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Growth-promoting effects of sustained swimming in fingerlings of gilthead sea bream (*Sparus aurata* L.)

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ABSTRACT

1 Fish growth is strongly influenced by environmental and nutritional factors and changing culture conditions can
2 help optimize it. The importance of early-life experience on the muscle phenotype later in life is well known. Here
3 we study the effects of five weeks of moderate and sustained swimming activity ($5\text{BL}\cdot\text{s}^{-1}$) in gilthead sea bream
4 during early development. We analysed growth and body indexes, plasma IGF-I and GH levels, feed conversion,
5 composition (proximate and isotopic ($^{15}\text{N}/^{13}\text{C}$)) and metabolic key enzymes (COX, CS, LDH, HOAD, HK, ALAT,
6 ASAT) of white muscle. Moderate and continuous exercise in fingerlings of gilthead sea bream increased plasma
7 IGF-I, whereas it reduced plasma GH. Under these conditions, growth rate improved without any modification to
8 feed intake through an increase in muscle mass and a reduction in mesenteric fat deposits. There were no changes
9 in the content and turnover of muscle proteins and lipid reserves. Glycogen stores were maintained, but glycogen
10 turnover was higher in white muscle of exercised fish. A lower LDH/CS ratio demonstrated an improvement in
11 the aerobic capacity of white muscle, while a reduction in the COX/CS ratio possibly indicated a functional
12 adaptation of mitochondria to adjust to the tissue-specific energy demand and metabolic fuel availability in
13 exercised fish. We discuss the synergistic effects of dietary nutrients and sustained exercise on the different
14 mitochondrial responses.
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24 **Key words:** exercise, white muscle, $\delta^{13}\text{C}/\delta^{15}\text{N}$, IGF-I/GH, RNA/DNA, COX/CS
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Introduction

1 For several species of fish, especially salmonids, moderate exercise (i.e. training) induced by a slow water current
2 enhances growth rate, being often accompanied by improved food conversion (Davison 1997). Previous studies
3 by our group observed similar effects of sustained swimming in juvenile gilthead sea bream (Ibarz et al. 2011;
4 Martín-Pérez et al. 2012), a fish species with rapidly increasing significance in the aquaculture of the
5 Mediterranean region (Aprumar 2014). Moreover, there is evidence that fish exposed to moderate water currents
6 exhibit less aggressive behaviour (Christiansen et al. 1992). Lower levels of circulating stress hormones would act
7 lowering the metabolic rate and exerting an energy-saving effect. In line with this theory, gilthead sea bream
8 expend 2.5-fold more energy under spontaneous (i.e. voluntary) activity than when they are forced to swim at a
9 speed of 0.5 body length per second ($BL \cdot s^{-1}$) (Steinhausen et al. 2010). It is clear that spontaneous swimming costs
10 may be considerably higher than those of a sustained swimming speed. In this regard, fish seem to adapt their
11 metabolism to nutritional regime and environmental conditions and depend on their ability to match fuel supply
12 to energy use in order to grow (Magnoni et al. 2013). Thus, sustained swimming can enhance the utilization of
13 dietary carbohydrates on a low-protein, high-carbohydrate regime, and spare the use of dietary proteins for muscle
14 growth in both rainbow trout, *Oncorhynchus mykiss* (Felip et al. 2012) and gilthead sea bream (Martín-Pérez et
15 al., 2012; Felip et al. 2013).

16 In general, the response of fish to chronic submaximal swimming is qualitatively similar to endurance exercise
17 training in mammals, and it results in the emergence of a more aerobic phenotype (Johnston and Moon 1980;
18 McClelland et al. 2006; Le Moine et al. 2010; and reviewed by McClelland and Scott 2013). This is consistent
19 with results observed in juvenile gilthead sea bream (Martín-Pérez et al. 2012). The selection of farmed fish on
20 the basis of growth performance, coupled with a sedentary life-style and high-energy diet, has resulted in
21 suboptimal production efficiency (Tørud and Hillestand 2004), given that these are symptoms of a reduced ability
22 to perform aerobic exercise (Claireaux et al. 2006). Enders et al. (2004) indicated that domestication could lead to
23 lower swimming capacity of fish. Since a hatchery provides a regular food supply and protection from predators,
24 it leads to a sustainable growing environment and allows individuals with low fitness levels to survive (Anttila et
25 al. 2008). Rearing units adjust water flow to maintain water quality and avoid pathologies. As a consequence, they
26 create an environment where fish experience few physical challenges in terms of their swimming capacity.

27 The importance of early-life experience on the muscle phenotype later in life has long been recognized, but only
28 recently has the influence of developmental exposure on muscle plasticity and swimming performance in adult
29 fish been explicitly demonstrated (Scott and Johnston 2012). Whether the beneficial effects of exercise on growth
30 performance can be induced during the early development of gilthead sea bream is not known. Nevertheless, we
31 observed that certain rearing conditions in three different hatcheries affected the growth rate of fingerlings of this
32 species and, more interestingly, that some of these characteristics were maintained for a long time, even under new
33 feeding regimes and culture conditions (Martín-Pérez et al. 2011). In that study we used, for the first time, the
34 potential of stable isotopes (SIA) of tissue deposits combined with metabolic and growth parameters to
35 discriminate fish seeds with a higher growth capacity and to relate it to their previous history.

36 The aim of the present work was to analyse the effects of sustained and moderate exercise in the early growth
37 period of gilthead sea bream. To achieve this, fingerlings were maintained under optimal farming conditions. One
38 group was forced to swim for five weeks and the other was maintained without induced water current, showing
39 only spontaneous swimming, acting as a control. Specifically, we analysed growth rate, food conversion rate, white
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1 muscle composition and metabolism (isotopic fingerprint of energy reserves, RNA/DNA content and key
2 metabolic enzymes activity). Plasma growth hormone (GH) and insulin growth factor (IGF-I) levels were also
3 analysed. Although there is no general consensus on the positive correlation between specific growth rate (SGR)
4 and plasma GH levels, it is well known that IGF-I mediates the growth-promoting effects of GH in teleost fish
5 (*reviewed* by Jönsson and Björnsson 2002). However, the effects of exercise on the GH/IGF-I axis are not well
6 known, and we related all these variables to the increased growth promotion observed in the exercised group.
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10 11 **Material and methods**

12 Experimental design

13 Five hundred and forty fingerlings of gilthead sea bream (5.05 ± 0.09 g body weight) obtained from a hatchery in
14 Northern Spain were held in the facilities of the Faculty of Biology (University of Barcelona, Spain). Fish were
15 randomly distributed into eight circular tanks equipped with a semi-closed recirculation system and physical and
16 biological filters at 23°C and with 15L:9D photoperiod at a stocking density of 1.5 kg/m³ (65-70 fish per tank).
17 Four 200-L tanks were kept in standard rearing conditions, with a water flow of 350 L/h and vertical water inflow.
18 In these conditions fish presented only spontaneous, voluntary movements and were used as a control group. Other
19 four 400-L tanks were equipped with a cylindrical tube in the central area, which resulted in a cylinder-toric living
20 area corresponding to an effective space of 200 L. These were maintained with a water flow of 700 L/h and a
21 circular, uniformly distributed flow induced by a perpendicular water inflow at the surface and an additional lateral
22 tube with holes to maintain the flux in the water column. The resulting flow was adjusted to a swimming velocity
23 of 5 BL·s⁻¹, in line with other studies (Ibarz et al. 2011; Martin-Pérez et al. 2012). The water current was measured
24 at three tank depths (near the surface, mid-tank and near the bottom) using a low-speed mechanical flow meter
25 (General Oceanics Inc., Miami, FL, USA). During the five-weeks experimental period, both groups were fed by
26 hand with a commercial diet (Gemma Diamond, Skretting) three times per day (7 a.m., 2 p.m. and 10 p.m.) until
27 apparent satiety. Feed intake was recorded on a daily basis for each tank and the food conversion ratio (FCR) was
28 calculated as fish biomass gained per feed administered. Body weight, standard length and total length were
29 measured at 21 and 35 days after the start of the trial. Specific growth rate ($SGR = 100 \cdot (\ln \text{ final body weight} - \ln$
30 $\text{ initial body weight}) \cdot \text{day}^{-1}$) was calculated for each tank biomass, weighing all fish from the tanks and averaging
31 fish weights at the beginning and the end of the experimental period (n=4 for each condition).
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45 Sample preparation

