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Early temperature effects on muscle growth dynamics and histochemical profile of muscle fibres of sea bass *Dicentrarchus labrax* L., during larval and juvenile stages

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Abstract

Recently, it has been found that the thermal experience during the earliest phases of development could determine the larval and postlarval growth characteristics of teleosts. In order to investigate the effects of the early temperature regime on the advanced stages of growth of sea bass, *Dicentrarchus labrax* L., this species was reared during the vitelline phase at two temperatures: natural temperature ($\cong 15$ °C) and 17.7 ± 0.1 °C, and then larvae transferred to common temperature (natural temperature). Muscle growth was studied by morphometric and histochemical techniques (mATPase and NADH-TR). Body length and body mass were also measured. During the vitelline phase, muscle growth was similar in both experimental groups, but at 25 days, both hypertrophy and hyperplasia of white muscle fibres were greater in the prewarmed group ($p < 0.05$). At the end of metamorphosis (80 days) and at 120 days, the average diameter of white muscle fibres, as well as the body length, were greater in the prewarmed group ($p < 0.05$), but the number of white fibres did not differ significantly between groups. The morphological mosaic of white muscle fibres was observed at the end of metamorphosis, and the histochemical mosaic appeared gradually since the early postlarval stages. Thus, at 120 days, some specimens in both experimental groups showed three or four different mATPase staining white fibres: low (L), moderate (M), high (H) and/or very high (vH), whereas in other specimens, only L or M mATPase activity fibres were observed. Early *T* influenced the histochemical maturity of the

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white muscle. By 120 days, the proportion of H and vH fibres was greater in the prewarmed group ($p=0.027$; $p=0.051$, respectively). By 154 days, the four mATPase staining profiles (L, M, H, vH) were observed in all specimens of both groups, but the proportion of vH fibres was still higher in the prewarmed group. By 188 days, 3774 °C-day for the prewarmed group and 3759 °C-day for the natural temperature group, only slight differences between groups were observed in the histochemical properties and no differences were found in the number and size of white muscle fibres, neither in the body length and body mass of specimens.

In conclusion, a slight increase of temperature during the vitelline phase of sea bass increased the muscle growth and body length of fish in subsequent larval and postlarval stages, and advanced the histochemical maturity of the white muscle.

Keywords: Sea bass; Fish; Muscle growth; Temperature; Histochemistry; mATPase

1. Introduction

In different teleost species, embryonic temperature influences muscle cellularity at hatching and/or in subsequent stages of development (herring, *Clupea harengus*, Vieira and Johnston, 1992; Johnston et al., 1995; salmon, *Salmo salar*, Stickland et al., 1988; Usher et al., 1994; Nathanailides et al., 1995a; sea bass, *Dicentrarchus labrax* L., Ayala et al., 2000, 2001). Particularly, in sea bass, the use of a slightly higher temperature during its short incubation period had a positive effect on the larval muscle growth, which was observed after 37–50 days posthatching (Ayala et al., 2000). The effect of the incubation temperature was also compared in two genetically different stocks (Atlantic and Mediterranean) of sea bass larvae (Ayala et al., 2001). A common result in both stocks was that higher incubation temperature had a positive effect on muscle growth at the end of metamorphosis, thus, the total myotomal area was higher in larvae incubated at 17 °C than at 15 °C. In these previous studies on sea bass, the temperature increase was maintained during the embryonic phase, being larvae transferred to a natural regime of temperature after hatching. Also, the long-term effect of the embryonic temperature increase was only studied until the beginning of the postlarval phase (90–120 days). On the basis of these previous results, one of the aims of this study was to find out the relevance of the use of a longer period of early temperature increase and the extension of sampling to a more advanced juvenile stage. These topics have been investigated in the present work by a temperature increase maintained until mouth opening (5–6 days posthatching), that coincides with reabsortion of yolk sac and a more advanced development of muscle fibres. In addition, we have extended sampling until 188 days.

In most teleost species, the myotomal organization of the axial musculature is commonly stratified in three layers: one located superficially (red muscle), another deeply (white muscle) and a third between the other two layers (intermediate or pink muscle), which varies between species, as regards extension and histochemical properties (Van Raamsdonk et al., 1974; Rowlerson et al., 1985; Mascarello et al., 1986; Romanello et al., 1987; Sänger et al., 1988; Scapolo et al., 1988; Ibabe et al., 2000). In many teleosts, the expression of adult myosin isoforms of slow-red, pink and fast-white

muscles is preceded during development by "embryonic" and "larval" isoforms (zebra fish, *Brachidanio rerio*, Van Raamsdonk et al., 1978; sea bass, Scapolo et al., 1988; herring, Crockford and Johnston, 1993; guppy, *Poecilia reticulata*, Veggetti et al., 1993). All these isoforms can be identified by their histo- and immunohistochemical profiles and are useful markers of the developmental stage reached by individual fibres (Veggetti et al., 1993). In sea bass, Scapolo et al. (1988) observed a sequence of developmental transitions of myosin isoforms in red, pink and white muscles during larval and postlarval growth. From about 80 days onward, the deep white muscle acquired the morphological mosaic appearance seen in transverse sections, and by 20 months, a histochemical mATPase mosaic was also observed. Ramírez-Zarzosa et al. (1995, 1998) and López-Albors et al. (1998) also studied fibre-type differentiation in the lateral musculature of sea bass larvae and postlarvae. The morphological mosaic of the white muscle was described from the end of the larval period, as reported by Scapolo et al. (1988), but the histochemical mATPase mosaic was defined at earlier stages of juvenile development (4–5 months).

The timing of the histochemical mATPase mosaic is an indicator of myotomal maturity and a consequence of postlarval muscle hyperplasia (Carpenè and Veggetti, 1981; Akster, 1983; Stickland, 1983; Romanello et al., 1987; Scapolo et al., 1988; Mascarello et al., 1995; Ramírez-Zarzosa et al., 1995). Temperature influences larval and postlarval muscle fibre hypertrophy and hyperplasia (Nathanailides et al., 1996; Johnston et al., 1996, 1998), and hence could induce changes in the myosin isoforms of muscle fibres (Gerlach et al., 1990), and consequently in their mATPase histochemical activity. The effect of the rearing temperature on the histochemical properties of muscle fibres was studied by Nathanailides et al. (1995b) in larvae and postlarvae of carp. The profile of acidstable mATPase activity of the white muscle fibres varied in fish reared at different temperatures. However, it has not been studied yet whether the early thermal experience (vitelline phase) of embryos and prelarvae, additionally to an effect on larval and postlarval muscle cellularity, could influence the histochemical profile of muscle fibres in these stages.

Sea bass has a relatively rapid growth and highly commercial value but early temperature effects on muscle growth and development are not well known on this species. In order to gain a better understanding of the early temperature effects on advanced stages of growth and development of this species, in the present study, a slight increase of temperature (2.7 ± 0.1 °C) was applied to embryos and prelarvae of sea bass, which were then transferred to natural temperature until the juvenile stage (188 days). The determination of the effects of the temperature on hypertrophic and hyperplastic muscle growth throughout larval and juvenile stages, as well as on the timing of the histochemical maturity of the myotome were the particular objectives of this study.

