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Changes in transcriptomic and behavioural traits in activity and ventilation rates associated with divergent individual feed efficiency in gilthead sea bream (*Sparus aurata*)

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A R T I C L E I N F O

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A B S T R A C T

Feed conversion ratio (FCR) is an important trait to target in fish breeding programs, and the aim of the present study is to underline how the genetic improvement of FCR in gilthead sea bream (*Sparus aurata*) drives to changes in transcriptional and behavioural patterns. Groups of fish with high (FCR+) and low (FCR-) individual FCR were established at the juvenile stage (161–315 dph) by rearing isolated fish on a restricted ration. Fish were then grouped on the basis of their individual FCR and they grew up until behavioural monitoring and gene expression analyses were done at 420 dph. The AEFishBIT datalogger (externally attached to operculum) was used for simultaneous measurements of physical activity and ventilation rates. This allowed discrimination of FCR+ and FCR- groups according to their different behaviour and energy partitioning for growth and locomotor activity. Gene expression profiling of liver and white muscle was made using customized PCR-arrays of 44 and 29 genes, respectively. Up to 15 genes were differentially expressed in liver and muscle tissues highlighting a different metabolic scope of FCR+ and FCR- fish. Hepatic gene expression profile of FCR- fish displayed a lower lipogenic activity that was concurrent with a down-regulation of markers of mitochondrial activity and oxidative stress, as well as a reallocation of body fat depots with an enhanced flux of lipids towards skeletal muscle. Muscle gene expression profile of FCR- fish matched with stimulatory and inhibitory growth signals, and an activation of energy sensors and antioxidant defence as part of the operating mechanisms for a more efficient muscle growth. These new insights contribute to phenotype the genetically mediated differences in fish FCR thanks to the combination of transcriptomic and behavioural approaches that contribute to better understand the mechanisms involved in a reliable FCR improvement of farmed gilthead sea bream.

1. Introduction

The aquaculture sector is the fastest growing human food producing system (Anon, 2020), but it is associated with increased environmental impact that needs to be considered to move towards a more environmentally-sustainable aquaculture sector (Bohnes et al., 2019). In this regard, the improvement of feed conversion ratio (FCR; the ratio of feed intake over body weight gain) is a highly desirable trait, as it increases industry profits (aquaculture feed accounts for 50–70% of production costs; Dossou et al., 2018), while decreasing at the same time the risk of eutrophication and the impact on climate change (Besson et al., 2014, 2016; de Verdal et al., 2018a). The main constraint is that FCR is a problematic trait to be included in aquaculture breeding programs, as it requires accurate measurements of body weight gain and feed intake (de Verdal et al., 2018a). The assessment of feed intake becomes especially challenging in aquatic environments, being now

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assessed in genetic studies by two main methods: i) meal video-recording of small groups of fish (10–15 per aquaria) fed with pellets provided one by one in different aquaria places to reduce fish competition (de Verdal et al., 2017), and ii) individual rearing on a restricted ration with a precise daily counting of uneaten pellets. These two approaches showed that there was individual variation in individual feed efficiency in Nile tilapia *Oreochromis niloticus* (de Verdal et al., 2018b) and European sea bass *Dicentrarchus labrax* (Besson et al., 2019), and that a significant part of this variation was heritable. However, there is no simple answer to guide the choice of the best method, though it appears that the use of video-recording for direct selection for FCR is clearly more efficient than indirect selection through growth to improve FCR in Nile tilapia (de Verdal et al., 2022). Likewise, the isolation method with restricted feeding has been proven an effective procedure for the genetic improvement of FCR in European sea bass (Besson et al., 2019). Another interesting approach could be to correlate individual feed efficiency with predictors, that could make selection more precise and/or easier (see review by de Verdal et al., 2018a). However, studies evaluating individual differences in feed efficiency on a significant number of fish are mostly recent, and thus studies correlating feed efficiency with other traits measured on the same fish are for the time being very scarce.

Recently, Besson et al. (2022) have also investigated the effect of genetic background on feed conversion traits in gilthead sea bream (*Sparus aurata*) differentially selected on the basis on their individual FCR. This has led to differentiate animals with high (FCR+) and low (FCR-) individual FCR at the juvenile stage, but the improvement of feed efficiency under restricted feeding is not associated with faster growth under ad libitum feeding in group-housed fish as clearly as with European sea bass. Besides, the general knowledge on selection of animals for feed efficiency suggests that selecting for leaner animals (reduced body fat content) would improve feed efficiency (Knap and Kause, 2018). This is because the energy cost of lipid deposition is higher than protein growth. In gilthead sea bream, the most efficient fish indeed tend to have less visceral fat (Besson et al., 2022), but further studies are needed to fully understand the physiological processes driving changes in FCR, and also how performance differences can be associated with a given behavioural and transcriptional trait. To achieve this goal, fish behaviour of group-housed sea bream from the study of Besson et al. (2022) were monitored using a smart small biologger (AEFishBIT) attached to the operculum for the simultaneous monitoring of physical activity and ventilation rates (Martos-Sitcha et al., 2019b). The usefulness of this device for the welfare assessment has been proven in gilthead sea bream, European sea bass and Atlantic salmon, bridging different activity and behaviour patterns with genetically- and environmentally-mediated changes in fish performance and welfare (Ferrer et al., 2020; Kolarevic et al., 2021; Perera et al., 2021; Rosell-Moll et al., 2021). Herein, the behavioural approach was complemented by the targeted gene expression profiling of liver and white skeletal muscle, using customized gilthead sea bream PCR-arrays with a wide-representation of selected markers of growth, lipid and energy metabolism, and antioxidant defence.

2. Materials and methods

2.1. 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#12550–20150717 18471859v9). 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.

2.2. Fish

A total of 458 juvenile gilthead sea bream from the Fermes Marines du Soleil (FMDS) breeding program (La Brée-les-Bains, France) were reared at the Ifremer Aquaculture Research Station in Palavas-les-Flots (France). They were classified as FCR+ or FCR- based on the individual measurements of weight gain and feed intake of isolated fish in experimental aquaria, in two consecutive periods of two weeks during the juvenile stage (161–315 dph) (Besson et al., 2022). During this period, each fish was fed daily a single meal with pellets corresponding to 70% of the standard ration, and uneaten pellets were collected and counted 1.5 h later, to calculate the individual feed intake. FCR+ and FCR- groups of fish were then tested for group feed efficiency in an experiment starting at the age of 323 dph (average weight = 176.4 g), for a total of 97 days in a recirculation system where water temperature was set at 22–23 ºC and photoperiod at 12 L:12D. Fish were fed with an automatic feeder delivering the daily ration in 20 portions between 5.30 a.m. and 8.35 a.m. (3 h after the onset of the light phase). At the end of the automatic delivery, if no pellets were found at the faecal trap, additional feed was given via a manual trigger until first pellets were collected in the trap. Feed was manufactured and formulated by Sparos LDA (Olhão, Portugal) to fulfil the gilthead sea bream nutritional requirements (Jobling, 2012) while maintaining a low fish meal content (Appendix 1). The timeline of the study for the measurements of individual or group feed efficiency is summarized in Fig. 1. The averaged individual and group-housed FCR was 1.22 and 1.23 for FCR- fish, whereas those of FCR+ were 1.74 and 1.28 (Besson et al., 2022).

2.3. Locomotor activity and metabolic traits (AEFishBIT)

Individual monitoring of whole organism traits in free-swimming FCR+ and FCR- fish was conducted by means of the smart device AEFishBIT. It is a small and light (14x7x7 mm; 1.1 g) sensor composed of a tri-axial accelerometer, a microprocessor, a battery and a RFID that is designed to be externally attached to fish operculum. This unique location serves to provide simultaneous measurements of activity patterns (signals of x- and y-axes) and respiratory frequency (z-axis signal) processed by on-board algorithms (Ferrer et al., 2020; Martos-Sitcha et al., 2019b).

The devices were externally attached to the operculum of anaesthetized (30 mg/L benzocaine) 420 dph gilthead sea bream (N = 12 per experimental group) using monel piercing fish tags (National Band & Tag Company) with a flexible heat shrink polyethylene tube (Eventronic) that is able to easily fit the device. This procedure has been demonstrated to be minimally invasive in gilthead sea bream and European sea bass, and in skilled hands the entire application procedure takes less than 30 s per fish. AEFishBIT devices were programmed for on-board calculation of respiratory frequency and physical activity over 2 min time windows each 15 min along two consecutive days. For each device, clock time drift was previously estimated for post-processing synchronization. This time drift was established to be constant for any given device in a temperature range of 4–30 °C. Fish remained unfed in their original tanks over the recording time. At the end of test, tagged fish were euthanized with 150 mg/L benzocaine for device recovery and retrieval of on-board processed data, as well as biometry and tissue collection. Muscle fat content was determined by the averaged measures on both sides of fish using a Distell fatmeter (Distell Ltd., UK) according to Haffray et al. (2005). Viscera and liver alone were dissected to calculate viscerosomatic index [VSI = 100 x (viscera weight/fish weight)] and hepatosomatic index [HSI = 100 x (liver weight/fish weight)]. Portions of liver and white skeletal muscle were excised and immediately put in RNA later (Thermo Fisher Scientific) at − 20 °C until extraction of total RNA for subsequent gene expression analysis.

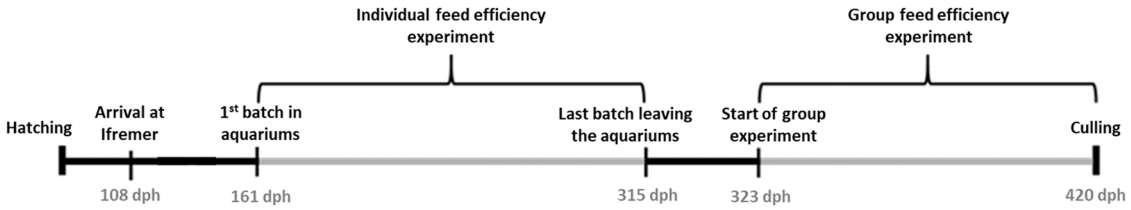

Fig. 1. Experimental timeline of individual and group feed efficiency experiments. Time is expressed as days post hatching (dph). Adapted from Besson et al. (2022).

2.4. Gene expression analysis

Tissue RNA was extracted using the MagMAX-96 total RNA isolation kit (Life Technologies) after tissue homogenization in TRI reagent following manufacturers' instructions. RNA quantity and purity was determined by Nanodrop (Thermo Scientific) with absorbance ratios at 260 nm/280 nm of 1.9–2.1. Reverse transcription (RT) of 500 ng of total RNA was performed with random decamers using the High-Capacity cDNA Archive Kit (Applied Biosystems). RT reactions were incubated for 10 min at 25 °C and 2 h at 37 °C. Negative control reactions were run without reverse transcriptase.

