

Temperature influence on the white muscle growth dynamics of the sea bass *Dicentrarchus labrax*, L. Flesh quality implications at commercial size

Octavio López-Albors^{a,*}, Isaac Abdel^b, M^a. Jesús Periago^c, M^a. Dolores Ayala^a, Alicia García Alcázar^b, Carmen Martínez Graciá^c, Cosmas Nathanailides^d, José Ma. Vázquez^a

^a Department Anatomía y Anatomía Patológica Comparadas, University of Murcia, Veterinary Faculty, 30100 Murcia, Spain

^b Centro Oceanográfico de Murcia, Instituto Español de Oceanografía, Ctra. de La Azohía s/n, Puerto de Mazarrón, 30860, Spain

^c Department Tecnología de los Alimentos, Nutrición y Bromatología, University of Murcia, Veterinary Faculty, 30100 Murcia, Spain

^d Department of Aquaculture and Fisheries, Faculty of Agricultural Sciences, Epirus Institute of Technological Educational, Igoumenitsa 46100, Greece

Received 12 July 2007; received in revised form 11 February 2008; accepted 13 February 2008

Abstract

Rearing temperature was used to monitor muscle growth patterns of sea bass *Dicentrarchus labrax*, L. from hatching to the commercial size (350 g, 30 cm). At this moment, the white muscle cellularity, proximate composition and organoleptic parameters of the fillet were analysed to evaluate the influence of temperature on the structure and quality of the commercial product. One ambient (A/A) and three warm regimes of temperature were compared. In warmed tanks temperature was raised to 19 °C: during the larval period (W/A), only during the cool seasons (winter and early spring) (A/W), or both during the larval period and cool seasons (W/W). High temperature during the larval period was positive for an advanced yield of fingerlings and early juveniles. Winter heating of juveniles also advanced the time to reach 200 g by 25 days. However, independently of the thermal regime all fish attained the commercial size at approximately 600 days. Temperature modulated the rates of hypertrophy and hyperplasia of white muscle fibres throughout the larval, fingerling and juvenile periods, such that at commercial size the number and size distributions of muscle fibres varied depending on the previous thermal story. Thus, the density of muscle fibres (number/mm²) was maximum in tank W/A (212.19±8.77) and minimum in W/W (151.95±8.7) ($p<0.001$). The proximate composition varied for moisture and protein content, but independently of temperature, all the sensorial attributes of cooked flesh (firmness, juiciness, fattiness, chewiness, flavour and odour) were similar. Thus, no correlation between muscle fibre size and flesh texture was found. Compensatory growth of fish reared at ambient temperature and the uniformity of the proximate composition and sensorial properties evaluated by panellists minimized the impact of temperature on both the time at harvest and the quality of the commercial product.

© 2008 Elsevier B.V. All rights reserved.

Keywords: Fish; Muscle growth; Temperature; Sea bass; Flesh quality

1. Introduction

The axial musculature is the edible part of most commercial fish accounting for approximately 60–70% of fish weight. It is arranged in a series of folded myotomes each one including a thin superficial layer of red muscle, enlarged to some extent at the level of the lateral line, and a deep stratum of white muscle which

comprises up to 80% of the trunk musculature thickness. Due to great variability in the size of muscle fibres, at microscopical level, a cross-section of the white muscle usually displays a typical image of mosaic. White muscle fibre size variability is the consequence of a double mechanism of muscle growth: hypertrophy and hyperplasia of muscle fibres. Hypertrophy is an increase in the size of the muscle fibres, whereas muscle fibre hyperplasia depends on the recruitment of new muscle fibres. The cellular basis of both processes is the existence of a population of myogenic progenitor cells, which after activation and several mitoses (proliferation), either add to the existing muscle fibres to

* Corresponding author. Tel.: +34 968 364694; fax: +34 968 364147.

E-mail address: albors@um.es (O. López-Albors).

keep their volumetric ratio of cytoplasm to nuclei within the physiologic limits (hypertrophy), or fuse to other myogenic cells to differentiate progressively into new muscle fibres (hyperplasia) (Johnston, 1999). In most teleost of a large final size, muscle fibre hyperplasia persists throughout the juvenile and even adult stages (Rowlerson and Veggetti, 2001).

Depending on endogenous as well as environmental factors, temperature may significantly influence the muscle growth patterns of fish by a modulation of the rates of hypertrophy and hyperplasia of muscle fibres (Johnston et al., 2003a). Consequently, the muscle cellularity, defined by the size distribution and total number of muscle fibres in the myotomes, may significantly vary throughout the lifecycle of fish. The relationship between temperature and muscle growth patterns has been investigated in embryos, larvae and postlarvae of several marine and fresh water species of farming interest such as Atlantic salmon, *Salmo salar*, L. (Stickland et al., 1988; Nathanailides et al., 1995, Johnston et al., 2003a), common trout *Salmo trutta* (Killeen et al., 1999), Atlantic cod *Gadus morhua* (Galloway et al., 1998), turbot *Scophthalmus maximus* (Gibson and Johnston, 1995), pacu *Piaractus mesopotamicus* (de Asis et al., 2004), etc. The influence of temperature on the muscle growth dynamics of sea bass has been studied during larval stages (Ayala et al., 2000, 2001, 2003; Wilkes et al., 2001; Alami-Durante et al., 2006), early postlarval (Nathanailides et al., 1996; López-Albors et al., 2003), and

advanced juvenile periods (≈ 210 mm, Alami-Durante et al., 2007). In this work, the whole productive lifecycle of sea bass is studied for the first time: from hatching to the usual market size (approximately 30 cm, 350–400 g). Investigations focused on the final growth period of fattening or on-growing are relevant for the fish farm industry. We were interested in monitoring the influence of the rearing temperature on the muscle growth dynamics and final cellularity of the fillet. The hypothesis was that sea bass white muscle cellularity at harvest can vary depending on the thermal regime of rearing.

Four regimes of temperature were used: ambient/ambient (A/A), warm/warm (W/W), warm/ambient (W/A) and ambient/warm (A/W) (Fig. 1). Warm water ($W=19\text{--}20^\circ\text{C}$) was used during the larval period and/or in the following winter and early spring seasons. Justification of thermal regimes is based on the following facts. Water heating during the larval period (20°C), has greatly improved sea bass husbandry in the last two decades (Moretti et al., 1999). Also, early temperature may affect muscle phenotype by immediate and even persistent effects on muscle cellularity of postlarvae (Johnston and Hall, 2004). On the other hand, probably due to seasonal influences and particular physiological conditions, somatic growth of juvenile sea bass is generally reduced in winter (Akbulut and Sahin, 1999; Abdel et al., 2004). Water heating during cold months could therefore be used to overcome somatic growth constraints by seasonal influence.

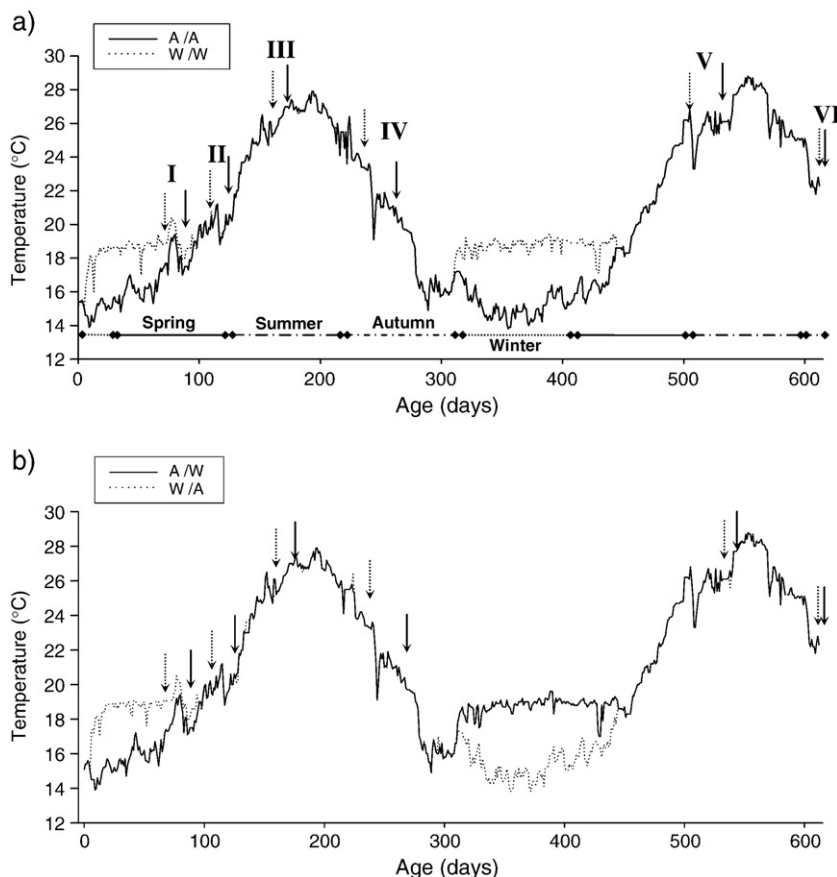


Fig. 1. Regimes of temperature and sampling. a) A/A and W/W tanks; b) A/W and W/A tanks. Solid and dotted arrows indicate samples for A/A and A/W tanks, and W/W and W/A tanks, respectively. Sampling points correspond to: end of the larval period (I), 4 g (II), 10 g (III), 50 g (IV), 200 g (V) and 350 g (VI, commercial size).

Sea bass, together with Gilthead sea bream *Sparus aurata*, L., is one of the main reared species in Mediterranean countries. In Spain, sea bass production reached a peak of 9438.78 tons in 2006 (www.mapa.es), and figures may increase during the following years due to reasonable price in markets and highly appreciated quality. Despite its economic relevance there is still limited scientific information about the influence of rearing conditions on the quality of the final product. Fish quality has been traditionally associated with freshness. However, nowadays fish quality is a wider concept involving attributes associated to chemical and nutritional composition, as well as to various organoleptic parameters such as flavour, odour, and textural properties. Flesh quality of cultivated fish can be influenced by physiological (biological age, growth rate, sexual maturation), environmental (water temperature, salinity, pressure, water flow) and dietary (feeding cycle, starvation, overfeeding, diet composition) factors (Haard, 1992). Changes in any of these factors can potentially modify the chemical composition or the nutritional and sensorial qualities of the flesh. Among the sensorial characteristics, texture is one of the most important parameters for fish producers, processors and consumers (Hyldig and Nielsen, 2001). Texture is influenced by several factors such as species, age, size, growth rate, nutritional stage and postmortem biochemical changes (Love, 1983; Barroso et al., 1998). In different teleost species, sea bass included, a correlation between texture and muscle fibre size has been demonstrated (Hatae et al., 1990; Hurling et al., 1996; Johnston et al., 2000a; Periago et al., 2005). Large muscle fibre size, or correspondingly low muscle fibre density, correlated with soft texture of the flesh. Recently, we found that sea bass texture of raw flesh was correlated with muscle fibre size, pH and collagen content (Periago et al., 2005). However, the relationship between muscle cellularity and quality parameters of cooked flesh remains to be investigated in sea bass. Considering these precedents and the former hypothesis written above, a supplementary hypothesis for the present study was that muscle cellularity differences induced

by rearing temperature may modify the sensorial properties of the cooked flesh.

