Anat. Histol. Embryol. **32,** 271–275 (2003)

ISSN 0340–2096

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Effect of Two Thermal Regimes on the Muscle Growth Dynamics of Sea Bass Larvae, *Dicentrarchus labrax* L.

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With 6 figures

Received January 2002; accepted for publication December 2002

Summary

Muscle growth was studied in larvae of sea bass, *Dicentrarchus labrax* L., reared at two temperatures: real ambient temperature (≅15°C during vitelline phase and increased gradually) and 19°C from fertilization until the end of larval development. Muscle cellularity, body length and body weight were measured.

Early temperature influenced larval development and so, pre-larval phase finished earlier at 19°C than at ambient temperature (4 and 6 days, respectively). Temperature also affected muscle growth such that at hatching and at mouth opening hypertrophy of muscle fibres was greater at 19°C ($P < 0.05$), whereas hyperplasia was similar in both groups. After 25 days, the cross-sectional area of the white muscle was greater at 19°C ($P < 0.05$), which was mainly associated with a higher proliferation of new white muscle fibres. At this stage the body length was also higher at 19°C. Metamorphosis finished earlier in fish reared at 19°C (52 days) than at natural temperature (82 days). At this developmental stage body length and cross-sectional area of the myotome were similar in both groups. However, muscle cellularity differed between groups. Thus, hypertrophy of muscle fibres was higher in fish reared at ambient temperature ($P < 0.05$), whereas proliferation of new muscle fibres was higher at 19°C ($P > 0.05$).

Introduction

Sea bass is a eurithermic teleost species widely distributed in the Mediterranean and Atlantic seas. This species is interesting in aquaculture because of its rapid growth and high commercial value. Muscle development and growth of sea bass have been characterized by several authors (Scapolo et al., 1988; Veggetti et al., 1990; Ramírez-Zarzosa et al., 1995, 1998; López-Albors et al., 1998). In many teleosts, muscle growth rate varies throughout development, and shows considerable plasticity with respect to feeding, environmental and genetic factors (Johnston and Mclay, 1997; Johnston, 1999). Temperature is a relevant environmental factor, which has been shown to influence the proliferation and growth of muscle fibres. Recently, we have studied the effect of the thermal regime on the larval muscle growth of different populations of sea bass and it was observed that high incubation and cultivation temperatures influenced muscle growth, the latter having higher influence (Ayala et al., 2000, 2001). High cultivation temperature significantly increased muscle growth and body length of larvae of sea bass, as previously reported in juveniles of this species by Nathanailides et al. (1995). High incubation temperature also influenced muscle growth of larvae of sea bass, but this effect was shown in a gradual way: during vitelline phase, embryonic temperature affected muscle growth not much, but in subsequent stages (metamorphosis) high embryonic temperature had a positive effect on muscle growth (Ayala et al., 2000, 2001).

The influence of temperature on muscle growth is also conditioned by genetic factors and thus differs among and within any teleost species. The intraspecific variations in muscle growth response to temperature have been reported for herring (*Clupea harengus*, L.; Johnston et al., 1996) and salmon (*Salmo salar*, L.; Johnston and Mclay, 1997; Johnston et al., 2000a,b), and were associated with genetic variation and/or differences in other factors that influence larval growth, such as egg size and quality (Johnston and Mclay, 1997). In this sense, two genetically different populations of sea bass larvae, Atlantic and Mediterranean, showed different responses to temperature on the relative contribution of muscle hypertrophy and hyperplasia to the total muscle growth during viteline phase (hatching and mouth opening) and at the end of the metamorphosis (scaling; Ayala et al., 2001).

Temperature effects on larval muscle growth and development show considerable heterogeneity and so, the aim of the present study is to complement results previously found in sea bass in order to obtain a better understanding of the thermal effect on this species. Thus, larvae of this species were maintained at two thermal regimes, being the experimental temperatures applied and were different to those in our previous work (Ayala et al., 2000, 2001).

Material and Methods

This experiment was carried out at the Instituto Español de Oceanografía (Centro Oceanográfico de Murcia, Mazarrón). Eggs were obtained in the beginning of March 2001 at ambient temperature (≅15°C) from spawners of Atlantic sea bass, which were adapted to life in captivity. Egg incubation as well as prelarval and larval cultivation was performed in cylindricoconical tanks (1 m^3).The experimental groups were maintained at two temperatures: real ambient temperature (≅15°C during vitelline phase and raised gradually) and 19°C from fertilization until the end of larval metamorphosis (Fig. 1). Sampling points were defined as follows: hatching, mouth opening (4 and