Real-time quantitative PCR was carried out with an Eppendorf Mastercycler Ep Realplex, using 96-well PCR array layouts designed for simultaneously profiling a panel of 44 genes for liver samples (Table 1), and 29 genes for muscle samples (Table 2). The liver array comprised of gene markers of GH/IGF system (9), lipid metabolism (15), energy metabolism (11), and antioxidant defence and molecular chaperones (9). Transcripts analyzed in muscle were associated with the GH/IGF system (12), muscle growth and cell differentiation (6), and energy sensing and oxidative metabolism (11). Specific primer pair sequences are listed in Appendix 2. Controls of general PCR performance were included on each array, and all the pipetting operations were performed by means of an EpMotion 5070 Liquid Handling Robot (Eppendorf) to improve data reproducibility. Briefly, reverse transcription reactions were diluted to convenient concentrations and the equivalent of 660 pg of total input RNA was used in a 25 μL volume for each PCR reaction. PCR-wells contained a 2 × SYBR Green Master Mix (Bio-Rad) and specific primers at a final concentration of 0.9 μM were used to obtain amplicons of 50–150 bp in length. The PCR amplification program consisted of an initial denaturation step at 95 °C for 3 min, followed by 40 cycles of denaturation for 15 s at 95 °C and annealing/extension for 60 s at 60 °C. The efficiency of the PCR reactions was consistently higher than 90% and similar among all genes. The specificity and efficiency of the reactions was verified by melting curve analysis (ramping rates of 0.5 °C/10 s over a temperature range of 55–95 °C) and linear regression of serial dilutions of RT reactions. PCR efficiency for target genes varied between 91% and 100%. Negative controls without a template were performed for each primer set. Gene expression was calculated using the delta-delta Ct method (Livak and Schmittgen, 2001). β-actin was tested for gene expression stability using GeNorm software (M score = 0.21) and it was used as housekeeping gene in the normalization procedure. For multigene analysis, all values in liver were referenced to the expression levels of *igfbp2a* of FCR- fish with an assigned value of 1.0; for skeletal muscle, values were referenced to those of *cpt1α* of FCR- fish.

2.5. Statistical analysis

Statistically significant differences on processed data were assessed by Student's t-test (group differences in a given gene and tissue) and Pearson correlation coefficients using the Sigmaplot suite (Systat Software Inc.). The daily rhythmicity of the time series analysis was further analyzed using a simple cosinor model (Refinetti et al., 2007). Recorded data from incomplete light and dark phases were excluded to avoid any temporal bias. Thus, analyzed rhythms typically comprised two complete dark phases and one complete light phase. Biometric data and AEFishBIT results were jointly analyzed by partial least-squares discriminant analysis (PLS-DA) using EZinfo v3.0 (Umetrics). The quality of the PLS-DA model was evaluated by the parameters R2Y (cum) and Q2 (cum), which indicate the fit and prediction ability, respectively. To assess whether the supervised model was being over-fitted, a validation test consisting on 500 random permutations was performed using the Bioconductor R package *ropls* (Thévenot et al., 2015). The list of factors contributing to group separation was determined by the minimum Variable Importance in the Projection (VIP) values. Discriminant factors were considered with a VIP threshold > 1.0 (Li et al., 2012; Kieffer et al., 2016).

3. Results

3.1. Leaner body shape for FCR- fish

Biometric data of sampled fish for analysis of gene expression and behavioural traits are shown in Table 3. FCR+ and FCR- fish did not share statistically significant differences in body weight, although body length of FCR- fish was significantly larger than that of FCR+ fish (25.2 vs 24.4 cm). This fact led to a significantly lower condition factor for FCR- fish (2.23 vs 2.36), pointing out a leaner body shape. This was concurrent with a significantly lower viscerosomatic index in FCR- fish (3.75 vs 4.36) that was coincident with a slight (non-statistically significant) increase of muscle fat content, whereas carcass and hepatosomatic (HSI) indexes remained almost unaltered.

3.2. AEFishBIT records allow discrimination by feed efficiency

AEFishBIT recording revealed pronounced daily rhythms of physical activity and respiratory frequency in FCR+ and FCR- groups, which rendered enhanced rates of physical activity during the feeding period after the onset of lights (Fig. 2). This enhanced activity was prolonged over time, and the cosinor acrophase (maximal value) was attained at the same time in both groups of fish (4:05 and 4:10 zeitgeber time in FCR+ fish and FCR- fish, respectively) with the mesor and amplitude of physical activity being slightly higher in FCR-. This trend was more clearly stated for respiratory frequency with mesor values increasing significantly from 1.70 in FCR+ to 1.96 in FCR- fish (Fig. 3). Also, the respiratory acrophase was moved later in day (6:57 h zeitgeber time) in FCR- fish, whereas that of FCR+ fish remained early in the day (2:23 h zeitgeber time) and more coupled to physical activity. This different energy partitioning of FCR- fish for locomotor activity and growth-related metabolic processes was further confirmed by regression analysis, with a regression slope of respiratory frequency against physical activity higher in FCR- fish than in FCR+ fish (Fig. 4).

3.3. Both biometric and behavioural traits contribute to FCR groups differentiation

Discriminant analysis (PLS-DA) of behavioural and biometric traits clearly separated FCR+ and FCR- fish groups along component 1, explaining by itself the 81% of total variance (Fig. 5A). The fit of the PLS-

Table 1
PCR-array layout for hepatic gene expression profiling.

Function	Gene	Symbol	GenBank
PERFORMANCE	Growth hormone receptor 1	*ghr1*	AF438176
Gh/Igf system	Growth hormone receptor 2	*ghr2*	AY573601
	Insulin-like growth factor-1	*igf1*	AY996779
	Insulin-like growth factor-2	*igf2*	AY996778
	Insulin-like growth factor binding protein 1a	*igfbp1a*	KM522771
	Insulin-like growth factor binding protein 1b	*igfbp1b*	MH577189
	Insulin-like growth factor binding protein 2a	*igfbp2a*	MH577190
	Insulin-like growth factor binding protein 2b	*igfbp2b*	AF377998
	Insulin-like growth factor binding protein 4	*igfbp4*	KM658998
LIPID	Elongation of very long chain fatty acids 1	*elovl1*	JX975700
METABOLISM	Elongation of very long chain fatty acids 4	*elovl4*	JX975701
	Elongation of very long chain fatty acids 5	*elovl5*	AY660879
	Elongation of very long chain fatty acids 6	*elovl6*	JX975702
	Fatty acid desaturase 2	*fads2*	AY055749
	Stearoyl-CoA desaturase 1a	*scd1a*	JQ277703
	Stearoyl-CoA desaturase 1b	*scd1b*	JQ277704
	Hepatic lipase	*hl*	EU254479
	Lipoprotein lipase	*lpl*	AY495672
	Adipose triglyceride lipase	*atgl*	JX975711
	85 kDa calcium-independent phospholipase A2	*pla2g6*	JX975708
	Cholesterol 7-alpha-monooxygenase	*cyp7a1*	KX122017
	Peroxisome proliferator-activated receptor α	*pparα*	AY590299
	Peroxisome proliferator-activated receptor β	*pparβ*	AY590301
	Peroxisome proliferator-activated receptor γ	*pparγ*	AY590304
ENERGY	Carnitine palmitoyltransferase 1 A	*cpt1a*	JQ308822
METABOLISM	Fatty acid binding protein, heart	*h-fabp*	JQ308834
	Citrate synthase	*cs*	JX975229
	NADH-ubiquinone oxidoreductase chain 2	*nd2*	KC217558
	NADH-ubiquinone oxidoreductase chain 5	*nd5*	KC217559
	Cytochrome c oxidase subunit I	*coxi*	KC217652
	Cytochrome c oxidase subunit II	*coxii*	KC217653
	Proliferator-activated receptor gamma coactivator 1 alpha	*pgc1α*	JX975264
	Sirtuin1	*sirt1*	KF018666
	Sirtuin2	*sirt2*	KF018667
	Uncoupling protein 1	*ucp1*	FJ710211
ANTIOXIDANT	Glutathione peroxidase 1	*gpx1*	DQ524992
DEFENCE	Glutathione peroxidase 4	*gpx4*	AM977818
	Peroxiredoxin 3	*prdx3*	GQ252681
	Peroxiredoxin 5	*prdx5*	GQ252683
	Superoxide dismutase [Cu-Zn]	*cu-zn-sod/ sod1*	JQ308832
	Superoxide dismutase [Mn]	*mn-sod / sod2*	JQ308833
	Glucose-regulated protein, 170 kDa	*grp170*	JQ308821
	Glucose-regulated protein, 94 kDa	*grp94*	JQ308820
	Glucose-regulated protein, 75 kDa	*grp75*	DQ524993

Table 2
PCR-array layout for white skeletal muscle gene expression profiling.

