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Muscle Cellularity at Cranial and Caudal Levels of the Trunk Musculature of Commercial Size Sea Bass, *Dicentrarchus labrax* (Linnaeus, 1758)

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With 4 figures and 2 tables

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Summary

In eight specimens of Atlantic sea bass of commercial size (≅350 g) muscle cellularity was studied at two selected sampling levels of the trunk axial musculature: caudal (anal opening) and cranial (fourth radius of the dorsal fin). The following parameters were quantified at both sampling levels: white muscle cross-sectional area, white muscle fibre diameter (900–1200 fibres), muscle fibre number and muscle fibre density. Results showed a higher total cross-sectional area at cranial than at caudal level ($P < 0.05$), what is related with their different gross morphology. However, the white muscle fibre size distribution, as well as the muscle fibre number and density did not show significant differences between them. This study contributes to typify muscle fibre sampling in sea bass of commercial size what is of great interest for morphometric studies where white muscle cellularity is commonly correlated with textural or organoleptic parameters.

Introduction

The axial trunk musculature of fish is divided into a series of myomeres and the muscle fibres inserted via short tendons into collagenous sheets called myosepta. The geometrical arrangement of the fibres is complex with individual fibres being arranged in helical trajectories between successive myomeres (Alexander, 1969). In most teleost species, the myotomal organization of the axial musculature is commonly stratified in three layers. One located superficially (red muscle), another deeply (white muscle) and a third between the other two layers (intermediate or pink muscle), which varies between species, as regards extension and histochemical properties (van Raamsdonk et al., 1974; Rowlerson et al., 1985; Mascarello et al., 1986; Romanello et al., 1987; Sänger et al., 1988; Scapolo et al., 1988; Ibabe et al., 2000). White muscle comprises the major edible part of the myotome and is composed of fast twitch fibres (Hudson, 1969; Johnston, 1980). The pattern of fibre number and fibre size distribution in a particular muscle section is commonly referred to as muscle cellularity (Johnston, 1999; Stoiber et al., 2003).

Growth of skeletal muscle occurs by both hyperplasia and hypertrophy of muscle fibres. In mammalian muscle, hyperplasia is restricted largely to the pre- and perinatal periods (Chiakulas and Pauly, 1965; Moss and Leblond, 1971; Goldspink, 1972; Schultz, 1974). By contrast, in teleost hyperplasia continues, together with hypertrophic growth, during the post-larval period. These processes give rise to a typical mosaic appearance of muscle fibres in transversal sections, with fibres of different diameter intermingled. The relative contribution of muscle fibre hypertrophy and hyperplasia to the total muscle growth varies markedly among different species (Greer-Walker, 1970; Weatherley and Gill, 1985; Weatherley et al., 1988) and in different muscle fibre types (Stickland, 1983; Kiessling et al., 1991). It also seems to depend on external factors like temperature (Ayala et al., 2000, 2001a,b, 2003; López Albors et al., 2003), photoperiod (Johnston et al., 2003b), exercise training (Johnston and Moon, 1980), and diets (Weatherley et al., 1980; Fauconneau et al., 1997). The plasticity of muscle growth under different production conditions determines different muscle cellularity, which is a major factor in determining quality, in particular the texture and processing characteristics of the flesh (Johnston, 1999). Although somatic growth can be easily measured in the form of body weight, it only gives an indirect estimation of the muscle growth. A long-established method to measure muscle growth, which provides useful quantitative data, is the measurement of muscle morphological parameters in a representative area of lateral muscle in fish of different ages, sizes or conditions (Johnston et al., 1998; Valente et al., 1999). Commonly, a whole cross-section of the body is used to measure the total transversal myotomal area. The size and number of muscle fibres in restricted areas of the cross-section is used to characterize muscle cellularity and to obtain an estimate of the size and number of muscle fibres (Weatherley and Gill, 1985; Rowlerson et al., 1995).

