were fed once in the morning with an automatic feeder. One and a half hours after feed delivering, uneaten pellets were counted and removed from the aquariums. Each day, for each fish, the total number of uneaten pellets eaten was converted into grams (1 pellet ≈ 0.01814 g) and subtracted to the fixed ration to estimate daily individual feed intake.

Among the 669 fish tested in the aquariums, 103 fish lost weight during the acclimation period. The remaining 566 sea bream completed the 42 days of individual evaluation. For these fish, the cumulated FCR (FCR), and the cumulated DGC (daily growth coefficient) were calculated:

$$\mathrm{FCR} = \frac{\mathrm{IFI}}{\mathrm{BWG}}$$

$$\mathrm{DGC} = \frac{\mathrm{BW3}^{\frac{1}{3}} - \mathrm{BW1}^{\frac{1}{3}}}{\mathrm{N\_DAYS}} \cdot 100$$

Where BWG is the body weight gain measured over periods 1 and 2 (BW3-BW1) and IFI the cumulated individual feed intake in periods 1 and 2. N_DAYS is the number of feeding days of periods 1 and 2 (23 or 24 days). Due to the skewed distribution of the data, DGC and FCR were log transformed to obtain log(DGC) and log(FCR). Additionally, we calculated the residual body weight gain (rBWG) as:

$$\mathrm{rBWG} = \mathrm{BWG} - (\beta_0 + \beta_1 \times \mathrm{MBW_i} + \beta_2 \times \mathrm{IFI})$$

$\mathrm{MBW_i}$ is the initial metabolic body weight ($\mathrm{BW1}^{0.8}$). β_0 is the regression intercept, β_1 is the partial regression coefficient of animal's BWG on metabolic weight and β_2 is the partial regression coefficient of animal's BWG on total feed intake.

Evaluation of group feed efficiency

Group constitution

Among the 538 fish well phenotyped in aquarium, we excluded fish with strong spinal malformations (N=80), with a negative cumulated FCR or FCR higher than 2.8. The 458 remaining fish were grouped following the procedure of Besson et al., (2019) in two steps:

- First, we made 7 groups of each 64 or 66 fish based on their relative daily feed intake (relative_DFI) calculated as:

$$\text{relative\_DFI} = \frac{\text{IFI}}{\text{BW1} \times \text{N}_{\text{DAYS}}} \cdot 100$$

- Second, within each group of 64-66 fish with similar DFI we divided the fish into two subgroups of 32 or 33 individuals, depending on the fact that their relative BWG ((BW3-BW1)/BW1) was higher (subgroup A) or lower (subgroup B) than the median of that group. Thus, the fish in subgroups A had a better (lower) FCR than their counterparts from subgroup B.

The aim of this grouping strategy was to compare groups of efficient fish to groups of less efficient fish at the same relative feed intake in aquarium. In total, 14 groups of 32 or 33 fish were distributed in 14 tanks of 2 m^2 covered by black plastic to reduce the amount of stress.

Experimental protocol

The group feed efficiency experiment started at the age of 323 dph (average weight = 176.4 g) and lasted a total of 77 days in a recirculation system where water temperature was set between 22 and 23ºC, with a 12L:12D photoperiod. First, 14 days were used for acclimation to the new environment, followed by one recording period of 21 days and 3 periods of 14 days. In each period, fish were not fed on the day of the biometry and two days before, and thus received 18 or 11 meals. On feeding days, fish were fed *ad libitum* with an automatic feeder delivering the daily ration in 20 portions between 5.30 a.m. and 8.35 a.m. The frequency of delivery was every 4 min for the first 10 portions, every 10 min for the following 5 portions, and then every 20 min for the last 5 portions. We used 4 mm pellet size for this experiment. All uneaten pellets were collected in a faecal trap. At the end of the automatic delivery, if no pellets were found, additional feed was given via a manual trigger until first pellets were collected in the faecal trap, meaning *ad libitum* was reached. Then, 60 minutes after feeding, all uneaten pellets were recovered, washed, photographed and analysed with the program ImageJ and the function "analyze particles" (Abràmoff et al., 2004). The picture was taken using backlighting with a 60x60 cm LED panel. This procedure allowed us to estimate the total surface covered by pellets on each photo measured in number of pixels. We chose to work with surface of pixels covered rather than the number of pellets because, after few hours in water, some pellets were

too soft and tented to break. Counting pellets would have resulted in an overestimation of the number of uneaten pellets, and then an underestimation of feed intake and feed conversion ratio.

The downside of working with surface of pixels is that the estimation depends on the focus of the camera which, in our case, was adapted every day. Hence, to avoid bias in the counting of pixels, we included a 5 cents euro coin on every photo (21.25 mm of diameter). Then, the surface of pixels occupied by pellets was divided by the number of pixels occupied by the coin to measure the surface of pellets in unit of coin surface. To convert a unit of coin surface into number of pellets, we took photos of eight batches of 200 pellets which gave us the average number of pellets per unit of coin surface. Finally, given the average weight of a single pellet, we could estimate the amount of uneaten feed per day and per period. Feed intake per tank (TFI) was calculated as the difference between the quantity of feed distributed and the quantity of uneaten feed over a certain period of time. All feeders were filled with a known quantity of feed at the start of a period, and were emptied to weigh the remainder feed at the end of each period, thus the amount of feed distributed per tank over the period could be precisely recorded.

Individual and group data available in the group experiment

All fish were weighed at the start of the experiment and at the end of each period. Thus, five body weight measurements were available for each fish. The measurements of all fish in a tank were added to calculate total weight gain for each tank and for each period of test (TWG). Then, from TWG we could calculate the TFCR per tank per period of test as:

$$\mathrm{TFCR} = \frac{\mathrm{TFI}}{\mathrm{TWG}}$$

Where TFI is the feed intake of a given tank.