2. Materials and methods

2.1. Fish rearing

Fish were cultivated in the Centro Oceanográfico de Murcia (Instituto Español de Oceanografía). On the 6th of February 2002, a selected spawn of 600,000 eggs was equally distributed in four cylindrical tanks (1 m³), each one aimed to experience a different thermal regime (Fig. 1): in tank A/A, water was kept at ambient temperature throughout the whole experiment (range of temperature 14 to 28 °C); in tank W/W, after the absorption of the yolk sac of larvae (mouth opening) temperature was raised to 19–20 °C, and kept constant until the beginning of May, when the ambient temperature reached 19 °C. In this tank temperature was increased again during the following winter and early spring seasons (from December the 18th of 2002 to April the 30th of 2003). In tank W/A, water was only heated during the larval phase; and finally, in tank A/W, water heating corresponded to winter and early spring seasons. Hence, from the beginning of the experiment to December the 18th (310 days), tanks A/A and A/W, as well as tanks W/W and W/A experienced the same regime of temperatures (duplicate tanks). The analysis of variance (Anova) and post-hoc Tukey test for the length and weight of fish throughout this period demonstrated the inexistence of differences between duplicates (no tank effect): $p_{A/A \text{ versus } A/W} = 0.415$ and $p_{W/W \text{ versus } W/A} = 0.081$ for length, and $p_{A/A \text{ versus } A/W} = 0.053$ and $p_{W/W \text{ versus } W/A} = 0.364$ for weight.

Initial larval rearing was done in darkness and without feeding until 160 °C-day. At that moment, a photoperiod of 16:8 h (light:dark) with 500 lux intensity was set up and larvae fed with nauplii of *Artemia salina*. Oxygen level was monitored with an oximeter (Oxiguard Mk III) and maintained over 6 ppm. Salinity was approximately 36‰. Weaning began at 35–40 days and larval feeding consisted of extruded commercial feed (Trouw, S.A). Postlarvae were transferred to rectangular tanks (2.5 m³) up to a weight of 15 g and subsequently to tanks of 7.5 m³ until commercial size (≈ 350 g). To monitor fish growth, the weight and length of 50 fish from each tank were recorded monthly. Fingerlings and juveniles were fed ad libitum with a commercial diet (Trouw, S.A) which contained: 45% protein, 11% ash and 22% fat.

The sampling strategy was defined for equivalent stages of development in the larval period — hatching and end of the larval metamorphosis (scaling) — and for defined weights in fingerlings (4 and 10 g) and juveniles (50 and 200 g), until a final stage of commercial size (350 g) (Table 1, Fig. 1). Fish of commercial size were sampled in two consecutive weeks to allow a simultaneous and optimum

Table 1
Fish sampling and corresponding average values for age, degree days, weight and length

| Sampling stage | Temperature regime | Age (days post fertilization) | Degree days (°C) | Weight (g) | Length (cm) |
|--------------------------------|--------------------|-------------------------------|------------------|--------------------------|-------------------------|
| Hatching | A/A and A/W | 3.75 | 59 | – | 0.319 |
| | W/A and W/W | 3.75 | 59 | – | 0.319 |
| End of metamorphosis (scaling) | A/A and A/W | 93 | 1676 | – | 1.33±0.35 ^a |
| | W/A and W/W | 82 | 1390 | – | 5.19±1.17 ^b |
| 4 g | A/A and A/W | 132 | 2567 | 4.24±0.15 ^a | 7.31±0.12 ^a |
| | W/A and W/W | 112 | 1953 | 3.35±0.14 ^b | 7.76±0.1 ^b |
| 10 g | A/A and A/W | 180 | 3790 | 10.86±0.13 ^a | 13.23±0.61 ^a |
| | W/A and W/W | 168 | 3262 | 11.05±0.13 ^a | 13.88±0.35 ^a |
| 50 g | A/A and A/W | 267 | 5883 | 50.15±0.65 ^a | 17.36±0.17 ^a |
| | W/A and W/W | 237 | 5058 | 52.32±1.03 ^a | 17.04±0.2 ^a |
| 200 g | A/A | 545 | 10,888 | 202.63±2.39 ^a | 25.92±0.24 ^a |
| | A/W | 520 | 10,630 | 201.54±2.18 ^a | 26.35±0.14 ^a |
| | W/A | 513 | 10,652 | 206.83±1.97 ^a | 26.08±0.21 ^a |
| | W/W | 507 | 10,532 | 192.35±1.25 ^b | 26.06±0.11 ^a |
| 350 g | A/A | 605 and 612 | 12,631 | 353.12±4.62 ^a | 31.12±0.31 ^a |
| | A/W | 605 and 612 | 13,060 | 348.98±4.5 ^a | 31.26±0.38 ^a |
| | W/A | 605 and 612 | 12,549 | 343.84±6.08 ^a | 31.03±0.3 ^a |
| | W/W | 605 and 612 | 13,262 | 345.49±4.9 ^a | 30.96±0.29 ^a |

Column values with different superscripts within each sampling stage are significantly different ($p < 0.05$).

processing of samples for muscle morphometry and flesh quality analysis (instrumental and sensorial). Sampling of larvae consisted of 50 specimens per tank, which were delivered alive in aerated containers to the Veterinary Faculty of Murcia and then overanaesthetized with clove oil (Guinama^R). Fingerlings and juveniles (up to 200 g) were anaesthetized with clove oil before sampling, and the selected specimens rapidly immersed in ice cold water (hypothermia). At commercial size fish were stunned with a blow to the head previous to immersion in ice cold water. After fish death, specimens of 350 g were washed with tap water, then the surface wiped with tissue and subsequently weighed, measured and eviscerated to weight the digestive tract, liver, visceral fat, gonads and carcass. Sex determination was done macroscopically. Within 3 h of harvest, all fingerlings and juveniles, each one inside a plastic bag, were submitted in a box with ice pellets to the Veterinary Faculty of Murcia. The number of fish per tank and sampling point was 10–12.

2.2. Sample processing for muscle morphometry

At hatching and scaling (end of metamorphosis), 20 larvae per tank were fixed in 2.5% glutaraldehyde in buffered 0.1 M cacodylate (pH 7.2–7.4) for 2 h at 4 °C and then embedded in Epoxy resin according to the routine protocol of the Microscopy University Service (University of Murcia). Semithin cross-

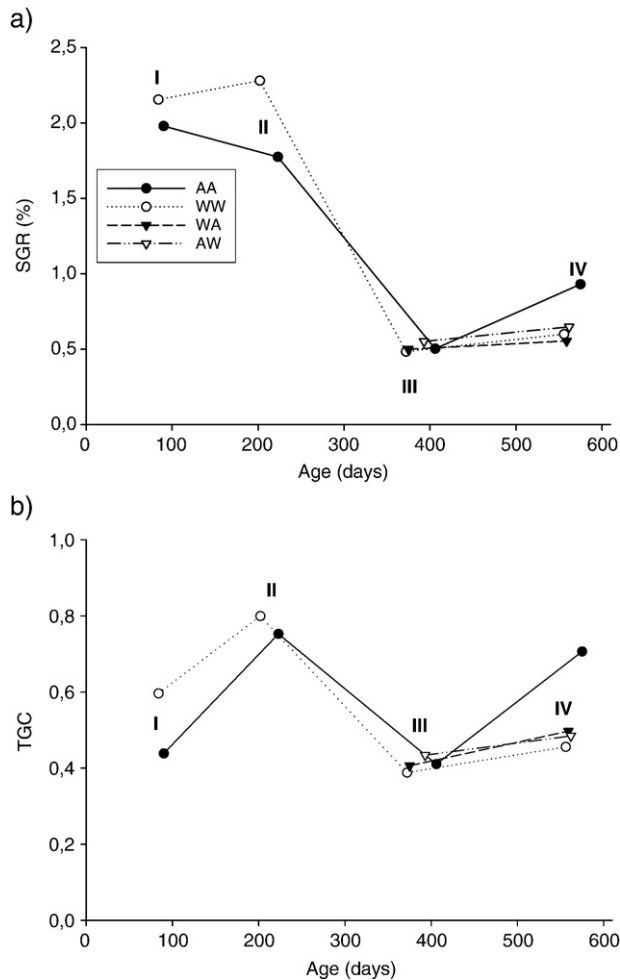


Fig. 2. Specific growth rate (SGR) a), and thermal growth coefficient (TGC) b), plotted against age. $SGR = (e^g - 1)100$, where $g = [\ln(W_2) - \ln(W_1)] / (t_2 - t_1)$; and $TGC = (W_2^{1/3} - W_1^{1/3})100 / (\sum \text{degree-days})$, where W_2 and W_1 were the average body weights (g) at t_2 and t_1 days, respectively (Nordgarden et al., 2003). Values have been represented at the midpoint of the time period over it is calculated: I, 4–10 g; II, 10–50 g; III, 50–200 g; IV, 200–350 g (fattening until commercial size). To simplify notation, in I, and II, A/A and W/W symbols correspond to the average value from tanks A/A–A/W and W/W–W/A, respectively.

Table 2

Muscle cellularity parameters for sea bass larvae reared at ambient (A/A and A/W) or increased (W/W and W/A) temperatures (mean \pm SEM)

| Stage | Muscle parameter | A/A and A/W (n=10) | W/W and W/A (n=9) |
|--------------------------------|------------------------|-------------------------------|-------------------------------|
| Hatching | TCA (mm ²) | 0.01 \pm 0.004 ^a | 0.01 \pm 0.001 ^a |
| | Φ (μ m) | 6.92 \pm 0.1 ^a | 7.33 \pm 0.17 ^a |
| | N | 255 \pm 8 ^a | 223 \pm 17 ^a |
| End of metamorphosis "scaling" | TCA (mm ²) | 1.4 \pm 0.08 ^a | 4.12 \pm 0.59 ^b |
| | Φ (μ m) | 18.9 \pm 0.36 ^a | 24.7 \pm 1.05 ^b |
| | N | 4516 \pm 166 ^a | 7056 \pm 952 ^b |

Total cross-sectional area (TCA), average diameter of muscle fibres (Φ), estimated number of muscle fibres (N). Different superscripts in values of the same row indicate the existence of significant differences ($p < 0.05$; p_{ANOVA} for TCA and N; $p_{Kolmogorov-Smirnov}$ for Φ).

sections (1 μ m thickness) were obtained at the level of the anal opening with a Reichert Jung ultramicrotome and stained with Toluidine blue after removal of the epoxy resin by overnight immersion of slices in sodium methyrate (30%) diluted in methanol (5% final dilution). In fingerlings and juveniles (4–350 g) a steak of 0.5–0.7 cm thickness was obtained at the level of the fourth ray of the cranial dorsal fin using a sharp knife. Then, the cross-section of the white muscle in the steak was traced onto acetate sheets using a fine permanent pen. By careful dissection, the white muscle from one side of the section was trimmed in a minimum of 2 (fingerlings of 4 g) and a maximum of 8 blocks of approximately 1 cm². Muscle blocks were covered with tissue freezing medium (Jung[®]), labelled and then frozen in 2-methyl butane cooled to near its freezing point (–159 °C) in liquid nitrogen. Frozen blocks were wrapped in foil and stored at –65 °C until sectioning. After temperature equilibration to –20 °C within the cryostat chamber (Leica CM 1850), 8 μ m thick sections were obtained from each block and then stained for Haematoxylin/Eosin.