2. Materials and methods

The experiment was carried out at the Instituto Español de Oceanografía (Centro Oceanográfico de Murcia, Mazarrón, España), using eggs from cultured spawners of

Atlantic sea bass. Eggs were obtained at the beginning of March of 2000 at natural temperature (15 °C). Eggs incubation as well as prelarvae and larvae cultivation were performed in cylindricoconical tanks (0.5 m^3), and postlarval cultivation was carried

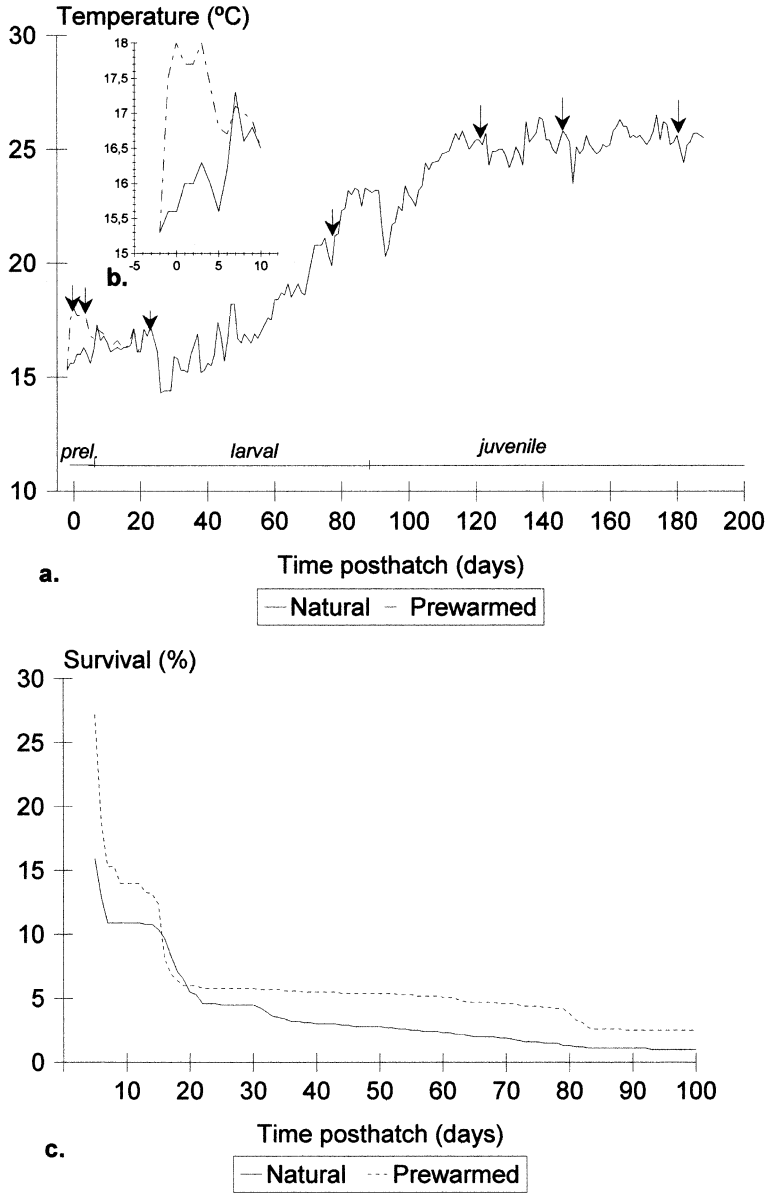

Fig. 1. (a) Temperature regimes of the prewarmed and natural *T* groups. (b) Detail of prelarval temperature regime. Prelarval (Prel), larval and juvenile phases are indicated. Arrows corresponds to sampling points. (c) Survival throughout the larval and postlarval stages in both experimental groups.

out in cylindrical tanks (1 m^3). Prelarvae were maintained in darkness during the vitelline phase until 140 °C-day and subsequently were exposed to continuous light. At 18 days, photoperiod was changed to 12:12 light/darkness. At the end of the darkness period, larvae were fed with *nauplii* of *Artemia salina*. Ten days later, *nauplii* were replaced by *Artemia metanauplii*. Around 40 days, these prey were gradually replaced by commercial feed (inert diet) and from 60 days, the fish were fed with inert diet ad libitum. Oxygen saturation was checked weekly and maintained over 80%.

Eggs were half separated in two experimental groups, maintained under different temperature (*T*) regimes (Fig. 1a,b): one group was reared at natural sea water *T* from fertilization until the end of the experiment (188 days), and a second group was reared at 17.7 ± 0.1 °C during embryonic and prelarval phases and then transferred at natural *T* until 188 days. The natural *T* group was maintained with a completely open seawater circulation system. Natural sea water *T* averaged 15 °C at the beginning of the experiment (spring) and rose gradually in summer months. The prewarmed group was reared in heated recirculated sea water with a biological filter. The water renewal rate was about 20% of tank volume/hour.

Sampling points were chosen according to predefined physiological and nutritional events (Table 1). Ten–twenty five specimens from each stage and tank were randomly chosen and delivered to the Veterinary Faculty of Murcia in opaque aerated containers. Fish were overexposed to the anaesthetic tricaine methanesulphonate (MS222, Sigma) and then 10 individuals for each sampling point and experimental group were processed as follows.

2.1. From hatching until the end of metamorphosis (80 days)

Larvae 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 Epon resin according to Wanson and Drochman's

Table 1
Sampling points related to developmental and nutritional events

Sampling points: physiological and nutritional events	Age (days posthatch)	Degree-days (°C)	
		Prewarmed	Natural *T*
Hatching (end of embryonic phase)	0 (incubation time: 2 days)	33	31
Mouth opening	5 (at 17.7 °C)	138	128
(end of prelarval and vitelline phase)	6 (at 15 °C)	155	143
Feeding with *nauplii* of *Artemia*	25	438	424
End of metamorphosis (scaling), Inert diet	80	1333	1319
Early postlarval phase	120	2127	2113
Juvenile phase	154	2957	2943
	188	3774	3759

Degree-days for both experimental groups are also indicated.

(1968) method. Semithin sections (1 μm thick) were cut transversely to the long body axis, at the point of the anal opening with a Reichert Jung ultramicrotome and stained according to Ontell's (1974) technique.

Muscle growth was quantified by means of a morphometric analysis system (KS-100 Kontron System). The following parameters were measured: total cross-sectional area of the red and white muscles; number of red and white muscle fibres; and cross-sectional diameter of the red and white muscle fibres. At hatching, all muscle fibres were measured. At mouth opening, the cross-section of the red and white muscles was measured, but due to poor quality of the embedded material, it was not possible to quantify the total number of fibres and the average fibrillar diameter. At 25 days and at the end of metamorphosis, due to the large number of white muscle fibres, the average diameter was estimated from those located at the intermediate sector of the myotome thickness, as well as at the epaxial and hypaxial quadrants. Most of red muscle fibres, with the exception of those located in the deep part of the lateral line triangle, were measured to obtain the average diameter of red fibres at these stages. The counts of fibres were carried out directly from photographs at an adequate magnification.

Survival was daily registered in both prewarmed and natural *T* groups (Fig. 1c). Body length of 10–12 specimens was measured at each sampling point in both groups, and the body weight of postlarvae was recorded at 154 and 188 days.

2.2. Postlarvae of 120, 154 and 188 days

Whole body slices of 5-mm thickness, cross-sectioned at the level of the anal opening were snap frozen in 2-methylbutane over liquid nitrogen. Subsequently, sections of 8-μm thickness were obtained from one half of those slices in a cryostat (Leyca CM 1850). Frozen quality was routinely checked at the beginning of sectioning by a rapid Haematoxylin/Eosin staining. Samples were stored at -25 °C and after thawing stained for myosin ATPase reaction (Mascarello et al., 1986) and NADH-TR (Dubowitz, 1985). By means of morphometric analysis, the total cross-sectional area of the red and white muscles and the average diameter of the red and white muscle fibres were measured. Red muscle fibres were measured similarly to the larval stage, whereas white muscle fibres were measured in the intermediate zone of the epaxial- and hypaxial limits of the myotome (Fig. 8a). The total number of white muscle fibres was estimated by the density of fibres (number of fibres $\times$ mm^{-2}) in both intermediate and axial zones, and then extrapolated to the whole white muscle area. The myosin ATPase (mATPase) technique was performed according to Mascarello et al. (1986), which is particularly appropriate to differentiate the mATPase activity of the intermediate muscle fibres (alkaline and acidstable), and in our experience, very useful to highlight the histochemical mosaic of the white muscle after acid preincubations (Ramírez-Zarzosa et al., 1995, 1998; López-Albors et al., 1998). As a particular modification of this method, acid preincubations (pH 4.6, 4.55, 4.4) were done in 0.05 M instead of 0.1 M Sodium Acetate. Besides, we have used very short preincubation times (15 and 30 s). The mATPase staining revealed three to four histochemical profiles of white muscle fibres: low activity (L); moderate (M); high (H) and very high (vH). These profiles of mATPase