Statistical analysis

To test potential difference in TFCR between subgroups A and B we used a two-sided paired t-test. This was done within each of the four periods and for the combined period. Then, as the mean weight of fish in a tank could affect TFCR, we also analysed body weight gain in each tank, corrected for feed intake and metabolic body weight in an ANCOVA analysis:

$$\mathrm{avg\_TWG}_{ij} = \beta_0 + \mathrm{subg}_i + \beta_1 \times \mathrm{avg\_MBW}_{ij} + \beta_2 \times \mathrm{avg\_FI}_{ij} + \varepsilon_{ij}$$

Where $\mathrm{avg\_TWG}_{ij}$ is the average body weight gain of a fish (TWG divided by the number of fish in the tank) from subgroup i in tank j during a predefined period. subg_i is the effect of subgroup A or B (i=1, 2). $\mathrm{avg\_MBW}_{ij}$ is the mean metabolic body weight of the fish in the same tank at the start of the period, $\mathrm{avg\_FI}_{ij}$ is the average feed intake of one fish in the same tank (TFI divided by the number of

fish in the tank) and ε_{ij} is the random residual. $\beta 0$ is the regression intercept, $\beta 1$ is the partial regression coefficient of avg_TWG_{ij} on avg_MBW_{ij} and $\beta 2$ is the partial regression coefficient of avg_TWG_{ij} on avg_FI_{ij}.

Phenotyping harvest traits

Harvest traits on experimental fish

After the group experiment, at the age of 420 dph, fish were euthanized with an overdose of benzocaine (150 mg/l). Then, we measured several harvest traits:

- Harvest weight (Harvest_W)
- Average muscle fat content from one measure on both sides of the fish using a Distell fatmeter (avg_Fat) according to Haffray et al. (2005)
- Left fillet weight (ribs and skin on)
- Half headless carcass weight (weight of the headless carcass after the left fillet was removed)
- Head weight (Head_W)
- Viscera weight (Viscera_W without liver)
- Liver weight (Liver_W)

From those base data, we calculated:

- Fillet weight (Fillet_W), calculated by multiplying the weight of left fillet by two
- Headless carcass weight (HC_W) representing the sum of the weight the left fillet and of the half carcass.

Harvest traits on sea-caged reared sibs

Additionally, 1112 fish from the same families reared in cages in Greece were harvested at 432 dph using ice. We measured:

- Harvest weight (Harvest_W_cage)
- Fat content (avg_FAT_cage) as reported above
- Viscera weight (Viscera_W_cage)
- Headless carcass weight (HC_W_cage)

Genetic analysis

Genotyping and parentage assignment

We genotyped the 89 parents and 750 offspring of sea bream of the "individual feed efficiency" groups using the Thermofisher SaurChip sea bream array of 60k SNP markers (Griot et al., 2021). SNP calling was done using ThermoFisher software AxiomAnalysisSuite™. Preliminary quality controls were applied with threshold values of 95% for SNP call rate and 90% for sample call rate. We could keep 50,417 effective SNP on 740 sea bream and their 89 parents. Then, we used a subset of 1,000 highly polymorphic SNP to retrieve the pedigree of the individuals using the R package APIS (Griot et al., 2020). 719 fish out of 740 could successfully be linked to a single parental pair (97.2%). For the group of fish sent to cages, among the 1112 fish harvested, 794 were genotyped on the same SNP array and all (100%) were successfully assigned to their parents.

Genetic parameters

Variance components for all traits were computed based on multivariate linear mixed animal models. In these multivariate models, three traits were included and we always included BW_136 as a "reference trait" as it has been measured on all animals of the individual feed efficiency group at the same time.

For traits measured in aquariums (log(FCR), log(DGC) and rBWG), fixed effects were the initial rearing tank (A or B), the batch (referring to the group of fish which started the phenotyping in aquarium together, 9 batches) and the deformities (presence or absence).

For all yield traits measured at harvest on experimental fish (Fillet_W, Head_W, Viscera_W and HG_W) and on cage-reared sibs (Viscera_W_cage and HC_W_cage), final body weight was always included (Harvest_W or Harvest_W_cage) as covariable in the model following practice from Kennedy et al. (1993) and Vandeputte et al. (2020). To highlight the fact that these regressed traits were representative of yields (weight of the body part adjusted to body weight), they were noted as rFillet_W, rViscera_W, rHC_W, with r standing for "residual".

The models were fitted by restricted maximum likelihood in AIREMLF90 (Misztal et al., 2002) to compute the classical heritabilities using pedigree and the genomic heritabilities using SNP data. We considered significant all correlations with an associated standard error lower than half the absolute value of the correlation. We applied the same rule for heritabilities.

Breeding values

The breeding values were also computed with classical pedigree-based BLUP (PBLUP) and genomic BLUP (GBLUP) using the genomic relationship matrix. The conventional pedigree-based EBVs were estimated using the following model:

$$y = Xb + Zu + e$$

Where **y** is the vector of phenotypes, **b** is the vector of fixed effects (batch, rack, line and column for the phenotypes measured in aquariums) and **X** an appropriate incidence matrix, **u** is the vector of random additive genetic animal effects, **Z** the appropriate incidence matrix and **e** is vector of random error variance. The additive (animal) genetic effects were assumed to follow $N(\mathbf{0}, V \otimes A)$, with **V** the genetic (co) variance matrix between traits and **A** the numerator relationship matrix relating all animals in the pedigree, while the residual effects were assumed to follow $N(\mathbf{0}, R \otimes I)$, **R** the residual (co) variance matrix between traits and **I** an appropriate identity matrix. To estimate the SNP based EBV (GEBV) we used GBLUP method where the relationships between fish are based on the genomic relationship matrix described by VanRaden (2008) (**G** matrix) instead of the classical pedigree-based relationship matrix (**A** matrix).