46 At the end of the experiment, 12 fish from each treatment were captured (three fish at random per tank, four tanks
47 per experimental condition), anaesthetized, weighed and sized. Samples of blood from caudal vessels were
48 obtained with heparinizing syringes. After centrifugation, plasma aliquots were frozen in liquid nitrogen and kept
49 at -80°C until IGF-I and GH analysis. The animals were then killed by sectioning the spinal cord and eviscerated.
50 The weight of mesenteric fat and liver was obtained and the percentage of the mesenteric-fat index, hepatosomatic
51 index and muscle-somatic index was calculated. Samples of epaxial white skeletal muscle under the dorsal fin
52 were dissected and frozen immediately in liquid nitrogen and kept at -80°C. The experiments complied with the
53 guidelines of the Council of the European Union (86/609/EU), the Spanish government (RD 1201/2005) and the
54 University of Barcelona (Spain) for the use of laboratory animals (DAAM 7644).
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1 Analytical procedures

2 Plasma GH and IGF-I analysis and muscle proximal composition

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4 Plasma GH levels were assayed using a homologous gilthead sea bream radioimmunoassay (RIA) in accordance
5 with the procedure described previously (Martínez-Barberá et al. 1995). This assay has a sensitivity of 0.15 ng/mL
6 and a mid-range of 1.8 ng/mL. Plasma IGFs were extracted by acid-ethanol cryoprecipitation (Shimizu et al. 2000),
7 and the IGF-I concentration was measured by means of a generic fish IGF-I RIA validated for Mediterranean
8 perciform fish (Vega-Rubín de Celis et al. 2004). The sensitivity and mid-range of the assay were 0.05 and 0.7-
9 0.8 ng/mL, respectively.

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13 Samples of white muscle were ground in liquid N₂ using a pestle and mortar to obtain a fine powder. Aliquots of
14 each sample were taken for use in isotopic analyses and to assess the lipid, protein, glycogen and water content.
15 Water content was determined gravimetrically after drying the samples at 95°C for 24 h. Lipids were extracted as
16 described by Blight and Dyer (1959), and lipid extracts were dried under a N₂ atmosphere and total lipids
17 determined gravimetrically. Proteins were purified from defatted tissue samples via precipitation with 10% (v/v)
18 trifluoroacetic acid. The extracts were dried using a vacuum system (Speed Vac Plus AR, Savant Speed Vac
19 Systems, South San Francisco, CA, USA) and the protein content was calculated from the total N content obtained
20 by elemental analysis (Elemental Analyser Flash 1112, ThermoFinnigan, Bremen, Germany), assuming 1 g of N
21 for every 6.25 g of protein. Glycogen was extracted and purified from tissues following alkaline hydrolysis by
22 boiling with 30% KOH and an alcoholic precipitation, as described by Good et al. (1933). Glycogen content was
23 then assessed using the anthrone colorimetric method described by Fraga (1956).
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31 Nucleic acid quantification and metabolic enzyme activity

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33 Muscle nucleic acid levels (RNA and DNA) were determined using the UV-based procedures for fish samples
34 described by Buckley and Bulow (1987). RNA and DNA from muscle samples were hydrolysed to nucleotides,
35 and their concentrations were calculated based on their absorbance at 260 nm. Nucleic acid concentrations were
36 expressed as µg of RNA or DNA per mg of wet tissue. An aliquot of supernatant was also used to determine
37 protein content (Bradford 1976).
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40 Enzyme activity was assayed from crude muscle extracts obtained by homogenizing frozen tissue (250 mg) in 2
41 mL of detergent solution (1.24 mM TRITON X-100, 1 mM EDTA, and 1 mM NaHCO₃) and stabilizing solution
42 (3.7 mM EDTA and 5 mM 2-β-mercaptoethanol), 1:1 v/v. Homogenates were centrifuged at 700 g at 4°C for 10
43 min. The microtitration assays to obtain maximal enzyme activity were performed at room temperature (20°C) in
44 96-well plates, as follows (final volume 200 µL): ASAT (aspartate aminotransferase, EC 2.6.1.1): 50 mM Tris-
45 HCl buffer (pH 7.4), 10 mM α-ketoglutarate, 0.3 mM NADH, 25 mM L-aspartate (substrate); ALAT (alanine
46 aminotransferase, EC 2.6.1.2): 50 mM Tris-HCl buffer (pH 7.4), 10 mM α-ketoglutarate, 0.3 mM NADH, 25 mM
47 L-alanine (substrate); GDH (glutamate dehydrogenase, EC 1.4.1.2): 50 mM imidazole-HCl buffer (pH 8.0), 100
48 mM ammonium acetate, 0.2 mM NADH, 1 mM ADP, 2IU LDH, 10 mM α-ketoglutarate (substrate); LDH (lactate
49 dehydrogenase, EC 1.1.2.4): 50 mM Tris-HCl buffer (pH 7.4), 0.16 mM NADH, 1 mM pyruvate (substrate). HK
50 (hexokinase, E.C. 2.7.1.1): 71.4 mM imidazole buffer (pH 7.4), 100 mM MgCl₂, 50 mM ATP, 8 mM NADP, 200
51 mM glucose 6-phosphate dehydrogenase (substrate). HOAD (3-hydroxiacyl CoA dehydrogenase, E.C 1.1.1.35):
52 71.4 mM imidazole buffer (pH 8.0), 2 mM NADH, 2 mM acetoacetyl-CoA (substrate).
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1 CS (citrate synthase, EC 2.3.3.1) activity was determined from absorbance increases at 412 nm of DTNB reagent,
2 using oxaloacetic acid as the substrate, following the method described by Srere (1969). COX (cytochrome-c-
3 oxidase, EC 1.9.3.1) activity was determined by adapting a commercial kit (CYTOC-OX1, Sigma-Aldrich Inc.,
4 St. Louis, MO, USA). This colorimetric assay measures the reduction in ferrocytochrome c absorbance caused by
5 oxidation of the latter by COX. Enzymatic activity measurements were performed in duplicate and expressed in
6 milliunits (mUI) per mg of wet tissue, one unit being the amount of enzyme that converts 1 μ mol of substrate per
7 min. Another aliquot of the supernatant was used to determine the protein content in accordance with the Bradford
8 method.
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10 Isotopic composition analysis ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$)

11 Samples of diet and white muscle were lyophilized and grounded into a homogenous powder for isotopic analysis.
12 Aliquots of the diet and their purified fractions (lipid and protein), and of white muscle, together with their purified
13 tissue fractions (glycogen, lipid and protein), which ranged from 0.3 mg to 0.6 mg, were weighed in small tin
14 capsules. Samples were analysed to determine the carbon and nitrogen isotope composition using a Mat Delta C
15 Isotope Ratio mass spectrometer (Finnigan MAT, Bremen, Germany) coupled to a Flash 1112 Elemental Analyzer.
16 Isotope ratios ($^{15}\text{N}/^{14}\text{N}$, $^{13}\text{C}/^{12}\text{C}$) determined by isotope-ratio mass spectrometry are expressed in delta (δ) units
17 (parts per thousand, ‰), as follows:
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$$25 \delta = [(R_{sa}/R_{st}) - 1] \times 1000$$

