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Muscle cellularity and flesh quality of wild and farmed sea bass, *Dicentrarchus labrax* L.

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Abstract

Sea bass (*Dicentrarchus labrax* L.) has been widely farmed in the last decade. In order to a better understanding of the final quality of this species, muscle cellularity and quality parameters of the flesh were studied on 14 specimens of wild and 11 farmed Atlantic sea bass, at approximate commercial size (weight 350 g, length 32 cm). White muscle cellularity was evaluated by means of the following parameters: number and diameter of muscle fibres, as well as the muscle fibre size distribution, throughout the total cross-section of the flesh. To ascertain the flesh quality, several physico-chemical parameters (moisture, protein, total fat, fatty acids, hydroxyproline, collagen and pH) were analyzed, and textural mechanical properties (hardness, springiness, chewiness, cohesiveness, gumminess) were determined objectively with a texturometer.

Muscle cellularity was different between both groups, such that muscle fibre density was higher for wild specimens ($p<0.05$). Farmed sea bass showed a higher content of moisture and protein ($p<0.01$), and a lower flesh pH, and hydroxyproline and collagen contents ($p<0.01$). Despite of the fact that the total fat did not show significant differences between both populations, saturated and monounsaturated fatty acids were significantly higher in farmed than in wild sea bass, whereas wild fish showed a higher content of polyunsaturated fatty acids ($p<0.05$). No significant differences were found in the total content of ω−3 fatty acids between both groups. All textural properties were significantly higher in wild than in farmed fish ($p<0.001$), all of them show a positive and significant correlation with muscle fibre density, pH, hydroxyproline and collagen contents. Changes in these parameters determined marked differences in the flesh quality of wild and farmed sea bass, whereas no relationship was found between muscle cellularity and nutritional composition of the sea bass. According to our results, genetic factors as well as the influence of extrinsic factors such as feeding regimes and/or exercise may determine significant variations of some structural and flesh quality parameters of the sea bass.

Keywords: Sea bass; Muscle cellularity; Texture; Flesh quality; Fatty acids; ω−3; PUFA; MUFA; SAFA

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1. Introduction

Muscle tissue is the main edible portion of fish and responsible of their nutritional value. Fish axial muscle is segmentally arranged into myotomes, with two main fibre types grouped into two muscle layers: the superficial (red muscle) and deep (white muscle) layers (Rowlerson et al., 1985; Scapolo et al., 1988; Veggetti et al., 1990). Also, an intermediate, thin layer (pink muscle) is usually present between them (Mascarello et al., 1986; López-Albors et al., 1998). Fish muscle growth commonly occurs by two possible mechanisms: hypertrophy and hyperplasia of muscle fibres. Hypertrophic growth occurs throughout post-embryonic life until muscle fibres reach a functional maximum diameter (Egginton and Johnston, 1982). Hyperplastic growth of muscle fibres refers to the increase in muscle fibre number due to the recruitment of new fibres. The rates of muscle fibre hypertrophy and hyperplasia to reach a given girth vary between species and different strains of the same species (Weatherley et al., 1979) and can be affected by controlled rearing conditions such as diet (Kiessling et al., 1991), exercise training (Johnston and Moon, 1980; Totland et al., 1987), and temperature (Nathanailides et al., 1996; Johnston et al., 1998, 2003a; Ayala et al., 2000, 2001; López-Albors et al., 2003). Hence, the cultivation of fish may produce a wide range of numbers and diameters of muscle fibres in the flesh (muscle cellularity), which is related to the growth history of the fish. Also, in wild fish the environmental and nutritional conditions may determine different muscle cellularities associated to their particular lifestyle.

White muscle cellularity is an important determinant of the textural characteristics of the flesh (Fauconneau et al., 1993; Hurling et al., 1996). Several studies have found a relationship between muscle fibre size and the firmness of the flesh (Hatae et al., 1990; Hurling et al., 1996), which could also influence on the taste and processing characteristics of the flesh (Johnston, 1999). This has already been demonstrated for the Atlantic salmon (*Salmo salar*, L.) where the firmness of smoked fillet and colour measured by Roche *Salmo*Fan™ were positively correlated with the muscle fibre density (Johnston et al., 2000a).

Flesh quality is a complex set of characters involving intrinsic factors such as texture, chemical composition, colour, fat content (Fauconneau et al., 1995), and is heavily influenced by extrinsic factors such as pre- and post-slaughter handling procedures (Dunajski, 1979; Gjerdrem, 1997). The quality characteristics of the sea food products, as of any other food items, are strictly dependent on factors involved in the production processes and vary between markets. The composition and sensorial parameters differ in general between wild and farmed fish (Børresen, 1992; Netteleton and Exler, 1992). The chemical parameters of wild fish are strongly influenced by the sea environmental conditions, which determine the nutrients availability. In farmed fish, feeding with artificial diets provides a wide range of nutrients and this fact, not only determines fish growth rate but flesh composition, in particular the lipid content, which may be quantitatively and qualitatively modified (Izquierdo et al., 2003). However, flesh protein content is less influenced by external feeding since it is mainly dependent on intrinsic factors such as the fish species, variety and size (Børresen, 1992; Shearer, 1994; Huss, 1999). Concerning the organoleptical properties, a high content of fat in the farmed fish could lead to a lower texture, but texture is also related to other factors, such as collagen content of the flesh and the muscle fibre size (Johnston et al., 2000a).

Farmed seafoods have an advantage over wild-caught fishery products since they are produced and harvested under controlled conditions, and for this reason the hazards associated with fish consumption might be reduced. Fish farming has registered a worldwide rapid expansion in the recent decades (FAO, 1998), showing the sea bass (*Dicentrarchus labrax*, L.) production a great increase. Murcia is the main sea bass farming region in Spain, with the 44% of the Spanish production (García-García et al., 2001). Parallelly to a higher production, the consumption of sea bass in Europe has significantly increased due to a lower price in markets and its desirable aroma and quality. Recently, the differences in the chemical composition between wild and farmed sea bass of Greece and Italy have been reported by Alasalvar et al. (2002) and Orban et al. (2002), however, no previous studies have compared

yet the differences in muscle cellularity between wild and cultured sea bass.