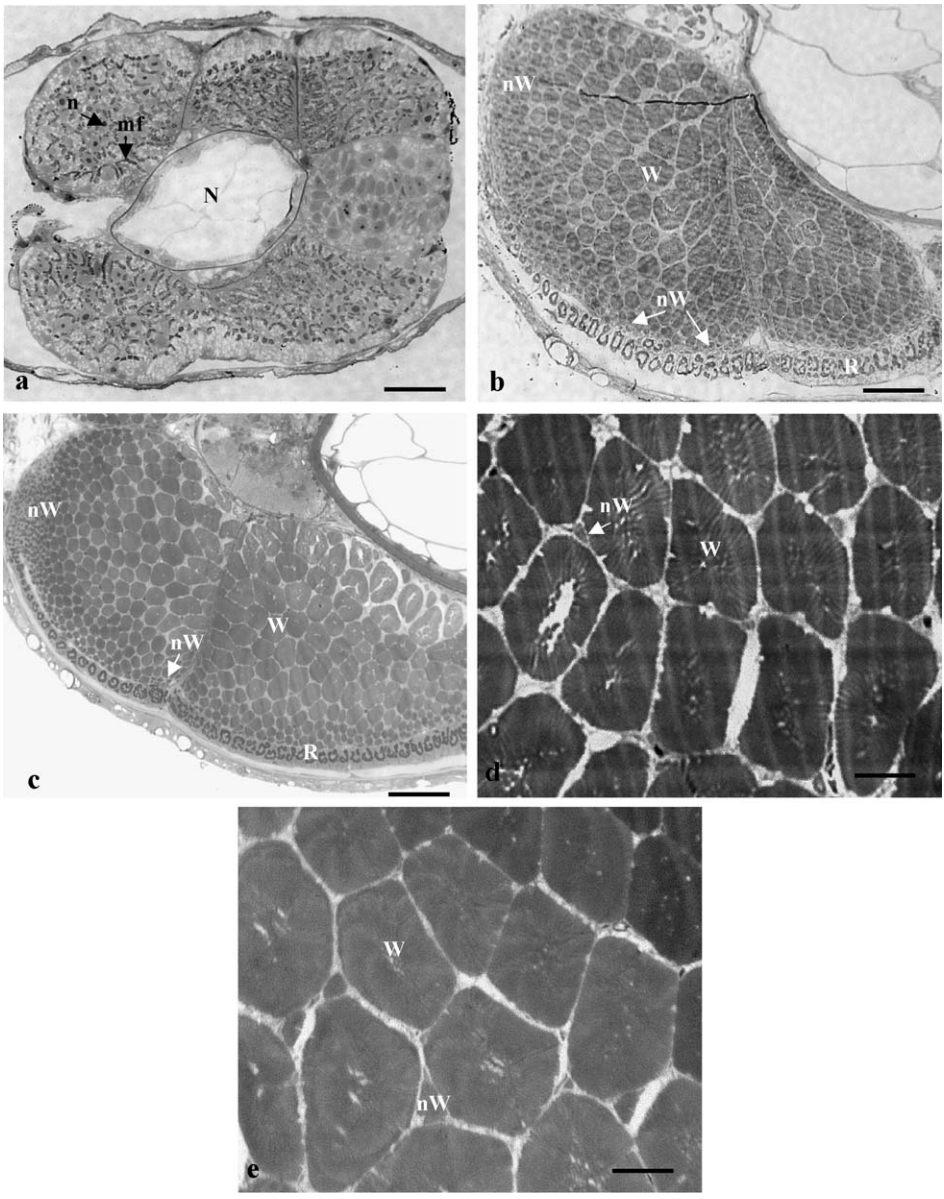

Fig. 2. Cross-sections of the lateral musculature of sea bass. (a) Newly hatched larva incubated at 17.7 °C. Bar: 20.27 μm. (b,c) Twenty-five-day larvae of sea bass of the natural *T* and prewarmed groups, respectively. Bar: 29.79 and 38.42 μm, respectively. (d,e) Eighty-day (end of metamorphosis) larvae of sea bass of the natural *T* and prewarmed groups, respectively. Bar: 12 and 13.33 μm, respectively. W: white muscle; R: red muscle; n: nucleus of myotube; mf: myofibrils; N: notochord; nW: new white muscle fibres.

activity of white muscle fibres correspond to those of the histochemical mosaic of the mature white muscle of sea bass defined in previous works by Ramírez-Zarzosa et al. (1998) and López-Albors et al. (1998). In order to determine whether there is any correlation between the mATPase activity profile and fibre size, the average diameter of white muscle fibres with different staining profile (L, M, H and vH) was separately quantified in six to seven specimens of each experimental group. Percentages of each muscle fibre subtype were calculated in order to monitor them throughout the postlarval phase.

2.3. Statistics

Measured morphometric parameters as well as body length and body mass were statistically analysed at each sampling stage by Analysis of Variance (ANOVA,

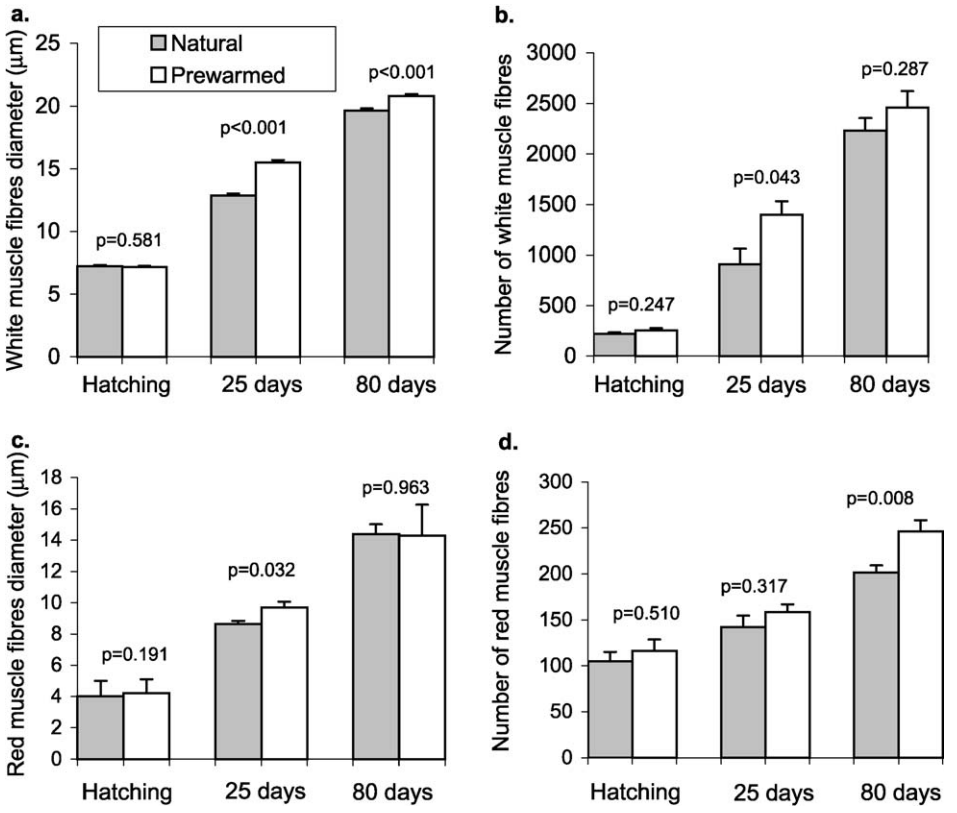

Fig. 3. Average diameter and total number of white muscle fibres (a,b) and red muscle fibres (c,d) at hatching, 25 and 80 days (end of metamorphosis: scaling). Estimation of significant differences (p) and standard error of the mean ($\pm$ S.E.M.) are also indicated. $n = 6-7$ Specimens per sample.