Randomly selected fields of white muscle fibres from all muscle blocks were measured using an image analysis system (SigmaScan Pro 5.0, SPSS Inc). The area and diameter of muscle fibres was recorded. The total number of measured fibres per fish for each sampling stage was: 70 (hatching), 300 (end of metamorphosis), 400 (4 g), 450 (10 g), 500 (50 g), 600 (200 g) and 1000 (commercial size, 350 g). Muscle fibre cross-sectional area values were used to calculate the muscle fibre density (muscle fibre number/mm²) for each fish (Johnston et al., 2000b). The cross-sectional area of the white muscle (mm²) was quantified from the acetates by image analysis, and the number of white muscle fibres estimated from values of muscle fibre density and the cross-sectional area of the white muscle (Johnston et al., 2000b). The rates of white muscle fibre hyperplasia (new muscle fibres/day) and hypertrophy (μ m/day) were calculated as the mean of the difference between the observed fibre number or fibre size for a defined stage, and the mean value of the fibre number or fibre size in the preceding stage, divided by the time period over which it is calculated (Johnston et al., 2003a).

2.3. Sample preparation for flesh quality assessment

In fish of commercial size, a second steak of 1 cm thickness was removed just caudal to the fourth ray of the dorsal fin for measuring of texture parameters (instrumental, see textural properties). Then, the caudal remnant of the trunk was dissected to obtain two fillets (right and left) of approximately 6 cm length and 6 cm height. The right side fillet was used for physicochemical analysis of raw flesh, whereas the left side fillet was used for sensory analysis.

2.4. Textural properties

Texture parameters were measured in raw flesh immediately after removal of the second transversal steak of 1 cm thickness from fish. Measurement was done by penetration of a probe perpendicularly to the cross-section of the 1 cm thick steak (in parallel to the muscle fibres). A texture analyzer TA-XT2 (Stable Micro Systems, Surrey, England) equipped with a 25-kg load cell was used. The probe was a flat ended stainless steel cylinder with a diameter of 10 mm and a

pace of 1.1 mm/s. Fillet temperature during compression was kept at 5–8 °C by storing the fillets in plastic bags inside a fridge until measurement. Compression curves were recorded and texture properties expressed as hardness (maximum compression force) and area under the curve (maximum compression work).

2.5. Physicochemical analysis

Right side fillets were minced with an Omnimixer (at 4000 rpm for 5 s) and the compound used for analysis of moisture, crude protein and total fat,

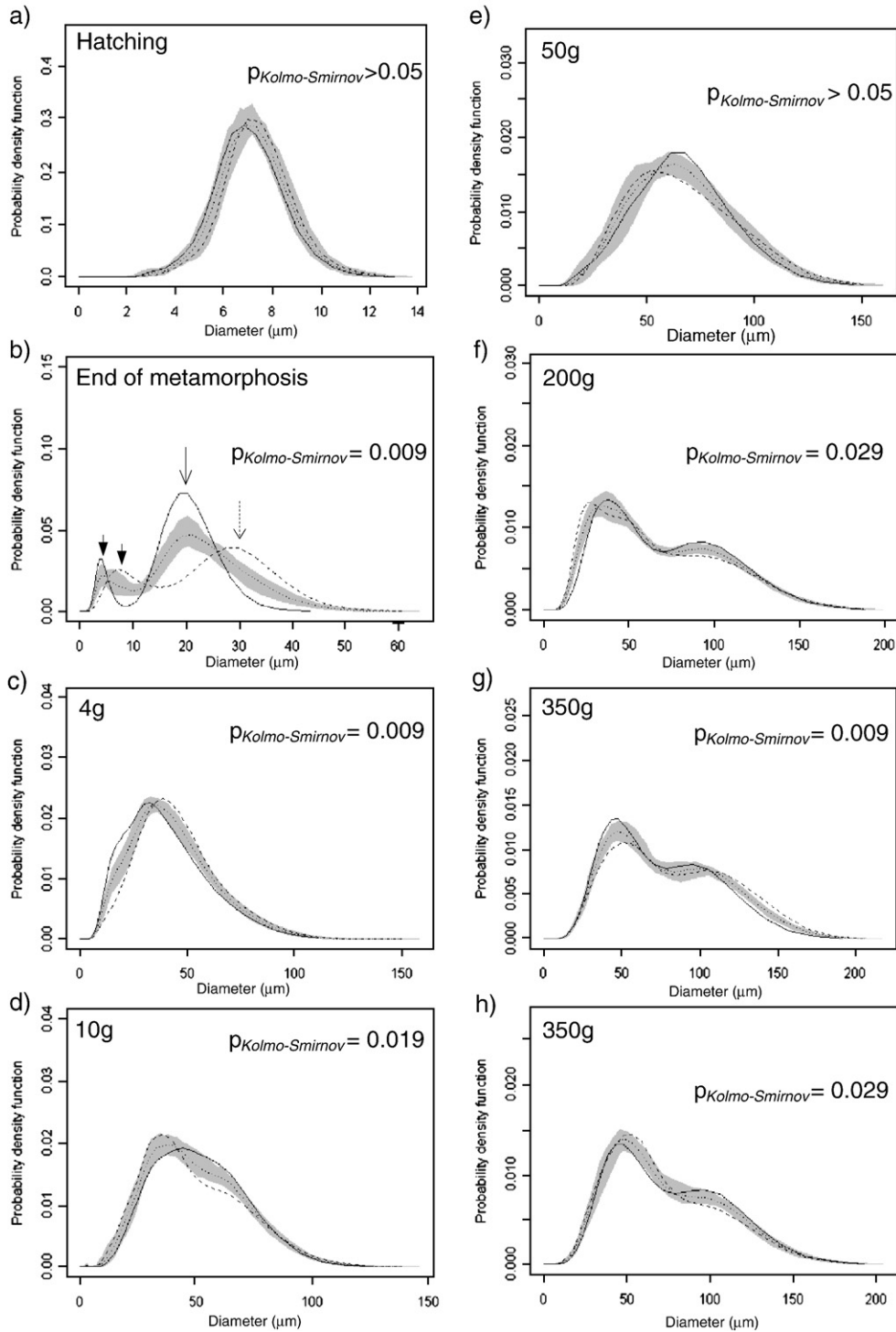


Fig. 3. Mean probability density functions (pdf) of white muscle fibre diameter for experimental groups. Solid and dotted lines correspond, respectively to: ambient (A/A and A/W) and warmed (W/W and W/A) groups in a), b), c), d) and e); to A/A and A/W groups in f); to A/A and W/W in g); and to A/A and W/A in h). The shaded polygon represents 100 bootstrap estimates of the combination of pooled groups, and the dotted line its mean probability density function. Regions where pdfs of experimental groups fall outside the shaded polygon provide graphical evidence for a statistical difference between them. In b) solid and dotted large arrows (↓) indicate peak frequencies for recruited muscle fibres by larval stratified hyperplasia in ambient and warmed groups respectively; solid and dotted short arrows (▼) indicate peak frequencies for small fibres recruited by mosaic hyperplasia.

according to MSC (1985). Hydroxyproline content was determined spectrophotometrically after acid hydrolysis of the sample (MSC, 1985). Flesh pH was measured with a pH-meter (micro-pH 2000 Crison) after mixing 10 g of compound with 50 ml of distilled water.

2.6. Sensory analysis

Sensory analysis was carried out in the day after sampling by a trained panel of 8 judges (3 females and 5 males, of 28–40 years) with previous experience in profile assessment of different food products. The dorsal half of each left fillet was divided into two portions of approximately 3×3 cm, wrapped in aluminium foil and kept in the fridge for 24 h. Samples were then cooked in a steam sauce pan for 5 min without any seasoning added. Fillet portions from fish of the four experimental tanks were cooked together. A preliminary training session was aimed to select the profile attributes, to establish the specific cooking and serving conditions, to determine the sample evaluation procedures and to define the score sheet. A quantitative descriptive analysis method was used and the selected attributes were: firmness, juiciness, fattiness, chewiness, flavour, odour and colour. The definition of each of them was taken from the Spanish specification for sensory analysis of foods (AENOR, 1997) and literature (Hyldig and Nielsen, 2001; Carbonell et al., 2002). According to sampling at commercial size (Table 1) the sensory analysis was carried out in two sessions, and to reduce the panellists' effect an analysis of repeated measurements was done. In each session, the 8 judges received four different portions of cooked flesh (one from each experimental tank), coded with three digital random numbers. Different code numbers were used for each session. Portions were evaluated for intensity of sensorial attributes from 1 (low intensity) to 5 (high intensity). Training and evaluation sessions were carried out in the morning (11.00–13.00) in a standardized test room equipped with a round table, 8 individual taste booths, controlled temperature (24 °C) and cool white light. Judges were instructed not to eat, drink or smoke for 1 h before sensory evaluation.

2.7. Statistical analysis

Most data were analyzed with the Statistical Package SPSS 11.5 version. The mean and standard error of each group of data were calculated. Experimental variables were checked for normality and homogeneous variance using the Kolmogorov–Smirnov and the Levene tests, respectively. Analysis of variance (ANOVA) was performed to evaluate the effects of temperature on experimental variables, and a post-hoc study (Tukey test) was used to establish the mean differences (at least for $p < 0.05$) between experimental groups. At commercial size, both ANOVA of muscle parameters using the sex as a fixed effect, and the Pearson correlation analysis among muscle cellularity, physico-chemical, texture (instrumental) and sensorial parameters were done.

