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Hyperplastic and hypertrophic growth of lateral muscle in blackspot seabream *Pagellus bogaraveo* from hatching to juvenile

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To understand better the growth mechanisms in the economically important fish *Pagellus bogaraveo*, in terms of muscle fibre hyperplasia *v.* hypertrophy, the lateral muscle of this fish was studied morphometrically from hatching to juvenile comparing rostral and caudal locations. Fish were sampled at 0, 5, 23, 40, 70, 100, 140 and 180 days. Fibre types were first identified by succinate dehydrogenase (SDH) and immunostaining with a polyclonal antibody against fish slow myosin (4–96). Morphometric variables were then measured in transverse body sections, at both post-opercular and post-anal locations, to estimate the following variables: total muscle area [A (*muscle*)], total fibre number [N (*fibres*)], fibre number per unit area of muscle [N_A (*fibres*, *muscle*)] and cross-sectional fibre area [$\overline{a}$ (*fibres*)] of the two main muscle fibre types (white and red). Overall, growth throughout the various stages resulted from increases both in the number and in the size of muscle fibres, paralleled by an expansion of the [A (*muscle*)]. Nonetheless, that increase was not significant between 0–5 days on one hand and 100–140 days, on the other hand. On the contrary, the [N_A (*fibres*, *muscle*)] declined as the body length increased. Analysis of the muscle growth kinetics suggested that, within the important time frame studied, hyperplasia gave the main relative contribution to the increase of white muscle [A (*white muscle*)], whereas red muscle [A (*red muscle*)] mainly grew by hypertrophy, with both phenomena occurring at a faster pace posteriorly in the body. Finally, when comparing rostral and caudal locations, a greater [N (*fibres*)] and [A (*muscle*)] of the posterior white and red fibres were the consistent features. It was also observed that the proportion of the cross-sectional area of the myotomal muscle comprised of white muscle was greater in the anterior part of the fish.

Key words: blackspot seabream; fish; hyperplasia; hypertrophy; morphometry; muscle.

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INTRODUCTION

The swimming muscle of fishes comprise *c.* 60% of the total body mass consisting of a number of almost identical units, the myotomes. In most species, the myotomal organization of the axial musculature is commonly stratified in three layers. One located superficially (red muscle), another deeply and responsible for most of the muscle mass (white muscle) and a third between the two former layers (intermediate or pink), which varies with species as regards to extent and histochemical properties (Rowlerson *et al.*, 1985; Scapolo *et al.*, 1988; Higgins & Thorpe, 1990; Veggetti *et al.*, 1993; Mascarello *et al.*, 1995).

Muscle growth in fishes, including early myotome expansion, is a plastic process involving a combination of enlargement of previously formed muscle fibres (hypertrophy) as well as recruitment of new ones (hyperplasia) (Rowlerson & Veggetti, 2001). Previous work has shown that muscle hyperplasia and hypertrophy require distinct populations of myogenic stem cells (Koumans & Akster, 1995). These cells may add new fibres to the myotome (hyperplasia) by apposition within peripheral proliferation zones ('stratified' type of growth) and by insertion between the already existing fibres ('mosaic' type growth) (growth terminology after Rowlerson & Veggetti, 2001). Stem cells may also enlarge existing fibres (hypertrophy) by fusion with them (Johnston, 1999). The relative importance of hypertrophy and hyperplasia of muscle growth varies both with fibre types and growth rate and ultimate body size of the species (Weatherley *et al.*, 1988). Because the largest tissue fraction in most fishes comprise myotomal muscle (Weatherley *et al.*, 1979), the plasticity of fish growth implies a corresponding effect in the muscle growth dynamics (Weatherley *et al.*, 1988).

The muscular differentiation, development and growth of *Pagellus bogaraveo* (Brünnich), a new fish species under consideration for intensive aquaculture in Southern Europe, is currently under study. The understanding of the effects of key rearing conditions on *P. bogaraveo* growth depends on the knowledge of its muscular growth mechanisms and dynamics during ontogeny. Hence, the muscle growth of *P. bogaraveo* was measured using a morphometric approach in order to: (1) determine the relative contributions of hyperplasia and hypertrophy for muscle growth from hatching (0 days) until the juvenile stage (180 days) and (2) compare the relative contributions in the anterior and posterior body regions, an aspect for which very few data are available for fishes in general.

MATERIALS AND METHODS

FISH

Eggs of *P. bogaraveo* were obtained from an adult stock adapted to life in captivity in a flow-through seawater system at Instituto Español de Oceanografía (IEO), Centro Oceanográfico de Vigo, Spain. Eggs were incubated at ambient temperature (*c.* 14° C) and hatching occurred 54 h thereafter. One day prior to mouth opening (115 h after hatching), larvae were transferred to culture tanks where temperature was gradually increased to 18° C, range ±1° C. Fish were fed rotifers *Brachionus plicatilis* (fish age: 5–35 days), *Artemia* sp. naupli (fish age: 30–35 days), *Artemia* sp. metanaupli (fish age: 35–45 days) and Gemma Micro diet (fish age: 45–60 days) (rotifers, *Artemia* sp.

naupli and metanaupli were raised by IEO; Gemma Micro diet was supplied by INVE Animal Health, SA, San Francisco, CA, U.S.A.). *Pagellus bogaraveo* were sampled at the following ages: 0, 5, 23, 40, 70, 100, 140 and 180 days. At each age, six fish were individually killed by over-anaesthesia in a 400 mgl^{-1} solution of MS-222 (Sigma-Aldrich, St Louis, MO, U.S.A.) (400 mgl^{-1}) and measured to estimate an instantaneous relative growth rate $G = 100(\ln S_2 - \ln S_1)(t_2 - t_1)^{-1}$, where S_1 and S_2 are initial and final mean total length (L_T) expressed in mm, and t_1 and t_2 are the times (days) of measurement (Forsythe & Van Heukelen, 1987).

For morphometric analysis, fish were fixed in 4% paraformaldehyde (Sigma-Aldrich) (0–5 days: 6 h; 23–40 days: 12 h; 100–180 days: 24 h) in phosphate buffer. Prior to fixation, larger individuals (140 and 180 days) were sliced, and the body cross-sections of interest were processed. The samples were then dehydrated in a graded ethanol series, cleared in xylol and finally embedded in paraffin. Perfect (*i.e.* without wrinkles, knife strikes or other potential defects) 10 μm-thick sections were cut transversely to the body axis at the point of the post-opercular and the post-anal levels, mounted in slides coated with aminopropyltriethoxysilane (APES) (Sigma-Aldrich), for improving section adhesion and then stained with haematoxylin–eosin before placing a cover slip.

