Physiological trade-offs associated with fasting weight loss, resistance to exercise and behavioral traits in farmed gilthead sea bream (*Sparus aurata*) selected by growth

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ARTICLE INFO

ABSTRACT

Three gilthead sea bream families representative of slow, intermediate and fast heritable growth in the Spanish PROGENSA® selection program were used to uncover the effects of such selection on energy partitioning through measurements of fasting weight loss, swimming performance and behavioral traits in one- and two-year-old fish. Firstly, selection for fast growth significantly increased fasting weight loss and decreased the hormonal ratio of circulating Igf-i/Gh in short-term fasting fish (17 days). This is indicative of a stronger negative energy balance that explains the reduced compensatory growth of fast-growing fish during the subsequent short-term refeeding period (7 days). Selection for fast growth also decreased the critical speed (U_{crit}, 6–7 BL s^{-1}) at which fish become exhausted in a swim tunnel respirometer. The maximum metabolic rate (MMR), defined as the maximum rate of oxygen consumption during forced exercise, was almost equal in all fish families though the peak was achieved at a lowest speed in the fast-growing family. Since circulating levels of lactate were also slightly decreased in free-swimming fish of this family group, it appears likely that the relative energy contribution of anaerobic metabolism to physical activity was lowered in genetically fast-growing fish. Selection for heritable growth also altered activity behavior because slow-growing families displayed an anticipatory food response associated with more pronounced daily rhythms of physical activity. Also, respiratory frequency and body weight showed and opposite correlation in slow- and fast-growing free-swimming fish as part of the complex trade-offs of growth, behavior and energy metabolism. Altogether, these results indicate that selective breeding for fast growth might limit the anaerobic fitness that would help to cope with limited oxygen availability in a scenario of climate change.

1. Introduction

Most of the historical gain on animal feed conversion ratio (FCR) has been obtained through selection for growth rates (Drouilhet et al., 2016; Emmerson, 1997; Nguyen et al., 2005), but such selection remains largely uncertain in fish due in part to the difficulties of measuring individual feed intake in the aquatic environment (Besson et al., 2020).

However, the prospects for improving FCR are promising for European sea bass using individual measurements of growth rates and food intake in isolation (Besson et al., 2019). Simultaneous selection for rapid growth and reduced body lipid percentage is also effective to improve FCR in European whitefish (Quinton et al., 2007) and rainbow trout (Kause et al., 2016; Knap and Kause, 2018). Likewise, selection for fasting weigh loss can be implemented in fish breeding programs to yield

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substantial gains in FCR, though it is difficult to establish a general pattern due to fish species differences in metabolic rates and substrate utilization (lipids *vs* proteins) as metabolic fuels (Besson et al., 2019; Dupont-Prinet et al., 2010; Grima et al., 2010; Mckenzie et al., 2014; Zeng et al., 2017). Thus, several attempts have been made to link the metabolic phenotype with changes in environmental conditions and other whole-organism traits such as dominance, aggression, stress coping style, and swimming performance (Metcalfe et al., 2016; Castanheira et al., 2017; Carbonara et al., 2015, 2019). However, a high percentage of genetic variance is expected to be unexplained (Knap and Kause, 2018). Therefore, a better understanding of genotype-by-environment interactions are of paramount importance for domestication and enhancement of fish health and welfare, and also to gain new knowledge on growth trajectories through the productive cycle (Gjedrem and Robinson, 2014; Janssen et al., 2017; Vandeputte et al., 2019). For instance, the PROGENSA® gilthead sea bream selection program favors a more continuous growth rather than a pronounced seasonality with enhanced growth spurts in summer (Perera et al., 2019).

The general thinking is that accelerated growth is associated with high rates of food intake and metabolic energy allocation to growth at the expense of energy expenditure on activity, predator avoidance, and reproduction (Sibly et al., 2015). A unified mechanistic theory for this energy partitioning remains elusive in most farmed fish and gilthead sea bream in particular (Ejbye-Ernst et al., 2016; Svendsen et al., 2015). However, Palstra et al. (2020) highlighted that critical swimming predicts fillet yield in both Atlantic salmon and gilthead sea bream, and other studies indicated that it can be used as a welfare indicator in different fish species (Carbonara et al., 2015; Zupa et al., 2015). The aim of the present study was to provide new insights on growth and energy metabolism trade-offs for the improvement of selective breeding programs in gilthead sea bream, a highly cultured fish in all the Mediterranean area. To do that, we evaluated in PROGENSA® fish families with different growth scores (fast, intermediate, low) a set of whole-organism traits: i) susceptibility to fasting weight loss, ii) exercise resistance in swim chambers, and iii) behavioral traits in free-swimming fish using tri-axial accelerometers externally attached to the operculum (Ferrer et al., 2020; Martos-Sitcha et al., 2019b).

2. Materials and methods

2.1. Fish

Families with different heritable growth value were produced and reared as described in Perera et al. (2019). Briefly, juveniles from the PROGENSA® selection program were grown out from early life stages in a sea water common garden system at the experimental facilities of IATS. Fish were fed near visual satiety with automatic feeders with BioMar (BioMar Process Innovation Technical Centre, Brande, Denmark) diets, formulated to meet all nutrient requirements. All individuals were tagged in the dorsal musculature with passive integrated transponders (ID-100A 1.25 Nano Transponder; Trovan, Madrid, Spain) for the individual tracking of analyzed parameters. The present study focused on three selected families, categorized as slow- (c4c3), intermediate- (e4e1) and fast- (e5e2) growing fish under the natural photoperiod and temperature conditions at IATS latitude (40° 5′N; 0° 10′E) (Perera et al., 2019). As a general indication, all measurements were conducted during the active feeding period of summer, with dissolved O_2 concentrations in water effluents always higher than 5.6 ppm, and ammonia concentration below toxic levels (< 0.05 mg L^{-1}).