Non-parametric statistical techniques were used to fit smoothed probability density functions (pdfs) to the measured diameters of white muscle fibres using a kernel approach (Johnston et al., 1999). Authors obtained the particular 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. Values for the smoothing parameter h were in the range 0.078 to 0.126 with no systematic differences between populations. Bootstrap techniques were used to distinguish underlying structure in the distributions from random variation. The Kolmogorov–Smirnov two sample statistical test 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 fifth, tenth, fiftieth, ninety-fifth and ninety-ninth percentiles of muscle fibre diameter were calculated from the distributions. A Kruskal–Wallis non-parametric test was used to test the hypothesis that the average value of the specified percentile was equivalent between experimental groups.

3. Results

3.1. Development and body growth

Hatching occurred in all experimental tanks at approximately 90 h postfertilization (59 degree-days). After mouth opening, temperature was progressively raised to ≈ 19 °C in tanks W/W and W/A. Consequently, in those tanks larval development was accelerated and the

end of the larval period (scaling) advanced 11 days. Larval length at the end of the metamorphosis was also higher in heated tanks (Table 1).

Body growth of fingerlings (4 and 10 g) and juveniles of 50 g was more rapid in tanks which had been heated during the larval period (W/W and W/A) (Table 1, Fig. 2a,b, stages I and II). Fish from those tanks showed both higher specific growth rates (SGR) and thermal growth coefficient (TGC) than fish reared at ambient temperature (AA and AW). Water heating during cool seasons —winter and early spring— was not so relevant for the subsequent fish growth (Fig. 2a,b, stage III), although fish from preheated tanks WW, WA and A/W achieved 200 g, 25–38 days earlier than fish always reared at ambient temperature (tank A/A) (Table 1). During the second summer (200 to 350 g, fattening period), fish growth was intense in all tanks but mainly in A/A, which showed compensatory growth during this period (Fig. 2a,b, stage IV). Thus, independently of the previous thermal story and the amount of degree-days all fish reached the commercial size (350 g, 30 cm) at an approximate age of 600 days (20.3 months).

3.2. Temperature influence on the muscle growth dynamics

Two regimes of temperature were compared during the larval period (Fig. 1): ambient (A/A and A/W tanks) and warm (W/W and W/A

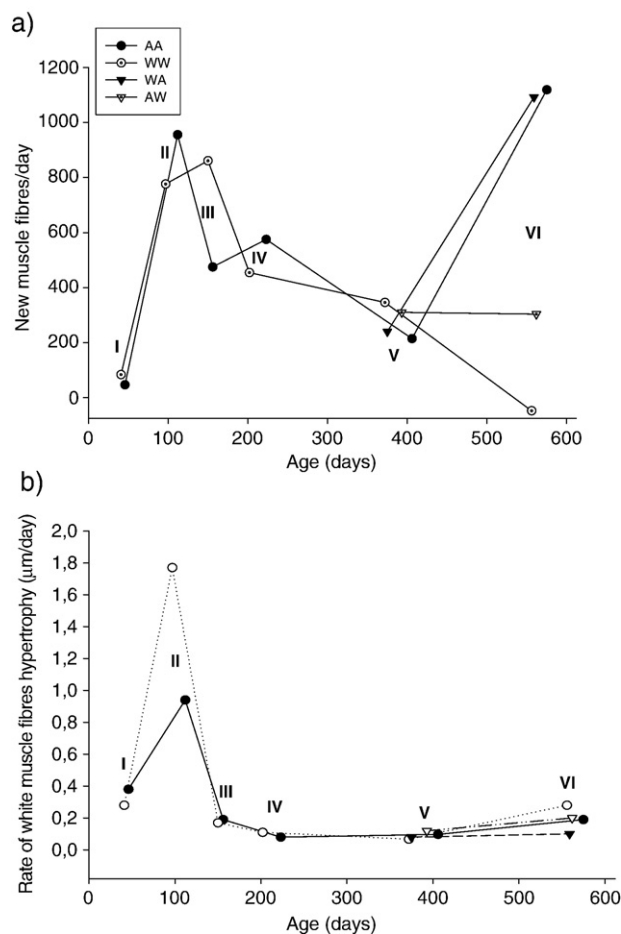


Fig. 4. The rates of muscle fibre hyperplasia a), and muscle fibre hypertrophy b), between successive sample points plotted against age. Values have been represented at the midpoint of the time period over it is calculated: I, hatching-end of metamorphosis; II, end of metamorphosis — 4 g; III, 4–10 g; IV, 10–50 g; V, 50–200 g; VI, 200–350 g (fattening until commercial size). To simplify notation, in I, II, III and IV, A/A and W/W symbols correspond to the average value from tanks A/A–A/W and W/W–W/A, respectively.

tanks). Larval heating started after mouth opening, so no differences between experimental tanks were observed for all muscular parameters at hatching (Table 2, Fig. 3a). During the larval period, white muscle thickness increased rapidly as a consequence of active hypertrophy of muscle fibres and recruitment of new fibres. Muscle fibre recruitment during the larval period consisted of successive phases of “stratified” and “mosaic” hyperplasia (Rowlerson and Veggetti, 2001) (Fig. 3b, arrows). In the stratified hyperplasia new white muscle fibres appeared at the dorso-ventral extremities of the myotome and between the red and white muscles. In contrast, in the mosaic hyperplasia phase new muscle fibres were scattered throughout the entire white muscle. White muscle hyperplasia was highly sensitive to temperature during the larval period. Thus, the onset of the mosaic hyperplasia phase was advanced (Fig. 3b) and the rate of muscle fibre recruitment 44% higher in fish from warm tanks (Fig. 4a, stage I tanks W/W and W/A). Contrarily, the rate of hypertrophy of white muscle fibres was slightly higher in larvae from ambient tanks (26%) (Fig. 4b). As a consequence of larval heating, muscle cellularity at the end of the larval metamorphosis (scaling) was significantly different in specimens from warm and ambient tanks (Table 2, Fig. 3b).

Following the larval period, all postlarvae experienced the same regime of temperature until the end of the autumn (Fig. 1). In fingerlings of 4 and 10 g, and juveniles of 50 g, muscle growth was always very intense, but depending on the previous regime of temperature (larval phase) a different muscle growth strategy was observed. Muscle growth was particularly high in early fingerlings. Thus, to reach 4 g fish from pre-warmed tanks (W/W and W/A) showed a very high rate of muscle fibre hypertrophy, whereas fish from ambient tanks (A/A and A/W) showed a higher rate of muscle fibre hyperplasia. Consequently, at 4 g the muscle fibre size distributions for ambient and pre-warmed groups were significantly different (Fig. 3c): the left hand curve in the ambient group at this stage denoted a recent period of intense mosaic hyperplasia. In fingerlings of 10 g, muscle cellularity differences between ambient and pre-warmed groups were less evident but still significant (Fig. 3d, $p=0.019$). As demonstrated by percentile values of the muscle fibre size distributions, differences occurred for small and medium size muscle fibres: P_5 (23 μm ambient, 19.15 μm pre-warmed, $p=0.059$), P_{10} (27.74 μm ambient, 24.06 μm pre-warmed, $p=0.04$), and P_{50} (49.91 μm ambient, 45.27 μm pre-warmed, $p=0.019$). Such differences corresponded to a higher rate of muscle fibre hyperplasia in the pre-warmed group (Fig. 4a, stage III). In contrast, the hypertrophic rate decreased significantly during this period in all fish (Fig. 4b). In juveniles of 50 g, no muscle cellularity differences were observed (Fig. 3e). Detailed values for the

muscle parameters of these fish are shown in Table 3. Therefore, the influence of the larval regime of temperature on the postlarval muscle growth dynamics was progressively lost, so that all fish reached 50 g with a similar muscle cellularity.

From 50–200 g (winter and spring months), tanks A/A and W/A experienced the ambient (cool) regime of temperatures, whereas temperature was kept at $\approx 19^\circ\text{C}$ in tanks W/W and A/W (Fig. 1). In this period, water heating determined an active recruitment of new muscle fibres but no increase in the rate of hypertrophy (Figs. 4a,b, stage V). Consequently, fish from tanks W/W and A/W showed the highest values for the total number of muscle fibres, and the lowest average diameter of muscle fibres at 200 g (Table 3). A comparison of the muscle fibre size distributions for A/A and A/W fish at stage 200 g is shown in Fig. 3f. Both groups exhibited a typical probability density function with two peaks where the muscle fibres included in the left hand curve are the most recently recruited. Due to water heating, fish from tank A/W showed lower values for percentiles P_5 (24.78 μm A/A, 20.01 μm A/W, $p=0.0001$), P_{10} (29.5 μm A/A, 24.26 μm A/W, $p=0.0001$), and P_{50} (67.12 μm A/A, 60.42 μm A/W, $p=0.035$).

In the short period between 200 and 350 g, all fish experienced a similar and favourable regime of temperatures (second summer). Throughout this period of rapid somatic growth (fattening), fish from unheated tanks during the previous winter (A/A and W/A) showed a very high rate of white muscle fibre hyperplasia (Fig. 4a, stage VI), which determined the highest muscle fibre numbers at commercial size (350 g) (Table 3). In contrast, in the same period, the rate of white muscle fibre recruitment did not increase or was apparently negative in fish from winter heated tanks (A/W and W/W, respectively) (Fig. 4a). At commercial size, those fish displayed the lowest number and densities of white muscle fibres (Table 3), but the highest average fibre size. Comparative muscle fibre size distributions between fish from tanks A/A and W/W evidenced such differences (Fig. 3g). Frequencies for small size fibres were higher in fish from tank A/A, while frequencies for medium size and big fibres were higher in fish from tank W/W: P_5 (30.16 μm A/A, 31.24 μm W/W, $p=0.326$), P_{10} (35.64 μm A/A, 37.81 μm W/W, $p=0.105$), P_{50} (71.27 μm A/A, 80.82 μm W/W, $p=0.005$), P_{95} (137.64 μm A/A, 154.38 μm W/W, $p=0.001$), and P_{99} (160.44 μm A/A, 178.49 μm W/W, $p=0.002$). Therefore, the regime of temperature during the cold seasons (winter and early spring) influenced the following muscle growth strategy in summer, such that water heating during winter months determined an immediate slight positive effect but a long-term negative effect on the recruitment of white muscle fibres. On the other hand, fish from tanks A/A and W/A also showed different muscle cellularities at commercial size