GWAS

We used the BLUPF90 suite of programs to perform GWAS under multi-marker linear regression models using GBLUP for individual feed efficiency traits (rBWG, log(FCR) and log(DGC)). The breeding values were estimated with BLUPF90 using the linear model described in the previous section. The p-values were obtained from POSTGSF90 (Aguilar et al., 2019). The –log10 of the p-values were compared to the chromosome-wide significance threshold and to the genome-wide significance threshold at 5% after Bonferroni correction for the average number of markers per chromosome and the total number of markers, respectively.

Estimation of accuracy via cross validation

To assess the potential interest of using genomic information for the genetic selection of feed efficiency traits, we performed cross-validation tests and estimated the accuracies of PBLUP and GBLUP models. These accuracies were assessed using a cross validation scheme which followed four steps:

1) First, we estimated the corrected phenotypes (Y). In this step, all performances recorded for rBWG were corrected for fixed effects using the PREDICTF90 software.
2) Second, we estimated the EBV for rBWG of all 516 fish while masking the phenotypes of a validation group composed of 20% of the fish (103 fish with phenotypes set missing). The EBVs were estimated with a bivariate model including rBWG and BW_136 (weight at 136 dph) using the BLUPF90 program. Here, on the same validation group set, we estimated GEBVs with genomic information in a GBLUP model and the EBVs (only with pedigree information) in a PBLUP model.
3) Then, we calculated the correlation between corrected phenotypes (Y) and predicted EBV ($r_{EBV,Y}$) for the 103 fish of the validation group.

4) The accuracies ($R_{EBV,BV}$) of PBLUP and GBLUP models was estimated using the following formula:

$$R_{EBV,BV} = \frac{r_{EBV,Y}}{\sqrt{h^2_{ped}}}$$

Where h^2_{ped} is the heritability of rBWG estimated using pedigree including all fish with phenotypes.

5) Finally, all steps from 2) were repeated 50 times to get 50 estimates of accuracy for GBLUP and PBLUP models. Each time, another 103 fish were randomly picked with phenotypes set to missing. We reported the average accuracy and its standard error.

Results

Individual feed efficiency in aquarium

Phenotypic and genetic parameters

We could obtain 538 reliable phenotypes from fish in the experiment in individual aquariums. The fish with reliable phenotypes were all fish with an FCR_tot below 2.8. This number includes fish with deformities (N = 74). Among those 538 fish, 520 were successfully assigned to their parents and used to estimate phenotypic and genetic parameters. Basic phenotypic statistics are given in table 1.

Table 1. Summary of individual phenotypes measured in aquariums on the 537 fish with reliable phenotypes. ADG refers to average daily gain during P2 and P3 periods of individual phenotyped. FCR_tot is the feed conversion ratio over P2 and P3. DGC is the daily growth coefficient over P2 and P3.

Trait	Mean	Median	s.d.	CV
ADG_P2 (in % of body weight per day)	0.94	0.97	0.27	29.2 %
ADG_P3 (in % of body weight per day)	0.94	0.98	0.28	30.5 %
FCR_tot	1.3	1.19	0.35	26.8 %
DGC	1.17	1.22	0.28	24.0 %

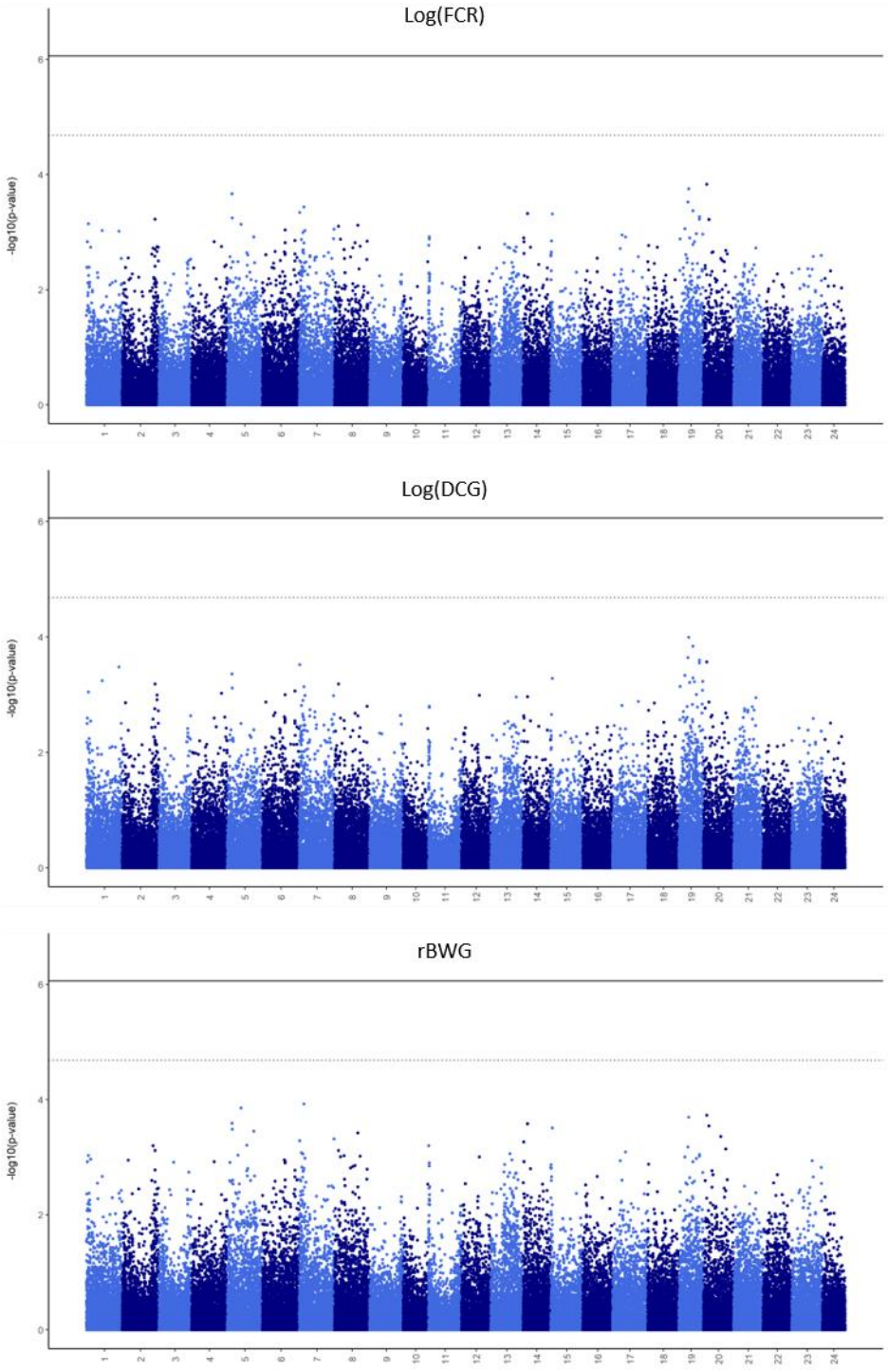
The genomic heritabilities estimated for individual feed efficiency traits were moderate, ranging from 0.17 to 0.25 (table 2). It was also clear that, in these conditions of restricted feeding in individual aquarium log(FCR) and log(DGC) were strongly negatively correlated (r = -0.93). A similar strong negative phenotypic correlation was also observed between log(FCR) and rBWG (r = -0.86). These results at phenotypic level were confirmed by the strong genomic correlations between all three traits (table 2).