2.2. Fasting weight loss and refeeding

In July 2018, one-year-old fish (100−200 g) from the three selected families (c4c3, 15 fish; e4e1, 41 fish; e5e2, 45 fish) were placed together in a 3000 L tank (6.4 Kg/m³) to ensure common environmental

conditions. Fish continued to be fed during one month prior to a fasting period of 17 days, followed by a refeeding of 7 days under natural water temperatures that increased from 24 to 27 °C throughout the experimental period. No mortalities occurred and fish were weighed and measured (standard length) at initial (10 days fasting), intermediate (17 days fasting) and final sampling points (7 days refeeding), using a FR-200 FishReader W (Trovan, Madrid, Spain) for data capture and pre-processing. At the end of the fasting period, six fish per family were randomly selected and anesthetized with 3-aminobenzoic acid ethyl ester (MS-222, 100 µg mL^{-1}; Sigma, St. Louis, MO, USA) and blood was taken by puncturing caudal vein with heparinized syringes. Blood was centrifuged at $3000 \times g$ for 20 min at 4 °C, and plasma was stored at −80 °C until hormone and metabolite analyses.

2.3. Swim tunnel respirometer

Also in July-August 2018, individual measurements of O_2 consumption rates (MO_2) were conducted in one-year-old fish from the three studied families, brought into an intermittent closed swim tunnel respirometer of 10 L water volume (swim chamber 10 × 10 × 40 cm, Model PA10500, Loligo® Systems, Viborg, Denmark) as reported in Martos-Sitcha et al. (2018). Fish subjected to the U_{crit} tests weighed (g) 130.5 ± 4.43 (c4c3), 149.1 ± 4.38 (e4e1) and 172.2 ± 4.96 (e5e2), and standard length (cm) was 16.75 ± 0.14 (c4c3), 17.26 ± 0.17 (e4e1) and 17.93 ± 0.16 (e5e2). Briefly, 10–12 overnight fasted fish from each studied family were lightly anaesthetized with 100 µg mL^{-1} MS-222 (Sigma, Saint Louis, MO, USA), and biometric data were obtained for respirometer swimming speed adjustment in order to avoid any solid blocking effect. Then, fish were transferred to the respirometer and acclimated to the swim chamber at a low swimming speed (0.50 body length per second, BL s^{-1}) until MO_2 reached a constant low plateau. This typically happened after 30−45 min with MO_2 around 220–230 mgO$_2$/kg/hr. At that time, water speed was increased in 0.50 BL s^{-1} steps until the fish was exhausted. A fish was considered exhausted when it rested at the back grid of the swim chamber for at least 10 s. Each swimming interval at a given speed lasted 5 min according to the manufacturer's specifications, consisting of "flush–wait–measurement" cycles (a 60 s flush interval to exchange the respirometer water = "flush"; a 30 s mixing phase in a closed mode = "wait"; and a representative 210 s MO_2 measuring period in a closed mode). During measurement intervals, O_2 saturation of the swim tunnel water was recorded every second, and MO_2 was automatically calculated by the AutoResp™ software. Critical swimming speed (U_{crit}) was calculated in absolute (cm s^{-1}) and relative terms (BL s^{-1}) using the equation described by Brett (1964): $U_{crit} = U_{high} + [(t_{exh}. t_{inter}^{-1}). U_{incr}]$ where U_{high} is the highest swimming speed maintained for the entire time interval; t_{exh} is the time interval spent at the exhaustion speed (min); t_{inter} is the time interval at each swimming speed (5 min); and U_{incr} is the speed increment in each new interval (0.50 BL s^{-1}).

2.4. Activity monitoring of free-swimming fish

In July 2019, two-year-old fish (237−514 g) from the three studied families, that had been reared in the common garden system, were placed together in a 3000 L tank (12 kg m^{-3}) feeding once per day at 13 h. Individual recording of behavioral traits was made by means of AEFishBIT, a stand-alone, small and light (1 g) motion embedded-microsystem based in a tri-axial accelerometer. The device was externally attached to the operculum (6–8 individuals per family) to monitor physical activity by mapping accelerations in x- and y-axes, while operculum beats (z-axis) serve as a measurement of respiratory frequency (Ferrer et al., 2020; Martos-Sitcha et al., 2019b). The accuracy of on-board algorithms was calibrated in the Loligo® Systems metabolic chamber, correlating the accelerometer outputs of exercised fish with data on swimming speed, O_2 consumption and operculum and body tail movements (Martos-Sitcha et al., 2019b). The attachment was made by

means of a non-invasive self-piercing procedure that is accomplished in less than 30 s (https://vimeo.com/325943543). The devices were programmed to acquire data during 2 min every 15 min during 2 recording days. Fish remained unfed during the recording time. After that, all devices were recovered and pre-processed data were downloaded for functional analyses. This included correlation analysis with data of muscle fat content, determined as the mean of two Fish Fat-Meter (FFM) measurements (Distell Fish Fat-meter, FM 692) on the left dorsal musculature.

2.5. Blood biochemistry

Plasma growth hormone (Gh) was determined by a homologous gilthead sea bream radioimmunoassay (RIA) (Martínez-Barberá et al., 1995). Sensitivity and midrange (ED50) of the Gh RIA assay were 0.15 and 1.8 ng mL^{-1}, respectively. Plasma insulin-like growth factors (Igfs) were extracted by acid-ethanol cryoprecipitation (Shimizu et al., 2000), and the concentration of Igf-i was measured by a generic fish Igf-i RIA validated for Mediterranean perciform fishes (Vega-Rubin de Celis et al., 2004). Sensitivity and midrange of the Igf-i RIA assay were 0.05 and $0.7-0.8$ ng mL^{-1}, respectively. Blood lactate was measured in deproteinized samples (8% perchloric acid) using a lactate dehydrogenase-based method (Instruchemie, Delfzijl, Netherlands).