26 where R_{sa} is the $^{15}\text{N}/^{14}\text{N}$ or $^{13}\text{C}/^{12}\text{C}$ ratio of samples and R_{st} is the $^{15}\text{N}/^{14}\text{N}$ or $^{13}\text{C}/^{12}\text{C}$ ratio of the international
27 standards (Vienna Pee Dee Belemnite for C and air for N). The same reference material analysed over the
28 experimental period was measured with $\pm 0.2\%$ precision. Nitrogen and carbon isotopic fractionation values
29 ($\Delta\delta^{15}\text{N}$ and $\Delta\delta^{13}\text{C}$) were calculated as the difference between the δ value in the tissue and their corresponding δ
30 value in the diet.
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36 Statistics

37 The results of tissue composition, nucleic acid contents, IGF-I and GH plasma levels, enzymatic activity and
38 isotopic analyses are presented as mean \pm standard error of the mean (SEM). A two-way ANOVA was used with
39 activity (exercise (E) or control (C) groups) as a fixed factor and tank as a random factor, after checking for normal
40 distribution of data and equality of variances using the Shapiro-Wilk and Levene's test, respectively. As there were
41 not significant effects of factor tank for any of the variables analysed, activity factor comparing 3 fish per 4 tanks
42 resulted in an n of 12 individuals per condition. Two-way ANOVA (activity and time) and Tukey's post-hoc test
43 were used to analyse fish body weight (n=4). All statistical analyses were performed using SPSS v.16 (SPSS Inc.,
44 Chicago, IL, USA).
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53 **Results**

54 The effects of sustained swimming on the growth performance and feed intake of gilthead sea bream fingerlings
55 are shown in Figure 1 and Table 1, respectively. Exercised fish significantly ($p < 0.05$) increased body weight after
56 20 days of moderate swimming, being 20% larger than control fish after five weeks of experiment ($p < 0.001$).
57 Although SGR showed the same tendency, no significant differences were observed ($p = 0.059$). Feed intake did
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not change significantly as a result of exercise, and exercised fish showed similar FCR with respect to the control group (Table 1). The hepato-somatic and muscle-somatic indexes did not differ significantly between groups, but the percentage of mesenteric fat significantly decreased in exercised fish compared to control fish (Table 1).

A slight but significant increase ($p < 0.01$) in plasma IGF-I levels was found in exercised fish (Figure 2a). At the same time, a significant two-fold decrease was found in GH levels ($p < 0.01$) (Figure 2b).

Table 2 shows the changes in nucleic acids contents, proximate composition and isotopic levels of the white muscle of gilthead sea bream fingerlings. White muscle RNA and DNA concentrations did not change significantly in response to exercise. Proximate composition of muscle (protein, lipid and glycogen content) did not differ between groups. $\delta^{13}\text{C}$ values of muscle glycogen in exercised group were significantly lower than those of control group, revealing turnover differences of this reserve between both groups. The isotopic composition of the other two energy stores did not change significantly. ^{15}N -fractionation in muscle tissue and in the protein fraction did not vary, which indicates that there was no change in the isotopic fractionation of the protein gained in white muscle in the exercised group.

The enzyme activities of aerobic (CS, COX) and anaerobic metabolism (LDH), protein catabolism (ALAT, ASAT, GDH), β -oxidation (HOAD) and glucose use (HK) are shown in Table 3. Muscle CS activity increased three-fold ($p < 0.001$), while COX activity decreased by 30% ($p < 0.05$) in exercised fish compared to control fish. No significant changes were observed in ALAT, ASAT, GDH or HOAD activities, but HK activity decreased significantly by 30% ($p < 0.05$) in exercised fish compared to control fish. Figure 3a shows the relationship between LDH and CS activity and reveals that the high anaerobic capacity of the white muscle of gilthead sea bream is maintained under exercise, but that the aerobic capacity of this muscle also increases. However, COX and CS did not show any correlation (as shown in Figure 3b). It is worth noting that individual variability in most of the studied variables, including COX, was much higher in the control group, than in the exercised group.

Discussion

Many studies have shown the positive effects of swimming activity on growth rate in adult fish, and McDonald et al. (1998) and Yagata and Oku (2000) also observed the growth-promoting effects of exercise in fingerlings of Atlantic salmon (*Salmo salar*) and yellowtail (*Seriola quinqueradiata*), respectively. In the present work, moderate and sustained exercise improved the growth performance of gilthead sea bream fingerlings, in line with previous findings in juveniles of this fish species (Ibarz et al. 2011; Martín-Pérez et al. 2012). As mentioned above, this is primarily due to the enhanced utilization of nutrients (Ibarz et al. 2011, Martín-Pérez et al. 2012), whereas the growth-promoting effects of aerobic training in salmonids is mostly driven by the increased feed intake (Davidson 1997; Felip et al. 2013).

It is important to note that the energy partitioning of dietary nutrients depends on feeding status and diet composition (Cho et al. 1982). When energy demand changes, as it occurs in exercise, the use of energy fuels depends on the intensity of the activity and the species (reviewed by Magnoni et al. 2013). In previous studies on gilthead sea bream, we observed that sugars are preferentially used as metabolic fuels in juveniles subjected to moderate activity and fed a low-protein/high-carbohydrate diet. In this scenario, COX activity increased in white muscle, whereas a parallel reduction in CS activity was reported (Martín-Pérez et al. 2012). This implies that the entry of amino acids into the tricarboxylic acid cycle is reduced and there is an increase in growth through a

1 protein- sparing effect. In the present study, fingerlings of gilthead sea bream were fed a high-protein diet under
2 sustained swimming. Therefore, in order to obtain energy, amino acid oxidation had to increase in the exercised
3 group, as reflected by the significant increase in mitochondrial CS activity. A meta-analysis of microarray gene
4 expression profiling shows that mitochondria are among the first responders to nutritional and environmental
5 stressors in gilthead sea bream (Calduch-Giner et al. 2014). For instance, the gene expression of CS is up-regulated
6 by thermal stress to cope with the enhanced oxidative capacity (Bermejo-Nogales et al. 2014). In contrast, the
7 same study showed that mitochondrial activity and CS in particular are down-regulated by multiple sensory
8 perception stressors. White muscle CS activity also increases under moderate activity in zebrafish (*Danio rerio*)
9 (McClelland et al. 2006), but a significant reduction has been observed in rainbow trout after four weeks at 1.5
10 BL·s⁻¹ (Morash et al. 2014), which demonstrates a complex and sometimes contradictory regulation. In exercised
11 brown trout (*Salmo trutta*), the COX activity of skeletal muscle also increased at a low velocity (42% of maximal
12 swimming capacity), but decreased when the swimming capacity increased to 83% of maximal capacity (Antilla
13 et al. 2010). In the present study, COX activity also decreased in exercised fingerlings, which emphasizes the fact
14 that the switch of metabolic flux towards aerobic and glycolytic metabolism varies with energy demand and the
15 oxidative capacity of the fish species at a given allostatic load. In that respect, the statistically significant reduction
16 in LDH/CS ratio in exercised fish would be indicative of an enhanced aerobic phenotype, but here it was concurrent
17 with the reduction in the COX/CS ratio in the white muscle of exercised gilthead sea bream. The enhanced aerobic
18 phenotype was also observed in the white muscle of zebrafish through increased CS activity (McClelland et al.
19 2006; Le Moine et al. 2010) and in the COX increase in the white muscle of juvenile gilthead sea bream (Martín-
20 Pérez et al. 2012). A possible explanation is that sustained exercise reduces energy wastage and decreases
21 mitochondrial respiration uncoupling, which serves as a safety valve when the energy supply exceeds the energy
22 demand or the oxidative capacity of the tissue. This futile cycle is mostly driven by uncoupling proteins that are
23 expressed at a higher level in the glycolytic skeletal muscle of gilthead sea bream than in highly oxidative muscle
24 tissues (e.g. cardiac muscle and red skeletal muscle) (Bermejo-Nogales et al. 2014). At the same time, the low
25 mitochondrial density and poor vascularization of fish white muscle (Johnston and Moon 1981) may limit maximal
26 rates of protein synthesis (Pelletier et al. 1993), and the increase in the mitochondrial enzyme activity of axial
27 muscle serves to meet the enhanced demand for aerobic ATP generation caused by increased growth rates (Goolish
28 and Adelman 1987). All of this demonstrates the great metabolic plasticity of fish to adjust tissue-specific demands
29 in response to exercise and energy availability. Indeed, a recent study on gilthead sea bream indicated that the
30 transcriptional regulation of almost all enzyme subunits of the mitochondrial oxidative-phosphorylation
31 (OXPHOS) pathway are regulated by fasting in a tissue-specific manner according to the different metabolic
32 capabilities of liver, cardiac muscle and glycolytic white skeletal muscle (Bermejo-Nogales et al. 2015).