$p < 0.05$) and post hoc Tukey test with the Systat 9.0 win and SPSS 10.0 programs. In addition, white muscle fibre diameter distributions were evaluated in both experimental groups (from 80 days on) by probability density functions (PDFs) constructed using the Kernel approach. The use of the Kernel method to evaluate and compare distributions of muscle fibre diameters in fish has been recently reviewed and justified by Johnston et al. (1999).

3. Results

3.1. Vitelline phase

In newly hatched larvae, myotomes showed two muscular layers: a superficial monolayer of fibres with high mitochondrial content and nuclei in a basal position, and a deep layer composed of larger diameter fibres with nuclei centrally placed. (Fig. 2). At hatching, no significant influence of the incubation T was observed on muscle parameters of larvae (Fig. 3). However, body length was significantly greater in larvae incubated at 17.7 °C than at natural T (3.5 ± 0.15 and 2.8 ± 0.09 mm, respectively). In the vitelline phase, the body length and the size of the white and red muscles slightly increased in both groups, but T effects were not significative for these parameters at mouth opening. Early T also influenced the developmental rate and survival, such that the vitelline phase finished earlier in the warm than in the natural T group (5 and 6 days posthatching, respectively), and survival was higher in the warm (27.2%) than the natural T group (12.9%) (Fig. 1c).

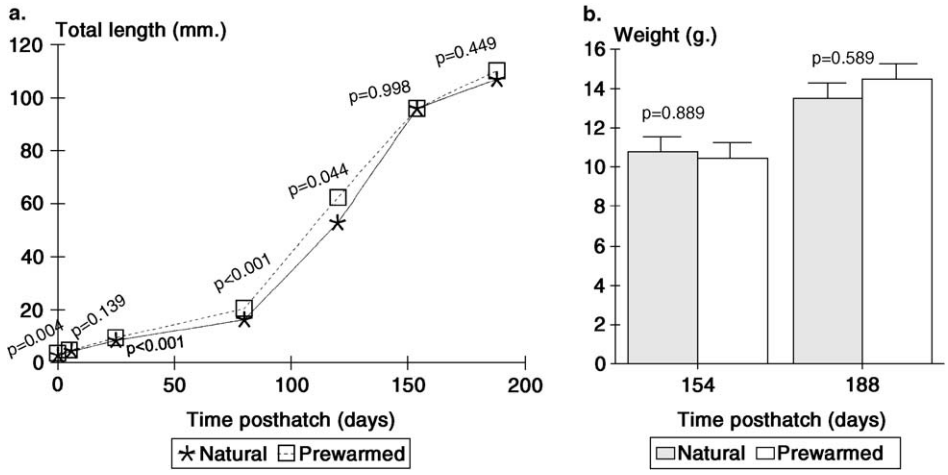

Fig. 4. Total length (a) and body mass (b) for the natural T and prewarmed groups. Values represent means ± S.E.M., $n = 10$ specimens per sample.

3.2. Larval phase

By 25 days posthatching, a great recruitment of new white muscle fibres was observed at the epaxial and hypaxial apices of the myotome and in the close zone just beneath the red muscle near the horizontal septum that spreads out dorsally and ventrally (Fig. 2b,c). New red muscle fibres were also observed near the horizontal septum. Hypertrophic and hyperplastic growth of white muscle was significantly greater in larvae maintained at warm *T* during the vitelline phase (prewarmed group) (Fig. 3a,b), and a similar tendency was observed for the red muscle ($p < 0.05$ for average diameter of red muscle fibres) (Fig. 3c,d), as well as for the body length (9.6 ± 0.15 mm in the prewarmed group, and 8.6 ± 0.16 mm in the natural *T* group). Survival at this stage was 4.5% and 5.8% in natural *T* and prewarmed groups, respectively (Fig. 1c).

The larval phase finished by 80 days posthatching in both groups (end of metamorphosis, scaling) and survival was higher in the prewarmed (3.8%) than in the natural *T*

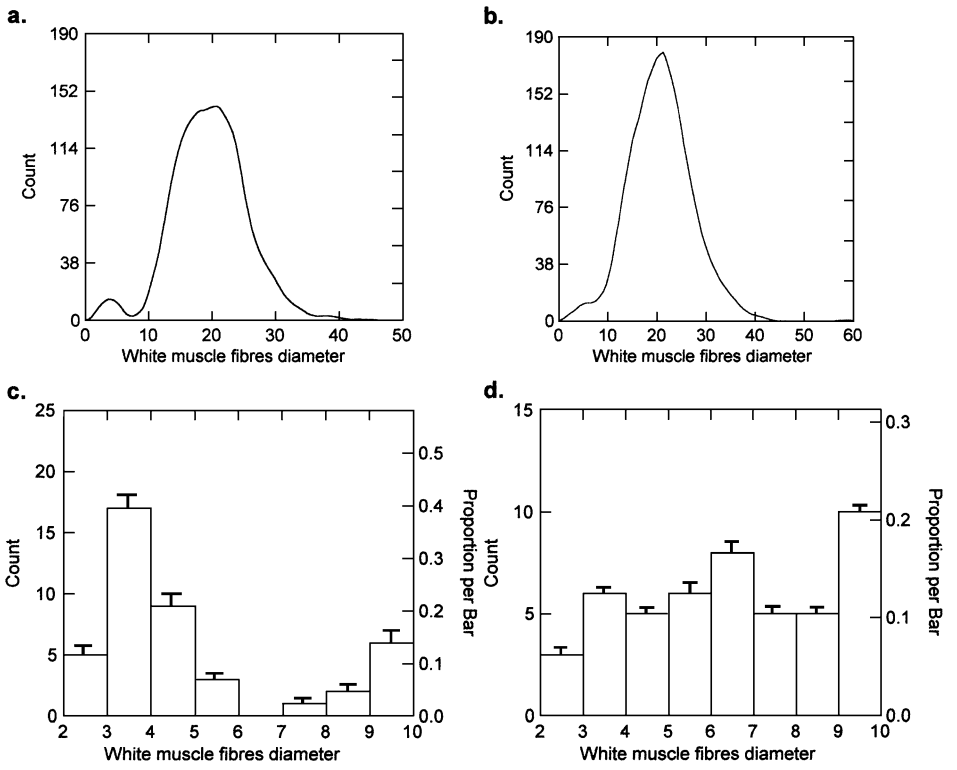

Fig. 5. (a,b) White muscle fibres size distributions (PDF) (μm) at the end of metamorphosis from six specimens of the natural *T* (a) and the prewarmed group (b). Histograms of the small new fibres (< 10 μm) with ± S.E.M. values are represented for the natural *T* (c) and prewarmed group (d).

group (1.3%). At this stage of development, most muscle parameters of the white and red muscles, as well as the body length were still greater in the prewarmed specimens (Figs. 3 and 4a). The *T* effects were significant for the average diameter of the white muscle fibres, the number of red muscle fibres and the body length. White muscle fibre size distributions (PDF) ranged widely in both experimental groups ($\cong 2-48$ μm) (Fig. 5a,b). New white muscle fibres (< 10 μm) were observed among the big white fibres in the depth, as well as at the apices of the myotome (Fig. 2d,e). Analysis of variance of these new fibres showed no significative differences between both groups ($p = 0.078$). However, comparative histograms of the white muscle fibres less than 10-μm diameter revealed that the