For histochemistry and immunohistochemistry, whole fish ($n = 3$ per age) were snap frozen in isopentane (Sigma-Aldrich) at −80° C. Before freezing, small individuals were combined with each other in composite blocks and sandwiched with two slices of pig liver. Using this method, it was possible to obtain a solid block, easy to cut, and larvae could be orientated to provide transverse sections. Also, identical treatment of samples with regards to staining was ensured. Sections were cut at 10 μm, mounted on slides treated with APES to improve section adhesion. Sections were then histochemically stained for succinic dehydrogenase (SDH) activity, which is a marker for mitochondrial content and oxidative metabolism or immunostained with 4–96: polyclonal anti-fish (*Sparus aurata* L.) slow myosin (Veggetti *et al.*, 1999). A sensitive streptavidin-biotin-peroxidase immunohistochemistry kit was used (Histostain Plus; Zymed, San Francisco, CA, U.S.A.), following the maker's instructions with minor adaptations. The endogenous avidin–biotin-binding activity was blocked (Avidin–Biotin Blocking Kit; Zymed). The peroxidase activity was developed using 0·05% 3,3′-diaminobenzidine (DAB) (Sigma-Aldrich), in phosphate buffered saline (PBS) and 0·03% H_2O_2, generating a brown end product. Once rinsed in tap water, sections were mounted in DPX (Sigma-Aldrich). Sections, for which the primary antibodies were omitted, showed no immunomarking (negative controls). Histochemistry of SDH activity was based on incubation of sections in a solution of sodium succinate at pH 7·4 in 0·2 M phosphate buffer. Nitroblue tetrazolium (Sigma-Aldrich) (1 mg ml^{-1}) was added to the solution prior to incubation; made in the dark until the stain developed (usually 120–180 min). Sections were mounted in glycerol gelatine (Sigma-Aldrich).

MORPHOMETRICAL ANALYSIS

The study was carried out using an interactive image analysis system (CAST-Grid version 1.6; Olympus, Tokyo, Japan), working with a live image captured by a CCD-video camera (Sony, Tokyo, Japan). The light microscope (BX50; Olympus) used was equipped with a fully motorized stage (Prior Scientific Inc., Rockland, MA, U.S.A.), thus allowing meander sampling with an (x–y axis) accuracy of 1 μm. For practical purposes, the muscle tissue was operationally divided into two morphologically well-differentiated zones: a main (and innermost) white fibre area and a thinner (and outermost) red fibre-rich area. Relative and absolute morphometric variables of the fish body section and components of interest were estimated as described below.

MUSCLE CROSS-SECTIONAL AREA

The cross-sectional (half) white muscle area [A (*white muscle*)] and red muscle area [A (*red muscle*)] were computed by the software after the operator interactively traced the physical limits of interest in the section. An estimate of the total area [A (*muscle*)] was

made by doubling the computed value (as pilot tests showed no left–right asymmetry differing >1%).

RELATIVE NUMBER (DENSITY) OF MUSCLE FIBRES

In larvae aged 0 and 5 days, all white fibres were directly counted, and the number of white muscle fibres per unit area within the respective muscle zone [N_A (*white*, *muscle*)] was then estimated as follows:

$$N_{\mathrm{A}}(white, muscle) = N(white\ fibres)[A(white\ muscle)]^{-1} \tag{1}$$

In older fish, a direct count was no longer possible due to the larger number of fibres. The number of white muscle fibres per unit area of fish aged 23–180, within the respective muscle zone [N_A (*white*, *muscle*)] was unbiasedly estimated as follows:

$$N_{\mathrm{A}}(white, muscle) = \sum N(white\ fibres)[\mathrm{a}\times\sum P(white\ muscle)]^{-1} \tag{2}$$

where $\sum N$(*white fibres*) is the total number of white fibres counted over all systematically sampled fields in a section, 'a' is the area of the counting frame used (23–40 days: 4,02E + 3 μm^2 and 70–180 days: 1,63E + 04 μm^2) when counting white fibres, and $\sum P$(white muscle) is the sum of the (one) frame associated point hitting the reference space (*i.e.* white muscle) across all sampled fields. In order to avoid the bias due to edge effects, an unbiased counting frame bearing forbidden lines was used (Gundersen, 1977); in accordance, fibres were counted only when they were within the counting frame or touching the inclusion lines, but in both cases not touching the forbidden lines or their extensions.

Except for the older fish, all red fibres were actually counted and the relative number of red muscle fibres was estimated as described above for the white muscle (equation 1). A sampling scheme similar to that defined for the white muscle (equation 2) was made for fish aged 180 days, using an adequate counting frame with an area of 1,63E + 04 μm^2.

When sampling was required for cell counting, such was made in systematically sampled fields and working either with the ×40 (23–40 days) or the ×20 (40–180 days) lens, according to the fish size, the respective final working magnification of the live image in the screen was ×1608 and ×799. Pilot approaches showed that, for the variables measured, the use of ×40 and ×20 lenses on all the fish produced equal results, therefore, the ×20 lens was selected for the sake of practical purposes and high sampling efficiency. After pilot trials, the stepwise stage *x*–*y* movement was defined according to the fish size in order to count the maximum number of fibres per section; the adopted steps varied from 50 to 1000 μm, for sampling white fibres and from 250 to 400 μm, for red fibres.

TOTAL NUMBER OF MUSCLE FIBRES

Except for the fish where all fibres were counted, the total number of (red or white) muscle fibres per cross-section (*N*) was estimated according to general principles for handling ratios and absolute values (Howard & Reed, 1998), namely by combining two previously estimated variable, and by using the following unbiased equation:

$$N(fibres) = N_A(fibres, muscle) \times A(muscle) \tag{3}$$

MEAN CROSS-SECTION FIBRE AREA

The mean individual, white and red, muscle fibre area [$\bar{a}$ (*fibres*)] was derived from *A* (*muscle*) and *N* (*fibres*), using an unbiased variable combination, as follows:

$$\bar{a}(fibres) = A(muscle)N(fibres)^{-1} \tag{4}$$

HYPERPLASIA *V.* HYPERTROPHY

The relative contribution of hypertrophy and hyperplasia to the increase of the cross-sectional area was estimated as follows (Valente *et al.*, 1999):

$$\Delta A(\mu m^2) = N_m \Delta \bar{a}(\mu m^2) + \bar{a}_m(\mu m^2)\Delta N \tag{5}$$

where Δ was calculated between two sampling times (t and $t + 1$) and N_m and $\overline{a}_m$ refer to the mean total number of fibres and fibre area at t.