Table 3
Muscle cellularity parameters for fish of 50, 200 and 350 g (mean \pm SEM)

| Stage | Muscle parameter | A/A | W/W | W/A | A/W |
|------------------|------------------|-----------------------------------|---------------------------------|---------------------------------|----------------------------------|
| 50 g ($n=8$) | TCA | 444 \pm 14 ^a | 410 \pm 5 ^a | 378 \pm 13 ^a | 425 \pm 16 ^a |
| | Φ | 68.24 \pm 3.24 ^a | 73.51 \pm 3.02 ^a | 61.58 \pm 1.55 ^a | 65.74 \pm 2.1 ^a |
| | N | 108812 \pm 5924 ^a | 89749 \pm 8170 ^a | 112881 \pm 10350 ^a | 117577 \pm 11061 ^a |
| | \bar{D} | 246 \pm 21 ^a | 217.75 \pm 17.2 ^a | 296.3 \pm 17.6 ^a | 274 \pm 18 ^a |
| 200 g ($n=10$) | TCA | 841 \pm 16 ^a | 850 \pm 8 ^a | 852 \pm 15 ^a | 871 \pm 10 ^a |
| | Φ | 71.42 \pm 1.43 ^{b,c} | 65.94 \pm 0.97 ^a | 72.71 \pm 0.77 ^c | 67.22 \pm 1.14 ^{a,b} |
| | N | 174042 \pm 8416 ^{a,b} | 194782 \pm 5397 ^b | 167654 \pm 3726 ^a | 192754 \pm 7282 ^b |
| | \bar{D} | 206.52 \pm 8 ^{a,b} | 229.39 \pm 7.32 ^b | 197.03 \pm 4.62 ^a | 221.03 \pm 7.49 ^{a,b} |
| 350 g ($n=10$) | TCA | 1259 \pm 45 ^a | 1264 \pm 20 ^a | 1270 \pm 43 ^a | 1235 \pm 23 ^a |
| | Φ | 75.37 \pm 1.45 ^{a,b} | 83.92 \pm 2.57 ^c | 71.23 \pm 1.64 ^a | 77.97 \pm 1.84 ^{b,c} |
| | N | 241159 \pm 13159 ^{a,b} | 191523 \pm 15619 ^c | 268109 \pm 11154 ^a | 218589 \pm 9648 ^{b,c} |
| | \bar{D} | 191.64 \pm 8.3 ^{a,b} | 151.95 \pm 8.7 ^c | 212.19 \pm 8.77 ^a | 177.9 \pm 9.67 ^{b,c} |

Total cross-sectional area (TCA, mm^2), average diameter of muscle fibres (Φ , μm), estimated number of muscle fibres (N) and density of muscle fibres (\bar{D} , n/mm^2). Different superscripts in values of the same row indicate the existence of significant differences ($p<0.05$; p_{ANOVA} for TCA, N and \bar{D} ; $p_{\text{Kolmogorov-Smirnov}}$ for Φ).

Table 4
Biometric parameters, body indices, sex percentage of sea bass at commercial size (350 g) (mean±SEM), number of fish per tank=10

| | A/A | W/W | W/A | A/W |
|-----------------------|------------|------------|------------|------------|
| Fork length (cm) | 31.12±0.31 | 30.96±0.29 | 31.03±0.3 | 31.26±0.38 |
| Dorsal height (cm) | 6.62±0.07 | 6.66±0.07 | 6.51±0.04 | 6.62±0.06 |
| K condition | 1.4±0.04 | 1.39±0.035 | 1.36±0.032 | 1.39±0.045 |
| Anal height (cm) | 6.18±0.05 | 6.22±0.08 | 6.25±0.06 | 6.21±0.04 |
| Hepatosomatic index | 2.01±0.08 | 1.88±0.09 | 1.97±0.06 | 1.89±0.12 |
| Visceral fat index | 8.1±0.78 | 8.34±0.49 | 7.94±0.49 | 7.78±0.60 |
| Digestive tract index | 1.35±0.084 | 1.42±0.050 | 1.43±0.059 | 1.31±0.035 |
| Gonadosomatic index | 0.29±0.032 | 0.20±0.05 | 0.21±0.031 | 0.27±0.042 |
| Sex (% males) | 10 | 50 | 40 | 30 |

No statistically significant differences among experimental tanks were obtained for all measured parameters ($p_{\text{ANOVA}} > 0.05$). K condition=100 weight/length³.

(Table 3, Fig. 3h), which suggests the existence of a subtle long-term effect of the larval regime of temperature on the muscle growth dynamics of sea bass which reach the commercial size during the early autumn of the second year of life.

3.3. Carcass and flesh quality parameters at commercial size

Results for biometric parameters and body indexes are shown in Table 4. No significant differences for any parameter were observed among experimental tanks. However, sex ratio was influenced by temperature as higher percentages of males were found in tanks of heated water. Warming during the larval period was most critical for sex determination, as the highest percentages of males were found in tanks W/W and W/A.

In Table 5 the chemical composition and textural parameters (instrumental) of the fillet are summarized. Fish from heated tanks during winter and early spring seasons (W/W and A/W) showed maximum values of moisture and protein content, whereas fish from tank W/A had the lowest protein content. The total fat, hydroxyproline and pH were not influenced by temperature. The total fat range was 5.4–7.33% and hydroxyproline 25.8–35.29 mg/100 g. Concerning the instrumental textural parameters, significant differences were found between tanks W/W (maximum value) and A/W (minimum). However, no correlation between texture and muscle cellularity parameters was found.

Results for the sensorial attributes of the flesh have been summarized in a spider web diagram (Fig. 5). No differences among experimental tanks were observed. Mean values for all evaluated

Table 5
Physicochemical and textural parameters (instrumental) of raw sea bass flesh at commercial size (mean±SEM), number of fish per tank=10

| Chemical composition | A/A | W/W | W/A | A/W |
|-----------------------------|--------------------------|-------------------------|--------------------------|--------------------------|
| Moisture (%) | 67.43±1.42 ^b | 72.32±0.49 ^a | 70.38±0.41 ^{ab} | 72.40±0.59 ^a |
| Protein (%) | 19.03±0.61 ^{ab} | 20.75±0.37 ^a | 18.77±0.33 ^b | 19.18±0.57 ^{ab} |
| Total fat (%) | 7.33±1.35 ^a | 5.40±0.87 ^a | 6.51±0.97 ^a | 6.00±0.88 ^a |
| Hydroxyproline (mg/100 g) | 28.56±4.30 ^a | 31.43±3.44 ^a | 25.80±3.57 ^a | 35.29±3.10 ^a |
| pH | 6.45±0.10 ^a | 6.41±0.08 ^a | 6.37±0.13 ^a | 6.39±0.07 ^a |
| <i>Textural parameters</i> | | | | |
| Hardness (kg) | 2.31±0.14 ^{ab} | 2.82±0.25 ^a | 2.24±0.14 ^{ab} | 2.04±0.11 ^b |
| Area under the curve (kg/s) | 21.27±2.25 ^{ab} | 32.29±4.19 ^a | 23.44±2.41 ^{ab} | 19.66±1.21 ^b |

Different superscripts in the same row indicate significant differences ($p < 0.05$).

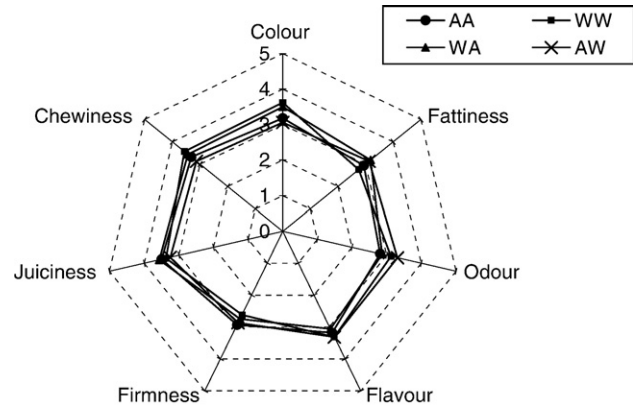


Fig. 5. Spider web diagram for scores (1–5) of the sensorial parameters of the fillet. Definition of sensorial parameters was done according to AENOR 1997: firmness (the force required to compress the sample between the molar teeth), juiciness (the total impression of succulence in the mouth just prior to swallowing), fattiness (fatty sensation in the mouth), chewiness (the total perceived effort required to prepares the sample for swallowing), flavour (the flavour associated with cooked fresh fish), odour (the aromatics associated with cooked fresh fish). No significant differences for all measured parameters were found ($p > 0.05$).

parameters ranged from 2.8 to 3.7, so none of the tested samples received too low or high punctuations. Positive correlations were observed between juiciness and chewiness ($r=0.59$, $p < 0.0001$), fattiness and juiciness ($r=0.36$, $p < 0.01$) and fattiness and chewiness ($r=0.43$, $p < 0.0001$). On the contrary, firmness was negatively correlated with juiciness ($r=-0.331$, $p < 0.01$). No correlations were found between muscle cellularity, texture parameters (instrumental) and texture attributes (sensorial).

4. Discussion

4.1. Temperature effect on sea bass growth

It has been extensively demonstrated that temperature has an intense effect on fish development and growth (Blaxter, 1988; Fukujara, 1990). In sea bass, high temperature reduces the embryonic period and anticipates the total absorption of the yolk sac, flexion of the notochord, completion of the fin ray counts and scaling (Ayala et al., 2000, 2001; López-Albors et al., 2003; Alami-Durante et al., 2006). Accordingly, in this work, larvae from warmed tanks scaled 11 days earlier than those reared at ambient temperature. The total length at different developmental stages of the larval life is also affected by temperature, but variable results have been found depending on the stock or the particular regime of temperature: Ayala et al. (2001) no difference, Alami-Durante et al. (2006) shorter larvae in warmed batches, and López-Albors et al. (2003) and this work longer larvae in heated tanks.

Postlarval growth of sea bass is also influenced by temperature. In this study, sampling of fingerlings and early juveniles (up to 50 g) was advanced in tanks where the larval temperature had been increased (Fig. 1, Table 1). This result may be of interest for hatcheries and nurseries, as it demonstrates that water heating during the larval period may be used to advance the obtaining of

fingerlings and early juveniles. Afterwards, however, fish reared at ambient temperature showed compensatory growth, which was particularly active during the fattening period (200–350 g) (Fig. 2a,b, stage IV). Hence, by the end of the experiment (≈ 600 days) all fish had attained the commercial size. Compensatory growth after thermal treatment during early life is common in the sea bass, and also in other teleosts such as the Arctic charr *Salvelinus alpinus*, L. and the Atlantic salmon (Mortensen and Damsgård, 1993; Maclean and Metcalfe, 2001; Johnston et al., 2003a). In sea bass, larvae exposed to 13 °C or 17 °C grew slower than at 21 °C, but 300 days after hatching all fish reached the same size (Mylonas et al., 2005). Also, larvae reared at low temperature (13 °C or 15 °C) showed slower growth than at 20 °C, but compensated for the growth retardation experienced during early life at a size of 21 cm length (Alami-Durante et al., 2007). Although the physiological mechanisms that mediate compensatory growth are not well understood, behaviour adjustments must be involved as higher growth rates require increased food intake (Maclean and Metcalfe, 2001). In Atlantic salmon, differences in the social status of individuals (dominant versus subordinate) and increased ability of dominants to monopolize the food patch in tanks of compensatory growth was observed (Maclean and Metcalfe, 2001). Sex differences may also be considered to explain the compensatory growth of the sea bass. Females of juvenile sea bass grow faster than males (Carrillo et al., 1995) and higher proportion of them are commonly obtained in tanks reared at low temperature during the larval period (Pavlidis et al., 2000). This was also the case of the present study. However, independently of the proportion of females, values of the gonadosomatic indices were similar in the four experimental tanks (Table 4), and this probably indicates that sexual maturation was not very active at commercial size both in males and females. With regard to previous work in sea bass (Gardeur et al., 2001; Mylonas et al., 2005; Alami-Durante et al., 2007), it is likely that compensatory growth in tanks at ambient temperature was not so dependent on the higher number and growth performance of females, but probably caused by social interactions of dominance, variation in the individual genetic growth potential, or by differences in appetite (Gardeur et al., 2001). In this sense, a higher food intake of AA fish during the warmest period of growth (summer and early autumn) is consistent with their higher SGR and TGC values and the necessity to attain a certain size to tackle any particular biological target, i.e. the gonads maturation.