In the present work, muscle cellularity and flesh quality parameters have been studied in two populations of sea bass: a farmed population obtained at the Instituto Español de Oceanografía (Centro Oceanográfico de Murcia) and one population of wild specimens captured on the Murcia's coast. The comparison of muscle cellularity between wild and farmed sea bass and its correlation with textural and physico-chemical parameters is important in order to find out any relationship between muscle structure, lifestyle and flesh characteristics. This study may contribute to understand the nutritional quality of sea bass, and the sensorial acceptance by consumers.

2. Materials and methods

2.1. Fish samples and growth conditions

Twenty five sea bass were studied: 11 farmed Atlantic sea bass, and 14 specimens captured on the Mediterranean coast of Murcia (wild population). Farmed specimens were obtained at the Instituto Español de Oceanografía (Centro Oceanográfico de Murcia) from a broodstock adapted to captivity. Eggs and larvae were maintained in cylindrical tanks (1 m^3) in darkness without feeding until 160 °C-day. At that moment, lighting conditions of 16:8 (L/D) photoperiod and 500-lx intensity were put in place and larvae fed with *nauplii* of *Artemia salina*. At 41 days, larvae feeding consisted of extruded commercial feed (Trouw, S.A.). Postlarvae were farmed 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). During the experiment, specimen cultivation was performed at ambient temperature—minimum of 13 °C in January, and maximum of 26 °C in August. Oxygen level was measured with an oximeter (Oxiguard Mk III) and maintained over 6 ppm, and salinity was 36‰. Fish were fed ad libitum with a commercial diet (Trouw, S.A.) which contained: 45% protein, 11% ash and 22% fat with the following fatty acids composition: myristic acid ($C_{14:0}$), 9.50%; palmitic acid ($C_{16:0}$), 24%; palmitoleic acid ($C_{16:1}$), 8%; estearic acid ($C_{18:0}$), 1.70%; oleic acid ($C_{18:1\omega9}$), 11%; vaccenic acid ($C_{18:1\omega7}$), 3%; linoleic acid ($C_{18:2\omega6}$), 6%; morotic acid ($C_{18:4\omega3}$), 3.10%; gadoleic acid ($C_{20:1\omega9}$), 1.50%; eicosapentanoic acid ($C_{20:5\omega3}$), 17%; cetoleic acid ($C_{22:1\omega11}$), 0.70%; clupanodonic acid ($C_{22:5\omega3}$), 2.10%; nervonic acid ($C_{24:1\omega9}$), 0.35%; docosahexanoic acid ($C_{22:6\omega3}$), 12%; SAFA, 35%; MUFA 25%; PUFA, 40%; ω_6, 6%; ω_3, 42%. Wild specimens were captured by net in March of 2002 on two different locations of Murcia's coast: Puerto de Mazarrón and San Pedro del Pinatar. After a selection of fish of approximate commercial size (≅ 350 g), farmed and wild specimens were kept in tanks without feeding for 24 h. Fish were anaesthetized with clove oil (Guinama^R), then immersed in ice cold water (hypothermia) and finally submitted in box with ice pellets to the Veterinary Faculty of Murcia, within 3 h of harvest.

2.2. Sample treatment

Fish were washed with tap water, then the surface wiped with tissue and subsequently weighted, measured and eviscerated to collect the weight of the digestive apparatus, liver, gonads, perivisceral fat and carcass. All data were used to calculate biometrical indexes (Pouey et al., 1993). Muscle samples for muscle cellularity and physico-chemical parameters were obtained as showed in Fig. 1.

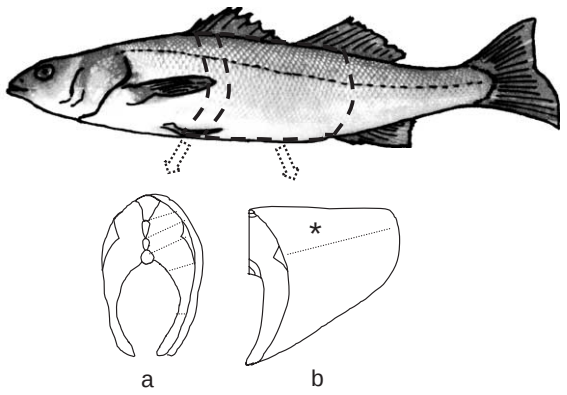

Fig. 1. Sea bass muscle sampling. (a) Transversal section of sea bass used for measurement of the total white muscle cross-sectional area. Muscle blocks used to measure the muscle fibre diameters have been approximately drawn on the left half of the total cross-section. (b) Flesh fillet used for textural and physico-chemical analysis. * indicates the approximate level of measurement of texture.

2.3. Muscle sample processing for morphometrical studies

A whole cross-section sample of the trunk musculature (0.5 cm thickness) was removed at the level of the fourth ray of the dorsal fin of each specimen (Fig. 1). These cross-sections were used to estimate the total area of white muscle by drawing it on acetate paper. Subsequently, the left half of each one of these samples was trimmed into 6–7 muscle blocks of approximately equal size. Blocks were covered with tissue freezing medium (Jung), frozen in 2-methylbutane (−80 °C), snap frozen over liquid nitrogen, and then stored in a −65 °C freezer until sectioning. Sections of 8 μm thickness were obtained in a cryostat (Leica CM 1850), and then stained with Haematoxylin/Eosin.