33 We previously observed that sustained and moderate swimming reduced the lipid content of white muscle in
34 gilthead sea bream juveniles (Ibarz et al. 2011). After four weeks of swimming, fingerlings of this species
35 presented no change in white muscle lipid content, HOAD activity (a marker of β -oxidation pathway) or $\delta^{13}\text{C}$
36 values of lipid deposits. All these results indicate that the turnover of lipid deposits did not change in the white
37 muscle of the exercised group compared to the control group, which is consistent with the results obtained for
38 brown trout submitted to five weeks of submaximal and continuous exercise (Antilla et al. 2010). Although aerobic
39 enzyme activity increases in the white muscle of zebrafish (McClelland et al. 2006) and brown trout (Antilla et al.
40 2010), HOAD levels remained unchanged, which shows that lipids are not the preferred fuel during any type of
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1 exercise. However, there are some conflicting results relating to muscle HOAD activity after training; endurance
2 training in the brook trout increases HOAD activity in red and white muscles, which would indicate an increase in
3 fatty acid oxidation (Johnston and Moon 1980), but the HOAD activity of zebrafish submitted to a progressive
4 training program from 2 to 5 BL/s remains unchanged (McClelland et al. 2006). With respect to the use of lipids
5 while swimming, some authors have observed a reduction in lipid content after training when the whole fish is
6 taken into account (red sea bream: Forster and Ogata 1996; rainbow trout: Lauff and Wood 1996). According to
7 these findings, we have observed a reduction in the mesenteric fat content in fingerlings of gilthead sea bream
8 under exercise. Therefore, the maintenance of muscle lipid content would indicate a good balance between dietary
9 lipid supply and its use as fuel for energy and growth, without the fat deposition presented by the control group.

10 The total glycogen store in white muscle was maintained in exercised fingerlings of gilthead sea bream, but the
11 $\delta^{13}\text{C}$ levels of this reserve decreased approaching the $\delta^{13}\text{C}$ values of the dietary protein. This is a good indicator of
12 a higher rate of *de novo* synthesis of glucose (and then of glycogen) with significant incorporation of carbon
13 skeletons from dietary proteins, which presented those lower $\delta^{13}\text{C}$ values.

14 Although gluconeogenesis from dietary amino acids should be increased in liver and thus glucose exported to
15 peripheral tissues, including muscle, plasma glucose levels were not modified by exercise (control: $2.7 \text{ mM} \pm 0.13$;
16 exercise: 2.9 ± 0.19). At the same time, HK activity in white muscle decreased compared to the control group.
17 Sánchez-Gurmaches et al. (2013) found lower insulin and higher IGF-I levels in the plasma of juveniles of gilthead
18 sea bream submitted to sustained swimming. The increase in plasma IGF-I concentration was also observed in the
19 present study, but insulin was not measured. The lower levels of muscle HK activity, possibly associated with
20 lower plasma insulin, may indicate that the use of dietary carbohydrates was not enhanced under the swimming
21 conditions of the present study, but it merits further study.

22 Regarding proteins, N fractionation (a good marker of protein turnover, Martín-Pérez et al. 2011) was unchanged
23 in exercised fingerlings compared to control fish. A similar observation was found in exercised juveniles (Martín-
24 Pérez et al. 2012). Moreover, exercise did not modify ASAT or ALAT activity. Therefore, a positive balance
25 between protein synthesis and degradation would explain the higher growth rate observed in exercised fingerlings
26 of gilthead sea bream, and may be promoted by the high levels of IGF-I. IGF-I and GH are the main regulatory
27 hormones of fish growth (Reindl and Sheridan 2012), and gilthead sea bream in particular (Mingarro et al. 2002),
28 although the role of these hormones in exercise is not fully understood. Thus, an increase in circulating levels of
29 plasma GH has been reported in coho salmon (*Oncorhynchus kisutch*) (Barrett and McKeown 1988a) and rainbow
30 trout (Barrett and McKeown 1988b; Nielsen et al. 1994) submitted to submaximal swimming, but a close
31 association between exercise-induced growth and circulating levels of GH and IGF-I has not been reported in masu
32 salmon (*Oncorhynchus masou masou*) (Azuma et al. 2002). This contrasts with the observations made in gilthead
33 sea bream, in which moderate exercise increased plasma IGF-I levels, both in this study and previous studies on
34 juvenile fish (Sánchez-Gurmaches et al. 2013). Moreover, we found that this endocrine feature was related to a
35 pronounced reduction in plasma GH levels, which was probably due to the negative feedback inhibition of systemic
36 IGF-I upon pituitary GH synthesis and release. Experimental evidence for this has previously been reported for
37 primary cultures of rainbow trout pituitary cells (Pérez-Sánchez et al. 1992). Therefore, the best growth
38 performance, in both salmonids and gilthead sea bream, is achieved with high levels of IGF-I and low levels of
39 GH, regardless of age and nutritional condition (Pérez-Sánchez and Le Bail 1999; Gómez-Requeni et al. 2004;
40 Benedito-Palos et al. 2007). In our experimental model, this was the case for the exercised fish. In this regard, the
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1 significant increase in body growth and the tendency towards higher muscle somatic index without changes in
2 DNA concentration might indicate that hypertrophic processes are produced in white muscle, which correspond
3 to a protein gain in white muscle, as observed in exercised rainbow trout (Houlihan and Laurent 1987). Moreover,
4 a tendency for the RNA content of exercised fingerlings to increase compared to control fish would indicate that
5 the synthetic capacity of white muscle is enhanced in the former. Both hypertrophy and a higher RNA content
6 were also observed in the white muscle of juveniles (Ibarz et al. 2011; Martin-Pérez et al. 2012), but further
7 histological studies are required to confirm this.
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10 Interestingly, this study also showed that moderate exercise reduces the individual variability for most of the
11 variables analysed, especially in terms of body weight and enzyme activities (LDH, COX). Jobling et al. (1993)
12 found that groups of salmonids exposed to water currents were less heterogeneous than groups held under static
13 water conditions. Thus, normal sedentary rearing conditions, as found in the control group, produce more
14 interactions between individual fish (Christiansen and Jobling 1990). The sudden changes in spontaneous activity
15 are associated with substantially higher energy costs compared with fish under sustained swimming at a constant
16 velocity (Steinhausen et al. 2010). The different levels of efficiency in the use of energy between the two groups
17 of fish affected their growth rates.
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20 In conclusion, moderate and continuous aerobic exercise improves body growth and nutrient use in gilthead sea
21 bream fingerlings, expands the aerobic capacity of white muscle and reduces mesenteric fat deposits. These
22 changes in muscle growth and metabolic signatures may occur at later stages if the training is maintained, although
23 further studies are required to fully explore the potential benefits of exercise in fish farming. In this sense,
24 considering that fingerlings and juveniles present similar response to swimming activity, it seems that the sooner
25 this is applied the better the benefits for sustainable production.
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40 University of Barcelona.
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51 The authors declare that they have no conflict of interest.