4.2. White muscle growth dynamics at ambient temperature

In this work, the growth mechanisms of the white muscle of sea bass have been described in detail from hatching to an advanced juvenile stage such as the commercial size (≈ 350 g, 30 cm). Although somehow restricted to the Southern Spanish Mediterranean Sea, where the annual regime of temperatures fluctuates between 14 and 28 °C, our results for fish reared at ambient temperature indicate a seasonal influence of the environmental temperature on the muscle growth patterns of this species. Hatching occurred in middle winter, and most of the larval period in spring. In agreement with previous works (Scapolo et al., 1988; Ayala et al., 2001; López-Albors et al.,

2003; Alami-Durante et al., 2006), muscle growth during the larval period was a consequence of continuous hypertrophy and active recruitment of new fibres (successive stratified and mosaic hyperplasia phases). In early summer, which approximately coincided with the beginning of the fingerling period, very high rates of white muscle fibre hyperplasia and hypertrophy were observed (Fig. 4a,b, stage II). This finding was also observed in previous works (Nathanailides et al., 1996; López-Albors et al., 2003; Alami-Durante et al., 2007) as it corresponds to a very active phase of mitotic activity and protein accretion favoured by maximum food availability. Thereafter, postlarval muscle growth dynamics described different tendencies for muscle fibre hyperplasia and hypertrophy.

Concerning the muscle hyperplasia, an influence by season and growth rate was observed (Fig. 4a): it decreased significantly in winter and spring seasons, but recovered and rose to a maximum during the second summer and following early autumn of postlarval life. Interestingly, this period of maximum hyperplasia was coincident with maximum values of SGR and TGC in tank AA (Fig. 2a,b, stage IV), and associated to the rapid growth period that enables sea bass to reach the commercial size (fattening). These results agree with those of Alami-Durante et al. (2007), who recently demonstrated that rapid postlarval growth of sea bass is highly influenced by season and linked to white muscle hyperplasia. Sex may also influence the recruitment of white muscle fibres. In the Atlantic halibut (*Hippoglossus hippoglossus*), even prior to sexual maturation and any evidence of dimorphism in body size the number of white muscle fibres was higher in females than in males (Hagen et al., 2006). Our results, however, do not deal with this study, as the ANOVA for the number of white muscle fibres at commercial size was similar in both sexes: $227,179 \pm 9968$ in males and $228,021 \pm 8486$ in females ($p=0.778$).

Contrarily to muscle hyperplasia, the postlarval hypertrophy of white muscle fibres decreased significantly by the end of the former summer, and persisted at a steady low level, independently of season, until the end of the experiment (Fig. 4b). Hypertrophy of white muscle fibres prior to commercial size was also unaffected by the sex. The size of the fibres was 77.87 ± 1.53 μm for males and 77.15 ± 1.33 μm for females at 350 g ($p=0.921$). A simple explanation for this result is that restrictions to increase in size are higher for large than for small diameter fibres, as large fibres have lower surface to volume ratio they also have lower capacity to assimilate nutrients (Weatherley et al., 1988). On the other hand, our results do not support the finding reported by Alami-Durante et al. (2007) of a shrinkage of the largest white muscle fibres during winter months. Actually, the P95 and P99 percentiles were always significantly lower in fish of 50 than 200 g: P95 109 μm and P99 126.9 μm at 50 g, versus P95 137.5 μm and P99 161.4 μm at 200 g.

4.3. Temperature influence on the patterns of white muscle growth

Manipulation of temperature during early stages of development has important effects on the cellularity of the myotomes of sea bass embryos and larvae. Incubation of embryos and

cultivation of yolk sac larvae at high temperature ($>15\text{ }^{\circ}\text{C}$) reduces (Alami-Durante et al., 2006), or hardly changes the number of white muscle fibres generated during the embryonic myogenesis (Ayala et al., 2001, 2003; López-Albors et al., 2003; Alami-Durante et al., 2006). Thereafter, in free swimming larvae, high temperature increases both the hypertrophy of white muscle fibres and the recruitment of new fibres by stratified and early mosaic hyperplasia (Ayala et al., 2001, 2003; López-Albors et al., 2003). Interestingly, in most previous works the number of white muscle fibres at equivalent stages of development such as the end of the larval metamorphosis was similar in larvae from warm and ambient tanks. Interactions between growth and development processes seem to regulate the proliferation of muscle fibres throughout the larval period. However, in the present work, larvae from warm tanks had a higher number of white muscle fibres at the end of the larval metamorphosis (scaling). This could be linked to the fact that, contrarily to the referred previous works, temperature was not increased until the stage of mouth opening, hence avoiding an effect of temperature on the embryonic myogenesis. In terms of muscle fibre proliferation water heating after yolk sac absorption stimulated the stratified and mosaic hyperplasia phases of larvae. Also, larval length at the end of the larval metamorphosis was increased by temperature. This effect was independent of the developmental program for other phenotypic characters such as scaling.

The positive influence of the larval temperature on the cellularity of the white muscle was extended throughout the fingerling period. Depending on the larval regime of temperature fish of 4 g and 10 g had different size distributions of muscle fibres (Fig. 3c,d). In fish of 4 g, differences were also observed for the number of muscle fibres: $41,750 \pm 1918$ and $30,346 \pm 1639$ for warm and ambient groups, respectively. Similarly, an increase of $2.7\text{ }^{\circ}\text{C}$ in the rearing temperature during the short period between hatching and mouth opening (5 days) led to higher size of white muscle fibres in postlarvae of 120 days (López-Albors et al., 2003). Long-term effects of the early temperature on the postlarval muscle growth patterns have also been described in the Clyde herring *Clupea harengus* L. (Johnston et al., 1998) and in different populations of Atlantic salmon (Johnston et al., 2000c, 2003a). The higher degree-days of fish from pre-warmed tanks (higher growth opportunity), the particular moment of the larval life when the increase of temperature is operated, or adaptive modifications in the response to temperature changes may be responsible for the elongated effect of the early temperature on the postlarval growth dynamics of the white muscle (Johnston et al., 2000c; López-Albors et al., 2003). In this sense, it has been suggested that early temperature may imprint changes in the proliferative capacity of the myogenic progenitor cells which will persist throughout life (Johnston et al., 2000c). This could be the reason for the observed highest number of muscle fibres at commercial size in fish from tank W/A (Table 3).

This is the first study evaluating the influence of water heating during winter and early spring seasons on the muscle growth dynamics of sea bass juveniles. Fish reared at $19\text{ }^{\circ}\text{C}$ during this period — tanks W/W and A/W — showed higher

muscle fibre hyperplasia (Fig. 4a, stage V). However, as shown in Fig. 4b, the hypertrophy of muscle fibres was not stimulated in this period. An explanation for this result might be that increased winter temperature acts selectively on the proliferation of muscle fibres (hyperplasia), with no significant effect on the hypertrophy. However, independently of the positive effect of temperature on the recruitment of muscle fibres, the lack of effect on the hypertrophy would rather be due to a reduced food intake, and/or to changes in the feeding pattern. In this sense, it is known that sea bass juveniles reduce food intake (Abdel et al., 2004) and tend to change feeding rhythms from diurnal to nocturnal pattern in winter months (Sánchez-Vázquez et al., 1998). As feeding regime was always diurnal in this experiment, it may have led to a reduction in food intake and consequently in muscle fibre hypertrophy during the winter period. Results were quite different in the following summer, as coinciding with the fattening period (200–350 g), sea bass from tanks W/W and A/W showed high muscle fibre hypertrophy, but null muscle fibre hyperplasia (Table 3, Fig. 4). Summing up, increased temperature ($19\text{ }^{\circ}\text{C}$) during winter and early spring had the capacity to stimulate muscle fibre hyperplasia of juvenile sea bass but in the following summer, when body growth is more favourable, those fish were unable to recruit more muscle fibres. This result, which indicates a change in the pattern of muscle fibre recruitment in fish which had previously experienced an apparently contradictory regime of temperature (raised during cold seasons), does not fit the common association between rapid body growth and high muscle fibre hyperplasia (Johnston et al., 2000b; Alami-Durante et al., 2007). A tentative explanation for that may be that water heating during the cool seasons not only increased the proliferative capacity of the myogenic precursors, but conditioned their subsequent behaviour. Thus, activated myogenic progenitor cells firstly preferentially fused to create new myotubes (hyperplastic immediate effect), but in the following summer rather were absorbed by mature muscle fibres (hypertrophic effect). Proliferation of myogenic cells may be induced by environmental factors such as temperature and photoperiod (Johnston et al., 2003a,b); however, their determination to form myotubes or to add to pre-existing fibres results from a complex regulating network where hormones, growth factors and transcription factors are involved (Johnston et al., 2003a). At the present moment, all these mechanisms have not yet been investigated in fish (Johnston, 2006), hence the muscle cellularity results for fish in tanks W/W and A/W during the fattening period (second summer) denotes the necessity of further investigations aimed at characterizing the proliferation, differentiation and postmitotic behaviour of the myogenic progenitor cells under environmental influence.

Our results confirmed that the muscle cellularity of the fillet of sea bass may be changed by the regime of temperature throughout the larval and postlarval periods (first hypothesis). Very few experiments have been carried out to study the effect of temperature on the muscle growth dynamics from early stages of development to advanced juvenile periods: Atlantic salmon (Johnston et al., 2003a) and sea bass (Alami-Durante et al., 2007). In the long period between hatching and marketing

many environmental as well as intrinsic factors influence muscle growth. As it has been found in this work, plasticity of myogenesis under environmental influence is not only described in larvae but in juveniles and adult fish (Johnston, 2006). Concerning the temperature effect on the myogenic plasticity, the choice of a fluctuant or constant regime of temperatures (Stoiber et al., 2002), the time and duration of the temperature changes (López-Albors et al., 2003), differences in the sensitivity of the fibre hyperplasia to temperature changes (Johnston et al., 1998) and interactions between temperature and mechanisms regulating muscle growth and other physiological processes such as sexual maturation (Johnston et al., 2006; Alami-Durante et al., 2007, this work) may result in different muscle growth patterns and diverse muscle phenotypes in fish delivered for the market.