All muscle cellularity parameters were measured in the white muscle since it comprises the major edible part of the myotome. Morphometric analysis was carried out by means of an image analysis system device (Qwin, Leica) connected to a light photomicroscope (Leitz Dialux 20). The whole white muscle cross-sectional area (mm^2) and diameters of a minimum of 600 white muscle fibres/fish were measured. These values were then used to calculate the white muscle fibre density (muscle fibre number/mm^2) for each fish. The total white muscle fibre number was estimated from values of muscle fibre density and the cross-sectional area of white muscle (Johnston et al., 2000b).

2.4. Textural parameters

Textural parameters were measured in the dorsal muscle of the fish fillet using a Texturometer mod.1011 (Instron) using a 25-kg load cell, a 5-mm spherical probe and test speed of 1 mm/s. Three measurements were made for each sample in dorsal muscle perpendicularly to the muscle fibres orientation. The distance, maximum force and maximum shear force values obtained from the texture profile curve of each sample were used to calculate the independent mechanical parameters (springiness, hardness and cohesiveness), and two dependent parameters (chewiness and gumminess) following the methodology described by Friedman et al. (1962).

2.5. Physico-chemical analysis

The flesh fillet of farmed and wild sea bass (Fig. 1) were homogenised separately in an Omni-mixer to obtain a homogeneous sample for the physico-chemical analysis. Fish homogenate was analyzed for moisture, crude protein, total fat and ash content according to AOAC methods (AOAC, 1999). Flesh pH was determined after mixing 10 g of sample with 50 ml of distilled water, measuring the pH value with a Crisson pH-meter (micro-pH 2000 Crison). Hydroxyproline and collagen content of the flesh fillet were determined after the acid hydrolysis of the sample and measuring spectrophotometrically the compound made with the *p*-dimethylaminobenzaldehide (MSC, 1985).

Fatty acids content was determined after the extraction of flesh lipids in accordance with Folch et al. (1957). Lipids were extracted with chloroform–methanol (2:1, v/v) and were obtained by decantation overnight. Lipids were dissolved in hexane and the fatty esters methylated by 2 N potassium hydroxide in methanol. The separation and quantification of the different fatty acids were made with gas liquid chromatography, using a Hewlett-Packard 5890 chromatograph with the following conditions: capillary column Supelco 10, helium as carrier gas with a flow of 1 ml/min, oven temperature 200 °C, injector temperature 250 °C, detector temperature 260 °C. Components peaks were identify by reference to the retention time of a standard PUFA-1 from marine source (Ref. No. 4-7033, Supelco). The relative proportion of each fatty acid in the sea bass flesh was expressed as a percentage of the total fatty acids. The sums of the saturated fatty acids (SAFA), the monounsaturated (MUFA), the polyunsaturated (PUFA), the total content and the ratio between ω3 and ω6 fatty acids were also calculated.

2.6. Statistical analysis

Most data were analyzed by the Statistical Package SPSS 10.1 version. For each group (wild and farmed sea bass), mean and standard deviation were calculated. Previous to any statistical analysis, all variables were checked for normality and homogeneous variance using the Kolmogorov–Smirnoff and the Levene tests, respectively. A Student's *t*-test was performed to evaluate mean differences (at least for $p<0.05$)

between wild and farmed sea bass. Pearson correlation, lineal regression and a Principal Component Analysis (PCA) were carried out to understand any relation between the analyzed parameters. PCA is a descriptive technique that summarizes pluridimensional information in a few uncorrelated "Factors". It is calculated with a procedure that explains the maximum variation in the data and generates a representation in two dimensional space. The rotated loading component obtained with a "varimax" rotation is an objective tool that can be used in conjunction with other subjective methods (knowledge from our experience) in the interpretation of the new "Factors".

Non-parametric statistical techniques were used to fit smoothed probability density functions (PDF) to the measured diameters of white muscle fibres using a kernel function. The statistical methods used were described by Silverman (1986) and Bowman and Azzalini (1997), and their application to the study of muscle fibre size distribution by Johnston et al. (1999). Authors obtained the software for this study from I.A. Johnston after 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 0.078–0.126 with no systematic differences between populations. Bootstrap techniques were used to distinguish underlying structure in the distributions from random variation (Bowman and Azzalini, 1997; Davidson and Hinkley, 1997; Johnston et al., 1999). The Kolmogorov–Smirnov two-sample test statistic was used to test the null hypothesis that the probability density functions of each experimental group were equal over all diameters ($P_{K-S} \geq 0.05$). 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 points.

3. Results

3.1. Biometric parameters

Table 1 shows biometric parameters in wild and farmed groups. Body size was very similar, the body length ranging between 32.04 and 32.82 cm, and the body weight between 360 and 365.53 g. Other biometric parameters also showed similar values in both populations, with no significant differences between the wild and farmed fish ($p \geq 0.05$).

Table 1
Mean values and standard deviation of biometric and muscle cellularity parameters in wild and farmed sea bass and significance levels between both populations

	Wild sea bass	Farmed sea bass	Significance
Body length (cm)	32.04 ± 0.48	32.82 ± 0.90	0.425
Body weight (g)	365.53 ± 15.00	360 ± 28.28	0.856
Cross-sectional area of the white muscle (mm^2)	887.29 ± 133.30	886.09 ± 135.74	0.983
Muscle fibre density (number of fibres/mm^2)	187.78 ± 7.42	126.31 ± 5.31	<0.001
White muscle fibres diameter (μm)	83.2 ± 1.65	101.11 ± 2.25	<0.001
Dressing index	90.93 ± 0.59	91.91 ± 0.74	0.305
Condition index	1.11 ± 0.03	1.03 ± 0.09	0.345
Perivisceral fat (g)	4.17 ± 0.43	3.92 ± 0.48	0.701
Hepatosomatic index	2.21 ± 0.15	1.68 ± 0.17	0.05
Gonadosomatic index	0.67 ± 0.17	0.39 ± 0.10	0.187
Digestosomatic index	2.16 ± 0.19	2.20 ± 0.18	0.874

Dressing index: (weight/ total weight) × 100; condition index: (total weight/total length^3) × 100; perivisceral fat: (weight perivisceral fat/total weight) × 100; hepatosomatic index: (weight liver/total weight) × 100; gonadosomatic index: (weight gonad/total weight) × 100; digestosomatic index: (digestive weight/total weight) × 100.