2.6. Statistical analysis

One-way ANOVA followed by Tukey tests was conducted using SigmaPlot version 14.0 (Systat Software, San Jose, CA, USA) with all *P*-values set to 0.05 for significance determination, to assess the family effects in fasting, blood biochemistry and exercise performance. All data were checked for normality and equal variance by Shapiro-Wilk and Levene tests, respectively. Recent research demonstrated the variable contribution of body mass, length, and MMR on *Ucrit* in several fish species (Rubio-Gracia et al., 2020), especially body length (Cano-Barbacil et al., 2020). Thus, the potential individual effects of family, body mass, length, and MMR on U_{crit} (in BL/s) were analyzed by multiple linear regression using GraphPad Prism 9.0 (GraphPad Software, Inc., San Diego, CA). Also, ANCOVA was applied to test for differences in relative *Ucrit* among fish families with all P-values set to 0.05 and body length as a covariate using SPSS Statistics for Windows v.27 (IBM Corp., Armonk, NY, USA). The relationships between U_{crit} and SGR, and between respiratory frequency and body weight, were described by regression and Pearson correlation analyses. Activity rhythms were assessed by a simple cosinor model (Refinetti et al., 2007), and the estimated midline or mesor (M), amplitude (A), acrophase (φ) and *P*-value of fit curves were obtained for each activity pattern.

3. Results

3.1. The trade-off between growth and fasting weight loss

At the beginning of the fasting period, the mean body weight of each group highly reflected the fish family differences in growth performance, with an average body mass (g, mean ± SEM) that varied from 164.9 ± 4.23 in c4c3 family to 175.4 ± 3.90 in e4e1 family, and 213.7 ± 3.43 in e5e2 family. After 10 days of fasting, the weight loss due to fasting (noted as negative SGR values) was 27.5 % lower ($p < 0.01$) in c4c3 family than in the other two families. However, no statistically significant differences in weight loss were found during the next 7 days of fasting. During the refeeding period (7 days), the compensatory growth (noted as positive SGR values) was 11 % higher ($p < 0.05$) in the slow-growing family (c4c3) than in the remaining ones (Fig. 1). At the level of circulating markers, the trend for the c4c3 family was a lower plasma Gh concentration at the end of the fasting period (Fig. 2A). Conversely, this family showed the highest plasma Igf-i concentration (Fig. 2B), being the Igf-i/Gh ratio higher ($p < 0.05$) in c4c3 than in the
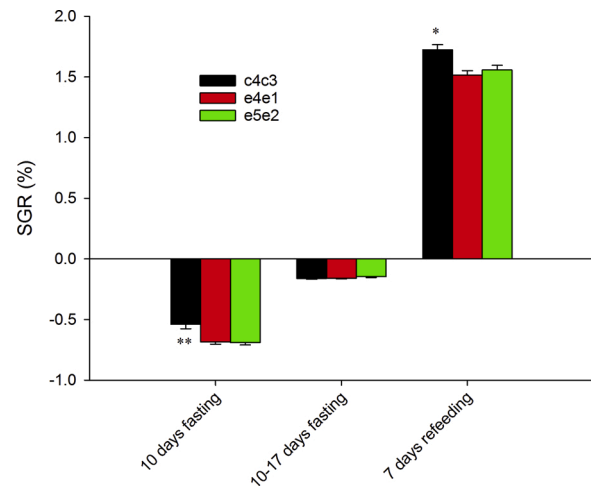

Fig. 1. Body weight changes during fasting and refeeding in one-year-old fish families with different heritable growth value. Values are mean ± SEM of 15-45 fish from families with slow- (c4c3, black bars), intermediate- (e4e1, red bars) and fast-growing phenotypes (e5e2, green bars). Asterisks indicate statistically significant differences between c4c3 and the other two groups (Tukey test, * $p < 0.05$; ** $p < 0.01$). SGR = 100 [ln final body weight - ln initial body weight] days^{-1}.

other two families (Fig. 2C). The trend was the same for blood lactate, though in this case no statistically significant differences were found (Fig. 2D).