Some previous studies have shown that the diameter and number of muscle fibres varies along the length of the body and in different regions of the same cross-section (Stickland, 1983; Kiessling et al., 1991; Rowlerson et al., 1995; Johnston, 2001a). For example, Kiessling et al. (1991) found marked differences between the ventral and dorsal region of the white epiaxial muscle indicating different growth areas within the muscle. However, previous works did not particularly focus on comparing muscle cellularity between distant zones of the trunk musculature that display different gross morphology. The myotomal cross-section at the level of the anal opening has an almost similar morphology and size of both the epaxial and hypaxial half, whereas at the level of the pectoral fin the epaxial half is clearly wider than the hypaxial half (Fig. 1). In some scombrids, i.e. the bluefin tuna, *Thunnus thynnus* L., or the Atlantic bonito, *Sarda sarda* B., the hypaxial half of the trunk musculature situated between the pectoral fin and the anal opening is individually sold under the name of loin. Such

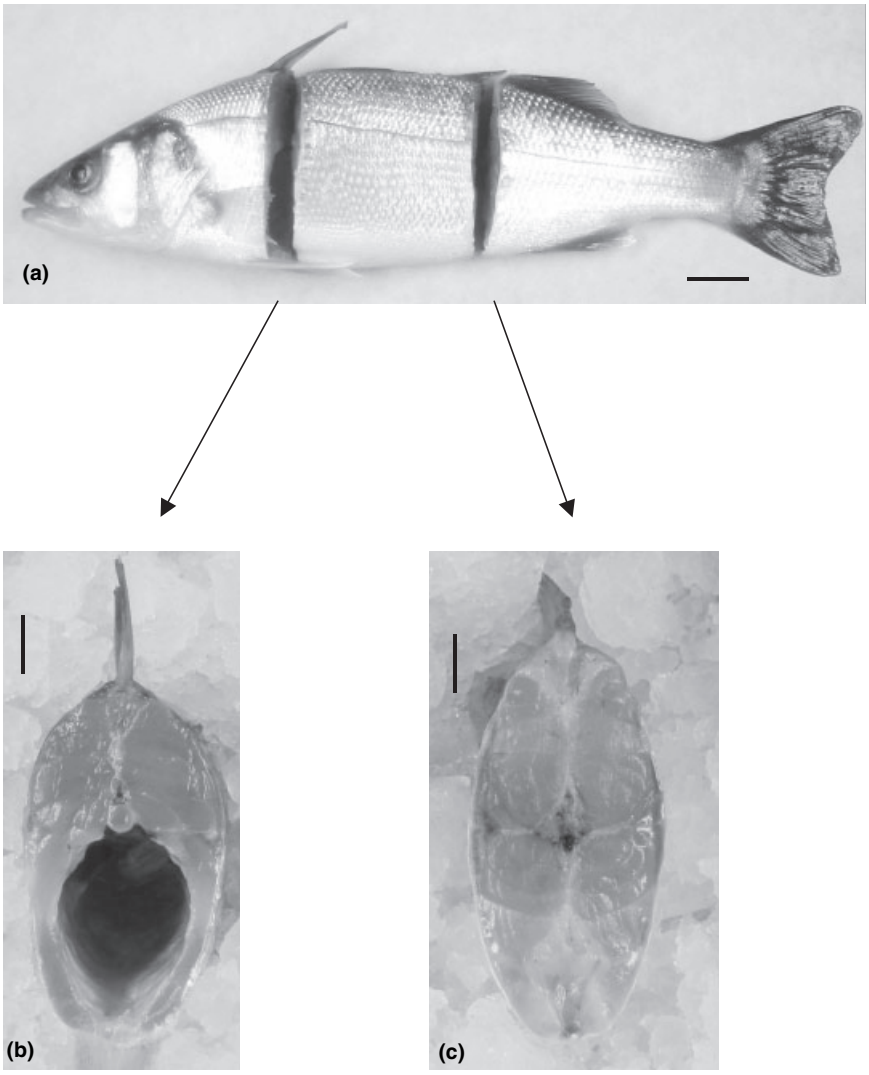

Fig. 1. (a) Cranial and caudal sampling levels for measurement of muscle cellularity, bar: 0.41 cm. (b) Body section at the level of the fourth ray of the dorsal fin (piece II), bar: 0.69 cm. (c) Body section just caudally to the anal opening (piece I), bar: 0.69 cm.

commercial piece is highly appreciated by its organoleptic properties.