3.2. Muscle cellularity

Muscle cellularity was different in wild and farmed specimens (Table 1). Wild sea bass showed a higher muscle fibre density ($p < 0.001$), together with lower value of the average white muscle fibre diameter ($p < 0.001$). Despite of their similar cross-sectional area (Table 1), the estimated number of white muscle fibres was 163,089 (±17,708) and 112,706 (±26,918) for the wild and farmed groups, respec-

tively (18.26% higher in the wild population, $p<0.001$).

The estimated probability density function (PDF) of white muscle fibre diameters for each particular fish and the mean PDF for all fish in the same group are shown in Fig. 2. In wild and farmed groups, the mean PDF was unimodal, resembling a Gaussian-like distribution of muscle fibre diameters. However, there were significant differences between groups concerning the muscle fibre size distributions (Fig. 2c). Fish of the wild group had higher cohorts of small muscle fibres (<10 μm) than farmed fish, what suggests the existence of higher rates of muscle fibre hyperplasia in wild specimens. The average PDF for each group hardly fell within the reference band for the combined groups, which indicates an overall existence of differences between them. This was supported by the non-parametric Kolmogorov–Smirnoff test for differences between groups. Besides, values for the percentiles 5th, 10th, 50th, 90th and 95th were always significantly lower in the wild than the farmed group (Table 2).

Table 2
The 5th, 10th, 50th, 95th, and 99th percentiles of white muscle fibre diameter calculated from the smoothed PDFs for wild and farmed groups

Percentiles (μm)	Wild sea bass	Farmed sea bass	Significance
P5	32.59	41.66	<0.001
P10	38.53	49.33	<0.001
P50	71.84	90.87	0.002
P95	144.79	165.32	0.002
P99	173.27	189.51	0.004

Mean values and significance level are shown.

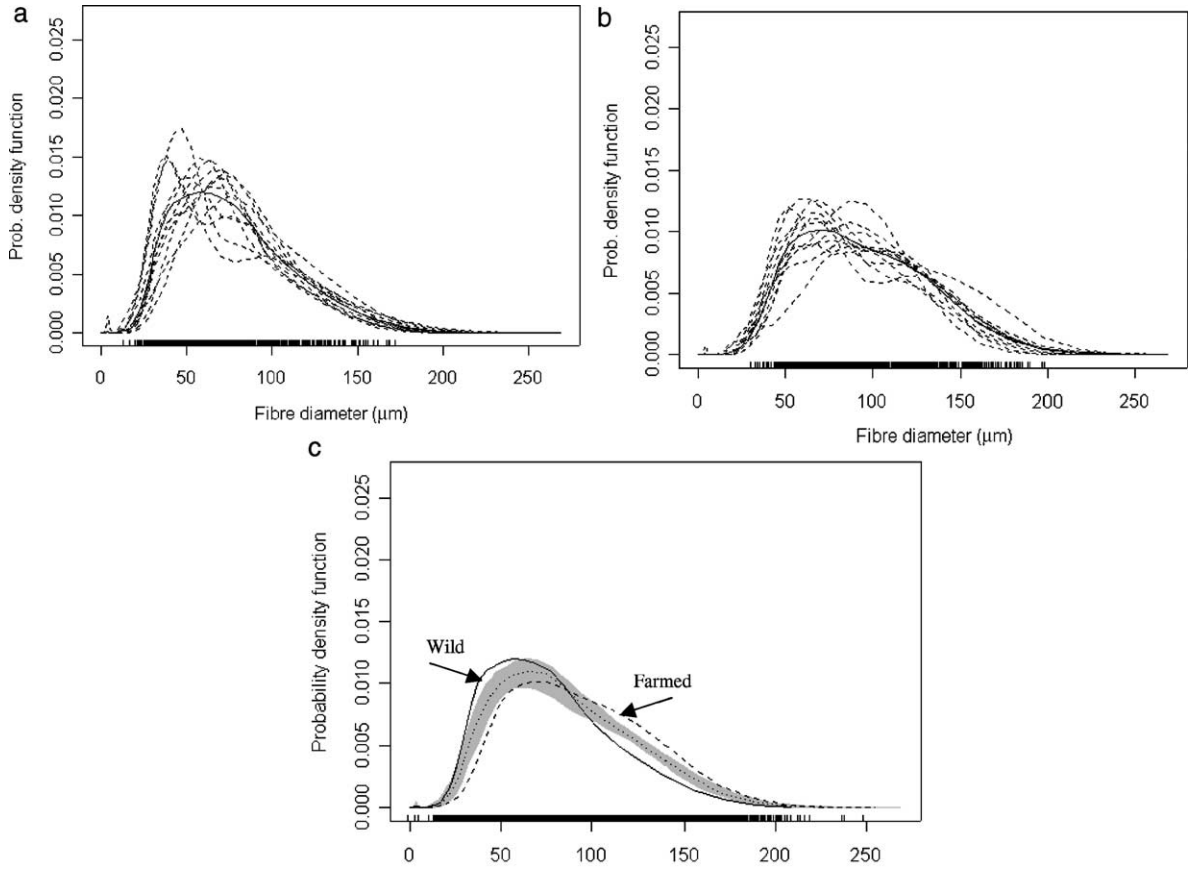

Fig. 2. Probability density functions (PDF) of white muscle fibre diameter (μm) for wild (a) and farmed (b) sea bass. Solid lines represent the average density estimate; dotted lines represent the distribution of muscle fibres of individual fish. (c) Lines represent the average density estimate for each group and shaded area corresponds to the variability band. $P_{K-S}=0.009$.