Table 2. Heritabilites and genomic correlations between individual feed efficiency traits. Heritabilites are on the diagonal, genomic correlations above the diagonal and phenotypic correlations below the diagonal. Standard error of estimates between brackets.

	log(FCR)	log(DGC)	rBWG
log(FCR)	0.20 (0.07)	-0.93 (0.56)	-0.95 (0.16)
log(DGC)	-0.93	0.17 (0.06)	0.90 (0.17)
rBWG	-0.86	0.70	0.25 (0.07)

411 *GWAS*

412 In the GWAS performed by GBLUP analysis, there were no markers with a p-values above the
413 genome-wide significance threshold nor above the chromosome-wide threshold for log(FCR),
414 log(DGC) or (rBWG) (Figure 3).



415

416 *Figure 3. Manhattan plot of -log10(*p-value*) obtained by GWAS for log(FCR), log(DGC) and rBWG (from top to bottom). The*
417 *horizontal full black line represents the genome-wide significance threshold while the dashed black line represents the*
418 *chromosome-wide significance threshold calculated with the Bonferroni correction.*

Accuracy of GBLUP VS PBLUP

The correlations between corrected phenotypes and predicted EBV ($r_{EBV,Y}$) for rBWG were 0.25 for the GBLUP model and 0.20 for the PBLUP model. Consequently, as the pedigree heritability for rBWG was 0.22, the corresponding accuracies were 0.53 for the GBLUP model and 0.42 for the PBLUP model (Figure 4). A pairwise t-test showed that the difference in accuracy between GBLUP and PBLUP models was significant (t_{49} = 6.1896, p < 0.001).

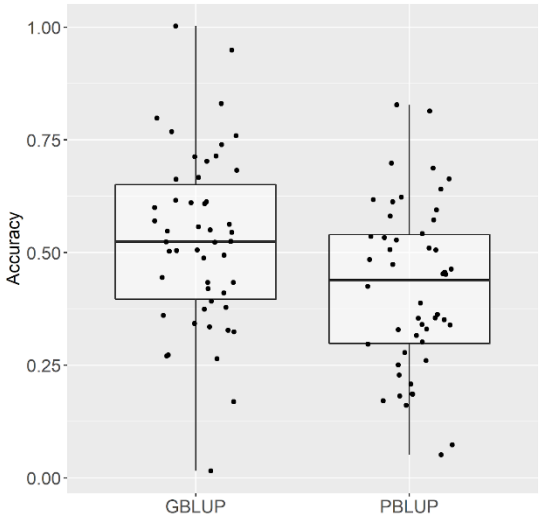


Figure 4. Boxplot of accuracy of GBLUP and PBLUP models for rBWG considering 50 runs with a validation group of 103 fish.

Feed efficiency in groups and growth rates across experiments

First, we compared the TFCR of the tanks from subgroup A and of the tanks from subgroup B for the five periods (four single periods and the full period) using a paired sample t-test. We showed a significant difference for the first period (t_6 = - 3.035, p = 0.023). In that period, subgroup B (composed of individuals with bad FCR in aquarium) had a higher FCR than subgroup A (composed of individuals with good FCR in aquarium). It meant that tanks of subgroups B were less efficient than tanks of subgroup A (Figure 5). We did not find significant differences for the other periods (period 2: t_6 = - 0.14, p = 0.89, period 3: t_6 = - 0.011, p = 0.99, period 4: t_6 = - 1.50, p = 0.18). In those periods,