Function	Gene	Symbol	GenBank
PERFORMANCE	Growth hormone receptor 1	*ghr1*	AF438176
Gh/Igf system	Growth hormone receptor 2	*ghr2*	AY573601
	Insulin-like growth factor-1	*igf1*	AY996779
	Insulin-like growth factor-2	*igf2*	AY996778
	Insulin-like growth factor binding protein 3a	*igfbp3a*	MH577191
	Insulin-like growth factor binding protein 5a	*igfbp5a*	MH577193
	Insulin-like growth factor binding protein 5b	*igfbp5b*	MH577194
	Insulin-like growth factor binding protein 6a	*igfbp6a*	MH577195
	Insulin-like growth factor binding protein 6b	*igfbp6b*	MH577196
	Insulin receptor	*insr*	KM522774
	Insulin-like growth factor receptor 1a	*igfr1a*	KJ591052
	Insulin-like growth factor receptor 2	*igfr2*	KM522776
MUSCLE	Myoblast determination protein 1	*myod1*	AF478568
GROWTH	Myogenic factor MYOD2	*myod2*	AF478569
	Myogenic factor 5	*myf5*	JN034420
	Myogenic factor 6	*myf6/mrf4/ herculin*	JN034421
	Myostatin/Growth differentiation factor 8	*mstn/gdf-8*	AF258448
	Follistatin	*fst*	AY544167
ENERGY	Sirtuin1	*sirt1*	KF018666
METABOLISM	Sirtuin2	*sirt2*	KF018667
	Sirtuin5	*sirt5*	KF018670
	Carnitine palmitoyltransferase 1 A	*cpt1a*	JQ308822
	Citrate synthase	*cs*	JX975229
	NADH-ubiquinone oxidoreductase chain 2	*nd2*	KC217558
	NADH-ubiquinone oxidoreductase chain 5	*nd5*	KC217559
	Cytochrome c oxidase subunit I	*coxi*	KC217652
	Cytochrome c oxidase subunit II	*coxii*	KC217653
	Uncoupling protein 3	*ucp3*	EU555336
	Proliferator-activated receptor gamma coactivator 1 alpha	*pgc1α*	JX975264

DA model was validated by a 500-random permutation test (Appendix 3). According to the VIP analysis of this projection (Fig. 5B), the most important parameters contributing to group separation (VIP > 1.0) between FCR+ and FCR- fish included biometric parameters such as length, condition factor, viscerosomatic index or body weight, as well as a number of descriptors informing of the daily cycle of respiratory frequency in free swimming individuals like mesor, acrophase and the respiratory phase shift. Relationship between behavioural and biometric traits was further assessed by correlation analysis, with FCR+ showing positive and significant ($p < 0.1$) correlations between HSI and the mesor and amplitude of the physical activity, whereas the respiratory mesor was negatively correlated with muscle fat content, and significant relative correlations were found for the physical activity acrophase and the phase shift with the condition factor and the carcass index (Appendix 4). Conversely, in FCR- fish the amplitude of respiratory frequency was positively correlated with the mesor and amplitude of locomotor activity, and negatively with muscle fat and carcass index (Appendix 5).

3.4. Gene expression analysis highlights a different metabolic scope

The hepatic expression profile of FCR+ and FCR- highlighted clear differences between both groups. Ten out of 44 analyzed genes were expressed at a significantly lower rate ($P < 0.1$) in FCR- fish than in FCR+ fish (Table 4). This down-regulation comprised genes related to growth (*igfbp2b*), lipid metabolism (*elovl6*, *fads2*, *scd1a*, *pparα*), oxidative metabolism (*coxi*, *coxii*, *h-fabp*) and antioxidant defense (*mn-sod/ sod2*, *grp94*). Regarding white skeletal muscle, gene expression was also altered between individual FCR groups, and a number of genes related to growth (*ghr2*, *igfbp5b*, *mstn/gdf-8*), energy sensing (*sirt2*), and mitochondrial respiration uncoupling (*ucp3*) were differentially regulated in FCR+ and FCR- fish (Table 5).

Table 3
Biometric parameters of FCR- and FCR+ analysed fish. Values are the mean ± SEM of 12 fish. Asterisks indicate significant differences between groups (*P < 0.05, Student's t-test).

Group	Weight (g)	Length (cm)	CF[a]	Carcass Index[b]	Muscle fat	HSI[c]	VSI[d]
FCR-	363.8 ± 14.1	25.2 ± 0.3 *	2.23 ± 0.04 *	0.840 ± 0.004	9.89 ± 0.53	1.07 ± 0.06	3.75 ± 0.17 *
FCR+	346.5 ± 13.8	24.4 ± 0.3	2.36 ± 0.04	0.844 ± 0.004	9.63 ± 0.53	1.02 ± 0.05	4.36 ± 0.23

[a] Condition factor, CF = 100 x (body weight/standard lenght^3)
[b] Carcass index = carcass/weight
[c] Hepatosomatic index, HSI = 100 x (liver weight/fish weight)
[d] Vicerosomatic index, VSI = 100 x (viscera weight/body weight)

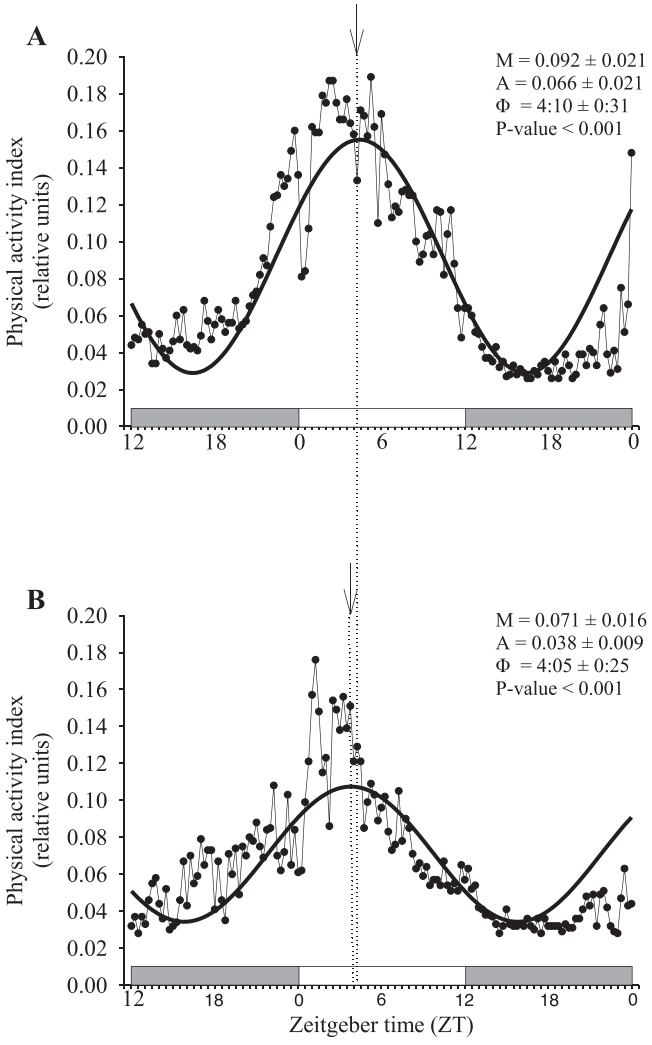

Fig. 2. Daily changes in physical activity in FCR- (A) and FCR+ (B) fish. The best-fit curves are obtained by cosinor analysis. Each point is the consensus of 10 individuals. Dark phase is labelled in grey (X-axis). The feeding period is indicated by a grey vertical box. Values of mesor (M), amplitude (A) and acrophase (Φ) are stated for each curve. Arrows indicate curve acrophase.

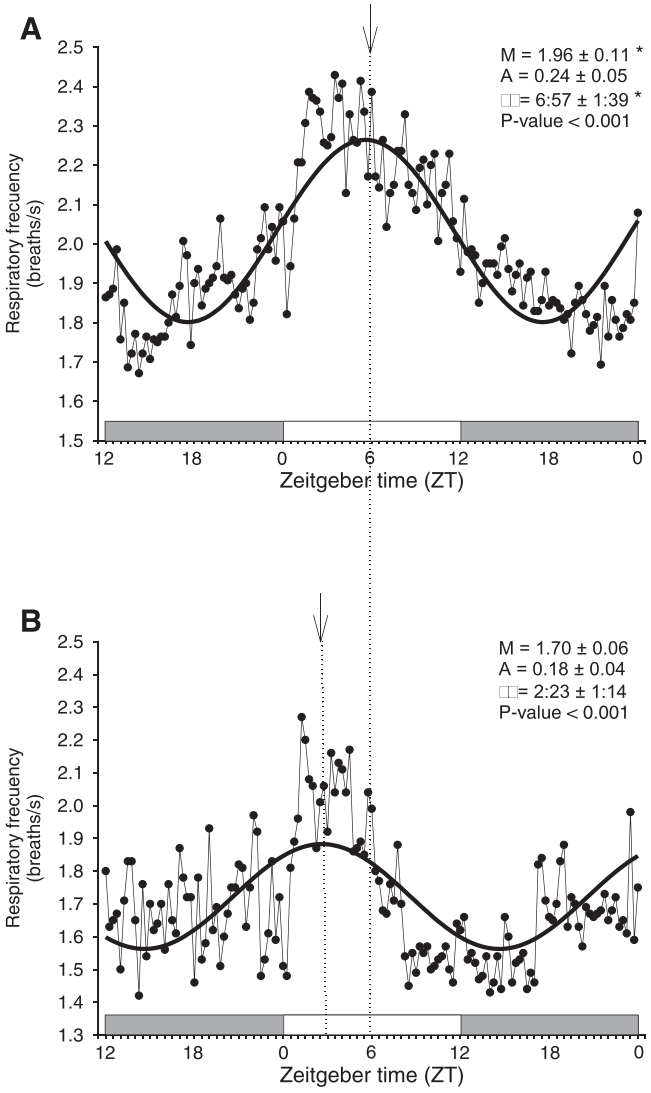

Fig. 3. Daily changes in respiratory frequency in FCR- (A) and FCR+ (B) fish. The best-fit curves are obtained by cosinor analysis. Each time point is the consensus of 10 individuals. Dark phase is labelled in grey (X-axis). The feeding period is indicated by a grey vertical box. Values of mesor (M), amplitude (A) and acrophase (Φ) are stated for each curve. Arrows indicate curve acrophase. Asterisks indicate significant differences between groups (*P < 0.05, Student-t-test).

4. Discussion

Individual selecting procedures for faster growing animals under restricted feeding were demonstrated to constitute an efficient method to genetically improve FCR in group-housed European sea bass (Besson et al., 2019). Similarly, several feed efficiency traits were estimated to be heritable also in gilthead sea bream, though a better FCR was not so clearly retained in grouped fish (Besson et al., 2022). Despite this, it appears that variation in individual feed efficiency under restricted feeding can reflect, at least partially, differences in FCR when fish are reared in groups without feed restriction. This assumption was further supported in the present work by an integrative approach combining data on transcriptomics and behavioural traits in swimming activity and ventilation rates. There is now an increasing interest for monitoring gilthead sea bream behaviour by means of accelerometer technology (Alfonso et al., 2021; Arechavala-Lopez et al., 2021; Føre et al., 2018;

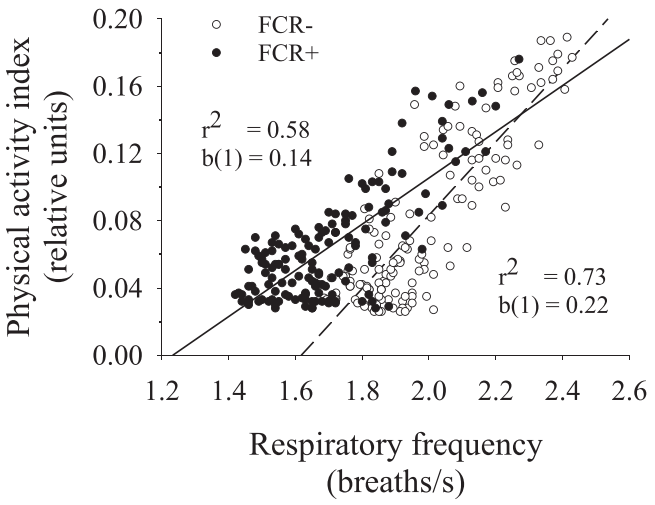

Fig. 4. Correlation analysis of individual measures of activity and respiration.