52 The experiments complied with the Guidelines of the European Union Council (86/609/EU), the Spanish
53 Government (RD 1201/2005) and the University of Barcelona (specific ethics approval number for the protocol
54 was CEEA-96/09) for the use of laboratory animals.
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References

- 1
2
3 Anttila K, Järvilehto M, Mänttari S (2008) The swimming performance of brown trout and whitefish: the effects
4 of exercise on Ca²⁺ handling and oxidative capacity of swimming muscles. *J Comp Physiol B* 178:465-475.
5
6 Anttila K, Jäntti M, Mänttari S (2010) Effects of training on lipid metabolism in swimming muscles of sea trout
7 (*Salmo trutta*). *J Comp Physiol B* 180:707-714.
8
9 APROMAR (2014) La acuicultura en España 2014. [http://www.apromar.es/content/la-acuicultura-en-espana-](http://www.apromar.es/content/la-acuicultura-en-espana-2014)
10 [2014](http://www.apromar.es/content/la-acuicultura-en-espana-2014). Accessed 03 August 2015.
11
12 Azuma T, Noda S, Yada T, Ototake M, Nagoya H, Moriyama S, Yamada H, Nakanishi T, Iwata M (2002) Profiles
13 in growth, smoltification, immune function and swimming performance of 1-year-old masu salmon, *Oncorhynchus*
14 *masou masou* reared in water flow. *Fish Sci* 68:1282-1294.
15
16 Barrett BA, McKeown BA (1988a) Sustained exercise increases plasma growth hormone concentrations in two
17 anadromous salmonids. *Can J Fish Aquat Sci* 45:747-749.
18
19 Barrett BA, McKeown BA (1988b) Plasma growth hormone levels in *Salmo gairderi*: studies on temperature and
20 exercise intensity/duration relationship. *Comp Biochem Physiol A* 94:791-794.
21
22 Benedito-Palos L, Saera-Vila A, Calduch-Giner JA, Kaushik S, Pérez-Sánchez J (2007) Combined replacement
23 of fish meal and oil in practical diets for fast growing juveniles of gilthead sea bream (*Sparus aurata* L.):
24 Networking of systemic and local components of GH/IGF axis. *Aquaculture* 267:199-212.
25
26 Bermejo-Nogales A, Nederlof M, Benedito-Palos L, Ballester-Lozano GF, Folkedal O, Olsen RE, Sitjà-Bobadilla
27 A, Pérez-Sánchez J (2014) Metabolic and transcriptional responses of gilthead sea bream (*Sparus aurata* L.) to
28 environmental stress: New insights in fish mitochondrial phenotyping. *Gen Comp End* 205:305-315.
29
30 Bermejo-Nogales A, Calduch-Giner JA, Pérez-Sánchez J (2015) Unraveling the molecular signatures of oxidative
31 phosphorylation to cope with the nutritionally changing metabolic capabilities of liver and muscle tissues in farmed
32 fish. *PLoS ONE* 10(4): e0122889. doi:10.1371/journal.pone.0122889.
33
34 Bradford M (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing
35 the principle of protein-dye binding. *Anal Biochem* 72:248-254.
36
37 Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* 37:
38 911-917.
39
40 Buckley LJ, Bulow FJ (1987) Techniques for the estimation of RNA, DNA and protein. In Summerfelt and Hall
41 (eds) *Age and Growth of Fish*. Iowa State University Press, Ames, Iowa, pp 345-357.
42
43 Calduch-Giner JA, Echassériau Y, Crespo D, Baron D, Planas JV, Prunet P, Pérez-Sánchez J (2014)
44 Transcriptional assessment by microarray analysis and large-scale amd meta-analysis of the metabolic capacity of
45 cardiac and skeletal muscle tissues to cope with reduced nutrient availability in gilthead sea bream (*Sparus aurata*
46 L.). *Mar Biotechnol* 16:423-435.
47
48 Cho CY, Slinger SJ, Bayley HS (1982) Bioenergetics of salmonid fishes: energy intake, expenditure and
49 productivity. *Comp Biochem Physiol B*, 73:25-41
50
51 Christiansen JS, Jobling M (1990) The behaviour and the relationship between food intake and growth of juvenile
52 Arctic charr, *Salvelinus alpinus* L., subjected to sustained exercise. *Can J Zool* 68:2185-2191.
53
54 Christiansen JS, Svendsen YS, Jobling M (1992) The combined effects of stocking density and sustained exercise
55 on the behavior, food intake, and growth of juveniles Arctic charr (*Salvelinus alpinus* L.,). *Can J Zool* 70:115-122.
56
57
58
59
60
61
62
63
64
65

1 Claireaux G, Couturier C, Groison AL (2006) Effect of temperature on maximum swimming speed and cost of
2 transport in juvenile European sea bass (*Dicentrarchus labrax*). J Exp Biol 209:3420-3428.

3 Davison W (1997) Training and its effects on teleost fish. Comp Biochem Physiol A 94:1-10.

4

5 Enders EC, Boisclair D, Roy AG (2004) Differences in the energetic cost of swimming in turbulent flow between
6 wild, farmed and domesticated juvenile Atlantic salmon *Salmo salar*. J Fish Biol 65:317.

7

8 Felip O, Ibarz A, Fernández-Borràs J, Beltrán M, Martín-Pérez M, Planas JV, Blasco J (2012) Tracing metabolic
9 routes of dietary carbohydrate and protein in rainbow trout (*Oncorhynchus mykiss*) using stable isotopes ($[^{13}\text{C}]$
10 starch and $[^{15}\text{N}]$ protein): effects of gelatinisation of starches and sustained swimming. Br J Nutr 107:834-844.

11

12 Felip O, Blasco J, Ibarz A, Martín-Pérez M, Fernández-Borràs J (2013) Beneficial effects of sustained activity on
13 the use of dietary protein and carbohydrate traced with stable isotopes ^{15}N and ^{13}C in gilthead sea bream (*Sparus*
14 *aurata*). J Comp Physiol B 183:223-234.

15

16 Forster IP, Ogata H (1996) Growth and whole-body lipid content of juvenile red sea bream reared under different
17 conditions of exercise training and dietary lipid. Fish Sci 62:404-409.

18

19 Fraga F (1956) Determination of glycogen in shellfish with the anthrone reagent. Investig Pesq 3:69.

20

21 Gómez-Requeni P, Mingarro M, Calduch-Giner JA, Médale F, Martin SAM, Houlihan DF, Kaushik S, Pérez-
22 Sánchez J (2004) Protein growth performance, amino acid utilisation and somatotropic axis responsiveness to fish
23 meal replacement by plant protein sources in gilthead sea bream (*Sparus aurata*). Aquaculture 232:493-510.