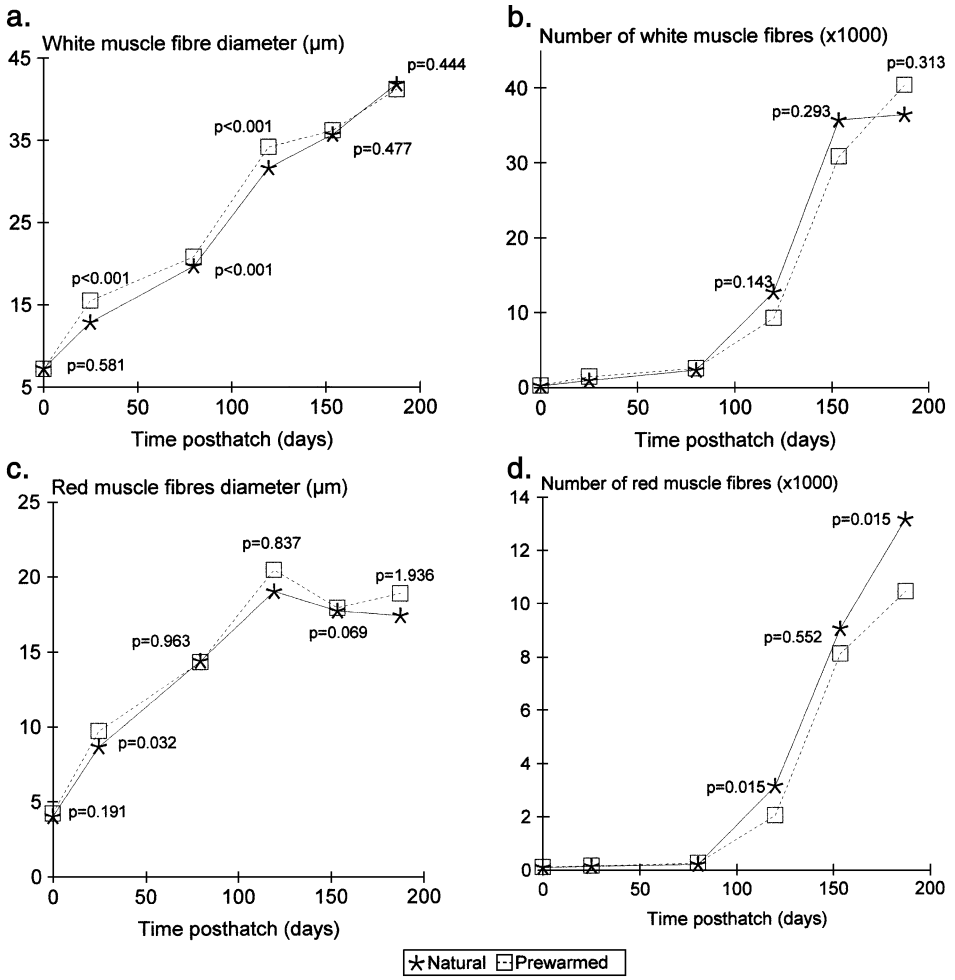

Fig. 6. Average diameter and number of white muscle fibres (a,b) and red muscle fibres (c,d) in the natural *T* and prewarmed groups from hatching to 188 days. *P*-value is indicated for each stage. Mean value from five to eight specimens.

proportion of fibres of 2–5 μm was higher in the natural *T* group than in the prewarmed group, while the proportion of fibres of 5–10 μm was higher in the prewarmed group (Fig. 5c,d).

3.3. Early postlarval stage (120 days)

The body length and the average diameter of white muscle fibres were greater in the prewarmed than in the natural *T* group ($p < 0.05$), that indicates that early *T* effects on growth are still present (Figs. 4a and 6a). On the contrary, white muscle fibre number did not show significant differences between both experimental groups, although it was slightly greater in the natural *T* group ($p = 0.143$) (Fig. 6b). The growth of the red muscle displayed a similar tendency to that of the white muscle (Fig. 6c,d).

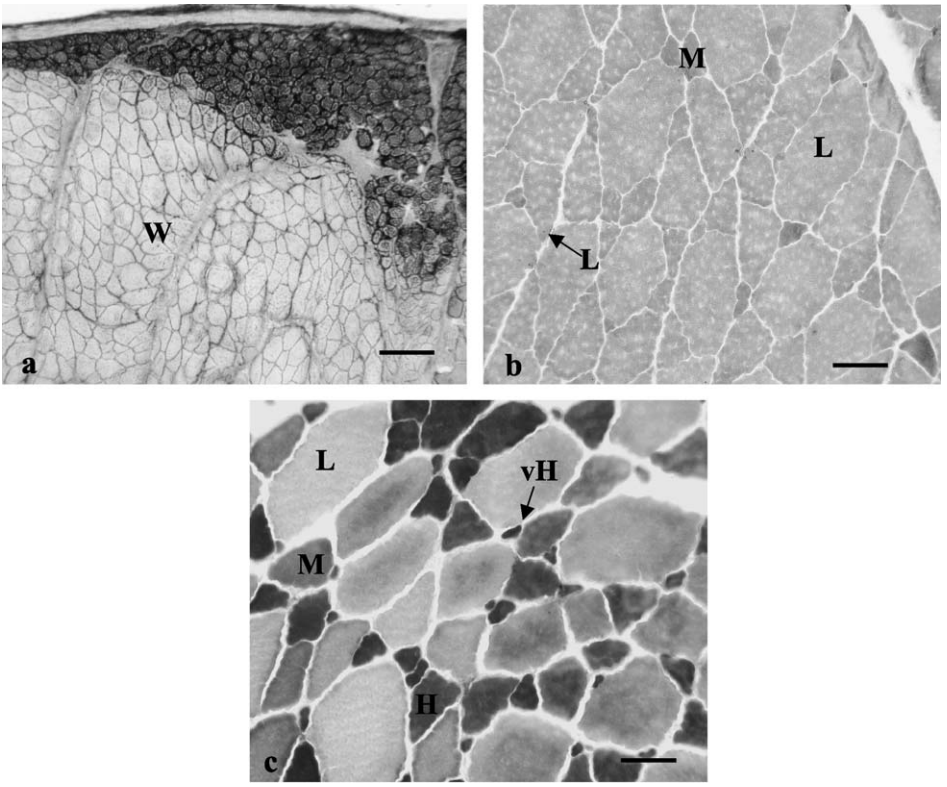

Fig. 7. Cross-section of the white muscle of sea bass of the prewarmed (a,c) and the natural *T* (b) groups at 120 days. (a) Oxidative NADH-TR reaction; bar: 181.875 μm. mATPase reaction after acid preincubation pH 4.55, 15 s (b) and pH 4.6, 30 s (c). Bar: 41.66 and 36.5 μm, respectively. W: white muscle; R: red muscle; P: pink muscle. L: low mATPase activity white muscle fibre; M: moderate mATPase activity white muscle fibre; H: high mATPase activity white muscle fibre; vH: very high mATPase activity white muscle fibre.

In both experimental groups, all specimens showed a great range of white muscle fibre sizes and a histochemical mosaic of the white muscle was observed close to the pink muscle in all specimens (mATPase pH 4.6 and 4.5; 15 or 30 s). The NADH-TR reaction displayed the typical staining for the red, intermediate and white muscle fibres, with high, intermediate and low oxidative capacity, respectively (Fig. 7a). No influence of the temperature on the oxidative capacity of muscle fibres was observed at any postlarval stage.

The early temperature (vitelline phase) influenced the mATPase staining of the white muscle fibres in the early postlarval stage. An extension of the histochemical mosaic over the whole myotome was mainly observed in specimens of the prewarmed group (Figs. 7 and 8). In these cases, three or four different mATPase staining fibres—low (L), moderate (M), high (H) and very high (vH)—were commonly observed (Fig. 7c). White muscle fibre size distributions (PDF) grouped by their staining profile (L, M, H or vH) showed