Palstra et al., 2020; Perera et al., 2021; Rosell-Moll et al., 2021), but to our knowledge this is one of the first fish studies addressing at the same time changes in behaviour and tissue-specific gene expression patterns.

AEFishBIT measurements were able to highlight differences in the activity patterns of FCR- and FCR+ fish on the basis of their different behaviour and energy partitioning for growth and locomotor activity. Indeed, FCR- fish showed higher respiratory rates during low physical activity periods (non-feeding periods), though swimming activity and respiratory rates were quite similar in both experimental groups during the fixed feeding period. In fact, feeding time is a relevant zeitgeber in a number of vertebrates (Hannay et al., 2020; Pickel and Sung, 2020), and a large body of evidence links circadian rhythms with feeding regimes of farmed fish to ensure that physiological processes are performed at the appropriate time of day (Sánchez-Vázquez and Madrid, 2001). Thus, transcriptomic profiling of gilthead sea bream whole-larvae on a daily basis revealed consecutive activation of canonical pathways of photo-transduction, intermediary metabolism, development, chromatin remodelling, and cell cycle regulation (Yúfera et al., 2017). This daily transcriptome resembles a cell cycle (G1/S, G2/M, and M/G1 transitions) in synchronization with multicellular processes, which is temporally organized in a 24-h cycle for maximizing fast growth during early life. Likewise, in the present study, the phase shift of respiratory frequency of FCR- fish is raising as a clock that matches with the post-prandial peak of ammonia excretion (2–3 h post-feeding) of fish of the same class of size under the same range of temperatures (Gómez-Requeni et al., 2003). This should represent a metabolic advantage, preparing the organism for the highly energy demanding process of digestion and nutrient absorption. In any case, the way how fish interact with the environment and their congeners is a major source of variation, and the general thinking is that fast growth and low activity are highly co-evolved through the evolution of modern teleosts (Rosenfeld et al., 2015; Sibly et al., 2015). This is because the enhanced energy cost of growth and maintenance is often supported by a higher feed intake and perhaps improved FCR, as the result of a reduced locomotor activity that does not offer a special advantage under intensive culture (Devlin et al., 2004; Killen et al., 2014). Certainly, selection for fast growth in the PROGENSA® gilthead sea bream selective breeding program comes at the expense of a reduced swimming performance and anaerobic fitness (Perera et al., 2021). In the present study, phenotypic selection for improved FCR on fish from the FMDS breeding program (Besson et al., 2022) also resulted in a reduced locomotor activity during non-feeding periods, but interestingly this low activity swimming behaviour was not extended to the feeding period. Moreover, the positive correlation between the mesor of respiratory frequency and the mesor and amplitude of locomotor activity in FCR- fish ensures a high

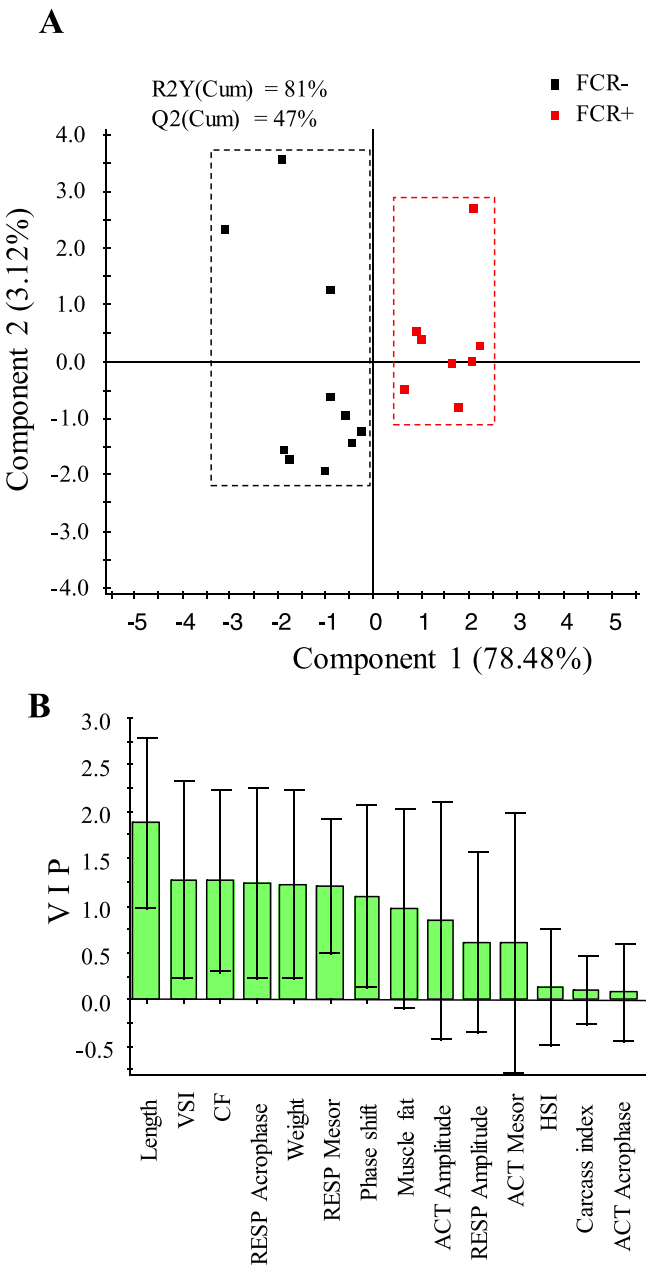

Fig. 5. (A) Discriminant analysis of FCR- and FCR+ fish for biometric and behaviour data. The two first components explain 81% of total variance. (B) Ordered list of variable importance (VIP) in the projection of PLS-DA model for group differentiation.

swimming activity when feed becomes available.

The above findings reinforce the usefulness of behaviour studies for the metabolic phenotyping of individual differences in energy metabolism in gilthead sea bream. This was also substantive in a previous study, in which metabolic rates inferred from respiratory frequency were negatively correlated with a different family susceptibility to fasting weight loss (Perera et al., 2021). It is also noteworthy that in FCR+ but not in FCR- fish the respiratory mesor was negatively correlated with muscle fat content, whereas HSI was positively correlated with the mesor and amplitude of the recorded free-swimming activity. Conversely, in FCR- but not in FCR+ , the amplitude of respiratory frequency was positively correlated with the mesor and amplitude of locomotor activity, and negatively with muscle fat and carcass index. Altogether, this suggests a different location and use of stored body fats for growth and locomotor purposes, as evidenced below by the hepatic

Table 4
Relative gene expression of hepatic genes in FCR- and FCR+ fish. Data are the mean ± SEM of 8–11 fish. All data values for each gene were in reference to the expression level of *igfbp2a* of FCR- fish with an arbitrary assigned value of 1.

	FCR-	FCR+	P-Value
ghr1	1.56 ± 0.14	1.82 ± 0.22	0.360
ghr2	1.07 ± 0.09	1.14 ± 0.22	0.812
igf1	5.49 ± 0.48	6.17 ± 0.74	0.484
igf2	1.99 ± 0.35	1.87 ± 0.35	0.816
igfbp1a	0.04 ± 0.01	0.06 ± 0.01	0.304
igfbp1b	1.55 ± 0.35	1.44 ± 0.37	0.842
igfbp2a	1.04 ± 0.11	1.16 ± 0.17	0.597
igfbp2b	1.71 ± 0.11	2.23 ± 0.14	**0.014**
igfbp4	0.5 ± 0.08	0.61 ± 0.07	0.331
elovl1	7.68 ± 0.51	8.4 ± 0.60	0.390
elovl4	0.11 ± 0.02	0.12 ± 0.01	0.635
elovl5	0.31 ± 0.12	0.3 ± 0.10	0.946
elovl6	0.19 ± 0.03	0.49 ± 0.13	**0.046**
fads2	0.72 ± 0.17	3.20 ± 1.10	**0.050**
scd1a	0.13 ± 0.02	0.35 ± 0.12	**0.090**
scd1b	0.06 ± 0.02	0.09 ± 0.02	0.343
hl	7.28 ± 0.96	8.41 ± 0.79	0.370
lpl	10.9 ± 1.35	10.4 ± 1.18	0.795
atgl	1.05 ± 0.27	1.43 ± 0.39	0.475
pla2g6	0.1 ± 0.01	0.11 ± 0.01	0.201
cyp7a1	0.39 ± 0.05	0.53 ± 0.10	0.290
pparα	1.2 ± 0.11	1.75 ± 0.19	**0.032**
pparβ	0.72 ± 0.08	0.83 ± 0.08	0.350
pparγ	0.29 ± 0.03	0.34 ± 0.03	0.439
cpt1a	0.71 ± 0.05	0.84 ± 0.10	0.346
h-fabp	27.60 ± 3.55	43.80 ± 7.96	**0.085**
cs	0.44 ± 0.02	0.48 ± 0.03	0.364
nd2	20.8 ± 2.65	27.8 ± 3.62	0.165
nd5	6.07 ± 0.57	7.63 ± 0.91	0.189
cox-i	27.3 ± 3.67	37.6 ± 4.03	**0.076**
cox-ii	14.8 ± 2.25	26.0 ± 4.51	**0.042**
pgc1α	0.07 ± 0.01	0.08 ± 0.02	0.630
sirt1	0.06 ± 0.00	0.07 ± 0.01	0.174
sirt2	0.10 ± 0.01	0.12 ± 0.01	0.142
ucp1	7.7 ± 1.33	9.26 ± 1.52	0.474
gpx1	0.99 ± 0.10	1.08 ± 0.08	0.466
gpx4	4.27 ± 0.85	4.29 ± 0.62	0.985
prdx3	0.58 ± 0.05	0.68 ± 0.04	0.144
prdx5	0.60 ± 0.03	0.68 ± 0.04	0.137
cu-zn-sod/ sod1	3.05 ± 0.47	3.72 ± 0.38	0.280
mn-sod / sod2	0.70 ± 0.07	0.93 ± 0.10	**0.095**
grp170	0.89 ± 0.06	1.97 ± 0.63	0.124
grp94	4.29 ± 0.45	14.7 ± 4.87	**0.066**
grp75	0.48 ± 0.03	0.60 ± 0.06	0.356

[1]P values result from Student t-test. Bold font in each row indicate significant differences or near to significance between FCR- and FCR+ fish groups ($P < 0.1$).