3.3. Physico-chemical parameters of the flesh

Table 3 shows the physico-chemical and textural parameters of the flesh in wild and farmed sea bass. The farmed group showed a higher content of moisture and protein, and a lower hydroxyproline and collagen contents. Total fat content was higher in wild than in farmed fish, with no significant differences between them. The flesh pH values were 6.44 in farmed and 6.75 in wild specimens, showing a statistical significance of $p<0.001$. All textural parameters were significantly higher in wild than in farmed sea bass.

The fatty acid composition of both sea bass groups is shown in Table 4 and Fig. 3. The origin—wild or farmed—of the fish determined the content of the most fatty acids, with the exception of estearic ($C_{18:0}$), oleic ($C_{18:1\omega9}$), euricic ($C_{22:1\omega9}$), docosapentanoic ($C_{22:5\omega3}$) and nervonic ($C_{24:1\omega9}$) acids (Fig. 3). The most abundant fatty acids were oleic ($C_{18:1\omega9}$), followed by palmitic ($C_{16:0}$), linoleic ($C_{18:2\omega6}$) and docosahexanoic ($C_{22:6\omega3}$) acids. The saturated fatty acids (SAFA) and monounsaturated fatty acids (MUFA) were significantly higher in farmed sea bass (Table 4), whereas wild sea bass showed a higher content of polyunsaturated fatty acids (PUFA). Wild specimens showed a higher content of fatty acids, but the total ω−3 fatty acids did not show significant differences between groups. Besides, wild sea bass showed a great content of linoleic ($C_{18:2\omega6}$) and docosahexanoic ($C_{22:6\omega3}$) acids (Fig. 3), which are considered as essential fatty acids for its beneficial effects for human health.

Table 3
Mean values and standard deviation of physico-chemical and textural parameters in flesh of wild and farmed sea bass flesh, and significance levels between both populations

Parameters	Wild sea bass	Farmed sea bass	Significance
Physico-chemical			
Moisture (%)	69.46 ± 1.54	72.63 ± 0.71	0.008
Protein (%)	17.64 ± 0.43	23.37 ± 1.67	0.006
Total fat (%)	9.19 ± 4.94	6.66 ± 1.57	0.119
pH	6.75 ± 0.06	6.44 ± 0.02	<0.001
Hydroxyproline (mg/100 g)	43.46 ± 6.69	32.77 ± 8.12	<0.001
Collagen (%)	0.34 ± 0.055	0.26 ± 0.089	0.004
Collagen/Total protein (%)	1.93 ± 0.31	1.11 ± 0.36	<0.001
Textural parameters			
Springiness (cm)	2.81 ± 0.02	2.07 ± 0.10	<0.001
Hardness (N)	50.57 ± 0.90	48.47 ± 0.60	<0.001
Cohesiveness (ratio)	0.52 ± 0.02	0.34 ± 0.07	<0.001
Chewiness (N cm)	74.43 ± 5.31	36.03 ± 9.98	<0.001
Gumminess (N)	26.44 ± 1.36	16.88 ± 3.57	<0.001

Table 4
Mean values and standard deviation of saturated, monounsaturated, polyunsaturated, total ω_6 and total ω_3 fatty acids of wild and farmed sea bass flesh, and significance levels between both populations

Parameters	Wild sea bass	Farmed sea bass	Significance
SAFA (mg/100 g fat)	25.66 ± 0.26	27.46 ± 0.25	<0.001
MUFA (mg/100 g fat)	37.61 ± 1.00	41.63 ± 0.29	0.002
PUFA (mg/100 g fat)	36.76 ± 0.91	30.90 ± 0.34	<0.001
Total ω_6 (mg/100 g fat)	14.21 ± 0.79	8.29 ± 0.11	<0.001
Total ω_3 (mg/100 g fat)	28.34 ± 0.66	28.99 ± 0.24	0.414
Ratio ω_3/ω_6	2.11 ± 0.17	3.49 ± 0.04	<0.001
PUFA/SAFA	1.43 ± 0.13	1.12 ± 0.07	<0.001

3.4. Relationship between muscle cellularity and physico-chemical and textural parameters

Table 5 and Figs. 4 and 5 show the relationship among textural parameters, muscle cellularity and physico-chemical parameters. Muscle fibre density showed a positive and significant correlation with collagen and hydroxyproline contents, and with textural parameters such as hardness, springiness, cohesiveness, gumminess and chewiness, whereas muscle fibre diameter showed a negative correlation (Table 5). Fig. 4 shows the relationship (linear regression) between muscle fibre density and springiness and chewiness. Textural parameters showed a significant linear dependence with the muscle fibre density. The values for each experimental group tended to be grouped separately due to their different muscle cellularity. However, within each group no or very low correlation between muscle fibre density and textural parameters was observed. The PCA plot (Fig. 5) allows a better view of the relationship among muscle cellularity, physico-chemical and textural parameters in both groups of sea bass. PCA was carried out with the more correlated variables in both populations. The total explained variance was of 74.5% (64.1% to Factor