4.4. Implications of the rearing temperature on the flesh quality

Temperature only influenced three parameters of the flesh: moisture, protein and hardness. The protein content tends to be very constant in fish; however, it can be slightly modified by rearing conditions and lifestyle (Haard, 1992; Periago et al., 2005). In the present work, protein content was significantly higher in sea bass from tank W/W, which could be related to the fact that these fish showed the highest rate of hypertrophy of muscle fibres during the fattening period (Table 3, Fig. 4), and it is known that muscle fibre hypertrophy requires very active protein synthesis. Total fat content did not show significant differences among the four tanks, which was probably caused by the high variability observed in fish from tank A/A (Table 5).

Texture parameters (instrumental) were different among the experimental tanks (Table 5). Both the hardness to compress the flesh and the area under the curve were maximum in sea bass harvested from tank W/W, and minimum in tank A/W. Also, no correlation between muscle fibre size and texture parameters was found, as the density of muscle fibres was similar in these two groups (Table 3). On the contrary, when differences in muscle fibre size were significant, similar texture was measured. These results disagree with our previous findings in sea bass where instrumental attributes of texture correlated with the density of fibres, collagen content and final pH (Periago et al., 2005). In that case, wild and farmed populations of sea bass were compared, and more than 40% of the variation in chewiness and springiness was explained by the density of muscle fibres. Although muscle fibre size plays an important role in texture of raw flesh (Johnston et al., 2000a), other factors could be responsible for the textural differences observed in the present study. Collagen content, measured as total collagen, hydroxyproline concentration, or alkaline-soluble or insoluble fractions have also been correlated with texture of raw flesh (Periago et al., 2005, Johnston et al., 2006). However, no differences in hydroxyproline concentration were found among our experimental groups, which was probably due to high interindividual variation within groups. Other factors influencing texture are processing and storage conditions of the fillets (Haard, 1992), season and body size (Bjørnevik et al., 2004), and also the instrumental methodology (Taylor et al., 2002). In salmon fillets, postmortem changes in texture varied depending on the orientation

of the muscle fibres, such that if the blade was perpendicularly orientated to the fibres postmortem changes in texture were estimated more accurately than if it ran in parallel to the fibres (Taylor et al., 2002). In raw beef, Swatland (1978) found that deformation in parallel to the fibres does not involve the muscle fibres but connective tissue breaks. Thus, measuring in parallel to the fibres is a rough estimator of fish flesh texture (Taylor et al., 2002). As we performed the shear test in parallel to the fibres, this methodological factor most probably conditioned texture instrumental results and hence the lack of correlation between texture and muscle cellularity parameters.

Panellists did not find significant differences between experimental groups for all the sensorial attributes of the fillet. As it was expected, odour, flavour and colour did not vary between groups because the rearing conditions that can modify them (feed and water quality) were always the same. Concerning texture, inter-correlation of some parameters was observed. Juiciness, chewiness and fattiness showed positive inter-correlation, whereas juiciness and firmness were negatively correlated. The observed correlations are easy to explain because high lipid content confers a juicy and fatty mouth feel, and low lipid content is rather related to firmness and grittiness (Nielsen et al., 2005). On the other hand, juiciness and firmness are conceptually opposite parameters. Thus, our results are consistent with the common sensory analysis of texture and can be partly explained by two main factors: first, the perception of one parameter will typically influence the perception of another (Christensen, 1984); second, many sensorial attributes are determined by the same underlying physical (muscle structure) or chemical factor (lipid, protein and moisture content) (Szczesniak, 1963).

One of the aims of this work was to test the hypothesis that muscle cellularity differences of sea bass fillets induced by rearing temperature, can modify the sensorial properties of cooked flesh. Muscle cellularity differences were found, but no correlation between muscle cellularity and sensorial parameters of texture was established after sensory analysis. According to Hatae et al. (1990) the main factors determining texture of cooked flesh are the presence of coagulated material in the interstitial spaces and the size of the muscle fibres. The interstitial material obstructs or impedes the movements of the fibres during mastication and results from thermal coagulation of sarcoplasmic and myofibrillar proteins (Ayala et al., 2005). Quantification of interstitial coagulated material was not done in this study, so its influence on the sensorial attributes of texture remains unknown. On the other hand, our results disagrees with those of Hatae et al. (1990) and Hurling et al. (1996), who found a negative correlation between muscle fibre size and texture of cooked flesh. In those studies, species with a wide range of differences in the size of muscle fibres were compared, hence allowing a sufficient degree of variation for the analysis of correlation. Similarly to us, Hurling et al. (1996) performed morphometry of the cross-section of the fibres and also sensory analysis of the flesh after cooking. The average diameters they compared ranged from 53 to 163 μm (Sword fish Xiphidae and Common dab *Limanda limanda*, respectively), but correlation was only found when the average diameter of the fibres differed by at least 60 μm . In this work, the regimes of temperature determined a shorter range of differences

in the size of muscle fibres between the four experimental tanks: lower than 13 μm . This range was probably not wide enough to be detected by panellists.

5. Conclusion

It is demonstrated that slight increases of temperature during the larval period and/or winter months have the capacity to modify the rates of hyperplasia and hypertrophy of white muscle fibres, such that depending on the previous thermal regime there were significant differences in the muscle cellularity of the fillet at commercial size. Such differences, however, had a limited influence on the physicochemical properties of the fillet and no effect on its sensorial attributes. These results together with the fact that all fish reached the commercial size at the same time minimized the impact of the rearing temperature on the yield and quality of sea bass.

Acknowledgements

This work was funded by the Ministerio de Ciencia y Tecnología (Project Ref. AGL2000-1738-C03-02). Authors are grateful to M. Moya for informatics assistance.

References

- AENOR, Spanish Association for Standardisation and Certification, 1997. Análisis sensorial. Asociación Española de Normalización (Ed.). Madrid. 253 pp.
- Akbulut, B., Sahin, T., 1999. Effect of weight loss occurring in winter season on growth of sea bass (*Dicentrarchus labrax*) reared in the Black Sea. *Turk. J. Mar. Sci.* 5 (1), 39–46.
- Abdel, I., López Albors, O., Abellán, E., García-Alcázar, A., 2004. The effect of the temperature regimes on feed response and growth performance during winter months in sea bass (*Dicentrarchus labrax*, L.). *Spec. Publ. - Eur. Aquac. Soc.* n° 34, 91–92.
- Alami-Durante, H., Rouel, M., Kentouri, M., 2006. New insights into temperature-induced white muscle growth plasticity during *Dicentrarchus labrax* early life: a developmental and allometric study. *Mar. Biol.* 149 (6), 1551–1565.
- Alami-Durante, H., Nellie, O., Rouel, M., 2007. Early thermal history significantly affects the seasonal hyperplastic process occurring in the myotomal white muscle of *Dicentrarchus labrax* juveniles. *Cell Tissue Res.* 327 (3), 553–570.
- Ayala, M.D., López-Albors, O., Gil, F., Latorre, R., Vázquez, J.M., García-Alcázar, A., Abellán, E., Ramírez, G., Moreno, F., 2000. Temperature effect on muscle growth of the axial musculature of the Sea Bass (*Dicentrarchus labrax* L.). *Anat. Histol. Embryol.* 29, 235–241.
- Ayala, M.D., López-Albors, O., Gil, F., García-Alcázar, A., Abellán, E., Alarcón, J.A., Álvarez, M.C., Ramírez-Zarzosa, G., Moreno, F., 2001. Temperature effects on muscle growth in two populations (Atlantic and Mediterranean) of sea bass. *Aquaculture* 202, 359–370.
- Ayala, M.D., López-Albors, O., García-Alcázar, A., Abellán, E., Latorre, R., Vázquez, J.M., Ramírez-Zarzosa, G., Gil, F., 2003. Effect of two thermal regimes on the muscle growth dynamics of sea bass larvae, *Dicentrarchus labrax* L. *Anat. Histol. Embryol.* 32, 271–275.
- Ayala, M.D., López-Albors, O., Blanco, A., García-Alcázar, A., Abellán, E., Ramírez-Zarzosa, G., Gil, F., 2005. Structural and ultrastructural changes on muscle tissue of sea bass, *Dicentrarchus labrax* L., after cooking and freezing. *Aquaculture* 250 (1–2), 215–231.
- Barroso, M., Careche, M., Borderías, A.J., 1998. Quality control of frozen fish using rheological techniques. *Trends Food Sci. Technol.* 9, 223–229.
- Bjørnevik, M., Espe, M., Beattie, C., Nortvedt, R., Kiessling, A., 2004. Temporal variation in muscle fibre area, gaping, texture, colour and collagen in triploid and diploid Atlantic salmon (*Salmo salar* L.). *J. Sci. Food Agric.* 84, 530–540.
- Blaxter, J.H.S., 1988. Pattern and variety in development. In: Hoar, W.S., Randall, D.J. (Eds.), *Fish Physiology*, vol. XI. Academic Press, San Diego, pp. 1–58.
- Carbonell, I., Izquierdo, L., Costell, E., 2002. Sensory profiling of cooked gilthead sea bream (*Sparus aurata*): sensory evaluation procedures and panel training. *Food Sci. Technol. Int.* 8 (3), 169–177.
- Carrillo, M., Zanuy, S., Prat, F., Cerda, J., Ramos, J., Mananos, E., Bromage, N., 1995. Sea bass (*Dicentrarchus labrax*). In: Bromage, N.R., Roberts, R.J., (Eds.), 1995. *Broodstock Management and Egg and Larval Quality*, Blackwell, Cambridge, pp. 138–168.
- Christensen, C.M., 1984. Food texture perception. *Adv. Food Res.* 29, 159–199.
- de Asis, J.M.F., Carvalho, R.F., Barbosa, L., Agostinho, C.A., Pai-Silva, M.D., 2004. Effects of incubation temperature on muscle morphology and growth in the pacu (*Piaractus mesopotamicus*). *Aquaculture* 237, 251–267.
- Fukujara, O., 1990. Effects of temperature on yolk utilization, initial growth, and behaviour of unfed marine fish-larvae. *Mar. Biol.* 106, 169–174.
- Galloway, T.F., Kjorsvik, E., Kryvi, H., 1998. Effect of temperature on viability and axial muscle development in embryos and yolk sac larvae of the Northeast Arctic cod (*Gadus morhua*). *Mar. Biol.* 132, 559–567.
- Gardeur, J.N., Lemarié, G., Coves, D., Boujard, T., 2001. Typology of individual growth in sea bass (*Dicentrarchus labrax*). *Aquat. Living Resour.* 14, 223–231.
- Gibson, S., Johnston, I.A., 1995. Temperature and development in larvae of the turbot *Scophthalmus maximus*. *Mar. Biol.* 124 (1), 17–25.
- Haard, N.F., 1992. Control of chemical composition and food quality attributes of cultured fish. *Food Res. Int.* 112, 321–332.
- Hatae, K., Yoshimatsu, F., Matsumoto, J.J., 1990. Role of muscle fibres in contributing firmness of cooked fish. *J. Food Sci.* 55, 693–696.
- Hagen, O., Solberg, Ch., Johnston, I.A., 2006. Sexual dimorphism of fast muscle fibre recruitment in farmed Atlantic halibut (*Hippoglossus hippoglossus*, L.). *Aquaculture* 261, 1222–1229.
- Hyldig, G., Nielsen, D., 2001. A review of sensory and instrumental methods used to evaluate the texture of fish muscle. *J. Texture Stud.* 32, 219–242.
- Hurling, R., Rodell, J.B., Hunt, H.D., 1996. Fibre diameter and fish texture. *J. Textural Stud.* 27, 679–685.
- Johnston, I.A., 1999. Muscle development and growth: potential implications for flesh quality in fish. *Aquaculture* 177, 99–115.
- Johnston, I.A., 2006. Environment and plasticity of myogenesis in teleost fish. *J. Exp. Biol.* 209, 2249–2264.
- Johnston, I.A., Hall, T.E., 2004. Mechanisms of muscle development and responses to temperature change in fish larvae. In: Govoni, J.J. (Ed.), *The development of form and function in fishes and the question of larval adaptation*, pp. 113–144.
- Johnston, I.A., Cole, N.J., Abercromby, M., Vieira, V.L., 1998. Embryonic temperature modulates muscle growth characteristics in larval and juvenile herring. *J. Exp. Biol.* 201, 623–646.
- Johnston, I.A., Strugnell, G., McCracken, M.L., Johnstone, R., 1999. Muscle growth and development in normal-sex-ratio and all-female diploid and triploid Atlantic salmon. *J. Exp. Biol.* 202, 1991–2016.
- Johnston, I.A., Alderson, R., Sandham, C., Dingwall, A., Mitchell, D., Selkirk, C., Nickell, D., Baker, R., Robertson, B., Whyte, D., Springate, J., 2000a. Muscle fibre density in relation to the colour and texture of smoked Atlantic salmon (*Salmo salar* L.). *Aquaculture* 189, 335–349.
- Johnston, I.A., Alderson, R., Sandham, C., Mitchell, D., Selkirk, C., Dingwall, A., Nickell, D.C., Baker, R., Robertson, W., Whyte, D., Springate, J., 2000b. Patterns of muscle growth in early and late maturing populations of Atlantic salmon (*Salmo salar* L.). *Aquaculture* 189, 307–333.
- Johnston, I.A., Mclay, H.A., Abercromby, M., Robbins, D., 2000c. Early thermal experience has different effects on growth and muscle fibre in spring- and autumn-running Atlantic salmon population. *J. Exp. Biol.* 203, 2553–2564.
- Johnston, I.A., Manthri, S., Alderson, R., Smart, A., Campbell, P., Nickell, D., Robertson, B., Paxton, Ch.G.M., Burt, M.L., 2003a. Freshwater environment affects growth rate and muscle fibre recruitment in seawater stages of Atlantic salmon (*Salmo salar* L.). *J. Exp. Biol.* 206, 1337–1351.
- Johnston, I.A., Manthri, S., Smart, A., Campbell, P., Nickell, D., Alderson, R., 2003b. Plasticity of muscle fibre number in seawater stages of Atlantic salmon in response to photoperiod manipulation. *J. Exp. Biol.* 206, 3425–3435.
- Johnston, I.A., Li, X., Vieira, V.L.A., Nickell, D., Dingwall, A., Alderson, R., Campbell, P., Bickerdike, R., 2006. Muscle and flesh quality traits in wild and farmed Atlantic salmon. *Aquaculture* 256, 323–336.