24

25 Good BCA, Cramer H, Somogy M (1933) The determination of glycogen. J Biol Chem 100:491.

26

27 Goolish EM, Adelman IR (1987) Tissue specific cytochrome-oxidase activity in largemouth bass: The metabolic
28 costs of feeding and growth. Physiol Zool 60:454-464.

29

30 Houlihan DF, Laurent P (1987) Effects of exercise training on the performance, growth, and turnover of rainbow
31 trout (*Salmo gairdneri*). Can J Fish Aquat Sci 44:1614-1621.

32

33 Ibarz A, Felip O, Fernández-Borràs J, Martín-Pérez M, Blasco J, Torrella JR (2011) Sustained swimming improves
34 muscle growth and cellularity in gilthead sea bream. J Comp Physiol B 181:209-217.

35

36 Jobling M, Baardvik BM, Christiansen JS, Jørgensen EH (1993) The effects of prolonged exercise training growth
37 performance and production parameters in fish. Aquaculture Int 1:95-111.

38

39 Johnston IA, Moon TW (1980) Exercise training in skeletal muscle of brook trout (*Salvelinus fontinalis*). J Exp
40 Biol 87:177-194.

41

42 Johnston IA, Moon TW (1981) Fine structure and metabolism of multiply innervated fast muscle fibers in teleost
43 fish. Cell Tiss Res 219:93-109.

44

45 Jönsson E, Björnsson BT (2002) Physiological functions of growth hormone in fish with special reference to its
46 influence on behaviour. Fish Sci 68:742-748.

47

48 Lauff RF, Wood CH (1996) Respiratory gas exchange, nitrogenous waste excretion, and fuel usage during aerobic
49 swimming in juvenile rainbow trout. J Comp Physiol B 166:501-509.

50

51 LeMoine CMR, Craig PM, Dhekney K, Kim JJ, McClelland GB (2010) Temporal and spatial patterns of gene
52 expression in skeletal muscles in response to swim training in adult zebrafish (*Danio rerio*). J Comp Physiol B
53 180:151-160.

54

55

56

57

58

59

60

61

62

63

64

65

- 1 Magnoni LJ, Felip O, Blasco J, Planas JV (2013) Metabolic fuel utilization during swimming: optimizing
2 nutritional requirements for enhanced performance. In Palstra AJ and Planas JV (eds) Swimming physiology of
3 fish. Springer, Berlin, pp 203-235.
- 4 Martínez-Barberá JP, Pendón C, Martí-Palanca H, Calduch-Giner JA, Rodríguez RB, Valdivia MM, Pérez-
5 Sánchez J (1995) The use of recombinant gilthead sea bream (*Sparus aurata*) growth hormone for radioiodination
6 and standard preparation in radioimmunoassay. *Comp Biochem Physiol A* 110:335-340.
- 7
8 Martín-Pérez M, Fernández-Borràs J, Ibarz A, Felip O, Gutierrez J, Blasco J (2011) Stable isotope analysis
9 combined with metabolic indices discriminates between gilthead sea bream (*Sparus aurata*) fingerlings produced
10 in various hatcheries. *J Agric Food Chem* 59:10261–10270.
- 11
12 Martín-Pérez M, Fernández-Borràs J, Ibarz A, Millán-Cubillo A, Felip O, de Oliveira E, Blasco J (2012) New
13 insights into fish swimming: A proteomic and isotopic approach in gilthead sea bream. *J Proteome Res* 11:3533-
14 3547.
- 15
16 McClelland GB, Craig PM, Dhekney K, Dipardo S (2006) Temperature- and exercise-induced gene expression
17 and metabolic enzyme changes in skeletal muscle of adult zebrafish (*Danio rerio*). *J. Physiol.* 577:739-751.
- 18
19 McClelland GB, Scott GB (2013) Muscle plasticity. In Evans, Clairbone and Currie (eds) *The Physiology of fishes*,
20 CRC Press, Boca Raton FL, pp 1-31.
- 21
22 McDonald DG, Milligan CL, McFarlane WJ, Croke S, Currie S, Hooke B, Angus RB, Tufts BL, Davidson K
23 (1998) Condition and performance of juvenile Atlantic salmon (*Salmo salar*): effects of rearing practices on
24 hatchery fish and comparison with wild fish. *Can J Fish Aquat Sci* 55:1208-1219.
- 25
26 Mingarro M, Vega-Rubín de Celis S, Astola A, Pendón C, Valdivia MM, Pérez-Sánchez J (2002) Endocrine
27 mediators of seasonal growth in gilthead sea bream (*Sparus aurata*): the growth hormone and somatolactin
28 paradigm. *Gen Comp End* 128:102-111.
- 29
30 Morash AJ, Vanderveken M, McClelland GB (2014) Muscle metabolic remodeling in response to endurance
31 exercise in salmonids. *Frontiers in Physiology*. doi: 10-3389/fphys. 2014.00452.
- 32
33 Nielsen ME, Boesgaard L, Sweeting RM, McKeown BA, Rosenkilde P (1994) Plasma levels of lactate, potassium,
34 glucose, cortisol, growth-hormone and triiodo-L-thyroxine in rainbow trout (*Oncorhynchus mykiss*) during exercise
35 at various levels for 24 h. *Can J Zool* 72:1643-1647.
- 36
37 Pelletier D, Guderley H, Dutil JD (1993) Does the aerobic capacity of fish muscle change with growth rates? *Fish*
38 *Physiol Biochem* 2:83-93.
- 39
40 Pérez-Sánchez J, Weil C, Le Bail PY (1992) Effects of human insulin-like growth factor-I on release of growth
41 hormone by rainbow trout, *Oncorhynchus mykiss*, pituitary cells. *J Exp Zool* 262:287–290.
- 42
43 Pérez-Sánchez J, Le Bail PY (1999) Growth hormone axis as marker of nutritional status and growth performance
44 in fish. *Aquaculture* 177:117–128.
- 45
46 Reindl KM, Sheridan MA (2012) Peripheral regulation of the growth hormone-insulin-like growth factor system
47 in fish and other vertebrates. *Comp Biochem Physiol A* 163:231-245.
- 48
49 Sánchez-Gurmaches J, Cruz-García L, Ibarz, A, Fernández-Borràs J, Blasco J, Gutiérrez J, Navarro I (2013)
50 Insulin, IGF-I, and muscle MAPK pathway responses after sustained exercise and their contribution to growth and
51 lipid metabolism regulation in gilthead sea bream. *Domest Anim Endocrin* 45:145-153.
- 52
53 Scott GR, Johnston IA (2012) Temperature during embryonic development has persistent effects on thermal
54 acclimation capacity in zebrafish. *Proc Natl Acad Sci USA* 109:14247-14252.
- 55
56
57
58
59
60
61
62
63
64
65

1 Shimizu M, Swanson P, Fukada H, Dickhoff W (2000) Comparison of extraction methods and assay validation
2 for salmon insulin-like growth factor-I using commercially available components. Gen Comp End 119:26-36.

3 Srere PA (1969) Citrate synthase. In Lowenstein, Kaplan and Colowick (eds) Methods of enzymology, Academic
4 Press, New York, 13:3-5.

5
6 Steinhausen MF, Steffensen JF, Andersen NG (2010) The effects of swimming pattern on the energy use of
7 gilthead seabream (*Sparus aurata* L.). Mar Freshw behav phy 43:227-241.

8
9 Tørud B, Hillestad M (2004) "Hjerterapporten". Rapport om hjertelidelser hos laks of regnbueorret.
10 <http://www.fiskerifond.no/files/projects/attach/hjerterapporten.pdf>.