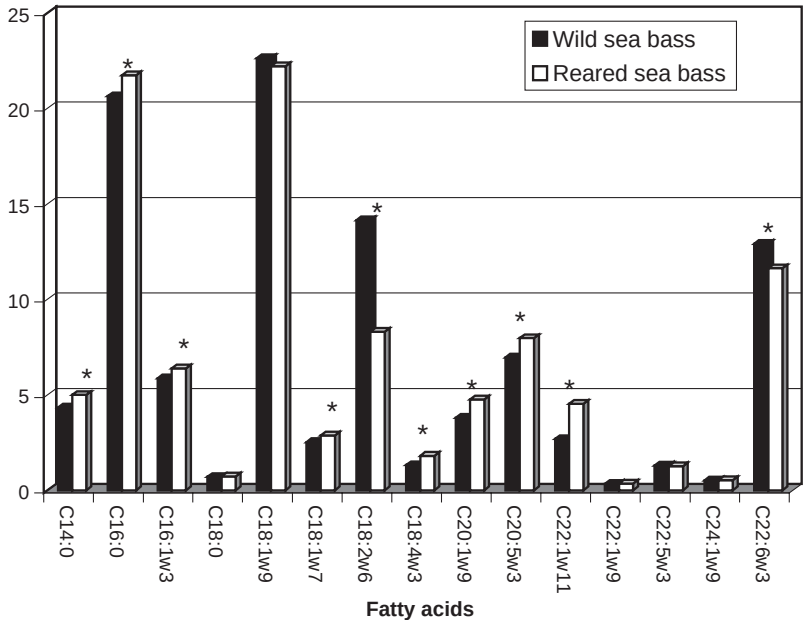

Fig. 3. Fatty acids content in wild and farmed sea bass, expressed as mg/100 g of fat. * indicates $p<0.05$.

1 and 10.4% to Factor 2). As have been described above, the mechanical texture of the flesh was correlated with the fibre number and density (Table 5; Fig. 5), and also with the collagen and the flesh pH (Fig. 5). The total protein and total fat were not correlated with other studied parameters, and the levels of different fatty acids were negatively

Table 5
Correlation coefficients and significance levels among muscle cellularity, physico-chemical and textural parameters of the sea bass

	Muscle fibre density (number/mm^2)		Muscle fibre diameter (μm)	
	Correlation	Significance	Correlation	Significance
Hardness	0.611	0.005	−0.591	0.008
Springiness	0.653	0.002	−0.647	0.003
Cohesiveness	0.656	0.002	−0.645	0.003
Chewiness	0.677	0.001	−0.671	0.002
Gumminess	0.667	0.002	−0.655	0.002
pH	0.685	0.000	−0.660	<0.001
Protein	−0.269	0.193	0.258	0.212
Fat	0.271	0.191	−0.252	0.225
Hydroxyproline	0.473	0.026	−0.449	0.036
Collagen	0.472	0.027	−0.449	0.036
SAFA	−0.635	0.001	0.681	<0.001
MUFA	−0.584	0.002	0.558	0.004
PUFA	0.738	0.000	−0.727	<0.001
ω_3/ω_6	−0.638	0.001	0.661	<0.001

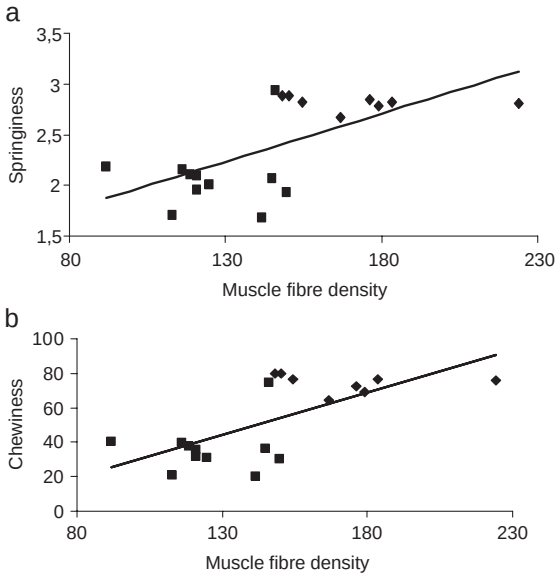

Fig. 4. Relationship between muscle fibre density (fibre number/mm^2) and textural parameters: springiness (a) and chewiness (b), of the sea bass. Rhombus and squares represent wild and farmed specimens, respectively. The data were fitted using a first-order linear regression. Springiness = 0.983 (± 0.398) + 0.0095 (± 0.003) * fibre density; R^2 adjusted = 0.427; $P = 0.002$. Chewiness = −1929.7 (± 1923.8) + 49.013 (± 12.9) * fibre density, R^2 adjusted = 0.46; $P = 0.001$.

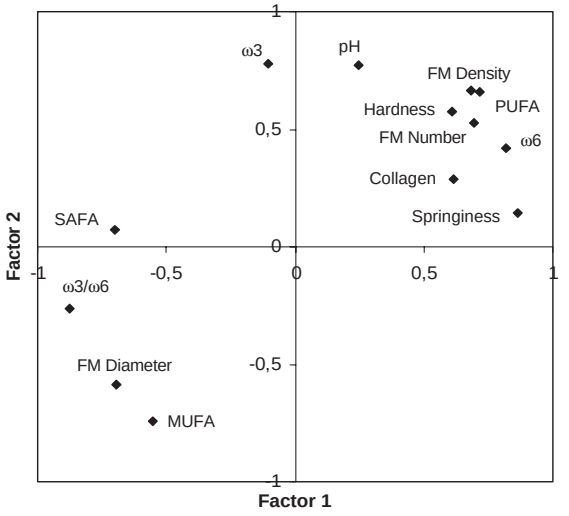

Fig. 5. Principal Component Analysis (PCA) for some structural, physico-chemical and textural parameters of wild and farmed sea bass.

correlated with textural parameters, with the exception of PUFA (Fig. 5).