Table 5
Relative gene expression of white skeletal muscle genes in FCR- and FCR+ fish. Data are the mean ± SEM of 8–11 fish. All data values for each gene were in reference to the expression level of *cpt1a* of FCR- fish with an arbitrary assigned value of 1.

	FCR-	FCR+	P-Value
ghr1	1.23 ± 0.09	1.24 ± 0.15	0.922
ghr2	1.70 ± 0.30	1.17 ± 0.13	**0.097**
igf1	0.09 ± 0.03	0.06 ± 0.01	0.217
igf2	0.60 ± 0.11	0.51 ± 0.06	0.429
igfbp3a	1.07 ± 0.11	1.10 ± 0.14	0.863
igfbp5a	0.18 ± 0.02	0.18 ± 0.02	0.994
igfbp5b	2.24 ± 0.17	1.74 ± 0.15	**0.048**
igfbp6a	0.01 ± 0.00	0.01 ± 0.00	0.380
igfbp6b	0.04 ± 0.01	0.04 ± 0.00	0.264
insr	0.29 ± 0.02	0.25 ± 0.02	**0.097**
igfira	0.29 ± 0.02	0.25 ± 0.02	0.192
igfr2	0.19 ± 0.01	0.18 ± 0.02	0.651
myod1	4.93 ± 0.40	4.60 ± 0.40	0.576
myod2	0.62 ± 0.11	0.58 ± 0.08	0.771
myf5	0.10 ± 0.00	0.10 ± 0.01	0.439
myf6/mrf4/herculin	0.11 ± 0.01	0.14 ± 0.02	0.409
mstn/gdf-8	2.78 ± 0.48	1.59 ± 0.29	**0.042**
fst	0.23 ± 0.04	0.18 ± 0.02	0.212
sirt1	0.09 ± 0.01	0.08 ± 0.01	0.165
sirt2	0.18 ± 0.01	0.14 ± 0.01	**0.006**
sirt5	0.23 ± 0.02	0.20 ± 0.01	0.202
cpt1a	1.09 ± 0.16	1.19 ± 0.18	0.691
cs	6.61 ± 0.57	5.76 ± 0.55	0.300
nd2	45.02 ± 6.51	43.21 ± 4.37	0.816
nd5	14.01 ± 1.65	13.40 ± 1.34	0.796
coxi	57.92 ± 6.88	55.82 ± 4.57	0.800
coxii	36.21 ± 7.44	32.93 ± 2.75	0.650
ucp3	5.60 ± 0.64	4.19 ± 0.45	**0.081**
pgc1α	0.54 ± 0.13	0.48 ± 0.09	0.289

[1]P values result from Student t-test. Bold font in each row indicate significant differences or near to significance between FCR+ and FCR- fish groups ($P < 0.1$).

and white skeletal muscle transcriptomic profiling.

Differences in hepatic expression pattern were indicative of the different mechanisms promoting FCR regulation in FCR- and FCR+ fish. For instance, Igfbps are emerging as highly regulated components of the Gh/Igf system that consequently have an impact on growth performance. Up to 11 *igfbp* variants, covering the full igfbp1–6 repertoire with paralogs pairs of igfbp1, 2, 3, 5 and 6 have been reported in gilthead sea bream (Pérez-Sánchez et al., 2018). The identity of these *igfbp* sequences have been corroborated by phylogenetic analyses, while gene expression analysis of adult fish indicated that mRNA transcripts of the *igfbp1/2/4* clade are highly represented in the liver tissue of gilthead sea bream, whereas the *igfbp3/4/5* clade is over-expressed in the skeletal muscle. Regarding Igfbp2, growth promoting and inhibitory actions have been reported in fish (Duan et al., 1999; Garcia de la Serrana and Macqueen, 2018), though gilthead sea bream data on *igfbp2b* expression mostly support a growth promoting action, which is substantiated by its up-regulation during the summer growth spurt and its depressed expression in fish with signs of essential fatty acid deficiencies (Pérez-Sánchez et al., 2018). In contrast to this, we found in the present study that the highest expression of *igfbp2b* was achieved in FCR+ fish, which might indicate that processes driving to FCR improvement are diverse and complex, and not only restricted to simple growth regulation at a given time.

Regarding key enzymes of lipid metabolism, the general idea is that starvation and reduced lipid storage rates during overwintering are related to a pronounced down-regulation of lipogenic enzymes, including fatty acid elongases and desaturases (Turyn et al., 2018; Benedito-Palos et al., 2014, 2016; Rimoldi et al., 2016). Conversely, the expression of *elov6*, *scd1* (Δ9 desaturase), and secondly *fads2* (Δ6 desaturase) is largely induced in gilthead sea bream by feeds formulated for deficiencies in long-chain n-3 PUFA (Perera et al., 2020), preventing this expression profile fatty livers and the lipotoxic effect of saturated fatty acids by favouring their conversion to more safely stored mono-unsaturated fatty acids (Li et al., 2009; Silbernagel et al., 2012). Since this up-regulated expression was already found in group-housed FCR+ fish fed diets that covered their nutritional requirements, it appears that this group of fish not only can share an impaired feed efficiency, but also a reduced tolerance to the high replacement of dietary fish meal by alternative raw materials. Certainly, fish nutrient deficiencies in sulfur amino acids, essential fatty acids or minerals drive to histological traits such as liver steatosis (Ballester-Lozano et al., 2015). Given that these nutrients are perhaps the most important limiting factors for the total replacement of marine feedstuffs in fish feeds, it is likely that selection for improved FCR also facilitates the use of new fish feed formulations based on alternative and more sustainable feed ingredients (i.e., terrestrial plants, insect proteins, single cell proteins, sea weeds, etc).

In concordance with the above transcriptional changes, FCR- fish showed a down-regulated hepatic gene expression of the lipolytic transcription factor *pparα*, intracellular fatty acid transporter *h-fabp*, and enzyme subunits of the mitochondrial respiratory chain (*coxi*, *coxii*).

Since lipogenesis is considered the major energy-demanding process in liver (Rui, 2014), this expression feature substantiates a reduced ATP-energy production and reduced risk of oxidative stress. Mitochondrial activity is a major source of reactive oxygen species (ROS), and their attenuated production in FCR- fish was associated with a reduced expression of antioxidant enzymes (*mn-sod/sod2*) and molecular chaperones (*grp94*) that are well-recognized markers of the fish response to stressors (Saera-Vila et al., 2009; Magnoni et al., 2017; Peixoto et al., 2019; Martos-Sitcha et al., 2019a). Overall, this hepatic expression pattern might support a better performance of FCR- fish in concurrence with a lower hepatic lipogenic activity and a reduced risk of oxidative stress.

The gene expression of white skeletal muscle was also altered by selection for individual FCR, highlighting the role of growth signals, energy sensors and antioxidant defense markers as part of the operating mechanism for an efficient muscle growth. The enhanced expression of *ghr2* in FCR- fish was particularly noticeable. This feature rendered a lower *ghr1/ghr2* expression ratio (0.66 in FCR- vs 1.05 in FCR+), which is viewed in gilthead sea bream as a local compensatory growth mechanism to mitigate growth derangements in fish facing nutritional deficiencies (Pérez-Sánchez et al., 2018). This was concurrent with the up-regulation of *igfbp5b*, the igfbp paralog with a higher expression level in the skeletal muscle of juveniles and adults of gilthead sea bream that supports its role as a growth-promoting factor in both sparids and salmonids (Garcia de la Serrana and Macqueen, 2018; Pérez-Sánchez et al., 2018).

Sirtuins (SIRT) exert their function by coupling protein deacetylation of histones and metabolic enzymes with the energy status of the cell via the cellular NAD+ /NADH ratio (Schwer and Verdin, 2008). This offers the possibility of different energy sensing mechanisms, dependent on the tissue and the intensity and nature of the energy-demanding process. Thus, the muscle expression of *sirt1* and *sirt5* are induced by fasting in gilthead sea bream (Simó-Mirabet et al., 2017), but in the present work and previous studies (Simó-Mirabet et al., 2018) *sirt2* was especially responsive to genetic improvement of FCR, with higher expression of *sirt2* in the white skeletal muscle of FCR- than in FCR+ fish, with no differences in muscle expression of *sirt1* between the two experimental groups. Studies in humans and rodents also indicate that SIRT2 integrates changes in energy status, lipid oxidation and redox homeostasis by increasing fatty acid oxidation and the activity of ROS-scavenging enzymes (Austin and St-Pierre, 2012; Krishnan et al., 2012). Moreover, a role as muscle stem cell proliferation and differentiation factor has been reported in humans (Dryden et al., 2003; Wu et al., 2014; Stanton et al., 2017), and single nucleotide polymorphism of SIRT2 has been associated with different body size traits in Quinchuan cattle (Gui et al., 2015). All this highly supports a conserved role of SIRT2 as key regulator of muscle growth, linking the availability of metabolic fuels with growth regulation.

Like SIRTs, mitochondrial UCPs act as markers of cell redox balance and oxidative stress, attenuating the production of ROS by the uncoupling of oxidative phosphorylation (OXPHOS) (Rial and Zardoya, 2009). This family of mitochondrial transporters is indeed widely distributed in plants and animal phyla, with one UCP orthologue in avian species (avian UCP) and a core group of three UCP variants (UCP1–3) in mammals and the lineage of modern fish (Emre et al., 2007; Hughes and Criscuolo, 2007). Each of these UCP transcripts have evolved with a different tissue-specific expression pattern, and consequently gilthead sea bream *ucp1* is mostly expressed in liver and secondly intestine, *ucp2* is more ubiquitous, and *ucp3* is specific of skeletal and cardiac muscle tissues with a higher expression level in glycolytic (white skeletal muscle) than in oxidative muscle tissues (red skeletal muscle, heart) (Bermejo-Nogales et al., 2010, 2014). Additionally, it is known that the increased flux of fatty acids towards muscle tissue enhances the expression of Ucp3 in a wide range of animal models, including gilthead sea bream (Schrauwen et al., 2001; Nabben and Hoeks, 2008; Bermejo-Nogales et al., 2011). In other words, Ucp3 acts as a muscle safety valve and changes in mRNA transcripts follow the switches in the oxidative capacity in order to match both energy demand and antioxidant defense. This dual role is probably closely related to the ancestral protein UCP function as an antioxidant agent that allows the use of Ucp3 as a lipotoxicity marker in ectothermic fish. Taking in mind all this, the up-regulated expression of *ucp3* in FCR- fish is viewed as part of the mechanisms that protect muscle cells against an excessive flux of fat when it surpasses the muscle oxidative capacity. This was supported by a lower condition factor and a reduced hepatic lipogenic activity of FCR- fish, linked to the redistribution of body fat depots with a diminished viscerosomatic index that was concurrent with a slight increase (non-statistically significant) of muscle fat content. Muscle lipid deposition is also an indicator trait of FCR in farmed rainbow trout (Kause et al., 2016), but in this case fish with genetically low body and muscle lipid content were the more efficient in turning ingested protein into protein weight gain. It appears, thereby, that selection for improved FCR operates differentially in gilthead sea bream and trout, although leaner individuals are typically more efficient, and enhanced hepatic lipogenesis or excessive levels of lipid deposition in viscera are not preferred traits in breeding programs for improved growth or FCR. This is confirmed by the negative genetic correlation of residual body weight gain (another indicator of feed efficiency) with viscero-somatic index in the total population of gilthead sea bream from which our FCR- and FCR+ fish were selected (Besson et al., 2022). In other words, prevention of excessive lipid deposition is considered beneficial for the improvement of FCR, although muscle fat content is sometimes a confusing leaner trait that requires normalization by body weight and other co-selected traits as stated before by other authors.