Sea bass (*Dicentrarchus labrax*) is an euryhaline species widely cultured in Mediterranean areas. In Spain, with an annual sea bass production of ≅3000 metric tonnes in 2002, aquaculture accounts for about 60% of this total (http://www.feap.org). It may be significant to mention that most previous works on sea bass muscle growth have been carried out with samples obtained at the level of the anal opening (Veggetti et al., 1990; Ramírez Zarzosa et al., 1995, 1998; Ayala et al., 2000, 2001a,b; López Albors et al., 2003). These studies were focused in larval and early post-larval stages, however there is a lack of quantitative muscle works on specimens of commercial size (≅350 g).

According to these precedents the aim of the present work was to find out whether the gross anatomical different shape between distant sampling areas of the axial musculature display different muscle cellularities.

Materials and Methods

Fish rearing

The axial skeletal muscle was studied in eight specimens of farmed Atlantic sea bass sampled at commercial size (366.25 ± 24.85 g). Fish were obtained at the Instituto Español de Oceanografía (Centro Oceanográfico de Murcia, Puerto de Mazarrón) from a broodstock of 50 Atlantic sea bass (7 years old) adapted to captivity and maintained in a 45 m^3 tank, with food based on pellets, squid and fresh fish. A batch of ≅380 000 eggs was obtained at ambient temperature (≅14.6°C) and natural photoperiod in March. Incubation and larval rearing were performed under natural conditions in cylindrical tanks (1 m^3), at an initial density of 86 eggs/l. Eggs and larvae were maintained in total darkness with no food until 160°C day, at which point lighting conditions of 16:8 (L:D) photoperiod and 500 lx intensity were put in place. At that moment food supply with *nauplii* of *Artemia* enriched with Selco (Inve, Ghent, Belgium) started. On day 41 post-hatch larvae were fed with commercial feed (inert diet) based on extruded feed. The size of particles was gradually increased from 0.1 mm.

Post-larval phase was performed in rectangular tanks (2.5 m^3) until a weight of 10–15 g and subsequently in tanks of 7.5 m^3 until commercial size (≅350 g). Fish were fed *ad libitum* twice daily with commercial pellets. The diet contained 45% protein, 22% fat and 11% ash. During the course of the experiment, dissolved oxygen levels were measured by an oximeter (OxyGuard MK III; OxyGuard International A/S, Birkerød, Denmark) and maintained at about 6.5 ppm.

Cultivation was performed at ambient temperature – minimum of 13°C in January, and maximum of 26°C in August. After 29 months of culture, sampled fish were anaesthetized with clove oil, killed by immersion in ice cold water (hypothermia) and despatched (with polystyrene box with ice pellets) to the Veterinary Faculty of Murcia, within 3 h of harvest.

Muscle sample preparation

Muscle samples were obtained by removal of two pieces (0.5 cm thickness) of the whole cross-section of the trunk musculature, just caudally to the anal opening (piece I, caudal) and at level of the fourth ray of the dorsal fin (piece II, cranial) (Fig. 1).

Pieces I and II were traced on acetate sheet using a fine permanent pen to identify the whole transversal area of the white muscle. Subsequently, the whole left half of pieces I and II were trimmed into six blocks (three epaxial and three hypaxial), that were frozen in 2-methylbutane (−80°C), snap frozen over liquid nitrogen, and then stored in a −65°C freezer (Nuaire Nu:6511 E) until sectioning. Sections of 8–10 μm thickness were obtained in a cryostat (Leica CM 1850; Leica Instruments, Heidelberg, Germany), and then stained with haematoxylin/eosin.