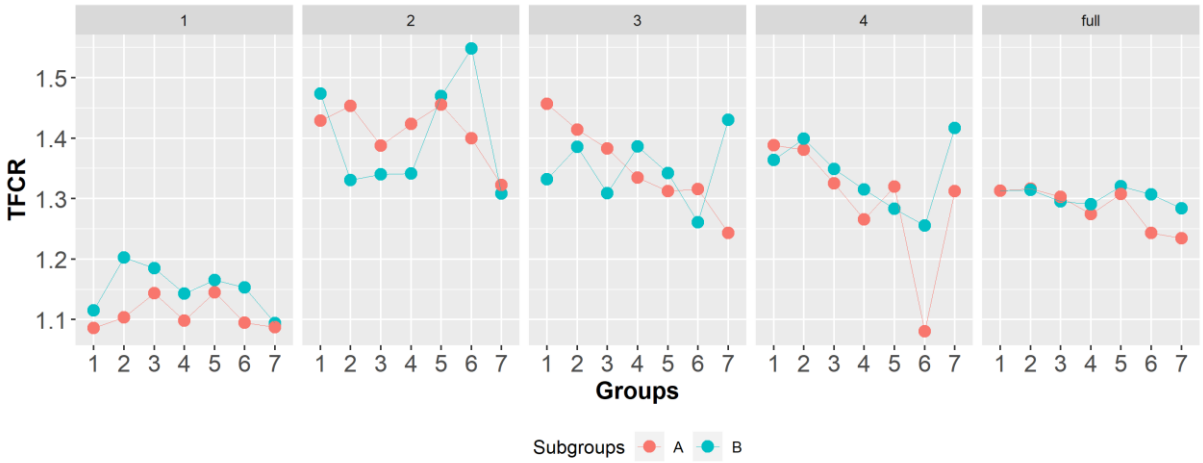
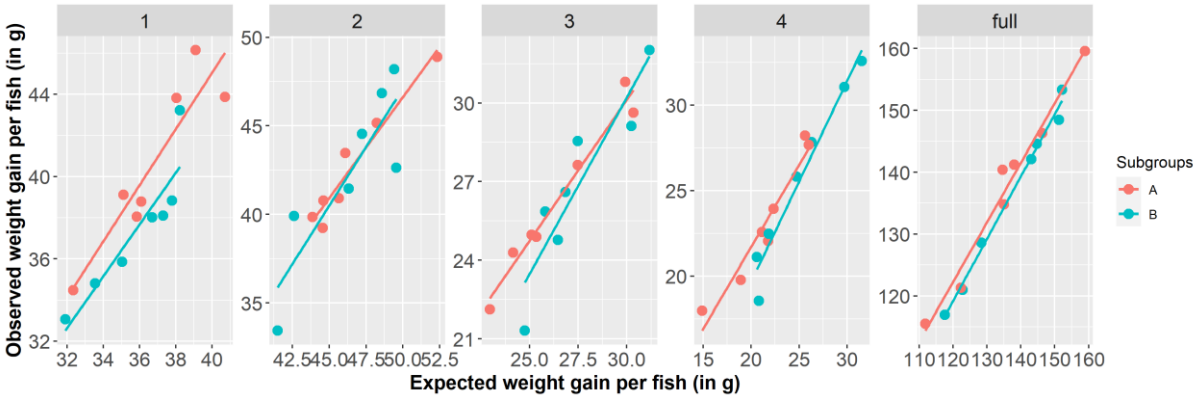


Figure 5. Results of TFCR for each tank for the four period of test plus the full period of 9 weeks. On the x-axis is the group of the tanks and the colors refers to the subgroups A (in red, tanks composed of fish with good FCR in aquariums) or B (in green, tanks composed of fish with bad FCR in aquariums).

there was always at least one tank of subgroup B that had better FCR than the corresponding tank of subgroup A (Figure 5). When all periods were combined, we also did not find significant differences (t_6 = - 1.68, p = 0.14) in TFCR between subgroup A and subgroup B.

To account for a possible effect of mean metabolic body weight on TFCR, body weight gain in each tank was evaluated for each subgroup, after adjustment for feed intake and metabolic body weight, in an analysis of covariance. This showed that there was a significant effect of subgroup on body weight gain during the first period ($F_{1,10}$ = 6.09, p = 0.033) and the full period of 9 weeks ($F_{1,10}$ = 6.26, p = 0.031). For those periods, the fish in tanks from subgroups A showed a higher body weight gain than fish from subgroups B, once corrected for the effect of feed intake and metabolic body weight (Figure 6). Hence, tanks of subgroups A were more efficient over the period of nine weeks. However, for periods 2, 3 and 4, there was no significant effect of subgroups on TFCR.

Figure 6. Regression of observed body weight gain on expected body weight gain (estimeatdestimated from feed intake and metabolic body weight) in tanks with fish from subgroups A (low individual FCR) and B (high individual FCR). The left panle show the regression for the first period of the experiment while de the right panel show the regression for the full experimental period of 9 weeks. The solid line is the linear regression and the color refers to the subgroups of each tanks.



Finally, we calculated the phenotypic and genomic correlations between DGC measured between 136 and 161 dph (log(DGC_juv)), DGC measured in aquariums (log(DGC)) and DGC measured during the 9 weeks of the group experiment (log(DGC_group)). First, the heritabilites of the three growth

rates where moderate (Table 3). Second, due to high standard error, none of the genomic correlations were significant, although they were much higher than the phenotypic correlations, which were all close to zero (Table 3). Only the phenotypic correlation between DGC_juv and DGC_group was found significant, but was surprisingly negative ($r = -0.17$, $F_{1,427} = 4.521$, $p = 0.0003$).