3.2. Swimming performance in exercise tests

Fish subjected to the U_{crit} tests weighed (g) 130.5 ± 4.43 (c4c3), 149.1 ± 4.38 (e4e1) and 172.2 ± 4.96 (e5e2), and standard length (cm) was 16.75 ± 0.14 (c4c3), 17.26 ± 0.17 (e4e1) and 17.93 ± 0.16 (e5e2), being representative of the different genetic growth potential of the families studied as revealed during the previous on-growing period from fry until one-year-old animals. The maximal MO$_2$ in exercised fish (termed MMR), remained almost invariant ($360-330$ mg O$_2$ kg^{-1} h^{-1}) among fish families, but the slow-growing family (c4c3) exhibited a more gradual increase in MO$_2$ as swimming speed increased (Fig. 3A). As a result of this phase-shift, the MMR was achieved at 5 BL s^{-1} in c4c3 fish or at 4.5 and 4.0 BL s^{-1} in e4e1 and e5e2 families, respectively (Fig. 3B). After that, all fish showed a sharp decrease in O$_2$ consumption until being exhausted at their own U_{crit}, which decreased significantly from approximately 7 BL s^{-1} in c4c3 family to 6 BL s^{-1} in the other two fish families. The same trend was found for the U_{crit} assessed in absolute terms (Fig. 3C). The potential individual effects of family, body mass, total length, and MMR on U_{crit} (in BL/s) were analyzed by multiple linear regression. Considering all factors, no significant effects were found for body mass (F = 0.32, P = 0.58), total length (F = 2.35, P = 0.14), or MMR (F = 2.09, F = 0.16), while significance was only found for the family effect (F = 7.86, P < 0.01). The model improved by removing MMR (difference in Akaike Information Criterion, AIC, of −0.71), and removing both MMR and body mass (difference in AIC of -2.91). With the simplest model, including only family and length, length had a barely significant effect (F = 4.38, P = 0.045) while family (F = 8.81, P = 0.0009) remained as the best predictor of U_{crit} (in BL/s). The same results were obtained when analyses were performed on absolute U_{crit} (cm/s), but in this case no size effect was found (F = 0.20, P = 0.65) with any model, and family was the only single predictor of absolute U_{crit} (F = 7.97, P = 0.0016). Thus, in our experimental model, absolute U_{crit} was considered to be not affected by differences in body weight and length resulting from unequal growth rate of families at the moment of experimentation, while relative U_{crit} was only slightly affected by difference in size. Due to this small but significant effect, ANCOVA was
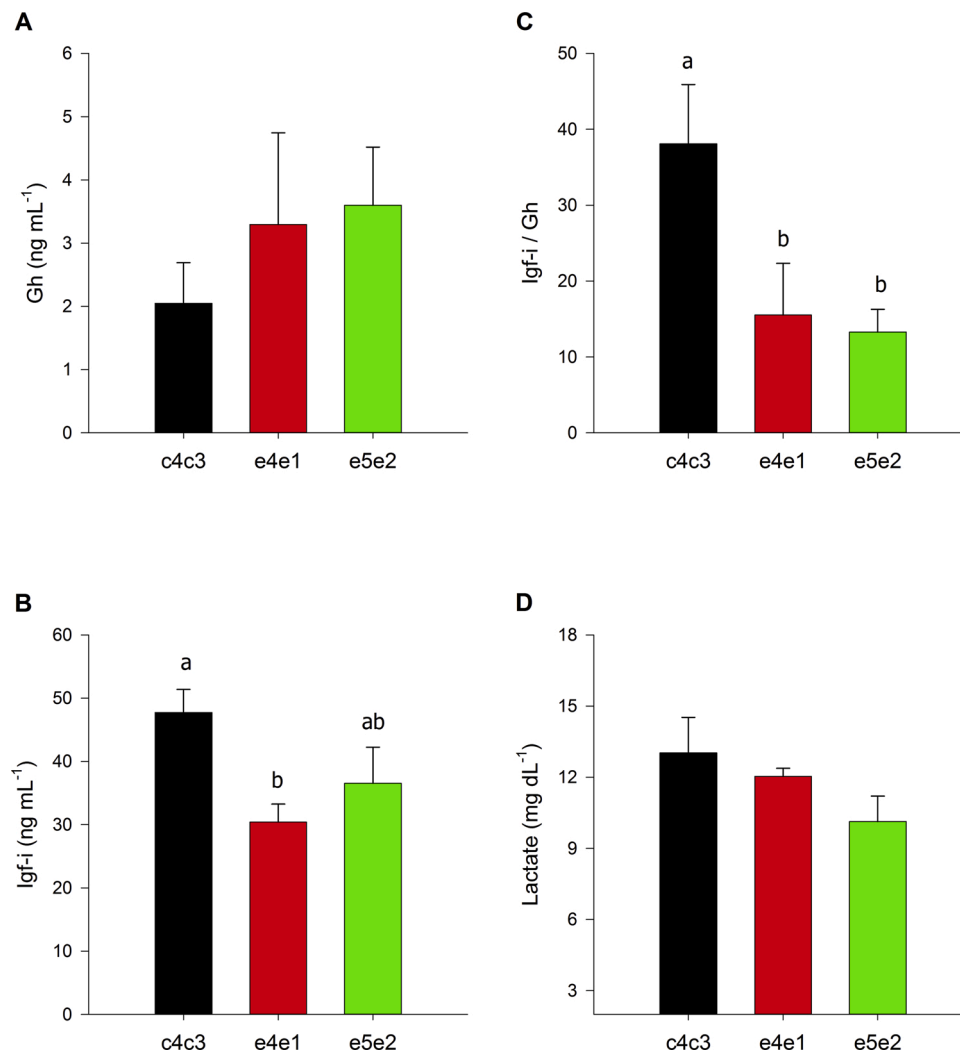
Fig. 2. Effect of fasting (17 days) on circulating levels of hormones and metabolites in one-year-old fish families with different heritable growth value. Values are mean ± SEM of 6 fish from families with slow- (c4c3, black bars), intermediate- (e4e1, red bars) and fast-growing genotypes (e5e2, green bars). Different letters indicate statistically significant differences among groups (Tukey test, $p < 0.05$).

applied to test for differences in relative U_{crit} among fish families using body length as the covariate. After accounting for length differences, a significant effect of family on relative U_{crit} was also evidenced (F = 8.81, P = 0.0009).

On the other hand, at the individual level, regression analysis revealed a close negative linear association between U_{crit} and the overall SGR across the productive cycle (Fig. 4), which reinforces the trade-off between growth and physical activity.

3.3. Behavioral traits in free-swimming fish

Family effects on energy metabolism were also inferred from behavioral traits in free-swimming fish. Thus, most of the two-year-old individuals from c4c3 and e4e1 families shared an averaged respiratory frequency that correlated negatively with body mass (Fig. 5A). By contrast, the subset of fish with body mass higher than 400 g was mostly represented by the e5e2 family, and intriguingly larger fish had a higher respiratory frequency than their smaller siblings (Fig. 5B). In both cases, the respiratory frequency was negatively correlated with muscle fat content, but a close positive association between body weight and muscle fat content was only found with fish weighing less than 400 g.