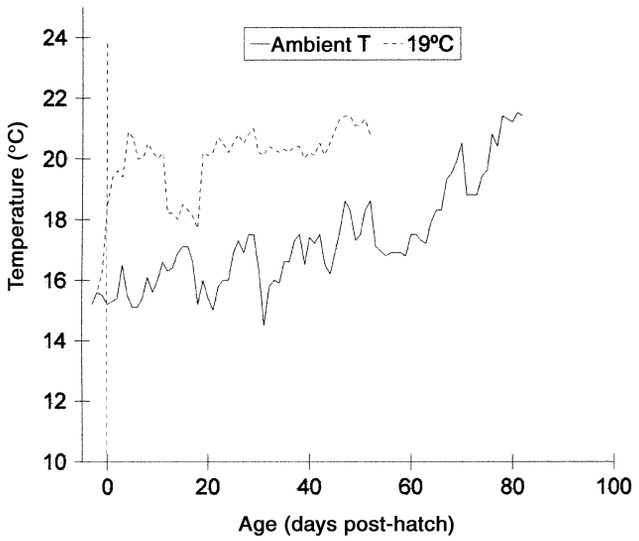

Fig. 1. Thermal regimes in both experimental groups.

6 days post-hatching at 19°C and real ambient temperature, respectively), 25 days and end of larval metamorphosis (scaling, 52 and 82 days post-hatching, at 19°C and real ambient temperature, respectively). Ten specimens from each sampling point and tank were randomly chosen. Fish were overexposed to the anaesthesic tricaine methanesulphonate (MS222, Sigma, St Louis, MO, USA) and then 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 epoxi resin according to Wanson and Drochman's (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 (Heiderberger, Germany) ultramicrotome and stained according to Ontell's (1974) technique. Muscle growth was quantified by means of a morphometric analysis system [KS 100, Kontron (Care Zeiss Vision GmbH), München, Germany] on five specimens from each group and stage. 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 and at mouth opening all muscle fibres were quantified and measured. After 25 days and at the end of metamorphosis, all fibres were counted but their average diameter was estimated from those fibres located at the intermediate sector of the myotome as well as at the epi- and hypo-axial quadrants (Fig. 6a). Body length of 10 specimens from each group was measured at each sampling point, whereas body weight of 10 specimens was recorded at the end of the metamorphosis. In order to determine the influence of thermal regime on muscle growth and body length in each stage, statistical analysis of results was carried out by analysis of variance (ANOVA, $P < 0.05$; SYSTAT 9.0 WIN program).

Results

Vitelline phase

High temperature influenced rate of development of prelarvae, such that the vitelline phase finished 4 and 6 days after hatching at 19°C and at real ambient temperature, respectively.

During the vitelline phase the transversal areas of the white and red muscles were similar in specimens of both experimental tanks (Fig. 2). However, muscle cellularity (size and number of muscle fibres) was influenced by thermal regimes and thus, white and red muscle fibre diameters were greater in larvae maintained at 19°C than at real ambient temperature ($P < 0.05$) at hatching and mouth opening (Fig. 3a and b). Number of white and red muscle fibres showed slight but no significant differences between both tanks in both stages (Fig. 3c and d).

Body length was higher at hatching in larvae incubated at 19°C (3.74 mm) than at real ambient temperature (3.54 mm) ($P < 0.05$). However, at mouth opening body length was higher in larvae reared under real ambient temperature than at 19°C (5.29 and 4.9 mm, respectively; $P < 0.05$; Fig. 4).

Twenty-five days

Muscle growth at this age stage is characterized by a strong increase in both hypertrophy and hyperplasia processes (Figs 5 and 6). Thermal regime influenced white and red muscle growth. The transversal area of the white muscle was higher in specimens reared at 19°C ($P < 0.05$; Fig. 2a). This was associated with a greater proliferation of new white muscle fibres in this group ($P < 0.05$; Fig. 5c), whereas hypertrophy

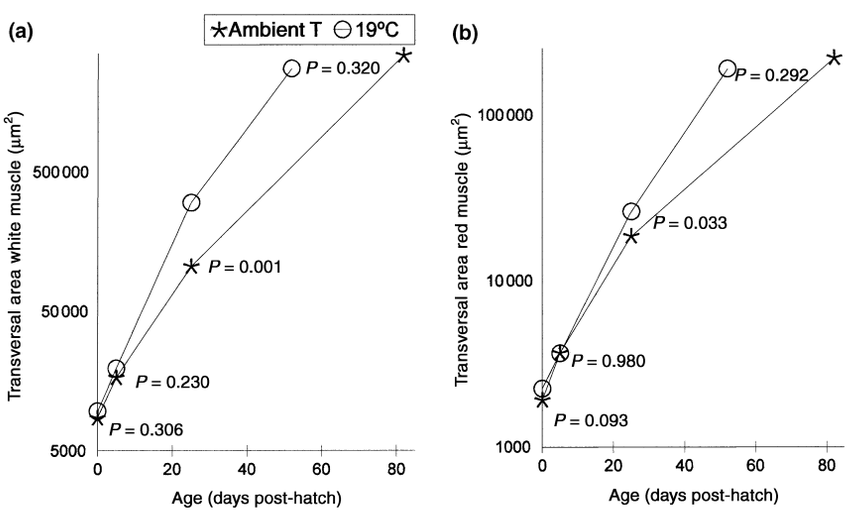

Fig. 2. (a) and (b) Semilogarithmic plot of the transversal area of both white and red muscles against age in larvae of sea bass reared at 19°C and at ambient temperature. Significant differences (p) between both groups are indicated for each stage.