4. Discussion

4.1. Muscle cellularity

Fish muscle cellularity at any given stage of growth is a consequence of the previous balance between muscle fibre hypertrophy and hyperplasia. These are plastic processes, whose balance in growing fish depends on both intrinsic (genotype) (Johnston and McLay, 1997; Johnston et al., 2000b), and extrinsic factors such as temperature (Nathanailides et al., 1995; Ayala et al., 2001; Johnston et al., 2000b, 2003a), photoperiod (Johnston et al., 2003b), diet (Kiessling et al., 1991) and ecological context (Johnston et al., 2000c,d). In the present work, white muscle cellularity was significantly different in wild and farmed sea bass of similar size (350 g and 32 cm) (Table 2; Figs. 1 and 2). The white muscle fibre density was significantly lower in farmed than in wild specimens (126.31 and 186.78, respectively), which reflects the existence of different patterns of muscle growth between them to reach the same body size. Previous studies have reported substantial differences in the relative contribution of hypertrophy and hyperplasia of muscle fibres between rapid and slow growing strains of the same species (Weatherley et al., 1979; Higgins and Thorpe, 1990; Alami-Durante et al., 1997; Valente et al., 1999; Johnston et al., 2000b) such that rapid somatic growth is commonly associated to a higher rate hyperplasia. In farmed specimens, the lower white muscle fibre density reflects a low rate of white muscle fibre hyperplasia, which could be determined by a relatively low velocity of body growth. Age of farmed specimens was 28 months, which indicates that their overall growth rate was quite slow compared with other stocks of sea bass farmed in Mediterranean regions, which commonly attain commercial size at 18–20 months. However, this period is very variable in farmed fish depending upon the specific cultivation conditions (diet, temperature, photoperiod, etc.) as well as of intrinsic factors (Gjerdrem, 1997; Kavadias et al., 2003). In this sense, Kavadias et al. (2003) reported that sea bass farmed in cages in the Ionian Sea (Greece) did not reach 400 g until 24 months. Concerning the wild sea bass, there is a lack of information about their genetic lineage, age as well as the environmental and nutritional parameters affecting these fish during ontogeny. This makes quite difficult to find a unique or direct explanation for their results of muscle cellularity. Wild sea bass showed a higher white muscle fibre density than farmed specimens, which should be associated to a higher rate of white muscle fibre hyperplasia to reach the defined sampling size (350 g). This may be determined by different endogenous and external factors affecting muscle growth dynamics such as genotype (probably wild sea bass were of Mediterranean lineage instead of Atlantic), age and other important factors related to lifestyle such as exercise, feeding habits, diet, etc. The present work is the former carried out to compare white muscle cellularity between wild and farmed sea bass. Our results demand further investigation aimed to monitor farmed and wild sea bass growth until commercial size. This topic is very difficult to undertake in wild specimens but is of great interest in order to make accurate comparisons between wild and farmed sea bass populations.

4.2. Physico-chemical parameters

There are scarce studies in the scientific literature comparing the physico-chemical composition between wild and farmed sea-bass. Alasalvar et al. (2002) and Orban et al. (2002) have studied the differences in the proximate composition of wild and farmed sea bass from Greece and Italy, respectively. They described that farmed fish show higher fat and lower moisture than wild specimens, whereas protein remain unchanged, due to high dietary fat level in the feed and a reduced activity. In our study the flesh of wild and farmed sea bass showed proximate composition values within the data reported by the scientific literature (Amerio et al., 1996; Alasalvar et al., 2002; Orban et al., 2002). However we observed a different behaviour, since significant differences were found in protein and moisture contents but not in fat content of both populations (Table 3). Fat content is related with moisture, since fat and water account for approximately the 80% of the total composition of the flesh (Huss, 1999) (79% in our results). Although sea bass is considered a non-fatty fish, the fat content in our specimens (6.66% to 9.19%) can be considered very high compared with the expected values reported in the literature (less than 5%), in particular for wild sea bass. These results may be explained at least in part because the total fat was determined using a homogenised portion of the flesh including the dorsal (epaxial) and ventral (hypaxial) areas, and not only the commonly used dorsal area (Suzuki et al., 1986). From a nutritional point of view, we have considered that the lipid analysis in a homogenised portion of the whole flesh, as an edible portion of fish, gives better information about the total fat. In this sense, Gallagher (1989) have also reported a high total fat content in wild striped bass (*Morone saxatilis*), when the dorsal and ventral areas of the flesh are used for analysis. In addition to these reasons, it should be considered that the proximate composition depends on genetic factors, feeding, fish size and environmental conditions (Huss, 1999), so during the production of farmed fish, these factors can be controlled, but not in wild fish where a higher variability is commonly found.

pH was significantly higher in wild sea bass, as previously described by Orban et al. (2002). Similarly, Ofstad et al. (1996) observed that fed cod show a lower flesh pH than wild cod. In addition, farmed specimens had a higher degree of muscle post-mortem degradation. pH is related to the post-mortem evolution of the flesh and is influenced by the species, feeding, station of the year, etc. (Linden and Lorent, 1996). In order to guarantee that all specimens were in the same phase of the post-mortem degradation, all specimens of the present study were slaughtered following the same procedure. Thus, our result concerning the pH differences between groups may be related to the nutritional status of specimens at capture. Farmed sea bass were fed until the day before sampling, whereas the nutrient intakes of wild sea bass depend on the availability of food in the marine environmental and on the frightened during the capture, which could have determined a lower glycogen reserve in the muscle of the wild specimens.

Collagen and hydroxyproline contents in fish flesh range from 0.28% to 0.79% and 30 to 98 mg/100 g, respectively, depending on the fish species (Morrisey and Fox, 1981). Both populations of this study are within this range, but the wild specimens show a higher collagen content. This fact may be related with their higher muscle fibre number which determines a greater content of collagen (Sikorski et al., 1984), as well as with other factors such as the swimming behaviour (Sato et al., 1986).