Morphometry and statistics analysis

Morphometric analysis was carried out by means of an image analysis system device (Qwin; Leica) connected to a light photomicroscope (Leica DMLB). The diameter of 900–1200 white muscle fibres representing fields from all regions of the left half myotomal cross section was measured in pieces I and II of each fish. Subsequently, the white muscle fibre density was calculated as the number of fibres per mm^2 of muscle cross-sectional area, and the total number of fibres estimated from the fibre density and the value of the total cross-sectional area of white muscle (mm^2).

Non-parametric statistical techniques were used to fit smoothed probability density functions (PDFs) to the measured diameters using a kernel function. The statistical method used was described by Silverman (1986) and Bowman and Azzalini (1997), and its application to the study of muscle fibre size distributions by Johnston et al. (1999). Authors of the present work obtained a copy of statistical package from I. A. Johnston on request. The programs are written in the PC language R, which is a dialect of Splus (Ihaka and Gentelman, 1996). Values for the smoothing parameter h (Bowman and Azzalini, 1997) were in the range 0.08–0.10, with no systematic differences between the two sampling points (caudal and cranial). Bootstrap techniques were used to distinguish underlying structure in the distributions from random variation (Johnston et al., 1999). The Kolmogorov–Smirnov two-sample test statistic was used to test the null hypothesis that the PDFs for each sampling level (caudal/cranial) were equal over all diameters. The 5th, 10th, 50th, 95th and 99th percentiles of muscle fibre diameter were calculated from the distributions. A Kruskal–Wallis non-parametric test was used to test the hypothesis that the median value of the specified percentile was equivalent between the sampling levels.

The total white muscle cross-sectional area, average muscle fibre diameter, muscle fibre density and estimated number of white muscle fibres were statistically compared by ANOVA ($P < 0.05$) with the SPSS 11.0 program.

Table 1. Muscle cellularity parameters. White muscle cross-sectional area, fibre diameter, fibre density and total number of fibres at caudal and cranial levels of the musculature in reared sea bass of commercial size

	Caudal	Cranial
White muscle cross-sectional area (mm^2)	721.62 ± 47.6*	855 ± 26.47*
Average muscle fibre diameter (μm)	93.74 ± 0.42	95.91 ± 0.39
Muscle fibre density (no. fibres/mm^2)	126.91 ± 7.37	122.71 ± 5.85
Total number of fibres	90606.88 ± 6002	105442.74 ± 6728

Values are given as mean ± SEM.
*Existence of significant differences ($P < 0.05$).

Results

Descriptive statistics (mean ± SEM) for muscle cellularity parameters are summarized in Table 1. There were only minor differences between caudal and cranial sampling levels. Only the total cross-sectional area was higher at the level of the fourth ray of the dorsal fin (cranial) than at the anal opening (caudal) ($P < 0.05$). However, average values for muscle fibre diameter, fibre density and total number of white fibres were similar in caudal and cranial levels sampling.

The estimated PDF of muscle fibre diameter for each particular fish and the mean PDF for all fish are shown separately for the caudal and cranial levels in Fig. 2. The variability band that represents the maximal area created by 100 bootstraps estimates of density is shown in Fig. 3. The construction of a variability band around the mean PDF using bootstrap techniques allows inference about the existence of unimodal, bimodal or trimodal distributions. At caudal level the mean PDF was bimodal with the highest peak between 67 and 69 μm and a second peak between 125 and 127 μm (Figs 2a and 3a). The largest cohort of muscle fibres was around 195.13 μm in diameter (P_{99}, Table 2) and the smallest at 41.97 μm (P_5, Table 2). At the cranial level there was evidence of a bimodal distribution of muscle fibre diameters (Fig. 2b and 3b). The main peak probability density was around 63 μm diameter and the P_{99} of the distribution was 195.48 μm (P_{99}, Table 2).