The physical activity was also dependent on the family genotype, with a clear rhythmicity pattern that differed between individuals of the two extreme families. Indeed, the amplitude of the activity rhythm in c4c3 fish (<400 g) was significantly higher than in the fast-growing e5e2 family, also showing slow-growing fish an anticipatory food response with acrophase values around 10:30 h (4.0 ZT) (Fig. 6A). By contrast, in e5e2 fish, the maximal activity peaked around 13:30 h (7.0 ZT), matching well with the expected mealtime (Fig. 6B).

4. Discussion

Selection for high heritable growth value in the PROGENSA® gilthead sea bream program also modified other productive and physiological traits, such as gut length and amount of body fat depots (Perera et al., 2019). Besides, families with a fast growth phenotype displayed a more plastic gut microbiota that adapts quickly to changes in diet composition (Piazzon et al., 2020). Earlier studies also evidenced that such selective breeding has an impact on body-composition and deformity traits (Lee-Montero et al., 2015; Navarro et al., 2009). However, the effects on energy metabolism and behavior remained elusive, so in order to guarantee the whole production chain levels in a challenging environment, we intended to provide new insights on these relevant traits to contribute to the improvement of selective breeding programs in gilthead sea bream, producing more robust and efficient fish.
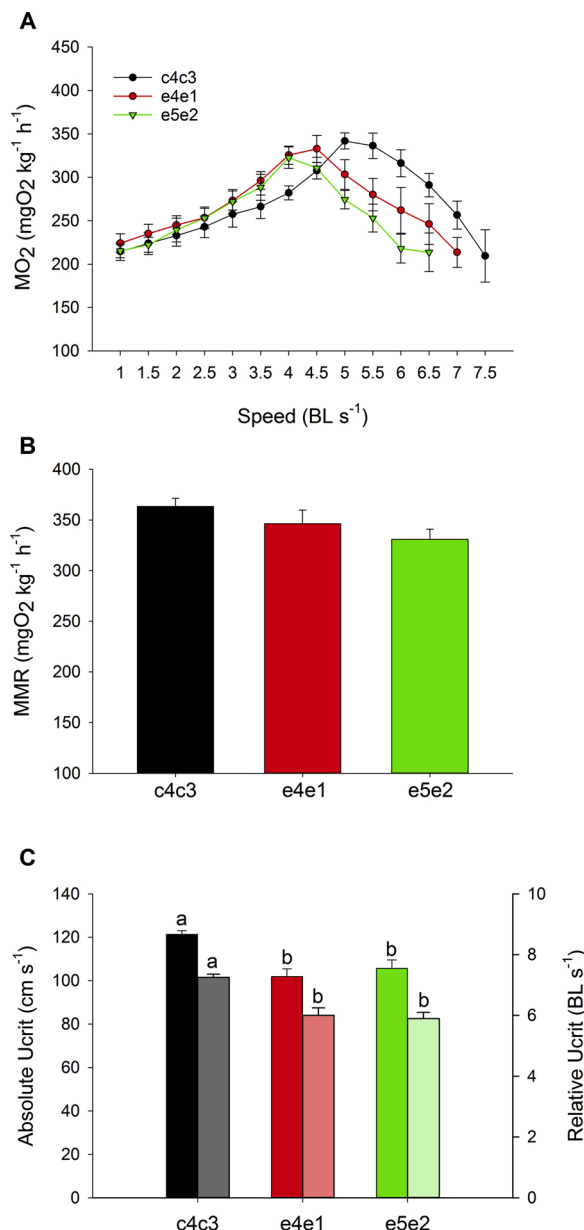
A

B

C

Fig. 3. Swim tests in one-year-old fish families with different heritable growth value. Slow-growing fish (c4c3) are in black, intermediate-growing (e4e1) fish in red and fast-growing (e5e2) fish in green. (A) Oxygen consumption (MO2). (B) Maximum metabolic rate (MMR). (C) Critical speed (U_{crit}). For each fish family, left bar indicates absolute U_{crit} (cm s^{-1}), and right bar indicates relative U_{crit} (BL s^{-1}).Values are the mean $\pm$ SEM of 11–13 fish. Different letters indicate statistically significant differences among groups (Tukey test, $p < 0.05$).

4.1. Selection by growth underlines a higher susceptibility to fasting weight loss

Selection for weight gain did not correlate with FCR in Nile tilapia, though increased weight loss at fasting can be implemented as a reliable criterion to yield substantial gains in FCR in this fish species (De Verdal et al., 2018). Conversely, studies in European sea bass indicated that individuals that lose less weight during fasting were fatter, but also more efficient to convert food into body mass (Besson et al., 2019; Grima et al., 2010). Mckenzie et al. (2014) also pointed out that the individual phenotypic variation in the tolerance to fasting might be due to both changes in metabolic rates and substrate utilization as metabolic fuels,
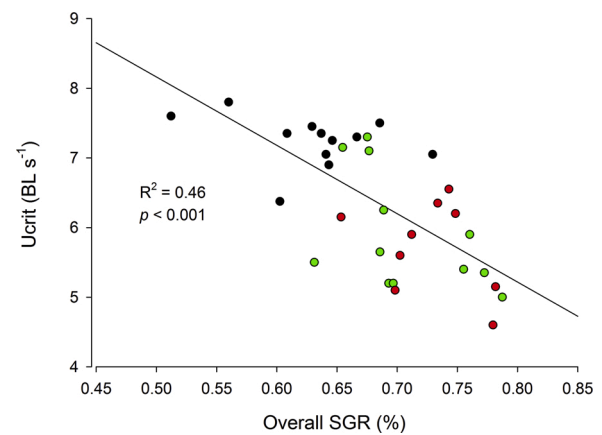