11
12 Vega-Rubin de Celis S, Gómez-Requeni P, Pérez-Sánchez J (2004) Production and characterization of
13 recombinantly derived peptides and antibodies for accurate determinations of somatolactin, growth hormone and
14 insulin-like growth factor-I in European sea bass (*Dicentrarchus labrax*). Gen Comp End 139:266-277.

15
16
17 Yogata H, Oku H (2000) The effects of swimming exercise on growth and whole-body protein and fat contents of
18 fed and unfed fingerling yellowtail. Fish Sci 66:1100-1105.

Figure captions

Fig. 1 Effect of moderate sustained swimming on body weight of gilthead sea bream fingerlings. Values are mean \pm SEM (n= 4). Significant differences were considered at $p<0.05$ (*), $p<0.001$ (***)

Fig. 2 Effect of moderate sustained swimming on plasma IGF-I (a) and GH (b) levels in gilthead sea bream fingerlings. Values are mean \pm SEM (n=12). Significant differences were considered at $p<0.01$ (**)

Fig. 3 Effect of moderate sustained swimming on LDH/CS (a) and COX/CS (b) relationships in muscle gilthead sea bream fingerlings. Values are mean \pm SEM (n=12). Pearson's correlation coefficients and p values: a) $r= -0.194$, $p=0.37$; b) $r=-0.285$, $p=0.19$

Table 1. Effect of moderate swimming activity on growth performance, feed intake and somatic indexes of gilthead sea bream fingerlings.

	Control	Exercise
Initial body weight (g)	4.97 ± 0.04	5.14 ± 0.20
Final body weight (g)	17.54 ± 0.46	20.28 ± 0.38 ***
^aSGR	3.41 ± 0.08	3.72 ± 0.13
Feed intake (g/fish·day)	0.37 ± 0.01	0.36 ± 0.01
^bFCR	1.17 ± 0.16	0.93 ± 0.06
^cHSI	1.12 ± 0.07	1.27 ± 0.08
^dMSI	34.89 ± 1.00	37.27 ± 0.99
^eMFI	1.37 ± 0.16	0.81 ± 0.15 *

Values are mean ± SEM (n=4 for initial and final body weight, SGR, feed intake and FCR) (n=12 for HSI, MSI and MFI).

^a Specific growth rate % =

$$= [100 (\ln \text{ final body weight} - \ln \text{ initial fish weight})] \text{ days}^{-1}.$$

^b Food conversion ratio = (Feed intake·daily weight gain⁻¹)

^c Hepato-somatic index (g liver·100g body weight⁻¹).

^d Muscle-somatic index (g muscle·100g body weight⁻¹).

^e Mesenteric fat index (g fat·100g body weight⁻¹)

Significant differences were considered at p<0.05 (*), p<0.001(***)).

Table 2. Effect of moderate swimming activity on nucleic acid, proximate and isotopic composition of muscle of gilthead sea bream fingerlings.

	Control	Exercise
Protein (% w.w)	19.47 ± 1.34	19.02 ± 1.47
Lipids (% w.w)	2.69 ± 0.43	2.66 ± 0.41
Glycogen (% w.w)	0.26 ± 0.02	0.30 ± 0.03
RNA (µg/mg prot)	4.88 ± 0.50	5.84 ± 0.47
DNA (µg/mg prot)	1.56 ± 0.20	1.26 ± 0.13
RNA/DNA	4.59 ± 0.43	4.02 ± 0.37
δ ¹³ C-muscle	-19.80 ± 0.07	-19.90 ± 0.09
δ ¹³ C-protein	-20.93 ± 0.33	-20.51 ± 0.05
δ ¹³ C-lipid	-24.84 ± 0.11	-24.84 ± 0.13
δ ¹³ C-glycogen	-19.14 ± 0.36	-21.05 ± 0.36 *
δ ¹⁵ N-muscle	11.87 ± 0.03	11.85 ± 0.02
δ ¹⁵ N-protein	12.84 ± 0.04	12.77 ± 0.07
^a Δ ¹⁵ N-muscle	2.33 ± 0.02	2.33 ± 0.04

Values are mean ± SEM (n =12).

^a Isotopic fractionation (δ¹⁵N-muscle- δ¹⁵N-diet).

δ¹⁵N-diet: 9.50 ± 0.02; δ¹³C-diet: -21.82 ± 0.04

Significant differences were considered at p<0.05 (*).

Table 3. Effect of moderate sustained swimming on the enzyme activities of muscle of gilthead sea bream fingerlings

	Control	Exercise	
Cox (UI/g w.w)	0.34 ± 0.03	0.25 ± 0.01	*
CS (UI/g w.w)	0.36 ± 0.04	1.07 ± 0.08	***
Cox / CS	0.84 ± 0.07	0.24 ± 0.02	*
LDH (UI/g w.w)	475 ± 25.7	467 ± 13.0	
LDH / (CS*1000)	1.18 ± 0.26	0.37 ± 0.03	*
ASAT (UI/g w.w)	15.23 ± 0.70	16.69 ± 0.7	
ALAT (UI/g w.w)	1.38 ± 0.06	1.35 ± 0.08	
GDH (UI/g w.w)	51.85 ± 2.80	52.76 ± 2.50	
HOAD (UI/g w.w)	0.14 ± 0.01	0.13 ± 0.01	
HK (mUI/g w.w)	91.8 ± 10.0	65.2 ± 5.0	*

Values are mean ± SEM (n =12).

Significant differences considered at p<0.05 (*), p<0.001 (***).

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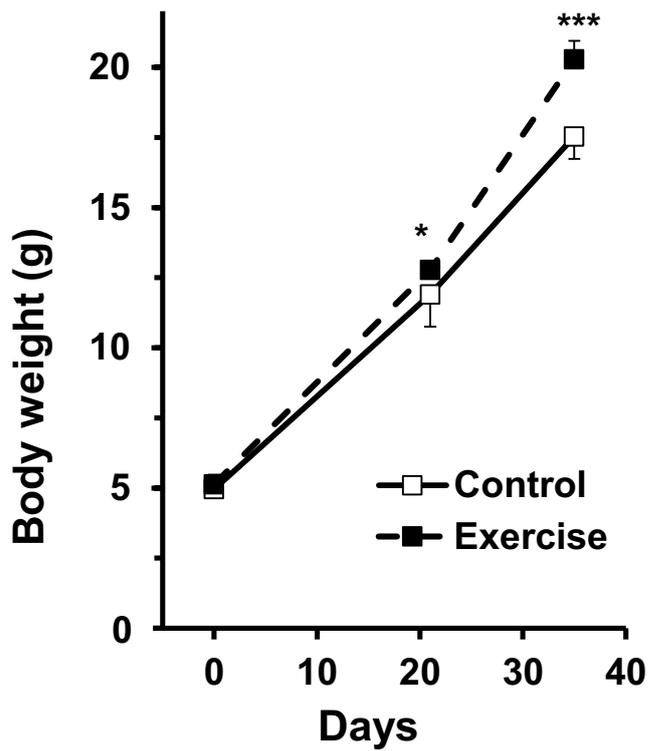