From our results, it is also conclusive that FCR- fish had a muscle expression profile that favoured an efficient muscle protein accretion, but not necessarily maximal growth due to the up-regulated expression of myostatin (*mstn*), a member of the transforming growth factor β (TGF-β) superfamily that acts as a suppressor of muscle mass through the cell surface receptor, activin receptor type II (de Caestecker, 2004). Thus, altering Mstn function through gene knockout, overexpression of inhibitors, or gene mutation edition prominently increases muscle mass in fish and other animal models of vertebrates (McPherron and Lee, 1997; Lee and McPherron, 2001; Khalil et al., 2017). According to this, maximum growth in FCR- fish should be under negative rather than positive control, with Mstn acting as a muscle growth suppressor but also as a causative agent of improved FCR. Indeed, the optimum FCR occurs in most fish species below maximum growth and feed intake (Storebakken and Austreng, 1987; Bureau et al., 2006). Moreover, the limitation of growth in FCR- fish might be also substantiated at systemic level by hepatic growth factors, such as the growth-promoting *ifgbp2b* that was consistently down-regulated in FCR- fish.

In summary, the achieved results are indicative of the complex trade-off between growth and feed efficiency, which also involves changes in social hierarchies and feeding behaviour as stated the use of AEFishBIT for the simultaneous monitoring of activity and metabolic traits (Fig. 6). In the practice, this new generated knowledge offers the possibility of a more suitable individual fish phenotyping. Altogether, this study is the proof of concept of a holistic approach that combines biometric, transcriptomic and behavioural tools for unravelling reliable indicator traits and/or mechanistic process participating in the FCR improvement of farmed gilthead sea bream. How all this can be applied in a consistent and a cost-effective manner remains to be established and improved before routine use by aquaculture stakeholders.

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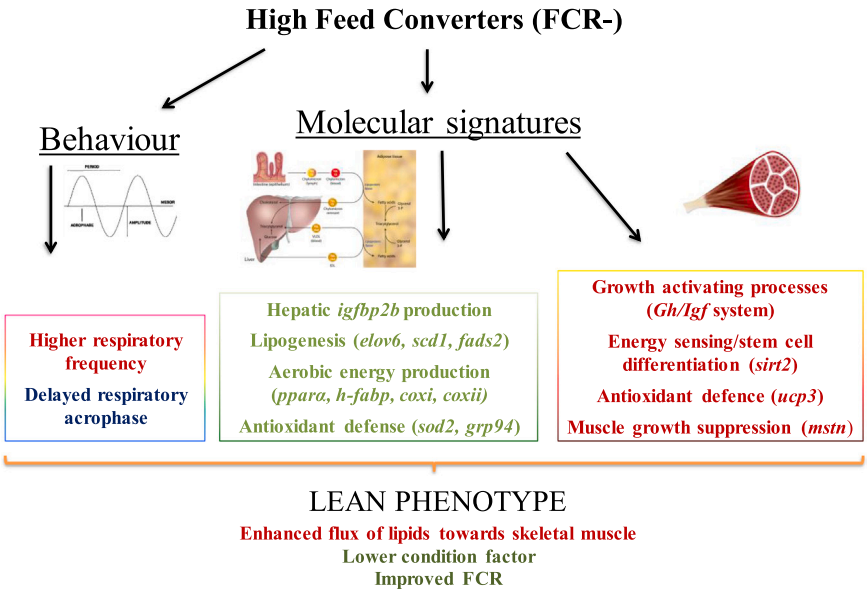

Fig. 6. Graphical abstract of main metabolic and behavioural traits of FCR- fish that lead to a leaner phenotype and improved FCR. Font colour in genes indicates up-regulation (red) or down-regulation (green).

any use which may be made of the information contained therein.

CRediT authorship contribution statement

Josep Calduch-Giner: Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Enrique Rosell-Moll:** Formal analysis, Investigation, Validation, Writing – original draft, Writing – review & editing. **Mathieu Besson:** Investigation, Writing – review & editing. **Alain Vergnet:** Investigation, Writing – review & editing. **Jean-Sébastien Bruant:** Investigation, Writing – review & editing. **Frédéric Clota:** Investigation, Writing – review & editing. **François Allal:** Investigation, Writing – review & editing. **Paul George Holhorea:** Investigation, Writing – review & editing. **Marc Vandeputte:** Conceptualization, Formal analysis, Funding acquisition, Writing – review & editing. **Jaume Pérez-Sánchez:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Validation, Resources, Supervision, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aqrep.2023.101476.

References

Alfonso, S., Zupa, W., Spedicato, M.T., Lembo, G., Carbonara, P.L., 2021. Mapping the energetic costs of free-swimming gilthead sea bream (*Sparus aurata*), a key species in European marine aquaculture. Biology 10, 1357. https://doi.org/10.3390/biology10121357.

Arechavala-Lopez, P., Lankheet, M.J., Díaz-Gil, C., Abbink, W., Palstra, A.P., 2021. Swimming activity of gilthead seabream (*Sparus aurata*) in swim-tunnels: acoustic accelerometry, oxygen consumption and body motion. Front. Anim. Sci. 2, 25. https://doi.org/10.3389/fanim.2021.679848.

Austin, S., St-Pierre, J., 2012. PGC1α and mitochondrial metabolism–emerging concepts and relevance in ageing and neurodegenerative disorders. J. Cell Sci. 125, 4963–4971. https://doi.org/10.1242/jcs.113662.

Ballester-Lozano, G.F., Benedito-Palos, L., Estensoro, I., Sitjà-Bobadilla, A., Kaushik, S., Pérez-Sánchez, J., 2015. Comprehensive biometric, biochemical and histopathological assessment of nutrient deficiencies in gilthead sea bream fed semi-purified diets. Br. J. Nutr. 114, 713–726. https://doi.org/10.1017/S0007114515002354.

Benedito-Palos, L., Ballester-Lozano, G.F., Pérez-Sánchez, J., 2014. Wide-gene expression analysis of lipid-relevant genes in nutritionally challenged gilthead sea bream (*Sparus aurata*). Gene 547, 34–42. https://doi.org/10.1016/j.gene.2014.05.073.

Benedito-Palos, L., Ballester-Lozano, G.F., Simó, P., Karalazos, V., Ortiz, A., Calduch-Giner, J.A., Pérez-Sánchez, J., 2016. Lasting effects of butyrate and low FM/FO diets on growth performance, blood haematology/biochemistry and molecular growth-related markers in gilthead sea bream (*Sparus aurata*). Aquaculture 454, 8–18. https://doi.org/10.1016/j.aquaculture.2015.12.008.

Bermejo-Nogales, A., Calduch-Giner, J.A., Pérez-Sánchez, J., 2010. Gene expression survey of mitochondrial uncoupling proteins (UCP1/UCP3) in gilthead sea bream (*Sparus aurata* L.). J. Comp. Physiol. 180D, 685–694. https://doi.org/10.1007/s00360-009-0441-6.

Bermejo-Nogales, A., Calduch-Giner, J.A., Pérez-Sánchez, J., 2014. Tissue-specific gene expression and functional regulation of uncoupling protein 2 (UCP2) by hypoxia and nutrient availability in gilthead sea bream (*Sparus aurata*): implications on the physiological significance of UCP1-3 variants. Fish. Physiol. Biochem. 40, 751–762. https://doi.org/10.1007/s10695-013-9882-7.

Bermejo-Nogales, A., Benedito-Palos, L., Calduch-Giner, J.A., Pérez-Sánchez, J., 2011. Feed restriction up-regulates uncoupling protein 3 (UCP3) gene expression in heart and red muscle tissues of gilthead sea bream (*Sparus aurata* L.). New insights in substrate oxidation and energy expenditure. Comp. Biochem. Physiol. A 159, 296–302. https://doi.org/10.1016/j.cbpa.2011.03.024.

Besson, M., Allal, F., Chatain, B., Vergnet, A., Clota, F., Vandeputte, M., 2019. Combining individual phenotypes of feed intake with genomic data to improve feed efficiency in sea bass. Front. Genet. 10, 219. https://doi.org/10.3389/fgene.2019.00219.

Besson, M., Aubin, J., van Arendonk, J.A.M., Komen, H., Poelman, M., Quillet, E., Vandeputte, M., de Boer, I.J.M. 2014. Environmental impacts of genetic improvement in growth rate and feed conversion in fish farming under density and nitrogen limitation. 9. International Conference on Life Cycle Assessment in the Agri-Food Sector (LCA Food 2014).

Besson, M., Aubin, J., Komen, H., Poelman, M., Quillet, E., Vandeputte, M., Van Arendonk, J.A.M., De Boer, I.J.M., 2016. Environmental impacts of genetic improvement of growth rate and feed conversion ratio in fish farming under rearing density and nitrogen output limitations. J. Clean. Prod. 116, 100–109. https://doi.org/10.1016/j.jclepro.2015.12.084.

Besson, M., Rombout, M., Salou, G., Vergnet, A., Cariou, S., Bruant, J.-S., Bestin, A., Clota, F., Haffary, P., Allal, F., Vandeputte, M., 2022. Potential for genomic selection on feed efficiency in gilthead sea bream (*Sparus aurata*), based on individual feed conversion ratio, carcass and lipid traits. Aquac. Rep. 24, 101132 https://doi.org/10.1016/j.aqrep.2022.101132.