Fig. 4. Growth performance parameters related to swim tests in one-year-old fish families with different heritable growth value. Correlation analysis between SGR across the trial (Sept 2017-July 2018) and the determined critical speed (U_{crit}) in July 2018. Slow-growing (c4c3) fish are in black, intermediate-growing (e4e1) fish in red and fast-growing (e5e2) fish in green. Values are the mean $\pm$ SEM of 11-13 fish.

which also evidences the different coping strategies across species and fish strains for facing bad nutritional periods over developmental and growth episodes (Vandeputte et al., 2019). Thus, in our selective breeding program for gilthead sea bream, the increased fasting weight loss of fast-growing families can be viewed as the physiological cost that these individuals pay to benefit rapidly of food when it becomes available. This adaptive feature might favor not only a more continuous growth trajectory, but also less individual size variation through the productive cycle (Perera et al., 2021). The drawback effect is an enhanced risk of negative growth during unstable or poorly predictable food availability, which requires metabolic and/or behavioral adaptations with prolonged food scarcity (Borowiec, 2018).

Family differences in the susceptibility to fasting weight loss were also supported in our study by a lower circulating fasting Igf-i/Gh ratio. This hormone quotient is a well-known marker of anabolic status in gilthead sea bream (Pérez-Sánchez et al., 2018; Simó-Mirabet et al., 2018), and their increased values in the slow-growing family would indicate a less harmful energy status at the end of fasting period, which in turn supported a more rapid compensatory growth during the subsequent short-term refeeding period. This can be also inferred on a seasonal basis, since PROGENSA® families with lower growth show a depressed growth and food intake during the cold season, followed by a normal or even enhanced catch-up growth in summer that serves to compensate, at least in part, their overall depressed growth (Perera et al., 2019). Other differential feature of this group of fish, represented herein by the c4c3 family, was a slight increase in circulating levels of lactate. This metabolite is a marker of anaerobic metabolism and it would support a swimming behavior powered by glycolytic white muscle fibers rather than slow muscle powered cruising fibers (Bale et al., 2015; Shadwick et al., 2013) (see below).

4.2. Fast-growth is co-related with impaired swimming performance

Our estimations of MMR in one-year-old juveniles of gilthead sea bream did not reveal differences between genetic families, being the achieved results in agreement with the values reported in previous studies in this species (Ejbye-Ernst et al., 2016; Steinhausen et al., 2010). A previous study also indicated that variations in aerobic scope (the difference between standard metabolic rate and MMR) and anaerobic capacity (determined by measurements of excess post-exercise O_2 consumption; EPOC) did not drive phenotypic variations of swimming performance in gilthead sea bream (Svendsen et al., 2015). In the same way, studies in European sea bass, zebrafish or the guppy *Poecilia*
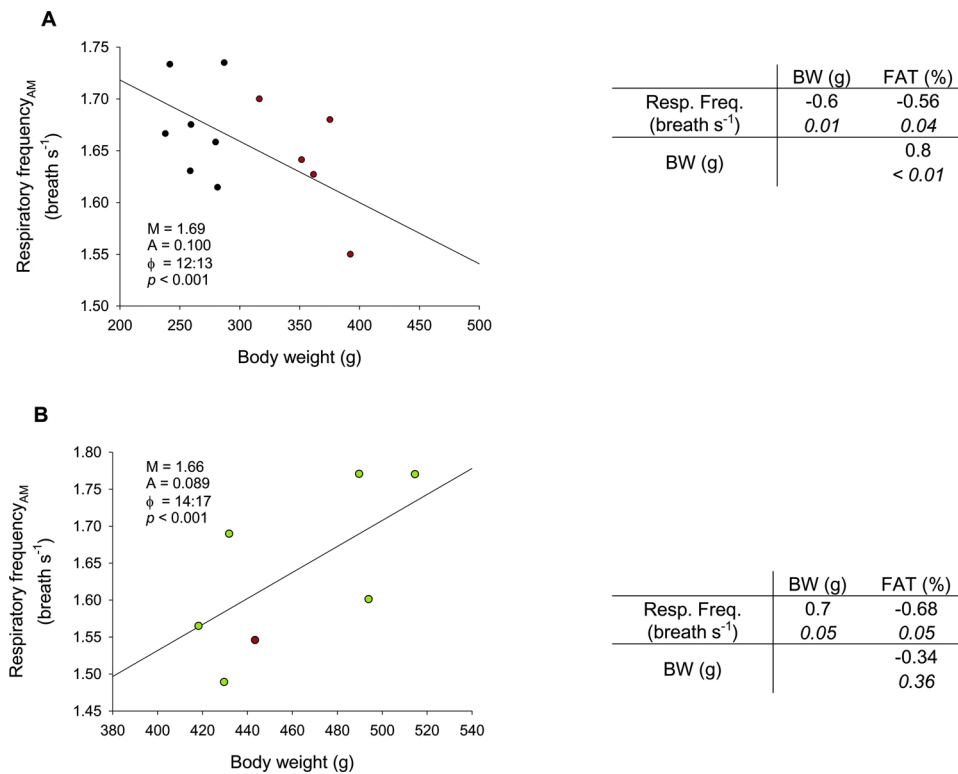
A

	BW (g)	FAT (%)
Resp. Freq. (breath s⁻¹)	-0.6 *0.01*	-0.56 *0.04*
BW (g)		0.8 *< 0.01*

B

	BW (g)	FAT (%)
Resp. Freq. (breath s⁻¹)	0.7 *0.05*	-0.68 *0.05*
BW (g)		-0.34 *0.36*