- Killeen, J.R., McLay, A., Johnston, I.A., 1999. Temperature and neuromuscular development in embryos of the trout (*Salmo trutta* L.). *Comp. Biochem. Physiol., A* 122, 53–64.
- López-Albors, O., Ayala, M.D., Gil, F., García-Alcázar, A., Abellán, E., Latorre, R., Ramírez-Zarzosa, G., Vázquez, J.M., 2003. Early temperature effects on muscle growth dynamics and histochemical profile of muscle fibres of sea bass *Dicentrarchus labrax* L., during larval and juvenile stages. *Aquaculture* 220, 385–406.
- Love, R.M., 1983. Texture and the fragility of fish muscle cells. Research at the Torry Research Station. *J. Texture Stud.* 5, 351–358.
- Maclean, A., Metcalfe, N.B., 2001. Social status, access to food, and compensatory growth in juvenile Atlantic salmon. *J. Fish Biol.* 58, 1331–1346.
- Moretti, A., Padini Fernandez-Criado, M.P., Cittolin, G., Guidastrì, R., 1999. *Manual on Hatchery Production of Seabass and Gilthead Sea Bream*. vol. 1. FAO, Rome. 194pp.
- Mortensen, A., Damsgard, B., 1993. Compensatory growth and weight segregation following light and temperature manipulation of juvenile Atlantic salmon (*Salmo salar* L.) and Arctic charr (*Salvelinus alpinus* L.). *Aquaculture* 114, 261–272.
- Mylonas, C.C., Anezaki, L., Divanach, P., Zanuy, S., Piferrer, F., Ron, B., Peduel, A., Ben Atia, I., Gorshkov, S., Tandler, A., 2005. Influence of rearing temperature the larval and nursery periods on growth and sex differentiation in two Mediterranean strains of *Dicentrarchus labrax*. *J. Fish Biol.* 67, 652–668.
- MSC. Ministerio de Sanidad y Consumo, 1985. *Análisis de Alimentos. Métodos Oficiales y recomendados por el Centro de Investigación y Control de la Calidad, Ministerio de Sanidad y Consumo* (Ed.). Madrid. 1015 pp.
- Nathanaelides, C., López-Albors, O., Stickland, N.C., 1995. Influence of pre-hatch temperature on the development of muscle cellularity in posthatch Atlantic Salmon (*Salmo salar*; L.). *Can. J. Fish. Aquatic Sci.* 52 (4), 675–680.
- Nathanaelides, C., López-Albors, O., Abellán, E., Vázquez, J.M., Tyler, D.D., Rowlerson, A., Stickland, N.C., 1996. Muscle cellularity in relation to somatic growth in the European sea bass *Dicentrarchus labrax* (L.). *Aquac. Res.* 27, 885–889.
- Nielsen, D., Hyldig, G., Nielsen, J., Nielsen, H.H., 2005. Liquid holding capacity and instrumental and sensory texture properties of herring (*Cuplea harengus*, L.) related to biological and chemical parameters. *J. Texture Stud.* 36, 119–138.
- Nordgarden, U., Oppedal, F., Taranger, G.L., Hemre, G.I., Hansen, T., 2003. Seasonally changing metabolism in Atlantic salmon (*Salmo salar* L.) I — growth and feed conversion ration. *Aquac. Nutr.* 9, 287–293.
- Pavlidis, M., Koumoundouros, G., Steriotti, A., Somarakis, S., Divanach, P., Kentouri, M., 2000. Evidence of temperature-dependent sex determination in the European sea bass (*Dicentrarchus labrax* L.). *J. Exp. Zool.* 287, 225–232.
- Periago, M.J., Ayala, M.D., López-Albors, O., Abdel, I., Martínez, C., García-Alcázar, A., Ros, G., Gil, F., 2005. Muscle cellularity and flesh quality of wild and farmed sea bass *Dicentrarchus labrax* L. *Aquaculture* 249, 175–188.
- Rowlerson, A., Veggetti, A., 2001. Cellular mechanisms of post-embryonic muscle growth in aquaculture species. In: Johnston, I.A. (Ed.), *Muscle development and growth*. Fish Physiology Series, vol. 18. Academic Press, London, pp. 103–141.
- Sánchez-Vázquez, F.J., Azzaydi, M., Martínez, F.J., Zamora, S., Madrid, J.A., 1998. Annual rhythms of demand feeding activity in sea bass: evidence of a seasonal phase inversion of the dial feeding pattern. *Chronobiol. Int.* 15, 607–622.
- Scapolo, P.A., Veggetti, A., Mascarello, F., Romanello, M.G., 1988. Developmental transitions of myosin isoforms and organisation of the lateral muscle in the teleost *Dicentrarchus labrax* (L.). *Anat. Embryol.* 178, 287–295.
- Stickland, N.C., White, R.N., Mescall, P.E., Crook, A.R., Thorpe, J.E., 1988. The effect of temperature on myogenesis in embryonic development of the Atlantic salmon (*Salmo salar* L.). *Anat. Embryol.* 178, 253–257.
- Stoiber, W., Haslett, J.R., Wenk, R., Steinbacher, P., Gollmann, H.-P., Sängler, A.M., 2002. Cellularity changes in developing red and white fish muscle at different temperatures: simulating natural environmental conditions for a temperate freshwater cyprinid. *J. Exp. Biol.* 205, 2349–2364.
- Swatland, H.J., 1978. Microstructure of cuts through the connective tissue framework of meat. *J. Inst. Can. Sci. Technol. Aliment* 11, 204–208.
- Szczesniak, A.S., 1963. Classification of textural characteristics. *J. Food Sci.* 28, 385–389.
- Taylor, R.G., Fjaera, S.O., Skjervold, P.O., 2002. Salmon fillet texture is determined by myofiber–myofiber and myofiber–myocommata attachment. *J. Food Sci.* 67 (6), 2067–2071.
- Weatherley, A.H., Gill, H.S., Lobo, A.F., 1988. Recruitment and maximal diameter of axial muscle fibres in teleosts and their relationship to somatic growth and ultimate size. *J. Fish Biol.* 33, 851–859.
- Wilkes, D., Xie, S.Q., Stickland, N.C., Alami-Durante, H., Kentouri, M., Steriotti, A., Koumoundouros, G., Fauconneau, B., Goldspink, G., 2001. Temperature and myogenic factor transcript levels during early development determines muscle growth potential in rainbow trout (*Oncorhynchus mykiss*) and sea bass (*Dicentrarchus labrax*). *J. Exp. Biol.* 204, 2763–2771.