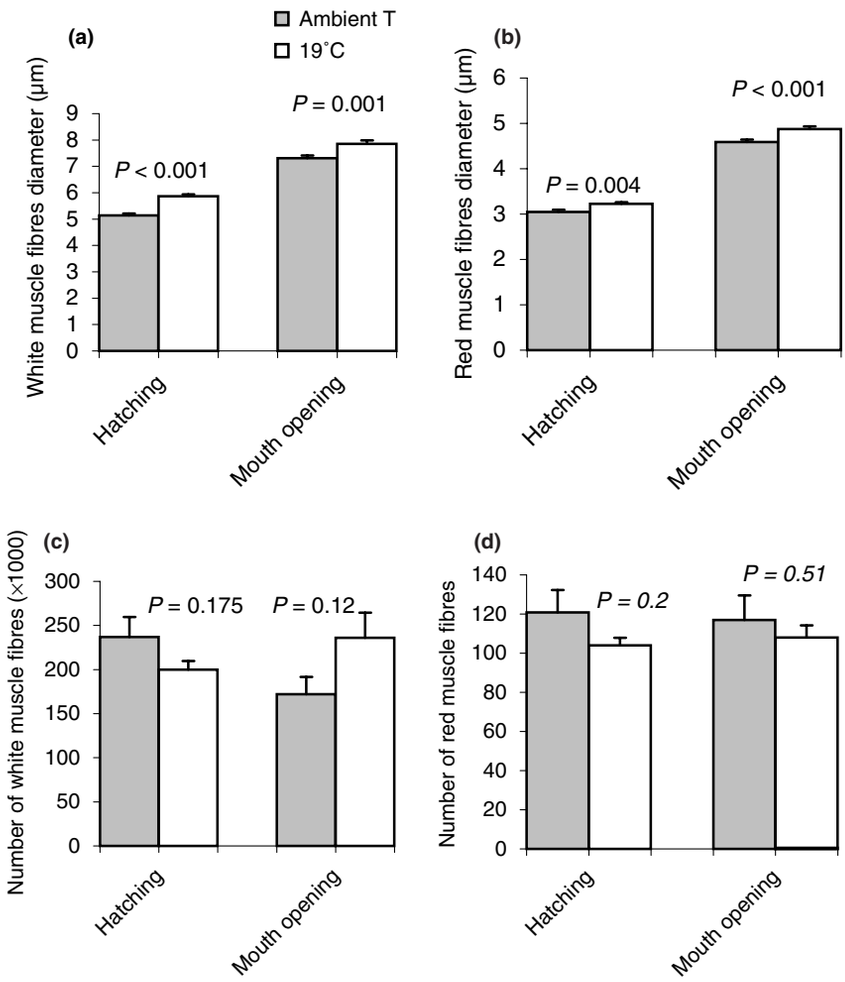

Fig. 3. White and red muscle fibre diameter (a) and (b) and number of white and red muscle fibres (c) and (d) at hatching and at the end of the pre-larval phase (mouth opening) in both experimental groups. Mean ± SEM and *P* values are indicated.

of white muscle fibres was similar in larvae of the two experimental groups (Fig. 5a). The transversal area of the red muscle was also greater in larvae reared at 19°C ($P < 0.05$; Fig. 2b), but associated with higher hypertrophy of the red muscle fibres ($P < 0.05$; Fig. 5b). No difference in number of red muscle fibres was observed between both groups at this stage (Fig. 5d). Body length was greater in larvae reared at higher temperature ($P < 0.05$; Fig. 4).

End of metamorphosis (scaling)

Larval development finished at 52 and 82 days, in tanks maintained at 19°C and real ambient temperature, respectively.

At this developmental stage, the total cross-sectional areas of white and red muscles did not show significant differences between both groups (Fig. 2). Body length (Fig. 4) and body weight of fish (134 and 132.7 mg at ambient temperature and 19°C, respectively, not shown) were also similar for both experimental groups. However, white and red muscle cellularity showed significant temperature effects. Thus, diameters of both white and red muscle fibres were higher at natural temperature ($P < 0.05$; Fig. 5a and b). On the contrary, the number of white and red muscle fibres (Figs. 5c and d) was higher in fish reared at 19°C.