Bohnes, F.A., Hauschild, M.Z., Schlundt, J., Laurent, A., 2019. Life cycle assessments of aquaculture systems: a critical review of reported findings with recommendations for policy and system development. Rev. Aquac. 11, 1061–1079. https://doi.org/10.1111/raq.12280.

Bureau, D.P., Hua, K., Cho, C.Y., 2006. Effect of feeding level on growth and nutrient deposition in rainbow trout (*Oncorhynchus mykiss* Walbaum) growing from 150 to 600 g. Aquac. Res. 37, 1090–1098. https://doi.org/10.1111/j.1365-2109.2006.01532.x.

de Caestecker, M., 2004. The transforming growth factor-β superfamily of receptors. Cytokine Growth Factor Rev. 15, 1–11. https://doi.org/10.1016/j.cytogfr.2003.10.004.

Devlin, R.H., D'Andrade, M., Uh, M., Biagi, C.A., 2004. Population effects of growth hormone transgenic coho salmon depend on food availability and genotype by environment interactions. Proc. Natl. Acad. Sci. USA 101, 9303–9308. https://doi.org/10.1073/pnas.0400023101.

Dossou, S., Koshio, S., Ishikawa, M., Yokoyama, S., Dawood, M.A., El Basuini, M.F., El-Hais, A.M., Olivier, A., 2018. Effect of partial replacement of fish meal by fermented rapeseed meal on growth, immune response and oxidative condition of red sea bream juvenile, *Pagrus major*. Aquaculture 490, 228–235. https://doi.org/10.1016/j.aquaculture.2018.02.010.

Dryden, S.C., Nahhas, F.A., Nowak, J.E., Goustin, A.S., Tainsky, M.A., 2003. Role for human SIRT2 NAD-dependent deacetylase activity in control of mitotic exit in the cell cycle. Mol. Cell. Biol. 23, 3173–3185. https://doi.org/10.1128/MCB.23.9.3173-3185.2003.

Duan, C., Ding, J., Li, Q., Tsai, W., Pozios, K., 1999. Insulin-like growth factor binding protein 2 is a growth inhibitory protein conserved in zebrafish. Proc. Natl. Acad. Sci. USA 96, 15274–15279. https://doi.org/10.1073/pnas.96.26.15274.

Emre, Y., Hurtaud, C., Ricquier, D., Bouillaud, F., Hughes, J., Criscuolo, F., 2007. Avian UCP: the killjoy in the evolution of the mitochondrial uncoupling proteins. J. Mol. Evol. 65, 392–402. https://doi.org/10.1007/s00239-007-9020-1.

FAO, 2020, The State of World Fisheries and Aquaculture 2020. Sustainability in action. https://doi.org/https://doi.org/10.4060/ca9229en.

Ferrer, M.A., Calduch-Giner, J.A., Díaz, M., Sosa, J., Rosell-Moll, E., Santana Abril, J., Santana Sosa, G., Bautista Delgado, T., Carmona, C., Martos-Sitcha, J.A., Cabruja, E., Afonso, J.M., Vega, A., Lozano, M., Montiel-Nelson, J.A., Pérez-Sánchez, J., 2020. From operculum and body tail movements to different coupling of physical activity and respiratory frequency in farmed gilthead sea bream and European sea bass. Insights on aquaculture biosensing. Comput. Electron. Agric. 175, 105531 https://doi.org/10.1016/j.compag.2020.105531.

Føre, M., Frank, K., Norton, T., Svendsen, E., Alfredsen, J.A., Dempster, T., Eguiraun, H., Watson, W., Stahl, A., Sunde, L.M., Schellewald, C., Skøien, K.R., Alver, M.O., Berckmans, D., 2018. Precision fish farming: a new framework to improve production in aquaculture. Biosyst. Eng. 173, 176–193. https://doi.org/10.1016/j.biosystemseng.2017.10.014.

Garcia de la Serrana, D., Macqueen, D.J., 2018. Insulin-like growth factor-binding proteins of teleost fishes. Front. Endocrinol. 9, 80. https://doi.org/10.3389/fendo.2018.00080.

Gómez-Requeni, P., Mingarro, M., Kirchner, S., Calduch-Giner, J., Medale, F., Corraze, G., Panserat, S., Martín, S.A.M., Houlihan, D.F., Kaushik, S., Pérez-Sánchez, J., 2003. Effects of dietary amino acid profile on growth performance, key metabolic enzymes and somatotropic axis responsiveness of gilthead sea bream (*Sparus aurata*). Aquaculture 220, 749–763. https://doi.org/10.1016/S0044-8486(02)00654-3.

Gui, L., Hao, R., Zhang, Y., Zhao, X., Zan, L., 2015. Haplotype distribution in the class I sirtuin genes and their associations with ultrasound carcass traits in Qinchuan cattle (*Bos taurus*). Mol. Cell. Probes 29, 102–107. https://doi.org/10.1016/j.mcp.2015.03.007.

Hannay, K.M., Forger, D.B., Booth, V., 2020. Seasonality and light phase-resetting in the mammalian circadian rhythm. Sci. Rep. 10, 19506. https://doi.org/10.1038/s41598-020-74002-2.

Hughes, J., Criscuolo, F., 2007. Evolutionary history of the UCP gene family: gene duplication and selection. BMC Evolut. Biol. 8, 306. https://doi.org/10.1186/1471-2148-8-306.

Jobling, M., 2012. National Research Council (NRC): Nutrient requirements of fish and shrimp. Aquac. Int. 20, 601–602. https://doi.org/10.1007/s10499-011-9480-6.

Kause, A., Kiessling, A., Martin, S.A.M., Houlihan, D., Ruohonen, K., 2016. Genetic improvement of feed conversion ratio via indirect selection against lipid deposition in farmed rainbow trout (*Oncorhynchus mykiss* Walbaum). Br. J. Nutr. 116, 1656–1665. https://doi.org/10.1017/S0007114516003603.

Khalil, K., Elayat, M., Khalifa, E., Daghash, S., Elaswad, A., Miller, M., Abdelrahman, H., Ye, Z., Odin, R., Drescher, D., Vo, K., Gosh, K., Bugg, W., Robinson, D., Dunham, R., 2017. Generation of myostatin gene-edited channel catfish (*Ictalurus punctatus*) via zygote injection of CRISPR/Cas9 system. Sci. Rep. 7, 7301. https://doi.org/10.1038/s41598-017-07223-7.

Kieffer, D.A., Piccolo, B.D., Vaziri, N.D., Liu, S., Lau, W.L., Khazaeli, M., Nazertehrani, S., Moore, M.E., Marco, M.L., Martin, R.J., Adams, S.H., 2016. Resistant starch alters gut microbiome and metabolomic profiles concurrent with amelioration of chronic kidney disease in rats. Am. J. Physiol. Ren. Physiol. 310, F857–F871. https://doi.org/10.1152/ajprenal.00513.2015.

Killen, S.S., Marras, S., McKenzie, D.J., 2014. Fast growers sprint slower: effects of food deprivation and re-feeding on sprint swimming performance in individual juvenile European sea bass. J. Exp. Biol. 217, 859–865. https://doi.org/10.1242/jeb.097899.

Knap, P.W., Kause, A., 2018. Phenotyping for genetic improvement of feed efficiency in fish: lessons from pig breeding. Front. Genet. 9, 1–10. https://doi.org/10.3389/fgene.2018.00184.

Kolarevic, J., Calduch-Giner, J., Espmark, Å.M., Evensen, T., Sosa, J., Pérez-Sánchez, J., 2021. A novel miniaturized biosensor for monitoring Atlantic salmon swimming activity and respiratory frequency. Animals 11, 2403. https://doi.org/10.3390/ani11082403.

Krishnan, J., Danzer, C., Simka, T., Ukropec, J., Walter, K.M., Kumpf, S., Mirtschink, P., Ukropcova, B., Gasperikova, D., Pedrazzini, T., Krek, W., 2012. Dietary obesity-associated Hif1a activation in adipocytes restricts fatty acid oxidation and energy expenditure via suppression of the Sirt2-NAD+ system. Genes Dev. 26, 259–270. https://doi.org/10.1101/gad.180406.111.

Lee, S.J., McPherron, A.C., 2001. Regulation of myostatin activity and muscle growth. Proc. Natl. Acad. Sci. USA 98, 9306–9311. https://doi.org/10.1073/pnas.151270098.

Li, H., Ma, M.L., Luo, S., Zhang, R.M., Han, P., Hu, W., 2012. Metabolic responses to ethanol in *Saccharomyces* cerevisiae using a gas chromatography tandem mass spectrometry-based metabolomics approach. Int. J. Biochem. Cell Biol. 44, 1087–1096. https://doi.org/10.1016/j.biocel.2012.03.017.

Li, Z.Z., Berk, M., McIntyre, T.M., Feldstein, A.E., 2009. Hepatic lipid partitioning and liver damage in nonalcoholic fatty liver disease: role of stearoyl-CoA desaturase. J. Biol. Chem. 284, 5637–5644. https://doi.org/10.1074/jbc.M807616200.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C}$ method. Methods 25, 402–408. https://doi.org/10.1006/meth.2001.1262.

Magnoni, L.J., Martos-Sitcha, J.A., Queiroz, A., Calduch-Giner, J.A., Magalhàes Gonçalves, J.F., Rocha, C.M.R., Abreu, H.T., Schrama, J.W., Ozorio, R.O.A., Pérez-Sánchez, J., 2017. Dietary supplementation of heat-treated *Gracillaria* and *Ulva* seaweeds enhanced acute hypoxia tolerance in gilthead seabream (*Sparus aurata*). Biol. Open 6, 897–908. https://doi.org/10.1242/bio.024299.

Martos-Sitcha, J.A., Simó-Mirabet, P., de las Heras, V., Calduch-Giner, J.A., Pérez-Sánchez, J., 2019a. Tissue-specific orchestration of gilthead sea bream resilience to hypoxia and high stocking density. Front. Physiol. 10, 840. https://doi.org/10.3389/fphys.2019.00840.

Martos-Sitcha, J.A., Sosa, J., Ramos-Valido, D., Bravo, F.J., Carmona-Duarte, C., Gomes, H.L., Calduch-Giner, J.À., Cabruja, E., Vega, A., Ferrer, M.Á., Lozano, M., Montiel-Nelson, J.A., Afonso, J.M., Pérez-Sánchez, J., 2019b. Ultra-low power sensor devices for monitoring physical activity and respiratory frequency in farmed fish. Front. Physiol. 10, 667. https://doi.org/10.3389/fphys.2019.00667.