Comparative plots of muscle fibre size distributions between the cranial and caudal sampling levels is shown in Fig. 4. The average PDF for each sampling level (cranial or caudal) fell within the reference band for the combined levels, that indicating an overall inexistence of differences between them. Besides, the Kolmogorov–Smirnoff test indicated no statistically significant differences between the muscle fibre size distributions at both sampling levels ($P = 0.9405$), neither the Kruskal–Wallis test for differences between the 5th, 10th, 50th, 95th and 99th percentiles (Table 2).

Discussion

The present work is one of the very few aimed to investigate muscle cellularity of fish related to sampling level (caudal and cranial). It is important to highlight that adequate sampling of muscle fibres is necessary for statistical significance of morphometric results. Both the overlapping of the different myotomes and the helical trajectories of most white muscle fibres determine their average number and diameter to vary in

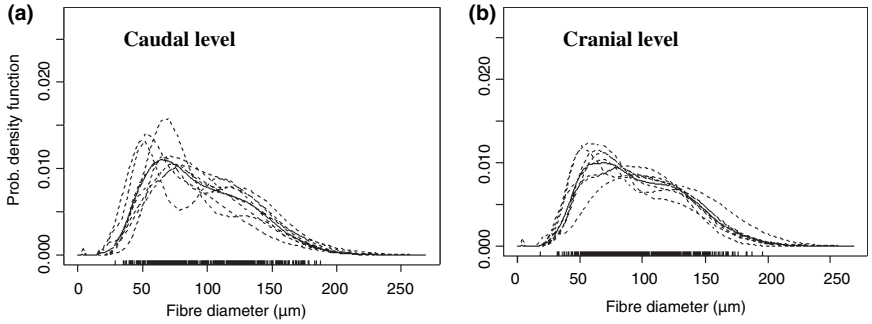

Fig. 2. The probability density function (PDF) of white muscle fibre diameters at the level of the anal opening (caudal) and fourth dorsal fin ray (cranial). Dotted lines illustrate the functions for individual fish and the solid line is the calculated mean value.

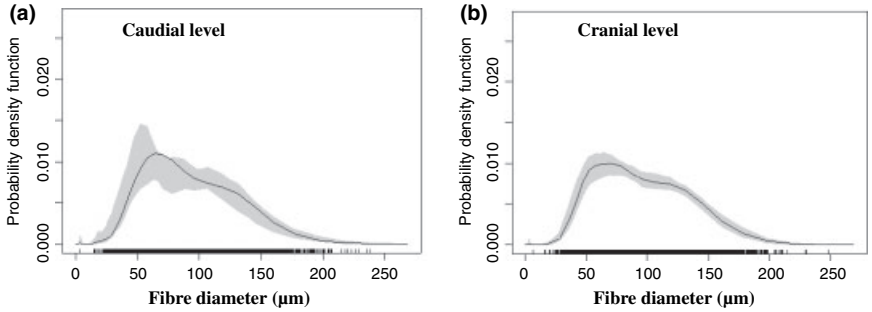

Fig. 3. Mean value and band variability (shaded area) of the probability density functions of muscle fibre diameter in caudal (a) and cranial (b) levels.

Table 2. Comparison of the percentiles for the mean probability density function of muscle fibre diameter in caudal and cranial sampling levels

Percentiles	Caudal	Cranial
P_5	41.97	42.73
P_{10}	49.05	49.66
P_{50}	89.25	92.17
P_{95}	169.24	170.63
P_{99}	195.13	195.48

No significant differences ($P > 0.05$) were observed, for Kruskal–Wallis test.