STATISTICS

Data were analysed using the software Statistica (version 6; StatSoft, Tulsa, OK, U.S.A.). The significance level was set at $\alpha = 0{\cdot}05$. All variables were checked for normality and homogeneity of variance, by using the Kolmogorov–Smirnoff and the Levene tests, respectively. Data were submitted to one-way ANOVA. After a significant ANOVA, pairs of means were compared by the Tukey honest significant difference test. For a particular age, and for each variable, the significance of the differences between rostral and caudal values was compared by the *t*-test for dependent samples, after checking the test assumptions as above. Each variable was plotted against L_T. Pair-wise *t*-tests were used to compare slopes of regression lines (Zar, 1996).

RESULTS

The distinction between white and red muscle was evident at very early stages (Fig. 1). At hatching, a superficial monolayer, lying just beneath the skin and along the lateral line, was clearly demarcated from the underlying white muscle mass. Subsequent growth led to the proliferation of the red monolayer fibres at the region of the lateral line. Pink muscle fibres were first apparent at 40 days, but only at 140 days a distinct muscle layer that would be suitable for morphometric analysis was formed. Because it was not always possible to distinguish between all muscle fibre types, the morphometric variable analysis was carried out for the fast white muscle (including pink muscle fibres) and for slow red muscle (including superficial monolayer fibres).

The mean L_T and the *G* of the fish during the trial are presented in Table I. The L_T increased linearly with age ($r^2 = 0{\cdot}95$). The larger growth rate was observed in the end of larvae life (between 5 and 23 days), and the smaller one was observed in the middle of juvenile life between 100 and 140 days of age (Table I).

FAST WHITE MUSCLE

At both locations, the N_A (*white*, *muscle*) was exponentially and negatively correlated with L_T, while all other morphometric variables measured were

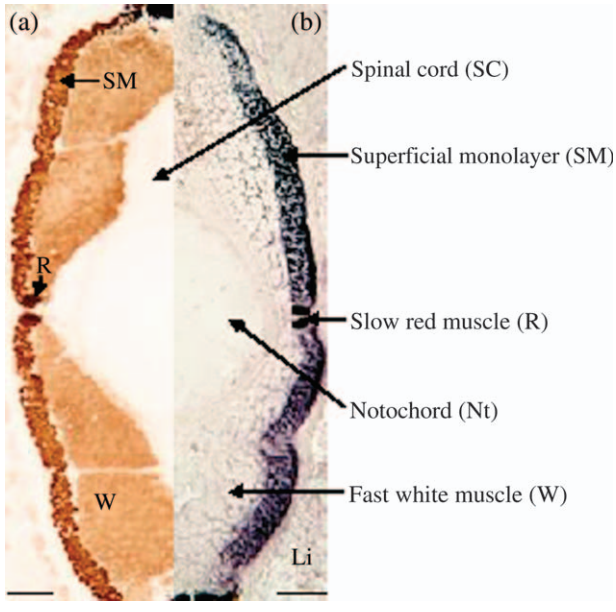

FIG. 1. Transverse sections of lateral muscle of *Pagellus bogaraveo* aged 23 days. (a) Immunostaining with 4–96 (polyclonal anti-fish, slow myosin) and (b) succinate dehydrogenase (SDH). The superficial monolayer fibres showed a strong reaction with 4–96 antibody and displayed strong SDH activities. A small group of red fibres appeared adjacent to the lateral line nerve with a particularly strong reaction with anti-fish slow myosin and SDH activity. Li, liver (used as support tissue when the larvae were snap frozen). Scale bar = 75 μm.

linearly and positively correlated with it. Only for N (*white fibres*) and A (*white muscle*) were differences between the slopes of the respective regression lines detected, with the post-anal level displaying the fastest growth (Table II).

The A (*white muscle*) increased over the larval period (anteriorly and posteriorly), but only significantly ($P < 0{\cdot}05$) from 5 to 23 days (Table III), a period in which the variable increased >10-fold. This increase was mainly due to the hyperplastic mechanism (Table IV). In postlarval life, there was a progressive

TABLE I. Water temperature, total length [L_T; mean (C.V.), $n = 6$] and instantaneous relative growth rate (G), *Pagellus bogaraveo* during the experiment over 180 days

Time (days)	Water temperature (° C)	L_T (mm)	G
0	14·0	3·6 (0·02)^{a}	—
5	16·7	6·0 (0·04)^{ab}	10·3
23	19·4	7·9 (0·002)^{ab}	1·5
40	19·6	12·0 (0·20)^{b}	2·5
70	19·2	23·7 (<0·01)^{c}	2·3
100	21·5	51·0 (0·11)^{d}	2·6
140	19·6	71·2 (0·06)^{e}	0·8
180	19·1	114·5 (0·07)^{f}	1·2

Means without a common superscript lower case letter differ significantly ($P < 0{\cdot}05$).

TABLE II. Relationships between total length (L_T) and total muscle area [A (*white muscle*)], number of fibres per unit area of muscle [N_A (*white, muscle*)], total number of fibres [N (*white fibres*)] and cross-sectional fibre area [$\bar{a}$ (*white fibres*)] in white muscle at post-opercular and post-anal locations in *Pagellus bogaraveo* (for all regressions $P < 0{\cdot}001$)

Variable	Location	Regression	r^2
A (*white muscle*) (μm^2)	Post-opercular	A (*white muscle*) $= -1{,}2E + 07 + 9{,}9E + 06^a \times L_T$	0·88
	Post-anal	A (*white muscle*) $= -1{,}3E + 07 + 1{,}2E + 07^b \times L_T$	0·91
N_A (*white, muscle*) (number mm^{-2})	Post-opercular	N_A (*white, muscle*) $= 6{,}6E + 03 \times e^{(-0{\cdot}32)} \times L_T$	0·75
	Post-anal	N_A (*white, muscle*) $= 7{,}5E + 03 \times e^{(-0{\cdot}33)} \times L_T$	0·69
N (*white fibres*)	Post-opercular	N (*white fibres*) $= -2319 + 3502^a \times L_T$	0·95
	Post-anal	N (*white fibres*) $= -2247 + 4574^b \times L_T$	0·97
$\bar{a}$ (*white fibres*) (μm^2)	Post-opercular	$\bar{a}$ (*white fibres*) $= 72{\cdot}03 + 267{\cdot}29^a \times L_T$	0·90
	Post-anal	$\bar{a}$ (*white fibres*) $= 122{\cdot}93 + 239{\cdot}39^a \times L_T$	0·87

For each variable, slopes followed by the same superscript lower case letters, did not differ significantly from each other ($P > 0{\cdot}05$).