McPherron, A.C., Lee, S.J., 1997. Double muscling in cattle due to mutations in the myostatin gene. Proc. Natl. Acad. Sci. USA 94, 12457–12461. https://doi.org/10.1073/pnas.94.23.12457.

Nabben, M., Hoeks, J., 2008. Mitochondrial uncoupling protein 3 and its role in cardiac- and skeletal muscle metabolism. Physiol. Behav. 94, 259–269. https://doi.org/10.1016/j.physbeh.2007.11.039.

Palstra, A.P., Roque, A., Kruijt, L., Jéhannet, P., Pérez-Sánchez, J., Dirks, R.P., 2020. Physiological effects of water flow induced swimming exercise in seabream *Sparus aurata*. Front. Physiol. 11, 610049 https://doi.org/10.3389/fphys.2020.610049.

Peixoto, M.J., Ferraz, R., Magnoni, L.J., Pereira, R., Gonçalves, J.F., Calduch-Giner, J., Pérez-Sánchez, J., Rodrigo, O., 2019. Protective effects of seaweed supplemented diet on antioxidant and immune responses in European seabass (*Dicentrarchus labrax*) subjected to bacterial infection. Sci. Rep. 9, 16134. https://doi.org/10.1038/s41598-019-52693-6.

Perera, E., Turkmen, S., Simó-Mirabet, P., Zamorano, M.J., Xu, H., Naya-Català, F., Izquierdo, M., Pérez-Sánchez, J., 2020. Stearoyl-CoA desaturase (scd1a) is epigenetically regulated by broodstock nutrition in gilthead sea bream (*Sparus aurata*). Epigenetics 15, 536–553. https://doi.org/10.1080/15592294.2019.1699982.

Perera, E., Rosell-Moll, E., Martos-Sitcha, J.A., Naya-Català, F., Simó-Mirabet, P., Calduch-Giner, J., Manchado, M., Afonso, J.M., Pérez-Sánchez, J., 2021. Physiological trade-offs associated with fasting weight loss, resistance to exercise and behavioral traits in farmed gilthead sea bream (*Sparus aurata*) selected by growth. Aquac. Rep. 20, 100645 https://doi.org/10.1016/j.aqrep.2021.100645.

Pérez-Sánchez, J., Simó-Mirabet, P., Naya-Català, F., Martos-Sitcha, J.A., Perera, E., Bermejo-Nogales, A., Benedito-Palos, L., Calduch-Giner, J.A., 2018. Somatotropic axis regulation unravels the differential effects of nutritional and environmental factors in growth performance of marine farmed fishes. Front. Endocrinol. 9, 687. https://doi.org/10.3389/fendo.2018.00687.

Pickel, L., Sung, H.K., 2020. Feeding rhythms and the circadian regulation of metabolism. Front. Nutr. 7, 39. https://doi.org/10.3389/fnut.2020.00039.

Refinetti, R., Cornélissen, G., Halberg, F., 2007. Procedures for numerical analysis of circadian rhythms. Biol. Rhythm Res. 38, 275–325. https://doi.org/10.1080/09291010600903692.

Rial, E., Zardoya, R., 2009. Oxidative stress, thermogenesis and evolution of uncoupling proteins. J. Biol. 8, 58. https://doi.org/10.1186/jbiol155.

Rimoldi, S., Benedito-Palos, L., Terova, G., Pérez-Sánchez, J., 2016. Wide-targeted gene expression infers tissue-specific molecular signatures of lipid metabolism in fed and fasted fish. Rev. Fish. Biol. Fish. 26, 93–108. https://doi.org/10.1007/s11160-015-9408-8.

Rosell-Moll, E., Piazzon, M.C., Sosa, J., Ferrer, M.A., Cabruja, E., Vega, A., Calduch-Giner, J.A., Sitjà-Bobadilla, A., Lozano, M., Montiel-Nelson, J.A., Afonso, J.M., Pérez-Sánchez, J., 2021. Use of accelerometer technology for individual tracking of activity patterns, metabolic rates and welfare in farmed gilthead sea bream (*Sparus*

aurata) facing a wide range of stressors. Aquaculture 539, 736609. https://doi.org/10.1016/j.aquaculture.2021.736609.
Rosenfeld, J., Van Leeuwen, T., Richards, J., Allen, D., 2015. Relationship between growth and standard metabolic rate: measurement artefacts and implications for habitat use and life-history adaptation in salmonids. J. Anim. Ecol. 84, 4–20. https://doi.org/10.1111/1365-2656.12260.
Rui, L., 2014. Energy metabolism in the liver. Compr. Physiol. 4, 177–197. https://doi.org/10.1002/cphy.c130024.
Saera-Vila, A., Calduch-Giner, J.A., Prunet, P., Pérez-Sánchez, J., 2009. Dynamics of liver GH/IGF axis and selected stress markers in juvenile gilthead sea bream (*Sparus aurata*) exposed to acute confinement. Differential stress response of growth hormone receptors. Comp. Biochem. Physiol. Part A 154, 197–203. https://doi.org/10.1016/j.cbpa.2009.06.004.
Sánchez-Vázquez, F.J., Madrid, J.A., 2001. Feeding anticipatory activity. In: Houlihan, D., Boujard, T., Jobling, M. (Eds.), Food Intake in Fish. Blackwell Science, London, pp. 216–232. https://doi.org/10.1002/9780470999516.ch9.
Schrauwen, P., Hoppeler, H., Billeter, R., Bakker, A.H.F., Pendergast, D.R., 2001. Fiber type dependent upregulation of human skeletal muscle UCP2 and UCP3 mRNA expression by high-fat diet. Int. J. Obes. 25, 449–456. https://doi.org/10.1038/sj.ijo.0801566.
Schwer, B., Verdin, E., 2008. Conserved metabolic regulatory functions of sirtuins. Cell Metab. 7, 104–112. https://doi.org/10.1016/j.cmet.2007.11.006.
Sibly, R.M., Baker, J., Grady, J.M., Luna, S.M., Kodric-Brown, A., Venditti, C., Brown, J. H., 2015. Fundamental insights into ontogenetic growth from theory and fish. Proc. Natl. Acad. Sci. USA 112, 13934–13939. https://doi.org/10.1073/pnas.1518823112.
Silbernagel, G., Kovarova, M., Cegan, A., Machann, J., Schick, F., Lehmann, R., Haring, H.U., Stefan, N., Schleicher, E., Fritsche, A., Peter, A., 2012. High hepatic SCD1 activity is associated with low liver fat content in healthy subjects under a lipogenic diet. J. Clin. Endocrinol. Metab. 12, E2288–E2292. https://doi.org/10.1210/jc.2012-2152.
Simó-Mirabet, P., Bermejo-Nogales, A., Calduch-Giner, J.A., Pérez-Sánchez, J., 2017. Sirtuin energy-sensing at the molecular level. Tissue-specific gene expression and fasting regulation of sirtuin family in gilthead sea bream (*Sparus aurata*). J. Comp. Physiol. 187, 153–163. https://doi.org/10.1007/s00360-016-1014-0.
Simó-Mirabet, P., Perera, E., Calduch-Giner, J.A., Afonso, J.M., Pérez-Sánchez, J., 2018. Co-expression analysis of sirtuins and related metabolic biomarkers in juveniles of gilthead sea bream (*Sparus aurata*) with differences in growth performance. Front. Physiol. 9, 608. https://doi.org/10.3389/fphys.2018.00608.
Stanton, D.A., Alway, S.E., Mohamed, J.S., 2017. The role of Sirtuin 2 in the regulation of myogenesis. FASEB Journal 31 (S1), 877.13. https://doi.org/10.1096/fasebj.31.1_supplement.877.13.
Storebakken, T., Austreng, E., 1987. Ration level for salmonids: II. Growth, feed intake, protein digestibility, body composition, and feed conversion in rainbow trout weighing 0.5–1.0 kg. Aquaculture 60, 207–221. https://doi.org/10.1016/0044-8486(87)90288-2.
Thévenot, E.A., Roux, A., Xu, Y., Ezan, E., Junot, C., 2015. Analysis of the human adult urinary metabolome variations with age, body mass index, and gender by implementing a comprehensive workflow for univariate and OPLS statistical analyses. J. Proteome Res. 14, 3322–3335. https://doi.org/10.1021/acs.jproteome.5b00354.
Turyn, J., Stojek, M., Swierczynsk, J., 2018. Up-regulation of stearoyl-CoA desaturase 1 and elongase 6 genes. Mol. Cell. Biochem. 345, 181–188. https://doi.org/10.1007/s11010-010-0571-x.
de Verdal, H., Haffray, P., Douchet, V., Vandeputte, M., 2022. Impact of a divergent selective breeding programme on individual feed conversion ratio in Nile tilapia *Oreochromis niloticus* measured in groups by video-recording. Aquaculture 548, 737572. https://doi.org/10.1016/j.aquaculture.2021.737572.
de Verdal, H., Vandeputte, M., Mekkawy, W., Chatain, B., Benzie, J.A.H., 2018b. Quantifying the genetic parameters of feed efficiency in juvenile Nile tilapia Oreochromis niloticus. BMC Genet 19, 105. https://doi.org/10.1186/s12863-018-0691-y.
de Verdal, H., Mekkawy, W., Lind, C.E., Vandeputte, M., Chatain, B., Benzie, J.A., 2017. Measuring individual feed efficiency and its correlations with performance traits in Nile tilapia, *Oreochromis niloticus*. Aquaculture 468, 489–495. https://doi.org/10.1016/j.aquaculture.2016.11.015.
de Verdal, H., Komen, H., Quillet, E., Chatain, B., Allal, F., Benzie, J.A.H., Vandeputte, M., 2018a. Improving feed efficiency by selective breeding: a review. Rev. Aquac. 10, 833–851. https://doi.org/10.1111/raq.12202.
Wu, G., Song, C., Lu, H., Jia, L., Yang, G., Shi, X., Sun, S., 2014. Sirt2 induces C2C12 myoblasts proliferation by activation of the ERK1/2 pathway. Acta Biochim. Et. Biophys. Sin. 46, 342–345. https://doi.org/10.1093/abbs/gmt151.
Yúfera, M., Perera, E., Mata-Sotres, J.A., Calduch-Giner, J., Martínez-Rodríguez, G., Pérez-Sánchez, J., 2017. The circadian transcriptome of marine fish *Sparus aurata* larvae reveals highly synchronized biological processes at the whole organism level. Sci. Rep. 7, 12943. https://doi.org/10.1038/s41598-017-13514-w.