Fig. 5. Correlation analysis between respiratory frequency and body weight in free-swimming two-year-old fish. Fish families are denoted by different colors (black points, c4c3; red points, e4e1; green points, e5e2). Respiratory frequency was assessed by the AEFishBIT biosensor attached to the operculum. Fish with a body weight lower than 400 g are included in panel A. Fish with a body weight higher than 400 g are included in panel B. For each group of fish, Pearson correlation coefficients between respiratory frequency and body weight or muscle Fat-meter measurements were reported with *p*-values in italics. In each panel, values of mesor (M), amplitude (A), acrophase (Φ) and *p*-values of best-fit curves from cosinor analysis are shown for the most extreme growing families (c4c3, e5e2).

reticulata did not support a trade-off between aerobic and anaerobic swimming performance (Marras et al., 2013; Oufiero and Garland, 2009; Seebacher and Walter, 2012). However, anaerobic metabolism occurs before reaching MMR (Svendsen et al., 2010; Teulier et al., 2013), and there is experimental evidence in gilthead seabream of a trade-off between aerobic- and anaerobic-locomotor traits that might have an effect on the achieved response in exercise tests (Ejbye-Ernst et al., 2016). Furthermore, Palstra et al. (2020) have recently evidenced that critical swimming predicts fillet yield in both Atlantic salmon and gilthead sea bream. The reported values for gilthead sea bream are in the range found in the present study, but it is noteworthy that large domesticated strains of Atlantic salmon (Anttila and Mänttäri, 2009; Yangfan et al., 2016) and rainbow trout (Bellinger et al., 2014; Claireaux et al., 2005) become athletically less robust that wild fish, which often display a higher cardiac pumping capacity (Claireaux et al., 2005) that improves the rate at which O_2 is transported from the environment to the tissue mitochondria (Weibel and Hoppeler, 2005). This issue also deserves investigation in Mediterranean farmed fish as they commonly exhibit deviations from normal heart morphology (Pombo et al., 2012).

The effect of selection on gill morphology is also worthy to be evaluated (Duthie and Hughes, 1987) due to its potential effects on endurance training and aerobic/anaerobic fitness, the two overarching factors linking the individual variations on swimming performance in livestock and competitive swimmers (Campos et al., 2017; Vasile, 2014). This can also be inferred from the results of the present study as MMR was attained at higher swimming speeds in the fish family growing at slow rates, which also resulted in higher U_{crit}. Hence, both in salmonids and non-salmonid fish, selection by growth might elicit a trade-off between aerobic and anaerobic swimming performance that primes different physiological processes (fast growth and aerobic swimming *vs.* slow growth and anaerobic swimming). Herein, this was also supported by the increased circulating levels of lactate in fish having slow-growing phenotypes. Altogether, results suggest that fast-growing strains of gilthead sea bream are likely to show a reduced capacity to cope with a low O_2 availability. This feature would help to establish an adequate relationship between farmed fish and their environment, which is the

result of a combined set of parameters (e.g., O_2 availability, rearing density, water current, light, temperature) acting synergistically (Herbert, 2013; Martos-Sitcha et al., 2019a). Also, it is well known that different exercise protocols are able to increase the growth performance of a wide range of farmed fish, including gilthead sea bream (Blasco et al., 2015; Vélez et al., 2016). However, further research is needed to know how swimming speed for optimal exercise-induced growth is modified by selective breeding (Davison and Herbert, 2013).

4.3. Behavioral traits reinforce the more proactive performance of slow growing phenotypes

Fish forced to swim show less aggressions to conspecifics with the benefit of lowering stress levels and collective improvements of growth and FCR at optimal swimming speed (Boesgaard et al., 1993; Jobling et al., 1993). Continuous circular schooling is, thereby, imposed by water currents in fish grown out in the circular tanks of our experimental facility at IATS. Fish typically align into flow (rheotactic orientation) and measurements conducted with the AEFishBIT smart device allow us to have simultaneous records of fish accelerations and breathing on a daily basis (Ferrer et al., 2020; Martos-Sitcha et al., 2019b). To minimize the disturbing effects of poorly controlled variables on behavioral traits (i.e., differences in food intake), measurements were carried out in fish kept together in the same tank without access to food over the course of the recording period. This steady state served to establish different patterns of physical activity and ventilation frequency that correlates with O_2 uptake in a wide range of fish, such as rainbow trout (Rogers and Weatherley, 1983) and the stoplight parrotfish *Sparisoma viride* (van Rooij and Videler, 1996). Indeed, both in gilthead sea bream and European sea bass, a close correlation between AEFishBIT measurements and O_2 uptake has been evidenced in forced exercised fish in swim chambers (Martos-Sitcha et al., 2019b). Herein the daily swimming activity of free-swimming c4c3 fish (225−275 g) exhibited a higher amplitude in comparison to e4e1 or e5e2 fish weighing more than 400 g. Additionally, the group of small fish elicited an anticipatory food response as part of an associated learning process that persisted during
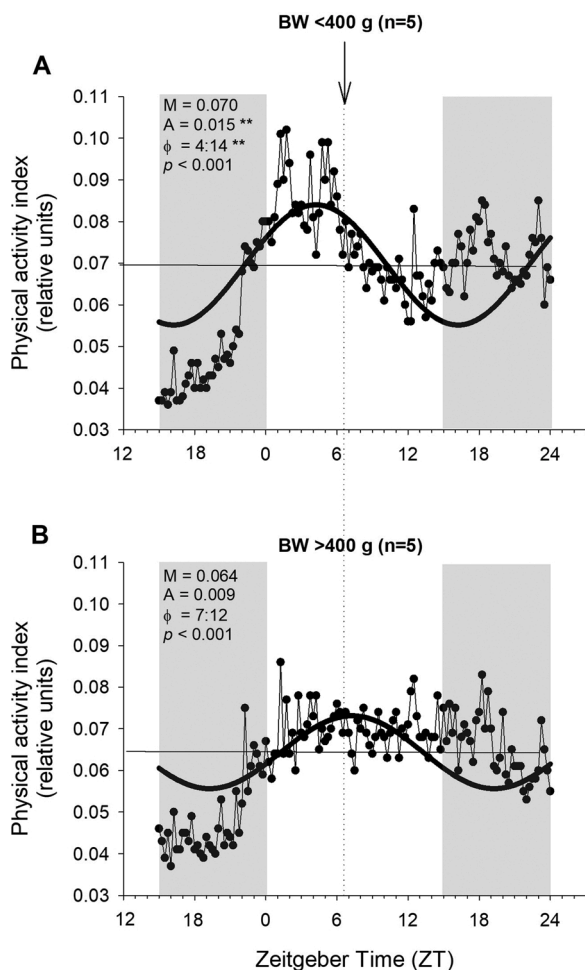
Fig. 6. Daily variations in physical activity of two-year-old free-swimming fish with extreme growth phenotypes. (A) Fish weight <400 g. (B) Fish weight >400 g. Swimming activity was assessed by the AEFishBIT biosensor attached to the operculum, and inferred from changes in acceleration integrated into a physical activity index. In each panel, the best-fit curve derived from cosinor analysis is shown as a continuous bold solid line, and represents the consensus of recordings from five individuals. Values of mesor (M), amplitude (A), acrophase (Φ) and *P*-value of best-fit curves are shown for each activity pattern. Statistically significant differences are indicated with asterisks (** *p* < 0.01, *t*-test). Grey shaded areas represent dark phases. Horizontal solid lines indicate the mesor, and vertical dotted line indicates the mealtime (6.5 ZT, local time 13:00 h).

