# SUSTAINED SWIMMING IMPROVES GROWTH BY HYPERPLASIA, HYPERTROPHY AND CAPILLARISATION OF WHITE MUSCLE IN FINGERLINGS OF GILTHEAD SEA BREAM (Sparus aurata)

A. Moya\*, J.R. Torrella, J. Fernàndez-Borràs, D. Rizo-Roca, A. Millán-Cubillo, E.J. Vélez, A. Ibarz, J. Gutiérrez, J. Blasco

Departament de Fisiologia i Immunologia, Facultat de Biologia, Universitat de Barcelona, 08028, Barcelona, Spain.

\*Corresponding author. E-mail: <u>albertomoya@ub.edu</u>

### Introduction

Exercise training leads to enhanced growth, mostly in salmonids (Davison 1997), but also in gilthead sea bream (*Sparus aurata*) (Ibarz et al. 2011; Martín-Pérez et al. 2012). The vast majority of studies have been carried out in juveniles or adult fish, but it is not known whether the beneficial effects of exercise on growth can be induced in the early stages of gilthead sea bream. Here we examine the effect of sustained, moderate swimming on the growth of gilthead sea bream fingerlings, and on their white muscle cellularity and capillarisation. This research was supported by a Spanish government grant (AGL2012-39768).

## **Material & Methods**

Fingerlings of gilthead sea bream (average body weight: 5 g) were distributed into six 200 l circular tanks equipped with semi-closed recirculation systems at 23°C and with a 15L:9D photoperiod at a density of 1.5 kg.m.<sup>-3</sup>. Three tanks were kept under standard rearing conditions, where fish movement was voluntary (control group, CT). In the other three tanks (exercise group, EX) the fish were forced to swim at 5  $BL \cdot s^{-1}$ . The fish were fed a commercial diet three times a day, to near satiety. Feed intake and growth were recorded during the experimental period of 5 weeks. Samples of epaxial white skeletal muscle from under the dorsal fin were removed, pre-cooled with isopentane and immediately frozen in liquid nitrogen. Serial transverse sections from each sample were cut in a cryostat (-20°C) and stained for endothelial ATPase and succinate dehydrogenase histochemical assays to reveal muscle capillaries and confirm the anaerobic characteristics of the muscle fibres. The RNA and DNA content were also measured.

### Results

The body weight of exercised fish was significantly enhanced (CT: 17.5 EX: 20.3 g, p<0.001) without any differences in feed intake. The muscle-somatic index did not differ significantly between groups (CT: 34.9% EX: 37.3% b.w.). A tendency towards higher RNA concentration in the exercised group, without any change in DNA content, was observed.

The fish under sustained swimming showed significant decreases in fibre cross-sectional area (FCSA) and in fibre perimeter (FPER), without any differences in fibre circularity (Table I). In the exercised group, the number of small fibres (FCSA <1500  $\mu$ m<sup>2</sup>) was significantly higher, whereas large fibres with FCSA >5500  $\mu$ m<sup>2</sup> were significantly fewer. The total muscle capillary density (CD, capillaries per mm<sup>2</sup>) and the individual

fibre capillarisation (CCA, number of capillaries per 1000  $\mu$ m<sup>2</sup> of fibre area) of the exercised fish were significantly increased (Figure 1).

**Table I**. Morphometrical fibre parameters and fibre density in muscles of control and exercised fish.

	Control	Exercise	
FCSA	<b>2935</b> ±669	<b>2325</b> ±447	*
FPER	<b>209</b> ±25	<b>188</b> ±16	*
SF	<b>0.698</b> ±0.02	<b>0.689±</b> 0.02	
FD	<b>302</b> ±76	<b>339</b> ±80	
Values are mean $\pm$ SD (n =10). Significant differences at			

(p < 0.05) (\*). FCSA: fibre cross-sectional area ( $\mu$ m<sup>2</sup>), FPER: fibre perimeter ( $\mu$ m<sup>2</sup>), SF: shape factor (circularity), FD: fibre density (fibres mm.<sup>2</sup>).



**Figure 1.** Values are mean  $\pm$  SD (n =10) Significant differences between CT and EX at (p < 0.05) (\*) and (p < 0.001) (\*\*\*). White: CD, black: CCA.

### **Discussion and conclusions**

Our study demonstrates that moderate and sustained exercise improves growth rate without modifying feed intake in fingerlings of gilthead sea bream, confirming previous observations in juveniles of this species (Ibarz et al. 2011; Martin-Pérez et al. 2012). The present work is the first attempt to study muscle cellularity and capillarisation in gilthead sea bream fingerlings. We found that exercised fish have smaller fibres than controls, in contrast to observations in juvenile gilthead sea bream (Ibarz et al. 2011). The enhanced body growth and the tendency towards higher MSI without changes in DNA concentration might indicate that hypertrophy processes also occur in white muscle. However, knowing that the exercised group grew more, the FCSA and FPER decreased, together with there being a larger amount of small fibres and higher fibre density in the exercised fish, could suggest the formation of new fibres in the white muscle (hyperplasia). Moreover, the moderate increase in RNA content measured in the exercised fish could indicate enhanced protein synthesis in accordance with findings in juveniles (Martin-Pérez et al. 2012).

White muscle is poorly capillarised compared with the red muscle. In this work, exercised fish showed a significant increase in capillarity (both CD and CCA). These results indicate that white muscle is remodelled to a more aerobic phenotype, in agreement with results for juvenile gilthead sea bream (Martín-Pérez et al. 2012).

In conclusion, moderate sustained aerobic exercise in gilthead sea bream fingerlings enhances body growth, initiates muscle fibre hyperplasia and increases total muscle and individual fibre capillarisation, leading to a more aerobic phenotype. So, moderate sustained swimming in the early stages of life in this species will improve growth capacity.

#### References

Davison W. 1997. Training and its effects on teleost fish. Comp. Biochem. Physiol. 94A, 1-10.

- Ibarz A., Felip O., Fernández-Borràs J., Martín-Pérez M., Blasco J. and Torrella J.R. 2011. Sustained swimming improves muscle growth and cellularity in gilthead sea bream. J. Comp. Physiol. B, 181, 209-217.
- Martin-Pérez M., Fernandez-Borras J., Ibarz A., Millan-Cubillo A., Felip O., de Oliveira E. and Blasco J. 2012. New insights into fish swimming: A proteomic and isotopic approach in gilthead sea bream. J. Proteome Res. 11, 3533-3547.