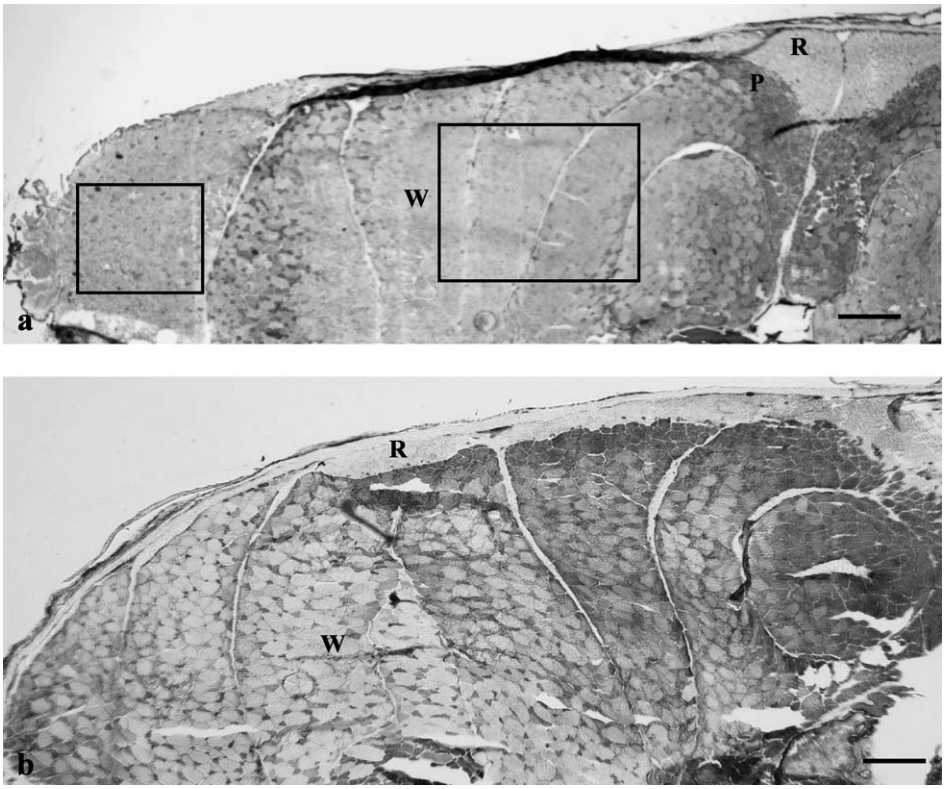

Fig. 8. Cross-section of the myotomes of sea bass of the natural *T* (a) and prewarmed group (b) at 120 days. (a) mATPase reaction after acid preincubation pH 4.6, 30 s, bar: 171.74 μm. (b) mATPase reaction after acid preincubation pH 4.6, 15 s; bar: 171.74 μm. Squared area was used to estimate white muscle fibres diameter. W: white muscle; R: red muscle; P: pink muscle.

that most of the L staining fibres were of the largest size, the M staining fibres had an intermediate size, whereas the H and vH staining fibres had the smallest diameters (Fig. 9). The relative frequencies of H and vH staining fibres were higher in the prewarmed than in the natural *T* group ($p=0.027$; $p=0.051$, respectively) (Table 2), whereas the proportion of L staining fibres was lower in the prewarmed group ($p=0.039$). The proportion of M fibres was similar in both experimental groups. Early temperature effects were also observed on the average diameter of the L, M, H and vH mATPase profiles of white muscle fibres, which was greater in specimens of the prewarmed group ($p<0.05$) (Table 2).

Regarding the small white muscle fibres (<10-μm diameter), not all of them showed high (H) or very high (vH) mATPase activity. Small white muscle fibres with low mATPase activity were commonly observed at 120 days (Figs. 7b and 9). The proportion of these fibres was lower in specimens of the prewarmed group than in specimens of the natural *T* group (0.66% and 1.59%, respectively, $p=0.102$).

Survival at 100 days was low in both experimental groups: ≅1% in the natural *T* group and 2.5% in the prewarmed group (Fig. 1c). Since all external parameters of the culture were under controlled conditions, the low survival larval rates were attributed to internal factors of spawners, such as advanced parental age.

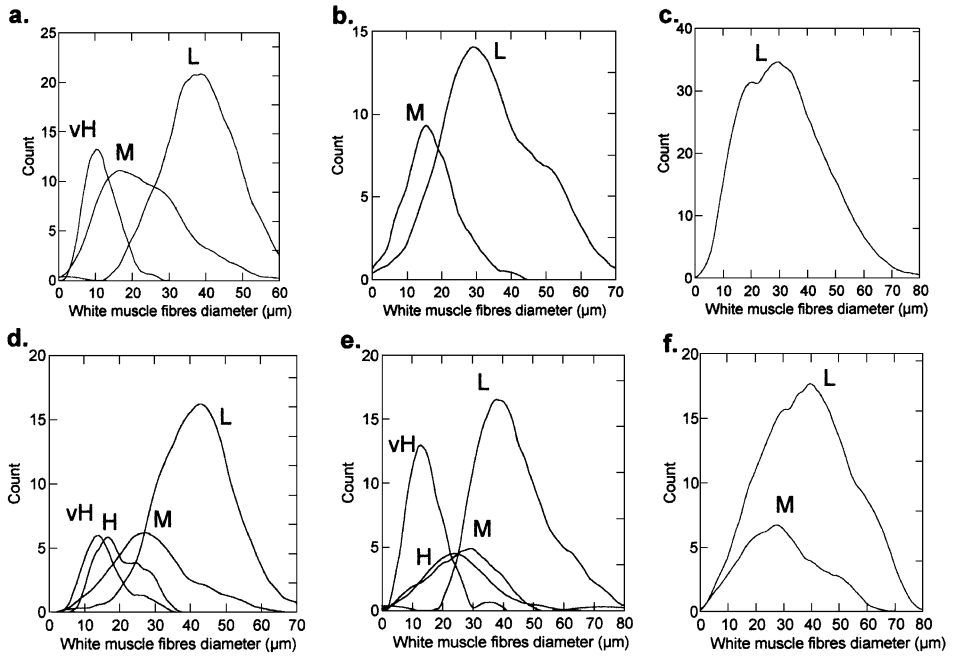

Fig. 9. White muscle fibre size distributions (PDF) of the different mATPase profiles (vH, H, M, L) at 120 days. Three specimens of the natural *T* (a–c) and prewarmed group (d–f) have been selected from six samples per group. Tukey test showed significant differences between all subtypes of fibres ($p<0.001$), except for the vH and H fibres of specimen represented in (d) ($p=0.406$), and the H and M fibres of specimen represented in (e) ($p=0.997$).

Table 2
Percentage of white muscle fibres (WMF%) and average diameter (WMFΦ) according to the mATPase activity: vH (++++), H (+++), M (++) and L (+) in the natural and prewarmed groups at 120, 154 and 188 days

		Staining intensity of white muscle fibres			
		vH (++++)	H (+++)	M (++)	L (+)
120 Days					
WMF%	Natural *T* group	3.73 (2.55)	0.63 (0.6)	18 (8.00)	78 (9.68)
(S.E.M.)	Prewarmed group	13.59 (3.6)	11.72 (4.2)	18.54 (6.98)	48.54 (5.2)
	p-value	0.051	0.027	0.451	0.039
WMF Φ,	Natural *T* group	11.97 (0.54)	12.05 (0.73)	23 (0.63)	34 (0.42)
μm (S.E.M.)	Prewarmed group	16.86 (0.50)	20.8 (0.65)	32.33 (0.70)	43.39 (0.55)
	p-value	0.004	<0.001	<0.001	<0.001
154 Days					
WMF%	Natural *T* group	12.02 (3.7)	20.69 (2.4)	34.61 (4.35)	32.68 (2.9)
(S.E.M.)	Prewarmed group	19.55 (4.07)	25.04 (2.62)	17.51 (1.83)	37.89 (2.57)
	p-value	0.197	0.243	0.003	0.203
WMF Φ,	Natural *T* group	15.55 (0.51)	23.32 (0.53)	34.61 (0.51)	52.36 (0.61)
μm (S.E.M.)	Prewarmed group	17.14 (0.41)	22.63 (0.51)	38.79 (0.81)	54.44 (0.59)
	p-value	0.015	0.351	<0.001	0.015
188 days					
WMF%	Natural *T* group	13.93 (1.45)	20.57 (1.69)	27.9 (1.96)	37.60 (2.7)
(S.E.M.)	Prewarmed group	22.96 (4.45)	13.89 (1.54)	22.26 (2.84)	40.91 (3.72)
	p-value	0.051	0.026	0.122	0.506
WMF Φ,	Natural *T* group	17.93 (0.62)	27.12 (0.5)	40.63 (0.62)	59.98 (0.71)
μm (S.E.M.)	Prewarmed group	18.54 (0.53)	30.14 (0.54)	40.17 (0.88)	58.98 (0.82)
	p-value	0.455	<0.001	0.658	0.353

Mean and *p*-values from six to seven specimens per sample are indicated. (± S.E.M. is indicated in parenthesis).