TABLE III. Total muscle area [*A* (*white muscle*)], number of fibres per unit area [N_A (*white*, *muscle*)], total number of fibres [*N* (*white fibres*)] and cross-sectional fibre area [$\overline{a}$ (*white fibres*)] measured in white muscle, in anterior and posterior part of *Pagellus bogaraveo* at different ages (0–180 days)

	Days							
	0	5	23	40	70	100	140	180
Post-opercular								
A (*white muscle*) (μm^2)	9,2E + 03 (0·45)^a	1,0E + 04 (0·19)^a	1,1E + 05 (0·33)^b	4,9E + 05 (0·47)^c	6,0E + 06 (0·33)^d	3,0E + 07 (0·28)^e	3,1E + 07 (0·18)^e	1,2E + 08 (0·14)^f
N_A (*white*, *muscle*) (number mm^{-2})	1,3E + 04 (0·24)^a	1,1E + 04 (0·09)^a	5,2E + 03 (0·13)^b	5,0E + 03 (0·27)^b	1,0E + 03 (0·09)^c	5,1E + 02 (0·25)^d	5,9E + 02 (0·03)^d	3,3E + 02 (0·08)^e
N (*white fibres*)	109 (0·13)^a	117 (0·19)^a	563 (0·30)^b	2210 (0·24)^c	6159 (0·30)^d	14 645 (0·27)^e	18 724 (0·21)^e	40 401 (0·13)^f
$\overline{a}$ (*white fibres*) (μm^2)	82 (0·30)^a	88 (0·10)^a	194 (0·13)^b	215 (0·29)^b	979 (0·09)^c	2065 (0·22)^d	1685 (0·03)^d	3015 (0·08)^e
Post-anal								
A (*white muscle*) (μm^2)	1,0E + 04 (0·26)^a	1,2E + 04 (0·14)^a	1,7E + 05 (0·30)^b	1,1E + 06 (0·33)^c	1,0E + 07 (0·43)^d	3,7E + 07 (0·23)^e	4,5E + 07 (0·16)^e	1,4E + 08 (0·13)^f
N_A (*white*, *muscle*) (number mm^{-2})	1,9E + 04 (0·3)^a	1,5E + 04 (0·13)^a	6,7E + 03 (0·13)^b	4,4E + 03 (0·31)^b	7,7E + 02 (0·08)^c	5,8E + 02 (0·09)^c	6,4E + 02 (0·06)^c	3,6E + 02 (0·09)^c
N (*white fibres*)	178 (0·05)^a	181 (0·13)^a	1146 (0·27)^b	4376 (0·12)^c	7832 (0·35)^d	21 422 (0·22)^e	28 547 (0·13)^e	50 901 (0·18)^f
$\overline{a}$ (*white fibres*) (μm^2)	56 (0·26)^a	66 (0·13)^a	152 (0·12)^b	245 (0·25)^b	1308 (0·08)^c	1725 (0·10)^d	1575 (0·06)^d	2794 (0·11)^e

Values are presented as means (C.V.), $n = 6$ per group. Within a row means without a common superscript lower case letter differ significantly ($P < 0·05$).

TABLE IV. Relative contribution of hyperplasia to white and red muscle growth of *Pagellus bogaraveo* at two different body locations

		Age period (days)						
Hyperplasia (%)	Location	0–5	5–23	23–40	40–70	70–100	100–140	140–180
White muscle	Post-opercular	50	76	97	33	55	100	59
	Post-anal	9	80	82	15	84	100	50
Red muscle	Post-opercular	29	45	98	40	23	78	35
	Post-anal	97	37	96	41	30	100	37

increase in *A* (*white muscle*), cranially and caudally, as the age increased (Table III). The *A* (*white muscle*) increased rapidly from 23 to 100 days ($P < 0{\cdot}05$), the 40 to 70 days period being the time where that increase was greater (*c.* 10-fold) (Table III). Such *A* (*white muscle*) increase then slowed down from 100 to 140 days ($P > 0{\cdot}05$), and rose again rapidly afterwards (three-fold rise from 140 to 180 days) ($P < 0{\cdot}05$) (Table III). At these stages, and at both body locations, with the exception of the period from 40 to 70 days, the main postlarval mechanism underlying the enlargement of *A* (*white muscle*) was hyperplasia (Table IV).

As to the N_A (*white*, *muscle*), no significant differences ($P > 0{\cdot}05$) were found between 0 and 5 days. The N_A (*white*, *muscle*) decreased 2·3-fold from 5 to 23 days ($P < 0{\cdot}05$) (Table III). In general, in postlarval life the N_A (*white*, *muscle*) decreased with age (Table III). No significant N_A (*white*, *muscle*) decrease ($P > 0{\cdot}05$), however, was observed from 23 to 40 and from 100 to 140 day periods, at the post-opercular location, and from 23 to 40 and 70 to 180 day periods at post-anal location (Table III).

The *N* (*white fibres*) did not increase ($P > 0{\cdot}05$) in the first 5 days post-hatch. By contrast, from 5 to 23 days the *N* (*white fibres*) increased rapidly ($P < 0{\cdot}05$), from 117 to 563 fibres and from 181 to 1146 fibres, at post-opercular and post-anal positions, respectively (Table III). There was a progressive increase in the *N* (*white fibres*) from 23 to 100 days ($P < 0{\cdot}05$), which slowed down from 100 to 140 days ($P > 0{\cdot}05$) and rapidly rose again (two-fold) from 140 to 180 days ($P < 0{\cdot}05$) (Table III).

The $\overline{a}$ (*white fibres*), reflecting hypertrophic growth, increased gradually over the larval period from 82 to 194 μm^2 at the post-opercular level and from 56 to 152 μm^2 at post-anal level. The $\overline{a}$ (*white fibres*) increased only modestly ($P > 0{\cdot}05$) from hatching to 5 days and doubled between 5 and 23 days ($P < 0{\cdot}05$) (Table III). During the postlarval life, the $\overline{a}$ (*white fibres*) rose from *c.* 194 to *c.* 3015 μm^2 at post-opercular level and from *c.* 152 to *c.* 2795 μm^2 at post-anal level (Table III). No significant increase, however, was observed in $\overline{a}$ (*white fibres*) in the beginning (23–40 days) and in the middle (100–140 days) of postlarval life ($P > 0{\cdot}05$) (Table III).