Figure 1

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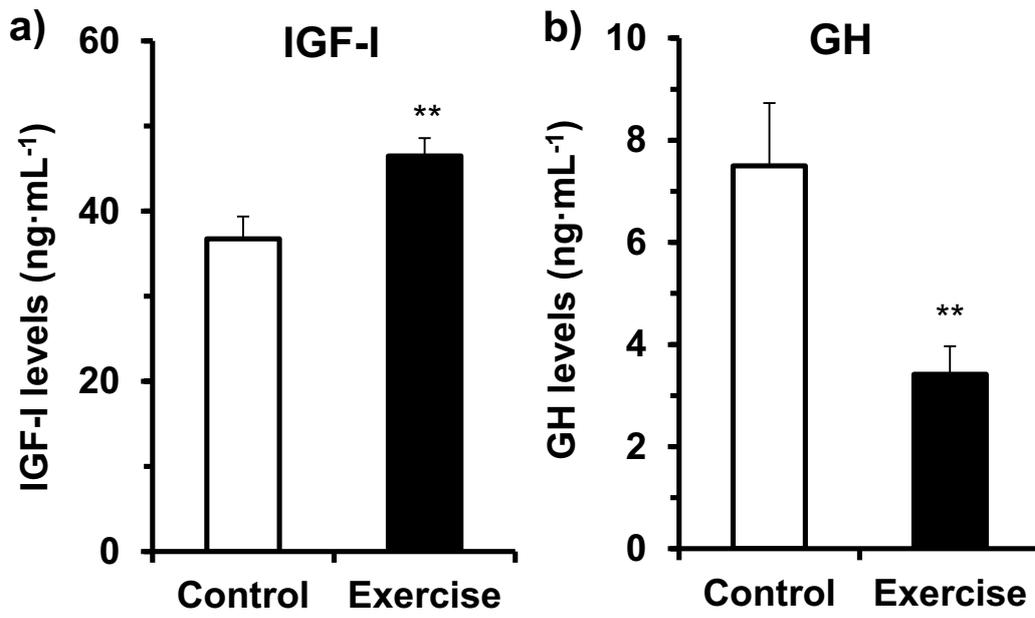
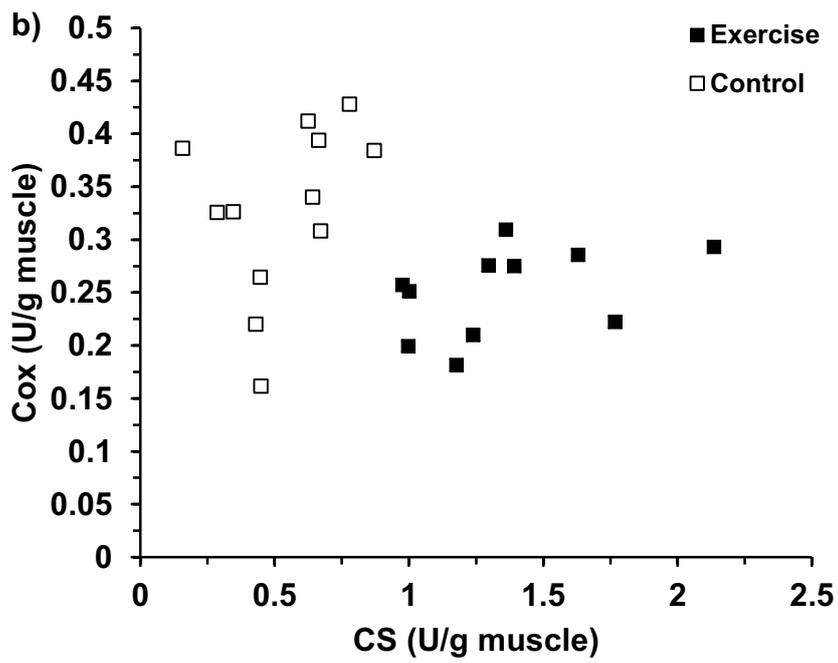
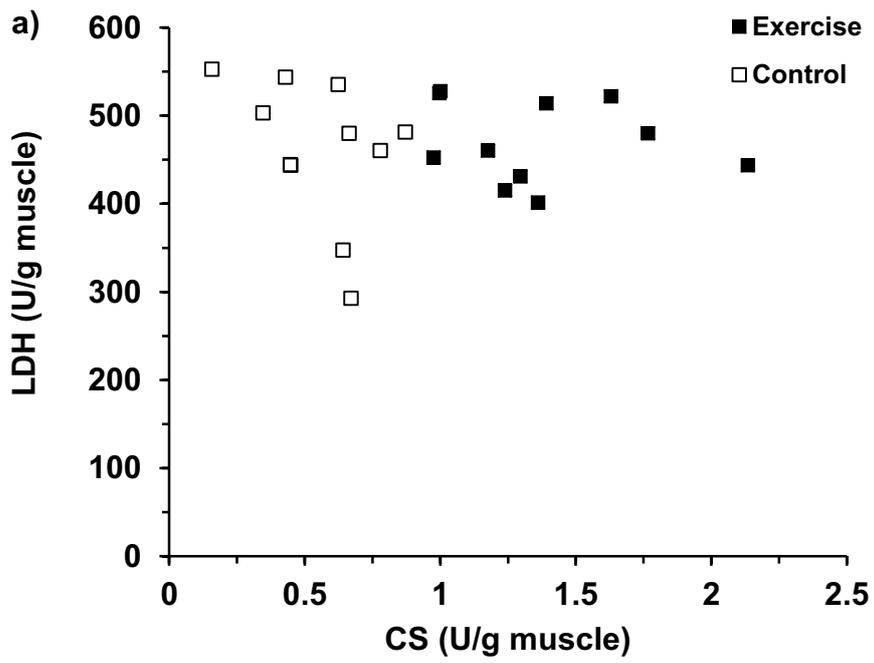


Figure 2



53 Figure 3a and 3b

Table 1. Effect of moderate swimming activity on growth performance, feed intake and somatic indexes of gilthead sea bream fingerlings.

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Values are mean ± SEM (n=4 for initial and final body weight, SGR, feed intake and FCR) (n=12 for HSI, MSI and MFI).

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RNA (µg/mg prot)	4.88 ± 0.50	5.84 ± 0.47
DNA (µg/mg prot)	1.56 ± 0.20	1.26 ± 0.13
RNA/DNA	4.59 ± 0.43	4.02 ± 0.37
δ ¹³ C-muscle	-19.80 ± 0.07	-19.90 ± 0.09
δ ¹³ C-protein	-20.93 ± 0.33	-20.51 ± 0.05
δ ¹³ C-lipid	-24.84 ± 0.11	-24.84 ± 0.13
δ ¹³ C-glycogen	-19.14 ± 0.36	-21.05 ± 0.36 *
δ ¹⁵ N-muscle	11.87 ± 0.03	11.85 ± 0.02
δ ¹⁵ N-protein	12.84 ± 0.04	12.77 ± 0.07
^a Δ ¹⁵ N-muscle	2.33 ± 0.02	2.33 ± 0.04

Values are mean ± SEM (n=12).

^a Isotopic fractionation (δ¹⁵N-muscle- δ¹⁵N-diet).

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Table 3. Effect of moderate sustained swimming on the enzyme activities of muscle of gilthead sea bream fingerlings

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Cox (UI/g w.w)	0.34 ± 0.03	0.25 ± 0.01	*
CS (UI/g w.w)	0.36 ± 0.04	1.07 ± 0.08	***
Cox / CS	0.84 ± 0.07	0.24 ± 0.02	*
LDH (UI/g w.w)	475 ± 25.7	467 ± 13.0	
LDH / (CS*1000)	1.18 ± 0.26	0.37 ± 0.03	*
ASAT (UI/g w.w)	15.23 ± 0.70	16.69 ± 0.7	
ALAT (UI/g w.w)	1.38 ± 0.06	1.35 ± 0.08	
GDH (UI/g w.w)	51.85 ± 2.80	52.76 ± 2.50	
HOAD (UI/g w.w)	0.14 ± 0.01	0.13 ± 0.01	
HK (mUI/g w.w)	91.8 ± 10.0	65.2 ± 5.0	*

Values are mean ± SEM (n =12).

Significant differences considered at p<0.05 (*), p<0.001 (***).