3.4. Juvenile phase (154 and 188 days)

The average diameter and estimated number of white muscle fibres did not show significative differences between both experimental groups (Fig. 6a,b). The body length and body weight were similar in both groups (Fig. 4). On the contrary, at 188 days, the number of red fibres was significantly greater in the natural *T* than in the prewarmed group (Fig. 6d).

The histochemical mATPase mosaic of the white muscle spread over the whole myotome in all specimens of both experimental groups (Fig. 10). After acid preincubations—pH 4.6 4.55 or 4.4—the three or four mATPase staining profiles—L, M, H and vH—were commonly observed in all specimens and associated to large, intermediate, small and very small fibres, respectively. In contrast to the previous stage (early postlarval, 120 days), small fibres (<10 μm) with low mATPase activity were not found at these stages. Fig. 11 shows the white muscle fibre diameter frequency distributions (PDF) for each experimental group in the juvenile phase. The mATPase activity that corresponds to each staining profile is expressed within a range of fibre sizes, thus indicating a gradual mATPase isoform transition from the vH staining of small fibres to the low staining of the adult white muscle fibres. Crossing-over of the PDF distributions indicates that there is not

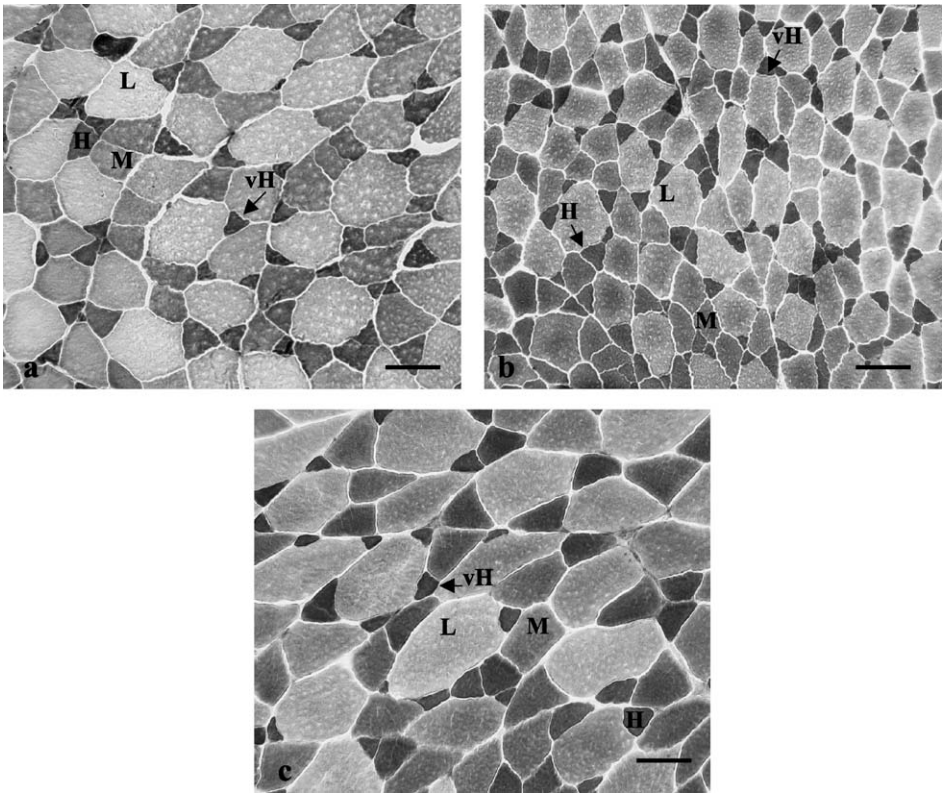

Fig. 10. Cross-sections of the white muscle of sea bass juveniles of the natural *T* group (a) and prewarmed group (b) at 154 days. mATPase reaction after pH 4.6, 15 s; bar: 58.4 μm (a) and 76.41 μm (b). (c) One-hundred eighty-eight-day sea bass juvenile of the natural *T* group after acid preincubation pH 4.6, 15 s; bar: 56.15 μm. L: low mATPase activity white muscle fibre; M: moderate mATPase activity white muscle fibre; H: high mATPase activity white muscle fibre; vH: very high mATPase activity white muscle fibre.

a scheduled size for the histochemical change between successive staining profiles, so many hybrid white muscle fibres with a mixture of at least two mATPase isoforms coexist in the myotomes of sea bass postlarvae. At 154 days, the average size of the different histochemically stained white fibres—L, M, and vH stained fibres—was greater in the prewarmed group ($p < 0.05$) (Table 2). However, by 188 days, this parameter was similar in both groups, except for the H staining fibres, which were bigger in the prewarmed group.

In juveniles, the proportion of L fibres decreased respect to the previous stage, mainly in the natural *T* group (78% at 120 days and 32–27% at 154–188 days). The proportion of H and vH fibres increased in both groups from 120 to 154 days, but the mean value was higher in the prewarmed group ($p > 0.05$). On the contrary, the proportion of M fibres was greater in the natural *T* group ($p < 0.05$) (Table 2). By 188 days, the proportion of vH was greater in the prewarmed group ($p = 0.051$), but the proportion of

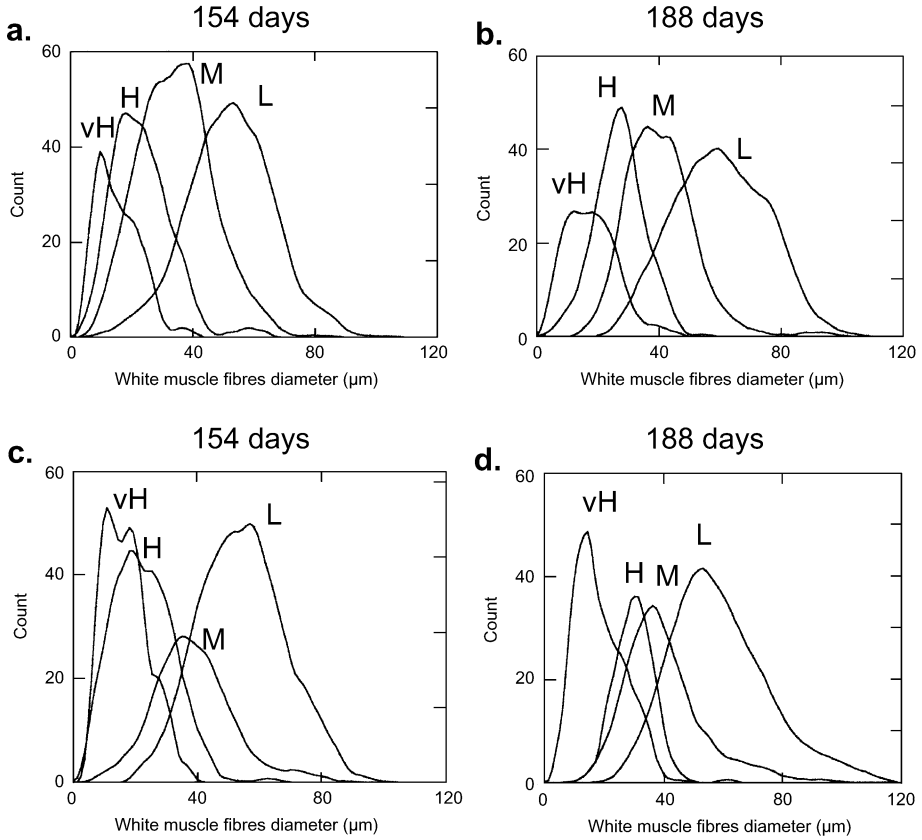

Fig. 11. White muscle fibre size distributions (PDF) of the different mATPase profiles (vH, H, M, L) at 154 and 188 days. Data from all measured specimens ($n = 6–7$) in the natural T (a,b) and prewarmed group (c,d). Tukey test showed significant differences between all subtypes of fibres ($p < 0.001$).