When comparing both muscle locations, at hatching, a larger *N* (*white fibres*) ($P < 0{\cdot}05$) was observed caudally. At 5 days, the *N* (*white fibres*) continued to be larger ($P < 0{\cdot}05$) at the post-anal level, but the fibres had now a smaller $\overline{a}$ (*white fibres*) ($P < 0{\cdot}05$). At this age the N_A (*white muscle*) was higher at the post-anal level ($P < 0{\cdot}05$). At the end of larval life (23 days), the differences

between locations were the same as the ones observed at 5 days, and now the *A* (*white muscle*) was also larger ($P < 0{\cdot}05$) caudally. Overall, from 40 to 140 days the *N* (*white fibres*) and the *A* (*white muscle*) were the only morphometric variables measured that were larger at the post-anal level ($P < 0{\cdot}05$). No significant differences ($P > 0{\cdot}05$) between locations were found at the end of the study (180 days).

SLOW RED MUSCLE

The *A* (*red muscle*) and N_A (*red*, *muscle*) were exponentially correlated with L_T at both muscle locations, positively and negatively, in this order. The other two morphometric variables measured, however, grew linearly and positively with L_T. The slope of the *N* (*red fibres*) regression line was higher at the post-anal level (Table V).

The *A* (*red muscle*) increased with age at both locations. Nonetheless, that increase was not significant from 0 to 5 or from 100 to 140 days ($P > 0{\cdot}05$) (Table VI). As shown in Table IV, the main contributor to growth, as evaluated by the increase in *A* (*red muscle*), in the above time periods and from 23 to 40 days, at post-anal level, was the hyperplastic mechanism, whereas in the other periods it was the hypertrophic one. The latter was true for the post-opercular level, except for the endogenous feeding period (0–5 days) where the *A* (*red muscle*) grew mainly by fibre hypertrophy.

The red slow muscle showed an exponential decrease in N_A (*red*, *muscle*) with age (Table V). Nevertheless, no significant differences ($P > 0{\cdot}05$) were found at both muscle locations in the following day periods: 0–5, 23–40 and 100–140.

As for the white muscle, the *N* (*red fibres*) increased with age. The *N* (*red fibres*) remained, however, fairly stable (Table VI) ($P > 0{\cdot}05$) in the first 5 days of life and in the middle of postlarval life (from 100 to 140 days).

The $\overline{a}$ (*red fibres*) was initially 10 μm^2 at both locations; gradually rising at 100 days to 454 and 531 μm^2, at post-opercular and post-anal levels,

TABLE V. Relationships between total length (L_T) and total muscle area [*A* (*red muscle*)], number of fibres per unit area of muscle [N_A (*red*, *muscle*)], total number of fibres [*N* (*red fibres*)] and cross-sectional fibre area [$\overline{a}$ (*red fibres*)] in red muscle at post-opercular and post-anal locations in *Pagellus bogaraveo* (for all lines $P < 0{\cdot}001$)

Variable	Location	Line	r^2
A (*red muscle*) (μm^2)	Post-opercular	$A = 1{,}6E+03 \times e^{(0{\cdot}81) \times L}$	0·80
	Post-anal	$A = 2{,}5E+03 \times e^{(0{\cdot}82) \times L}$	0·79
N_A (*red*, *muscle*) (number mm^{-2})	Post-opercular	$N_A = 5{,}4E+04 \times e^{(-0{\cdot}42) \times L}$	0·83
	Post-anal	$N_A = 4{,}8E+04 \times e^{(-0{\cdot}40) \times L}$	0·79
N (*red fibres*)	Post-opercular	$N = -42 + 286^a \times L$	0·93
	Post-anal	$N = -288 + 545^b \times L$	0·91
$\overline{a}$ (*red fibres*) (μm^2)	Post-opercular	$\overline{a} = -63{\cdot}88 + 98{\cdot}64^a \times L$	0·90
	Post-anal	$\overline{a} = -45{\cdot}79 + 91{\cdot}44^a \times L$	0·90

For each variable, slopes followed by the same superscript lower case letters, did not differ significantly from each other ($P > 0{\cdot}05$).

TABLE VI. Total muscle area [*A* (*red muscle*)], number of fibres per unit area [N_A (*red*, *muscle*)], total number of fibres [*N* (*red fibres*)] and cross-sectional fibre area [$\overline{a}$ (*red fibres*)] measured in red muscle, in anterior and posterior part of *Pagellus bogaraveo* at different ages (0 to 180 days)

	Days							
	0	5	23	40	70	100	140	180
Post-opercular								
A (*red muscle*) (μm^2)	3,7E + 02 (0·18)[a]	4,9E + 02 (0·23)[a]	2,1E + 03 (0·15)[b]	6,0E + 03 (0·10)[c]	1,7E + 05 (0·24)[d]	6,4E + 05 (0·13)[e]	8,4E + 05 (0·14)[e]	3,8E + 06 (0·21)[f]
N_A (*red*, *muscle*) (number mm^{-2})	1,1E + 05 (0·13)[a]	8,7E + 04 (0·17)[a]	3,9E + 04 (0·15)[b]	3,8E + 04 (0·10)[b]	6,1E + 03 (0·15)[c]	2,4E + 03 (0·28)[d]	2,1E + 03 (0·15)[d]	8,8E + 02 (0·19)[e]
N (*red fibres*)	38 (0·13)[a]	42 (0·18)[a]	82 (0·06)[b]	226 (0·07)[c]	1000 (0·21)[d]	1507 (0·27)[e]	1815 (0·25)[e]	3231 (0·15)[f]
$\overline{a}$ (*red fibres*) (μm^2)	10 (0·12)[a]	12 (0·17)[a]	26 (0·15)[b]	27 (0·09)[b]	167 (0·14)[c]	454 (0·27)[d]	481 (0·16)[d]	1171 (0·17)[e]
Post-anal								
A (*red muscle*) (μm^2)	4,7E + 02 (0·31)[a]	6,4E + 02 (0·38)[a]	5,0E + 03 (0·29)[b]	1,2E + 04 (0·38)[c]	2,0E + 05 (0·32)[d]	1,2E + 06 (0·24)[e]	1,3E + 06 (0·17)[e]	6,6E + 06 (0·31)[f]
N_A (*red, muscle*) (number mm^{-2})	1,0E + 05 (0·24)[a]	1,0E + 05 (0·19)[a]	3,0E + 04 (0·21)[b]	2,9E + 04 (0·26)[b]	6,1E + 03 (0·10)[c]	1,9E + 03 (0·10)[d]	2,6E + 03 (0·24)[d]	9,4E + 02 (0·16)[e]
N (*red fibres*)	47 (0·30)[a]	61 (0·32)[a]	146 (0·15)[b]	341 (0·31)[c]	1177 (0·22)[d]	2324 (0·18)[e]	3174 (0·23)[e]	6223 (0·30)[f]
$\overline{a}$ (*red fibres*) (μm^2)	10 (0·28)[a]	10 (0·22)[a]	34 (0·22)[b]	36 (0·24)[b]	164 (0·10)[c]	531 (0·09)[d]	414 (0·29)[d]	1084 (0·17)[e]