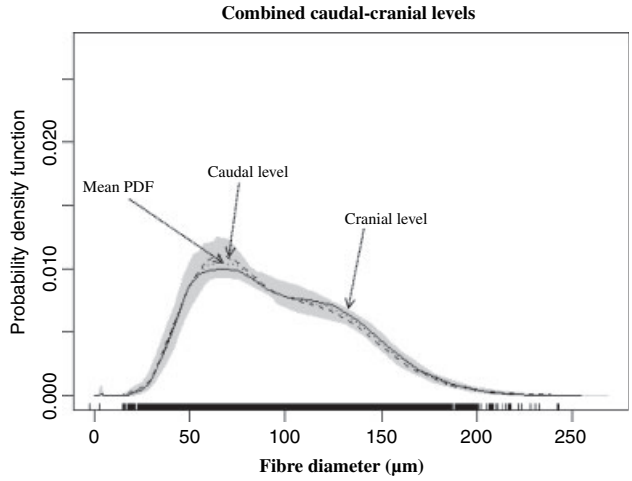

Fig. 4. The mean probability density function of muscle fibre diameter for cranial (solid line) and caudal (dashed line) levels in reared sea bass. The shaded polygon (reference band) represents 100 bootstrap estimates of the combined sampling levels and the dotted line represents the mean probability density function of the pooled groups.

different areas of the transversal trunk section (Johnston, 2001a). This topic is essential to obtain an appropriate estimate of the muscle fibre size distribution in the whole cross-section of the trunk musculature. When measuring a low quantity of muscle fibres or sampling restricted areas of the cross-section results for each particular species may not be representative enough to lead to conclusions. This was suggested by Kiessling et al. (1991) after comparing their results with those of Stickland (1983) in rainbow trout (*Oncorhynchus mykiss*). In 17 specimens, Stickland (1983) measured 100 white muscle fibres in three different regions (superficial, middle and deep) at the level of the horizontal septum from relatively caudal myotomes. More cranially, Kiessling et al. (1991) studied muscle fibre size distribution in dorsal, lateral and ventral areas of the cross-section. The discrepancy between the results found in these studies as regards white muscle growth dynamics may have a number of explanations including the importance of counting a statistically representative number of fibres as well as possible differences in sample localization (Johnston, 2001a). In this sense, we have carried out morphometry upon the whole cross-section of the trunk musculature and measured a large number of white muscle fibres (900–1200). This methodology was based on previous studies of Johnston et al. (1999, 2000a,c, 2003a,b), where comparisons of muscle fibre size distributions between two different experimental groups are systematically carried out. Despite of being a hard, time-consuming task, such methodology seems to be the most appropriate to characterize muscle cellularity in teleost fish.

Descriptive and non-parametric statistical techniques have been used to compare morphometric muscle parameters in sea bass. It is relevant that results for the average muscle fibre diameter, number of fibres and fibre density did not show significant differences between the two sampling levels (Table 1). Muscle fibre size distributions were also similar in

caudal and cranial levels as shown in Fig. 4, and demonstrated by the Kolmogorov–Smirnov and Krustal–Wallis tests ($P > 0.05$). These results compared with previous similar studies were unexpected. In gilthead sea bream larvae (*Sparus aurata*, L.) of 20 days, Rowlerson et al. (1995) found significant differences between the muscle fibre size distributions at the level of the anal opening and 2 mm more caudally. In Atlantic salmon (*Salmo salar*, L.), Johnston (2001a) found different muscle fibre size distributions on the basis of comparative histograms at three points along the body side. On the contrary, we have demonstrated that despite gross anatomical differences, the axial musculature of sea bass of commercial size (≅350 g) displayed an overall similar muscle cellularity between the caudal and cranial levels. As muscle cellularity is correlated with textural and organoleptic parameters of the flesh (Hatae et al., 1990; Hurling et al., 1996; Johnston et al., 2000b; Johnston, 2001b) this fact may be of great interest for future meat quality studies of sea bass. To find out whether our results are only valid for this particular species or body size would need further investigations.

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