Table 3. Heritabilities and genomic correlations between growth rates across experiments. Heritabilities are on the diagonal and genomic correlation are above the diagonal.

	log(DGC_juv)	log(DGC)	log(DGC_group)
log(DGC_juv)	0.15 (0.05)	-0.02 (0.38)	-0.44 (0.32)
log(DGC)		0.17 (0.06)	0.76 (0.48)
log(DGC_group)			0.21 (0.07)

Harvest traits and individual feed efficiency on experimental fish

Harvest traits could be measured on 451 fish previously phenotyped for their individual feed efficiency performances in aquarium. The phenotypic results for each trait are presented in table 4.

Table 4. Summary of phenotypes measured at harvest.

Fish group	Trait	Mean	CV
Fish with individual feed efficiency phenotypes (n = 451)	Harvest_W	363.0 g	20.2 %
	avg_FAT	9.5%	26.3 %
	Fillet_W	207.1 g	22.2 %
	HC_W	251.9 g	21.7%
	Head_W	81.3 g	17.5 %
	Viscera_W	14.9 g	28.9%
Cage-reared sibs	Harvest_W_cage	330.8 g	22.5 %
	avg_FAT_cage	12.8%	27.7%
	Viscera_W_cage	28.5 g	31.1%
	HC_W_cage	232.4 g	24.0 %

Among harvest traits measured on experimental animals in sea cage, the strongest phenotypic correlations were observed between Harvest_W and avg_fat ($r = 0.58$, $F_{1,432} = 224.1$, $p < 0.001$), rFillet_W and rHead_W ($r = -0.70$, $F_{1,432} = 451.1$, $p < 0.001$) and between rCarcass_W and rViscera_W ($r = -0.74$, $F_{1,432} = 528.1$, $p < 0.001$). All harvest traits displayed moderate to high heritability (table 5).

Table 5. Heritabilities and genomic correlations between harvest traits measured on the experimental fish. Heritabilities are underlined on the diagonal and genomic correlation are above the diagonal. In bold only are the genetic correlations close to significance.

	rBWG	BW_136	Harvest_W	avg_FAT	rFillet_W	rHC_W	rHead_W	rViscera_W
rBWG	*0.25 (0.07)*	0.11 (0.22)	0.41 (0.25)	-0.17 (0.34)	-0.04 (0.35)	0.13 (0.26)	0.25 (0.30)	**-0.41 (0.22)**
BW_136	-0.10	*0.43 (0.06)*	**0.56 (0.13)**	0.32 (0.21)	-0.29 (0.23)	-0.18 (0.20)	0.35 (0.21)	0.07 (0.15)
Harvest_W	0.23	0.60	*0.26 (0.07)*	0.21 (0.32)	-0.49 (0.32)	**-0.51 (0.25)**	**0.81 (0.23)**	0.23 (0.21)
avg_FAT	0.007	0.36	0.59	*0.24 (0.08)*	0.03 (0.46)	0.24 (0.41)	0.14 (0.68)	0.26 (0.24)
rFillet_W	-0.15	-0.08	0	0.32	*0.21 (0.07)*	**0.95 (0.12)**	**-0.99 (0.12)**	-0.29 (0.24)
rHC_W	0.03	0.03	0	0.03	0.40	*0.29 (0.08)*	-0.92 (0.70)	**-0.51 (0.22)**
rHead_W	0.17	0.11	0	-0.31	-0.70	0.03	*0.25 (0.07)*	0.24 (0.35)
rViscera_W	-0.11	-0.06	0	0.01	-0.19	-0.75	-0.1	*0.44 (0.07)*

Furthermore, weak but significant phenotypic correlations were observed between Harvest_W and rBWG ($r = 0.23$, $F_{1,432} = 23.94$, $p < 0.001$), rFillet_W and rBWG ($r = -0.15$, $F_{1,432} = 9.808$, $p = 0.001$), rHead_W and rBWG ($r = 0.17$, $F_{1,432} = 11.54$, $p < 0.001$) and between rViscera_W and rBWG ($r = -0.11$, $F_{1,432} = 5.686$, $p = 0.017$) (table 5). However, only few genomic correlations were not statistically different to zero. For the first time in this species, we report a very high genetic correlation between rFilet W and rHC_W ($r_g = 0{,}95 \pm 0.12$). We found a close to significance negative genetic correlation between rBWG measured in aquariums and rViscera_W ($r_g = -0.41 \pm 0.22$). It means that the most efficient fish had less viscera (and hence less visceral fat) than less efficient fish at the same weight. Although not significant, the genetic correlation between rBWG and muscle fat measured with the Distell fatmeter was also negative ($r_g = -0.17 \pm 0.34$). Finally, the genomic correlation between Harvest_W and rBWG ($r_g = 0.41 \pm 0.25$) was positive and close to significance.

Harvest traits and individual feed efficiency on cage-reared sibs

The genetic parameters are shown in Table 6. Although none of the traits was significantly genetically correlated with feed efficiency (measured as rBWG), the signs of the correlations of sib traits with rBWG were similar to those of the traits recorded on the feed efficiency animals themselves. The correlation was positive with Harvest_W_cage (0.22) and rHW_cage (0.55), and negative with avg_FAT_cage (-0.20) and rVisceral_W_cage (0.17).