short-term fasting (Sánchez-Vázquez and Madrid, 2001). These changes in swimming behavior after growth selection would be related with the selection of specific coping styles, although this has to be tested across time and context (e.g. different stoking densities, risk-taking tests) (Carbonara et al., 2019; Alfonso et al., 2020). This type of behavior (i.e. anticipatory food response) would require a greater contribution of anaerobic metabolism to the total energy demanding processes, which become adaptive to cope with a high food intake in summer with the reduction of O_2 availability (Perera et al., 2019) or the increase of rearing density that potentiates the drawback effects of mild hypoxia (Martos-Sitcha et al., 2019a). In any case, from our results it was also conclusive that breathing frequency was negatively correlated with muscle fat depots, which would highlight a consistent increase of aerobic metabolic rates with the increase of muscle energy demand for growth, maintenance, and exercise regardless of genotype. Certainly, gilthead sea bream strains with a fast-growing genotype generally exhibited a skeletal muscle metabolically more active and efficient than that of their small counterparts (Simó-Mirabet et al., 2018).

The general thinking is that there is a positive relationship between metabolism and food availability (Reid et al., 2011; Rosenfeld and Taylor, 2009). However, there is also evidence that this relationship varies among studies and fish species. Thus, the relationship between growth and standard metabolic rates can change from being positively to negatively correlated with a decrease in food availability (Auer et al., 2015; Liu and Fu, 2017). Different types of rearrangements between growth and metabolism also occurred in our common garden system, being respiratory frequency and body weight negatively and positively correlated in fish having slow- and fast-growing phenotypes, respectively. This revealed a complex trade-off that can occur in order to prime growth in genetically fast-growing fish, or swimming performance (and maybe escape reactions) in genetically slow-growing fish. However, the co-existence of these two opposite strategies is at risk to disappear progressively after prolonged selective breeding for accelerated growth in farmed fish. This loss of physiological plasticity requires, thereby, further research through development and productive cycle to mitigate the drawback effects of reduced swimming performance, including changes in social hierarchies, and limited capacity to cope with a changing environment (Rasmussen et al., 2013).

5. Conclusions

Accelerated growth in the PROGENSA® gilthead sea bream program comes at the cost of an enhanced susceptibility to fasting during food deprivation, a reduced swimming performance and perhaps anaerobic fitness. Such selective breeding also modifies behavioral traits of free-swimming fish (physical activity and respiratory rates), further supporting a lower capacity of larger fish to cope with a reduced O_2 availability. All these findings contribute to better understand the relationships between fish and their environment, though new approaches and tools are required to mitigate the drawback effects of the trade-off between aerobic and anaerobic fitness in a context of climate change with increasing water temperatures and reduced O_2 availability.

Ethics

All procedures were carried out according to Institute of Aquaculture Torre de la Sal (IATS) and CSIC Review Boards, European (2010/63/EU) animal directives and Spanish laws (Royal Decree RD53/2013) on the handling of experimental animals.

Funding

CRediT authorship contribution statement

Erick Perera: Formal analysis, Investigation, Validation, Writing - original draft, Writing - review & editing. **Enrique Rosell-Moll:** Formal analysis, Investigation, Validation, Writing - review & editing. **Juan Antonio Martos-Sitcha:** Formal analysis, Investigation, Validation, Writing - review & editing. **Fernando Naya-Català:** Formal analysis, Investigation, Validation, Writing - review & editing. **Paula Simó-Mirabet:** Formal analysis, Investigation, Validation, Writing - review & editing. **Josep Calduch-Giner:** Formal analysis, Investigation, Writing - review & editing. **Manuel Manchado:** Conceptualization, Resources, Writing - review & editing. **Juan Manuel Afonso:** Conceptualization, Resources, Writing - review & editing. **Jaume Pérez-Sánchez:**

Conceptualization, Formal analysis, Funding acquisition, Investigation, Validation, Project administration, Resources, Supervision, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgements

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