Values are presented as means (C.V.), $n = 6$ per group. Within a row means without a common superscript lower case letter differ significantly ($P < 0·05$).

respectively (Table VI). This increase, however, was not significant until fish mouth opening (5 days). Between 100 and 140 days, the $\overline{a}$ (*red fibre*) did not differ significantly ($P > 0{\cdot}05$). In small juveniles, the $\overline{a}$ (*red fibres*) rose again to reach *c.* 1100 μm^2 at 180 days at both locations ($P < 0{\cdot}05$) (Table VI).

At hatching, no differences between locations were found for all morphometric variables. At 5 days after hatch, a larger *N* (*red fibres*) existed caudally. In postlarval life, a higher *N* (*red fibres*) and *A* (*red muscle*) of the caudal red fibres were the consistent features. No differences were found, however, between both muscle locations at 70 days.

DISCUSSION

Changes in muscle fibre number during growth have only been determined in a relatively few species including: *Oncorhynchus mykiss* (Walbaum) (Stickland, 1983; Valente *et al.*, 1999), *Cyprinus carpio* L. (Koumans *et al.*, 1994), *S. aurata* (Rowlerson *et al.*, 1995) and *Clupea harengus* L. (Johnston *et al.*, 1998). Thus, this study is one of the few aimed at investigating the muscle recruitment between the hatching and beginning of the juvenile fish life. The application of this research lies in the understanding of the growth process of *P. bogaraveo*, a species new for aquaculture.

GROWTH MECHANISMS IN LARVAE

The morphometric data of both fibre types indicated that during the larval period *P. bogaraveo* muscle grew mainly by recruitment of new muscle fibres (hyperplasia). A different situation was found in *C. harengus* larva in which growth from 8 to 16 mm L_T involved a three-fold increase in muscle cross-sectional area largely due to the hypertrophy of the embryonic red and white muscle fibres (Johnston *et al.*, 1998). Five days after hatching, at the end of the endogenous feeding period, slow growth occurring by hyperplasia and hypertrophy of both fibre types was observed. A pause in hyperplasia also occurred in the first few days after hatching in some other species (Johnston, 1993; Gibson & Johnston, 1995; Johnston *et al.*, 1998; Galloway *et al.*, 1999; Veggetti *et al.*, 1999). The differences found among species in the duration of that pause, however, are not similar and may be related to variations in egg quality including the amount of yolk, the amino acid content and the concentration of maternal growth factors (Johnston *et al.*, 1998). Between the end of the endogenous feeding period (5 days) and the end of the larval period (23 days), both a gradual hypertrophy, of red and white fibres already present at hatching and an intense hyperplastic phase, mainly of the white fibres were detected. It is believed that the observed increase of the number of fibres corresponded to the stratified hyperplastic growth, also observed in *S. aurata* (Rowlerson *et al.*, 1995).

POSTLARVAL GROWTH MECHANISMS

In the juvenile stages, white muscle growth occurred simultaneously by hypertrophy, and by marked hyperplasia in both muscle locations. Also in

Salmo salar L. juveniles, Higgins & Thorpe (1990) reported a higher proportion of small diameter muscle fibres during the late summer when fish were growing at their fastest rate, consistent with increased muscle fibre recruitment. Between 70 and 100 days, a distinct hyperplastic process started up in the fast white muscle, resulting in a three-fold increase in the total number of fibres over that period. This was reflected in a wide range of fibre diameters displaying the mosaic appearance shown in transverse sections. This hyperplastic mosaic growth confirmed the result obtained with *P. bogaraveo*, in a previous histochemical and immunostaining study (Rowlerson *et al.*, 2004; Silva *et al.*, 2008) in which, at this age, new small fibres in the white muscle, which differed from the large diameter ones only in their ATPase reactivity, were observed. The relative timing of the mosaic hyperplastic processes in relation to the life cycle varies amongst species (Romanello *et al.*, 1987; Veggetti *et al.*, 1990; Brooks & Johnston, 1993; Mascarello *et al.*, 1995; Stoiber & Sänger, 1996; Johnston *et al.*, 1998; Veggetti *et al.*, 1999). Mosaic hyperplasia resulted in a large increase in the total fibre number during juvenile growth, being, therefore, very important for commercial aquaculture. According to the mathematical formula used to obtain a general idea of the contributions of hyperplasia and hypertrophy to the muscle area (Valente *et al.*, 1999), in the middle of the juvenile life, the white muscle area grew mainly by hyperplasia being the one and only mechanism between 100 and 140 days. The hyperplasia, however, was not enough to produce statistically significant differences in the fibre number in the above-mentioned period. This fact seems to be related to the thermal environment experienced by *P. bogaraveo*, during ontogeny. Seasonal changes in growth rate are apparent in many fishes, with water temperature the major factor. At temperatures below the optimum range, metabolic rate slows down. Lower metabolism means reduced feed intake and slower growth (Jobling, 1997). In the present study, it was verified that growth rate slowed down between 100 and 140 days when rearing temperatures fell from 21·5 to 19·6° C. The growth rate decrease observed in this period could explain the pronounced negative influence of the lower temperature on muscular growth and the consequent absence of significant differences in the number and the size of muscle fibres between those ages.