Table 6. Heritabilites and genomic correlations of processing traits on cage-reared sibs with individual residual body weight gain measured in aquariums. Heritabilites are on the diagonal and genomic correlations are above the diagonal. Genetic correlations significantly different from zero in bold.

	rBWG	Harvest_W_cage	avg_fat_cage	rHC_W_cage	rVisceral_W_cage
rBWG	0.23 (0.07)	0.22 (0.56)	-0.20 (0.35)	0.55 (0.44)	-0.17 (0.34)
Harvest_W_cage		0.23 (0.08)	**0.59 (0.24)**	**0.98 (0.12)**	**0.68 (0.18)**
avg_fat_cage			0.25 (0.08)	0.16 (0.48)	0.002 (0.25)
rHC_W_cage				0.16 (0.07)	**-0.67 (0.31)**
rVisceral_W_cage					0.48 (0.1)

Discussion

Investigating the genetic background of feed efficiency in fish started in the 90s. However, measuring individual feed efficiency of fish living in groups in a 3D water column is not an easy task, because phenotyping for individual feed intake in such conditions is not straightforward. If selective breeding for growth rate (which is done in virtually all breeding programs) would generate indirect selection gain in feed efficiency, that would make genetic improvement of feed efficiency feasible without going into complex methods to evaluate individual feed intake. Although all studies performed so far on selection for growth agreed that selection for growth rate in fish leads to animals with higher feed intake, the link between the increase in growth rate and the improvement of feed efficiency remains uncertain with some studies showing positive association, while others show no association between growth rate and feed efficiency (Ogata et al., 2002; Sanchez et al., 2001; Silverstein et al., 2005; Thodesen et al., 1999; Yamamoto et al., 2015). It is therefore likely that feed conversion ratio and growth rate are either not correlated or weakly negatively correlated (a negative correlation of growth and FCR implies that selecting for fast growth will reduce FCR and thus improve feed efficiency). When feed conversion ratio and growth rate have a genetic correlation comprised between 0 and -0.45, integrating a specific evaluation of FCR in a breeding program would largely improve economic returns and reduce environmental impacts, while if the correlation is more strongly negative (<0.45), selecting only for growth also provides the benefits of improved feed efficiency (Besson et al., 2020). Developing an efficient method of phenotyping individual feed efficiency is thus essential to breed for more efficient fish without being dependent on the potential correlation that feed efficiency could have with growth rate.

In this regard, the method we developed using restricted feeding of fish in individual aquariums is promising. We were able to phenotype more than 500 sea bream over a period of 5 months. The phenotyping of 500 juvenile European sea bass was already achieved by Besson et al. (2019) in this system, and thus we showed that it was reproducible for another fish species. Individual FCR values measured for sea bream were in the same range as the ones obtained on sea bass, with a mean of 1.30 while it was 1.38 in the sea bass experiment (Besson et al., 2019). The heritability of individual FCR was moderate (0.16) but lower than other heritability estimates for individual FCR in aquatic animals, which were 0.32 in Nile tilapia *Oreochromis niloticus* (de Verdal et al., 2019, 2018), 0.47 in sea bass (Besson et al., 2019) and 0.58 to 0.69 in Pacific white shrimp *Litopenaeus vannamei* (Dai et al., 2017). A reason that could explain this lower heritability is a potential confusion between genetic and environmental effects. Indeed, to avoid phenotyping big fish in the 10L aquariums, we picked the biggest fish from the holding tank at the start of each batch, hence generating some confusion between batch and body weight effects. Such confusion could have reduced the apparent genetic variance of FCR and thus reduced the heritability, especially if FCR and growth rate are correlated. From a

technical point of view, the experimental procedure could thus be improved by increasing the number of aquariums allowing to phenotype more fish at the same time, and/or by starting to phenotype earlier to avoid issues with fish too large for the system.

The phenotypic and genetic correlations between log(DGC) and log(FCR) were as strong as in the sea bass experiment with the same system (-0.93 in sea bream vs -0.78 in sea bass for the phenotypic correlation and -0.93 vs -0.98 for the genetic correlation). It confirms that growth in individual tanks under restricted feeding is a good proxy of FCR measured in these conditions. However, feed efficiency in aquarium (measured as log(DGC) or rBWG) was not correlated to the *ad libitum* growth rate measured during the group experiment. Our results may have been different if we have estimated this correlation under restricted feeding regime. However, from harvest data, there was a trend for a positive genetic correlation between harvest weight and rBWG (0.41 ± 0.25). Nevertheless, it should be noted that harvest weight was measured on the same animals after individual feed efficiency phenotyping. As individual feed efficiency was measured under restricted feeding, the most efficient fish were also necessarily those that grew the most during this individual housing phase. Thus, the positive link between harvest weight and rBWG might also be partly an artefact. In order to avoid such artefacts, we also measured harvest traits on a group of cage-reared sibs and family links enabled the estimation of genetic parameters. In this population, the link between rBWG and harvest weight was not significantly different than zero (0.22 ± 0.56) although correlations were of the same sign as those observed on the fish with individual feed efficiency phenotypes. These results are consistent with the results presented in Besson et al. (2019) in sea bass, and rather similar to those of Pang et al. (2017) on crucian carp *Carassius auratus*, which also found a low (0.15) correlation between initial weight and individual feed efficiency. It suggests that feed efficiency measured under restricted feeding in aquariums is not necessarily associated with faster growth under classical rearing condition and individual feed efficiency might be independent of growth rate, which is not the general view (Knap and Kause, 2018; Thodesen et al., 1999). Hence, the phenotyping procedure involving restricted feeding may not select for the animals with the best growth. This was hypothesized by Cameron et al. (1994) who suggested that restricted feeding may select for animals with higher partitioning of energy toward protein deposition rather than fat deposition but may not select for animals with the highest overall protein deposition. The results obtained on harvest traits tend to support this hypothesis. Although the genetic correlations between rBWG and harvest traits (measured on the same animals or on sibs reared in cage) were very uncertain due to their high standard error, they showed a trend for a negative correlation between rBWG and viscera yield. The most efficient fish would have lower viscera yield and it was shown in farmed European sea bass that viscera are mostly composed of visceral fat (Saillant et al., 2009). This is in line with the general knowledge on selection of animals for feed efficiency which suggests that selecting for leaner animals would improve feed efficiency (Knap and Kause, 2018). However, the direct genetic correlation between FCR and percentage of

intramuscular fat in our experiment was not significant, although again being positive, as expected under that hypothesis. We could not highlight other significant genetic correlations between rBWG and processing traits probably because of the small number of phenotypes collected (n = 451 individuals with both efficiency and harvest phenotypes).