The lipid composition of the sea bass flesh is quite variable since it depends on the dietary lipid sources, the period of the year, water temperature, salinity, photoperiod, spawning etc. (Codier et al., 2002). The pattern of fatty acids in both groups showed significant differences (Table 4; Fig. 3) for the main groups of fatty acids. SAFA content of sea bass was under 30% in both populations, as has been reported previously by Ackman (1989). MUFA content was very similar to PUFA in the wild specimens, however in farmed sea bass, the main fatty acids were MUFA followed by PUFA. Consequently, the PUFA/SAFA ratio was higher in wild than in farmed sea bass, what coincides with results of other authors (Alasalvar et al., 2002; Orban et al., 2002, 2003). The fatty acids profiles were very similar to the common values reported for sea bass (Amerio et al., 1996; Pirini et al., 2000; Alasalvar et al., 2002; Orban et al., 2002, 2003; Saglik et al., 2003), being the palmitic acid ($C_{16:0}$) the primary SAFA, the oleic acid ($C_{18:1\omega9}$) the main MUFA, and linoleic ($C_{18:2\omega6}$), eicosapentanoic ($C_{20:5\omega3}$) and docosahexanoic ($C_{22:6\omega3}$) acids the most

abundant PUFA. Concerning the total ω−6 fatty acids, wild sea bass had higher levels, what is mainly due to their higher content of linoleic acid ($C_{18:2\omega6}$). This fatty acid is present in vegetable oils which are used in the formulation of artificial diets, hence farmed sea bass usually have higher levels of this fatty acid than wild sea bass (Alasalvar et al., 2002; Orban et al., 2002). Our results could be related to the low content of linoleic acid in the feed of the farmed group (6% in the total content of fat), whereas wild specimens received natural diet and so the proportion of the different nutrients is unknown. In addition, this fatty acid is accumulated largely unchanged in the lipids of marine fish due to their reduced capacity for chain elongation and desaturation (Yamada et al., 1980). Both populations were a good source of ω−3 fatty acids, particularly the eicosapentanoic acid ($C_{20:5\omega3}$) and docosahexanoic acid ($C_{22:6\omega3}$), without significant differences between both groups. However, farmed specimens showed a higher ω−3/ω−6 ratio as a consequence of their lower content of ω−6. Although this result is in disagreement with those reported previously for sea bass (Krajnovic-Ozretic et al., 1994; Alasalvar et al., 2002) and other fish species (Suzuki et al., 1986), different authors have described the same behaviour in this ratio, suggesting that the artificial diet with an optimal formulation of marine oils allows to guarantee a specific pattern of fatty acids, particularly the ω−3 PUFA level in the flesh (Pirini et al., 2000; Orban et al., 2002, 2003).

4.3. Relationship among muscle cellularity, physico-chemical and textural parameters

Fish muscle cellularity has important implications for the end-product quality because its influence on textural characteristics (Johnston et al., 2000a). In the present study muscle fibre density showed a positive correlation with textural parameters (hardness, springiness, chewiness and gumminess) (Table 5; Fig. 4). Thus, wild specimens, which showed higher muscle density than farmed specimens, presented higher values of textural parameters ($p<0.001$). However, the variability in muscle fibre density within each population did not determine any correlation with the textural parameters (Fig. 4). This was probably due to the fact that not only muscle cellularity but also other chemical parameters, such as collagen and pH, are involved in flesh texture properties. Johnston et al. (2000a) also compared two populations of Atlantic salmon (*S. salar* L.) and found a significant positive correlation between muscle fibre density and all four measures of texture of smoked fillet: chewiness, firmness, mouth-feel and dryness. Hatae et al. (1984, 1990) and Hurling et al. (1996) described that species with thinner fibres showed higher firmness after cooking. In the present study, textural and structural parameters were only measured in raw flesh, thus further studies aimed to elucidate the final characteristics of the cooked flesh of sea bass are required.

Texture is also determined by physico-chemical parameters such as the collagen content and pH. The distribution of connective tissues between the myomeres and around fibres is very specific to each species (Ando et al., 1992). In the present study, collagen and hydroxyproline contents showed a positive correlation with muscle fibre density, which coincides with the one suggested by other authors (Dunajski, 1979; Johnston et al., 2000a). Thus, a muscle with a high fibre density presents higher surface-to-volume ratio of the muscle fibres and so the connective tissue surrounding each fibre would be relatively more abundant than in a muscle with low fibre density. This could explain the higher connective tissue content in the muscle with higher fibre density and hence their contribution to a firmer texture. As expected from this relationship, wild specimens of sea bass of this study showed higher collagen and hydroxyproline content because of their higher fibre density.

Flesh pH was also correlated with texture (Table 5; Fig. 5). The post-mortem decrease of the pH leads to a denaturalization of the sarcoplasmic proteins which reduces the water holding capacity. Besides, the post-mortem pH may determine the rate and extent of myofibrillar protein degradation by the calpain system (Linden and Lorent, 1996). In fact, Delbarre-Labrat et al. (2004) indicated that most post-mortem degradation in sea bass flesh occurred during the first hours of post-mortem storage in relation to the calpain activity. All these biochemical changes affect the tenderness of the flesh, as has been largely demonstrated in mammals meat (Linden and Lorent, 1996), as well as in fish (Davie and Sparksman, 1986; Delbarre-Labrat et al., 2004).

The relationship between some of the analyzed parameters is summarized in Fig. 5. In our opinion,

Factor 1 could be considered as a descriptor of parameters associated to the lifestyle of sea bass. Thus, from the left to the right, the number and density of muscle fibres, the collagen content, PUFA, ω−6 fatty acids, springiness and hardness increased, whereas SAFA and MUFA contents, ω−3/ω−6 and diameter of muscle fibre decreased. Wild sea bass showed higher values with scores upper zero for Factor 1 and Factor 2, whereas farmed sea bass showed higher values for the parameters opposite plotted. On the basis of these results, it can be suggested that some structural and flesh quality parameters of the sea bass may be associated to the genetic origin and lifestyle factors, such as swimming behaviour, feeding, etc.

5. Conclusions

Muscle cellularity, collagen content and pH were significantly different in wild and farmed specimens of sea bass. The resulting variation in these parameters determined significant differences on textural parameters, with higher values for the wild specimens. Thus, muscle cellularity parameters influenced texture but not the nutritional composition of the raw flesh of sea bass. Since cooking procedures can affect structural parameters of the flesh and its final acceptance by consumers, further studies should be done to find out the relationship between muscle tissue parameters and organoleptical properties after cooking.

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