As a result of the hyperplastic growth, at the juvenile stages, the mean white fibre area increased modestly from the end of larval life to 40 days; increased intensely (*c.* five-fold) between 40 and 70 days, when the very small-area fibres disappeared; and then underwent a small change after 70 days when those fibres reappeared. By 180 days, at the end of this study, neither hyperplastic nor hypertrophic growth had ceased. Further studies will be needed to confirm the persistence of hypertrophic growth throughout juvenile life up to adult stages, even after hyperplastic growth had ceased, as described for a variety of fishes, including *O. mykiss* (Weatherley *et al.*, 1980; Kiessling *et al.*, 1991), *C. carpio* (Koumans *et al.*, 1993), *Dicentrarchus labrax* (L.) (Veggetti *et al.*, 1990), *S. aurata* (Rowlerson *et al.*, 1995) and *Anguilla anguilla* (L.) (Romanello *et al.*, 1987).

As to the red muscle growth in *P. bogaraveo* juveniles, it seemed to be due to hypertrophy rather than to hyperplasia, as shown by a progressive increase in mean fibre area and in the proportion values of hypertrophy contribution to

red muscle area. In *O. mykiss*, the proportional increase in fibre number was also lower for red than for white fibres with recruitment stopping at significantly shorter L_T (Stickland, 1983).

Overall, it will how be possible to better understand how the factors that influence growth mechanisms in *P. bogaraveo* juveniles can be exploited for hyperplasia without detriment to hypertrophic growth.

MUSCLE LOCATION INFLUENCES

As far as is known, the present work is the first to ever investigate muscle cellularity of fishes considering two body locations (caudal and cranial) during growth. Another study with *D. labrax* also compared the cellularity in the anterior and posterior part of the fish but only in commercial-sized samplings (Abdel *et al.*, 2005). It has been here demonstrated that muscle growth dynamics were the same in the cranial and caudal parts of the fish, and that, overall, a greater total fibre number and muscle area existed at post-anal level, for both muscle types. Additionally, and as observed in other species (Sänger & Stoiber, 2001), the proportion of the cross-sectional area of the myotomal muscle comprised of white muscle was greater in the anterior part of the fish. The mechanistic reason for *P. bogaraveo* muscle displaying a different cellularity between the two body locations is not yet clear, but the data strongly support there are different regulatory mechanisms for establishing the total number of muscle fibres, according to location.

For the first time the muscle fibre growth kinetics of larvae, fry and juvenile of *P. bogaraveo* have been characterized. Growth throughout the various stages resulted from both hypertrophy and hyperplasia of muscle fibres. The N (*fibres*) and the $\overline{a}$ (*fibres*) of both fibre types were linearly and positively correlated with L_T. At 70–100 days, the white mosaic hyperplastic growth type was already underway as shown by the increase in fibre number and appearance of many very small fibres. Overall, hyperplasia provided the main relative contribution to the increase of white muscle cross-sectional area, but the red muscle area mainly benefited from hypertrophy, with both phenomena occurring at a faster pace posteriorly in the body. Therefore, it was proved that cranial–caudal differences existed in fibre cellularity, with a consistently greater number of fibres and muscle area being found at the caudal level, for both fibre types. The existence of seasonal cycles of muscle recruitment found in this study may be important to the design of feeding regimes and diets for optimizing production and minimizing environmental effects and feeding costs. In fact, the chance of using larval growth conditions to potentiate the mosaic hyperplastic growth phase is very attractive because a higher recruitment of fibres endows the fish with the potential to accomplish further growth by fibre enlargement. This has immediate practical interest as it brings the fish to commercial size. The economic goal of further research on *P. bogaraveo* muscle growth will be the study of factors of potential relevance to fish farming (*e.g.* photoperiod, exercise, diet composition and feeding regimes) in the fibre recruitment and hypertrophy.

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References

Abdel, I., López-Albors, O., Ayala, M. D., García-Alcazar, A., Abellán, E., Latorre, R. & Gil, F. (2005). Muscle cellularity at cranial and caudal levels of the trunk musculature of commercial size sea bass, *Dicentrarchus labrax* (Linnaeus, 1758). *Anatomia, Histologia, Embryologia: Journal of Veterinary Medicine Series C* **34,** 280–285. doi: 10.1111/j.1439-0264.2005.00613.x

Brooks, S. & Johnston, I. A. (1993). Influence of development and rearing temperature on the distribution, ultrastructure and myosin sub-unit composition of myotomal muscle-fibre types in the plaice *Pleuronectes platessa*. *Marine Biology* **117,** 501–513.

Forsythe, J. W. & Van Heukelen, W. F. (1987). Growth. In *Cephalopod Life Cycles, Comparative Reviews* (Boyle, P. R., ed.), pp. 135–156. London: Academic Press.

Galloway, T. F., Korsvik, E. & Kryvi, H. (1999). Muscle growth and development in Atlantic cod larvae (*Gadus morhua* L.) related to different somatic growth rates. *Journal of Experimental Biology* **202,** 2111–2120.

Gibson, S. & Johnston, I. A. (1995). Scaling relationships, individual variation and the influence of temperature on maximum swimming performance in newly settled turbot *Scophthalmus maximus*. *Marine Biology* **121,** 401–408.

Gundersen, H. J. G. (1977). Notes on the estimation of the numerical density of arbitrary profiles: the edge effect. *Journal of Microscopy* **111,** 21–23.

Higgins, P. J. & Thorpe, J. E. (1990). Hyperplasia and hypertrophy in the growth of skeletal muscle in juvenile Atlantic salmon, *Salmo salar* L. *Journal of Fish Biology* **37,** 505–519. doi: 10.1111/j.1095-8649.1990.tb05884.x

Howard, C. V. & Reed, M. G. (1998). *Unbiased Stereology. Three-dimensional Measurements in Microscopy*. Oxford: Bios Scientific Publishers.

Jobling, M. (1997). Temperature and growth: modulation of growth rate via temperature change. In *Global Warming: Implications for Freshwater and Marine Fish* (Wood, C. M. & McDonald, D. G., eds), pp. 225–252. Cambridge: Cambridge University Press.

Johnston, I. A. (1993). Temperature influences muscle differentiation and the relative timing of organogenesis in herring (*Clupea harengus*) larvae. *Marine Biology* **116,** 363–379.

Johnston, I. A. (1999). Muscle development and growth: potential implications for flesh quality in fish. *Aquaculture* **177,** 99–115.

Johnston, I. A., Cole, N. J., Abercromby, M. & Vieira, V. L. A. (1998). Embryonic temperature modulates muscle growth characteristics in larval and juvenile herring. *Journal of Experimental Biology* **201,** 623–646.

Kiessling, A., Storebakken, T., Asgard, T. & Kiessling, K.-H. (1991). Changes in the structure and function of the epaxial muscle of the rainbow trout (*Oncorhynchus mykiss*) in relation to ration and age. I-Growth dynamics. *Aquaculture* **93,** 335–356.