H fibres decreased in this group, being significantly lower than in the natural T group (Table 2).

4. Discussion

White and red muscle growth dynamics of sea bass were influenced by the early T regime and responses observed varied throughout the larval and postlarval periods. During the vitelline phase, there were no significant T effects on muscle growth, that agrees with previous results in other stock of this species (Ayala et al., 2000) and with data from herring (Johnston et al., 1998). By 25 days, both hypertrophy and hyperplasia of white muscle fibres were significantly greater in the prewarmed group. At the end of metamorphosis (80 days) and early postlarval stage (120 days), hypertrophic growth of white muscle fibres was still greater in this group, but the T influence was not significative

for the number of white fibres. Previous studies in sea bass showed that higher incubating *T* slightly influenced muscle growth throughout larval stages, but following metamorphosis no *T* effects were observed (Ayala et al., 2000, 2001). The longer *T* effect found in the present study compared to previous experiments may be due to the longer period of high thermal experience: until hatching in Ayala et al. (2000, 2001), but until first live feeding in this study. Besides, body length was greater in specimens of the prewarmed than of the natural *T* group until the early postlarval stage (120 days), whereas in previous studies, incubation *T* hardly influenced this parameter throughout the larval development.

Similarly to a previous work on sea bass (Ayala et al., 2001), hypertrophy of white muscle fibres is the main muscle parameter influenced by high early *T*, whereas hyperplasia is influenced to lesser extent. Thus, we have found that the positive effect of the high early *T* on the recruitment of new white muscle fibres ceased by the end of the larval period, whereas the average diameter of the white muscle fibres was higher in the prewarmed specimens until the postlarval stages (120 days). The long-term effect of the early temperature on the muscle growth dynamics of sea bass can be compared with results found in other teleosts. In herring maintained at 5, 8 or 12 °C until first feeding and then transferred to common *T*, both hypertrophy and hyperplasia increased in postlarvae previously maintained at higher early *T* (Johnston et al., 1998). Similarly, in one population of salmon, Johnston et al. (2000a,b) found that early *T* experience influenced hyperplasia and hypertrophy at subsequent stages of the life cycle. Upon these results, the authors indicated that *T* prior to hatching can influence the number of satellite cells and hence the relative importance of fibre recruitment and hypertrophy to muscle growth at subsequent stages of the life cycle. However, the effects of the early thermal experience on the long-term growth of fish should be carefully compared among teleost species, since biological characteristics as well as cultivation conditions are different, i.e., the vitelline phase in sea bass is very rapid (5–10 days), whereas in herring and salmon, it is longer (2–4 weeks) and hence, early thermal experience seems to influence more significantly in these species than in sea bass.

In juvenile stages of sea bass, new white muscle fibres are formed around and in close contact with large diameter mature fibres, giving the muscle a mosaic appearance in cross-section. In the mature histochemical mosaic of sea bass, the small white muscle fibres have different histochemical mATPase activity than the medium and large fibres (Scapolo et al., 1988; Ramírez-Zarzosa et al., 1998; López-Albors et al., 1998). Small white muscle fibres have high mATPase activity after both acid and alkaline preincubations, whereas larger muscle fibres have low mATPase activity. In our study, the beginning of the morphological mosaic of the white muscle was observed by the end of metamorphosis (80 days), whereas the histochemical mosaic was gradually observed from early postlarval stages on. At 120 days, two different histochemical profiles of the small white fibres were observed. Some small white fibres showed H or vH mATPase activity, whereas other showed L mATPase activity. Different histochemical profiles of the small white fibres were also found in sea bass by Scapolo et al. (1988): the small white fibres of the larval and early postlarval ages only reacted positively against anti-fast sera and had no acidstable mATPase activity, whereas in adult specimens, small white fibres had a positive reaction both against anti-fast and anti-slow seras, and had acidstable mATPase activity. These authors suggest that the developmental change in histo- and immunohistochemical profile of the small fibres in

the deep muscle may reflect correspondingly different mechanisms of histogenesis: small fibres produced in the earlier phase (larval and postlarval, 80 days) probably derive from the original myoblast population and then develop rapidly into typical large white fibres, whereas those produced later are derived from satellite cells, acquire a characteristic myosin isoform similar to that in small white fibres of other teleosts and then slowly transform into the adult white type as they increase in diameter. Our results agree with Scapolo's et al. (1988) hypothesis since two patterns of mATPase staining were observed in the small white muscle fibres of early postlarval sea bass: the former small fibres of the white mosaic appeared by the end of metamorphosis and expressed the same (low acidlabile) mATPase activity of the large white fibres. This first population faded out progressively, such that at 154 days, no small fibres with this mATPase staining were observed. Small white fibres with high acidstable mATPase activity appeared in the postlarval stages, from 120 days on, and progressively transformed into large white muscle fibres with low mATPase activity. Therefore, medium diameter white fibres with moderate mATPase activity should be a transitional form with a mixture of myosin isoforms. The proposed mechanism of histogenesis for the small muscle fibres of the white mosaic in sea bass has also been suggested in the eel, *Anguilla anguilla*, and carp (Rowlerson et al., 1985; Romanello et al., 1987; Alfei et al., 1994). However, it can not be extended to other teleosts since the mATPase activity and immunoreactivity of the small diameter fibres in the white mosaic vary widely between species. In the trout, *Oncorhynchus mykiis*, and salmon, they are not different from large diameter fibres (Rowlerson et al., 1985; Higgins, 1990); whereas in mullet, *Mugil capito* (Carpenè and Veggetti, 1981; Rowlerson et al., 1985), and the gilthead sea bream, *Sparus aurata* (Mascarello et al., 1995), they only differed in their mATPase activity.

Early *T* influenced the establishment of the adult histochemical mosaic such that it was advanced in the prewarmed group. Thus, the proportion of fibres with H and vH mATPase activity was greater in the prewarmed group ($p \leq 0.05$), whereas the percentage of L mATPase activity fibres was significantly greater in the natural *T* group. The earlier histochemical maturity of the prewarmed specimens could be explained in terms of a higher rate of muscle development. Nathanailides et al. (1995b) reared carp at different temperatures during larval and juvenile periods and reported a more advanced appearance of the pink muscle and different mATPase activity of the small white muscle fibres in specimens reared at higher temperature. However, in our study, the experimental stocks were not reared under different thermal regimes during larval and juvenile periods, since the temperature increase (2.7 °C) was only operated in a short and early period of development. At the end of experiment, the degree-days difference between both experimental stocks was of only 15 °C. Therefore, the cause of the earlier histochemical maturity in the prewarmed specimens seems unlikely to be the overall degree-days difference between the experimental stocks, but the critical moment of the thermal increase. Embryonic temperature has a profound effect on development in fish (Blaxter, 1988), and particularly in myogenesis (Johnston, 1993; Johnston et al., 1995, 1997). In herring, temperature during early development was able to alter the sequential expression for individual myofibrillar components (Crockford and Johnston, 1993) and determined the relative timing and degree of expression of the myogenic program (Johnston et al., 1997). In sea bass, our results show that the early temperature increase influenced

positively the larval and early postlarval muscle growth dynamics (as previously discussed), as well as the postlarval activation of the satellite cells population, which is responsible of the histochemical maturity of the white muscle. However, it is not possible from our results to elucidate the mechanisms implicated in myogenesis regulation. Understanding of the factors which control the proliferation of the myogenic cells which give rise to the mosaic hyperplastic growth phase would be of great importance for research and of practical interest for aquaculture (Rowlerson and Veggetti, 2001).

5. Conclusion

The use of a slight temperature increase during the vitelline phase of sea bass improved larval and early postlarval muscle growth dynamics and body length, without higher mortality. Muscle development was also affected by early thermal experience, and thus, histochemical maturity of white muscle was advanced in prewarmed specimens.

In mediterranean markets, reared sea bass are commonly sold at about 300–400 g (18–20 months). In order to obtain practical benefits from studies of the early thermal experience, we believe that by increasing the early temperature for a longer period (larval development), muscle growth and survival of fish could be probably improved under fish farming conditions.

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