In this context, where a limited number of animals can be phenotyped, genomic data showed their advantages compared to pedigree to estimate breeding values. Indeed, the prediction of EBV was more accurate using GBLUP models (using SNP data) than PBLUP models (using only pedigree) by 26%. This result is in line with previous results on fish for several other traits such as disease resistance (Aslam et al., 2020; Aslam et al., 2020; Vallejo et al., 2017; Yoshida et al., 2019), growth and quality traits (Blay et al., 2021; Palaiokostas et al., 2018; Tsai et al., 2016) or reproduction traits (D'Ambrosio et al., 2020). With GBLUP, the improvement of the accuracy was however lower than the 48% increase in accuracy we obtained for feed efficiency in European sea bass (Besson et al., 2019), but was in the range of the values in the previously cited studies. Our results therefore confirm the major potential benefits of genomics for improving genetic gain in complex traits for which only few animals can be phenotyped. These results are all the more important as we could not identify any QTL associated to individual feed efficiency traits, suggesting a highly polygenic architecture of those traits. These results differs however from previous results from Pang et al. (2017). In their study, they were able to highlight several QTL for feed efficiency of individually reared carp. Such QTL were also found for terrestrial farmed animals such as pig (Delpuech et al., 2021) or cattle (Seabury et al., 2017). In our case, the lack of QTL detection could be caused by the low number of animals phenotyped and genotyped and also by the family structure: among the 112 full-sib families present in our dataset, 31 were represented by only one individual.

To be applicable in a commercial breeding program this phenotyping method in aquarium should be able to identify fish that would be the most efficient when reared in group conditions, as group rearing is the normal way of farming fish. A validation experiment in groups is therefore essential. With such validation in groups, for instance, Rodde et al. (2020) showed that individual measurements of feed efficiency in aquarium for Nile Tilapia *Oreochromis niloticus* were not correlated to feed efficiency in groups. Hence, phenotyping feed efficiency in aquarium is probably not an efficient method to improve feed efficiency of Nile tilapia. Conversely, in shrimp, Dai et al. (2019) found a good genetic correlation (0.79 ± 0.11) between feed efficiency measured in isolated shrimp or in small groups of 10 shrimp. Our results for sea bream showed a link between individual and group feed efficiency but the results were less straightforward than those in sea bass (Besson et al., 2019). A significant difference in TFCR between subgroups A (most individually efficient fish) and B (least individually efficient fish) was only found for the first period of 2 weeks (out of 4 periods) and for the full period of 9 weeks. Several reasons that may explain this result. First, when looking at short time periods, TFCR

could fluctuate between periods due to social dynamics within the tanks or due to environmental conditions. This variance at small scale would be smoothed when looking at longer time periods such as 9 weeks. The lack of difference could also be due to health issues that affected the fish. During group experiments, fish suffered from an unexplained occurrence of bulging eyes, that could neither be related to nitrogen supersaturation nor to pathogens by veterinarians. This may have caused welfare issues, and perturbed their feeding pattern, as shown by the sharp increase in FCR, from 1.13 to 1.4, between periods 1 and 2 of the group feed efficiency experiment. Although the results are not straightforward, it still seems reasonable to consider that in sea bream, variation in individual feed efficiency under restricted feeding can be at least partly reflected in differences in feed efficiency when fish are reared in groups with *ad libitum* feeding.

Conclusion

The present study confirms the potential of phenotyping individual feed efficiency of fish in individual aquariums under restricted feeding. With this method, similar to what was previously shown in sea bass, several feed efficiency traits were shown to be heritable in sea bream, suggesting that genetic improvement for individual feed efficiency is feasible. Considering a selection index pressure of 10% and an accuracy of 0.53, the genetic gain could enable reduction of FCR of 1% per generation, from 1.29 to 1.276 in the first generation. At the Mediterranean scale, where 228,000 tonnes of sea bream were produced in 2018, such reduction of FCR would decrease the use of feed by 3,192 tonnes per year, with a direct impact on economic and environmental sustainability of sea bream production. The accuracy estimated in this research is however only an estimate and, ideally, the true response to selection should be measured with an experimental approach.

Acknowledgement

The authors are grateful to Sparos (Olhão, Portugal) for optimizing the formulation of the experimental feed and for producing it.

Funding

This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement no 727610 (PerformFISH). This output reflects the views only of the author(s), and the European Union cannot be held responsible for any use which may be made of the information contained therein.

Appendices

Appendix 1. *Composition of the feed*

Ingredients	**%**
Fishmeal Super Prime	5.00
Soy protein concentrate	25.00
Wheat gluten	7.50
Corn gluten	25.00
Rapeseed meal	11.00
Wheat meal	2.00
Pea starch	1.80
Fish oil	10.90
Rapeseed oil	5.70
Soy lecithin	1.65
PERFORMFISH WP1 Premix 1%	1.00
Guar gum	0.20
Monocalcium phosphate	2.10
L-Lysine	0.90
DL-Methionine	0.01
L-Taurine	0.24
Total	**100.00**
Analysis results	
Moisture (g/100g)	**8.2**
Crude protein (g/100g)	**42.5**
Fat (g/100g)	**20.3**
Crude ash (g/100g)	**6.2**
Crude fibre (g/100g)	**2.1**
Total phosphorus (g/100g)	**1.1**

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