Koumans, J. T. M. & Akster, H. A. (1995). Myogenic cells in development and growth of fish. *Comparative Biochemistry Physiology A* **110,** 3–20.

Koumans, J. T. M., Akster, H. A., Booms, G. H. R. & Osse, J. W. M. (1993). Growth of carp (*Cyprinus carpio*) white axial muscle; hyperplasia and hypertrophy in relation to the myonucleus/sarcoplasm ratio and the occurrence of different subclasses of myogenic cells. *Journal of Fish Biology* **43,** 69–80. doi: 10.1111/j.1095-8649.1993.tb00411.x

Koumans, J. T. M., Akster, H. A., Witkam, A. & Osse, J. W. M. (1994). Numbers of muscle nuclei and myosatellite cell nuclei in red and white axial muscle during growth of the carp (*Cyprinus carpio*). *Journal of Fish Biology* **44,** 391–408. doi: 10.1111/j.1095-8649.1994.tb01220.x

Mascarello, F., Rowlerson, A., Radaelli, P. & Veggetti, A. (1995). Differentiation and growth of muscle in the fish *Sparus aurata* (L): I. Myosin expression and organization of fibre types in lateral muscle from hatching to adult. *Journal of Muscle Research and Cell Motility* **16,** 213–222.

Romanello, M. G., Scapolo, P. A., Luprano, S. & Mascarello, F. (1987). Post-larval growth in the lateral white muscle of the eel, *Anguilla anguilla*. *Journal of Fish Biology* **30,** 161–172. doi: 10.1111/j.1095-8649.1987.tb05742.x

Rowlerson, A. & Veggetti, A. (2001). Cellular mechanisms of post-embryonic muscle growth in aquaculture species. In *Fish Physiology*, Vol. 18 (Johnston, I. A., ed.), pp. 103–140. San Diego, CA: Academic Press.

Rowlerson, A., Scapolo, P. A., Mascarello, F., Carpenè, E. & Veggetti, A. (1985). Comparative study of myosin present in lateral muscle of some fish: species variations in myosin isoforms and their distribution in red, pink and white muscle. *Journal of Muscle Research and Cell Motility* **6,** 601–640.

Rowlerson, A., Mascarello, F., Radaelli, G. & Veggetti, A. (1995). Differentiation and growth of muscle in the fish *Sparus aurata* (L): II. Hyperplastic and hypertrophic growth of lateral muscle from hatching to adult. *Journal of Muscle Research and Cell Motility* **16,** 223–236.

Rowlerson, A. M., Silva, P., Rocha, E., Olmedo, M. & Valente, L. M. P. (2004). Muscle development in cultured blackspot seabream *Pagellus bogaraveo*: preliminary histochemical and immunohistochemical data on the fibre types. Poster abstract. *Journal of Fish Biology* **65** (Suppl. A), 334. doi: 10.1111/j.0022-1112.2004.559at.x

Sänger, A. M. & Stoiber, W. (2001). Muscle fibre diversity and plasticity. In *Fish Physiology*, Vol. 18 (Johnston, I. A., ed.), pp. 187–250. San Diego, CA: Academic Press.

Scapolo, P. A., Veggetti, A., Mascarello, F. & Romanello, M. G. (1988). Development transitions of myosin isoforms and organisation of the lateral muscle in the teleost *Dicentrarchus labrax* (L.). *Anatomy, Histology and Embryology* **178,** 287–295.

Silva, P., Rowlerson, A. M., Valente, L. M. P., Olmedo, M., Monteiro, R. A. F. & Rocha, E. (2008). Muscle differentiation and growth in blackspot seabream (*Pagellus bogaraveo*, Brunnich): histochemical and immunohistochemical study of the fibre types. *Tissue & Cell* **40,** 447–458. doi: 10.1016/j.tice.2008.05.001

Stickland, N. C. (1983). Growth and development of muscle fibres in rainbow trout (*Salmo gairdneri*). *Journal of Anatomy* **137,** 323–333.

Stoiber, W. & Sänger, A. M. (1996). An electron microscopic investigation into the possible source of new muscle fibres in teleost fish. *Anatomy and Embryology* **194,** 569–579.

Valente, L. M. P., Rocha, E., Gomes, E. F. S., Silva, M. W., Oliveira, M. H., Monteiro, R. A. F. & Fauconneau, B. (1999). Growth dynamics of white and red muscle fibres in fast and slow growing strains of rainbow trout. *Journal of Fish Biology* **55,** 675–691. doi: 10.1111/j.1095-8649.1999.tb00710.x

Veggetti, A., Mascarello, F., Scapolo, P. A. & Rowlerson, A. (1990). Hyperplastic and hypertrophic growth of lateral muscle in *Dicentrarchus labrax* (L.). An ultrastructural and morphometric study. *Anatomy and Embryology* **182,** 1–10.

Veggetti, A., Mascarello, F., Scapolo, P. A., Rowlerson, A. & Carnevali, C. M. D. (1993). Muscle growth and myosin isoform transitions during development of a small teleost fish, *Poecilia reticulata* (Peters) (Atheriniformes, Poeciliidae): a histochemical, immunohistochemical, ultrastructural and morphometric study. *Anatomy, Histology and Embryology* **187,** 353–361.

Veggetti, A., Rowlerson, A., Radaelli, G., Arrighi, S. & Domeneghini, C. (1999). Posthatching development of the gut and lateral muscle in the sole, *Solea solea* (L.). *Journal of Fish Biology* **55** (Suppl. A), 44–65. doi: 10.1111/j.1095-8649.1999.tb01045.x

Weatherley, A. H., Gill, H. S. & Rogers, S. C. (1979). Growth dynamics of muscle fibres, dry weight, and condition in relation to somatic growth rate in yearling rainbow trout (*Salmo gairdneri*). *Canadian Journal of Zoology* **57,** 2385–2392.

Weatherley, A. H., Gill, H. S. & Rogers, S. C. (1980). The relationship between mosaic muscle fibres and size in rainbow trout (*Salmo gairdneri*). *Journal of Fish Biology* **17,** 603–610. doi: 10.1111/j.1095-8649.1980.tb02792.x

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. *Journal of Fish Biology* **33,** 851–859. doi: 10.1111/j.1095-8649.1988.tb05532.x

Zar, J. H. (1996). *Biostatistical Analysis*. London: Prentice-Hall International.