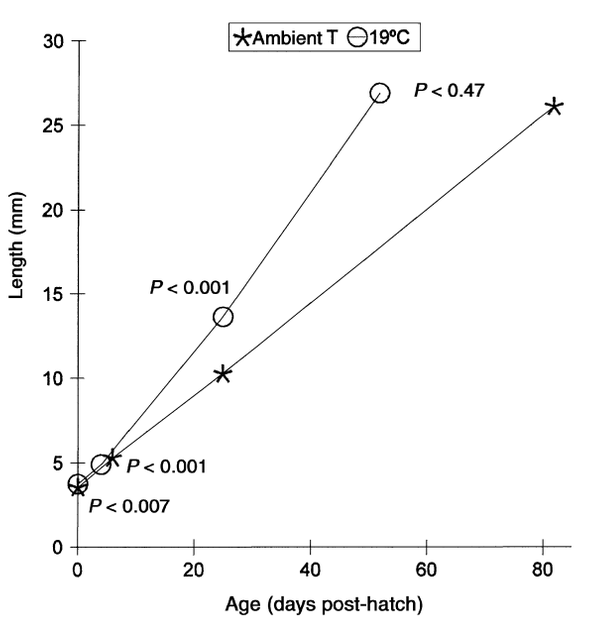

Fig. 4. Body length of larvae of sea bass reared at 19°C and ambient temperature. *P*-value is indicated.

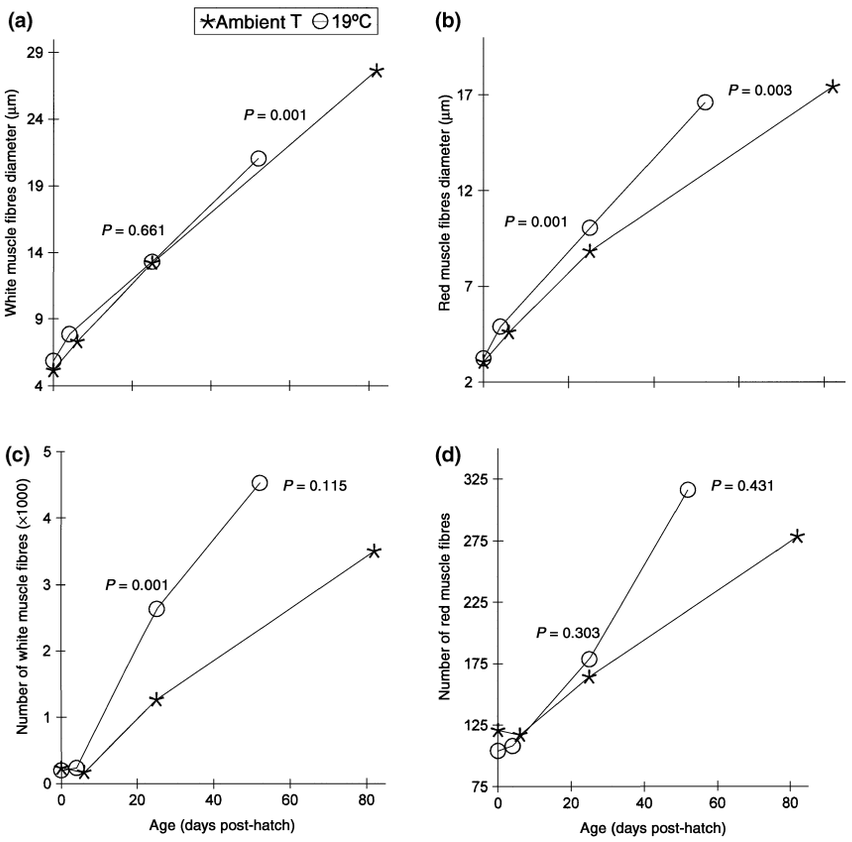

Fig. 5. White and red muscle fibre diameter (a) and (b) and number of white and red muscle fibres (c) and (d) from hatching to the end of larval metamorphosis. *P*-value is indicated.

Discussion

Muscle growth of sea bass larvae is a consequence of both hypertrophy and hyperplasia of muscle fibres (Scapolo et al., 1988; Veggetti et al., 1990; Ramírez-Zarzosa et al., 1995). Both mechanisms of muscle growth coexisted throughout the larval development of sea bass, but their relative contribution to the total growth varied. During the vitelline phase (hatching and mouth opening) muscle growth was minimal and mainly associated with hypertrophy of muscle fibres, which was significantly greater at higher temperature. Results in this work are similar to those obtained in two stocks (Atlantic and Mediterranean) of sea bass (Ayala et al., 2001) whereas in an other Mediterranean stock of this species temperature did not influence the muscle growth dynamic during the vitelline phase (Ayala et al., 2000). The intraspecific variation in muscle growth response to environmental temperature can be attributed to

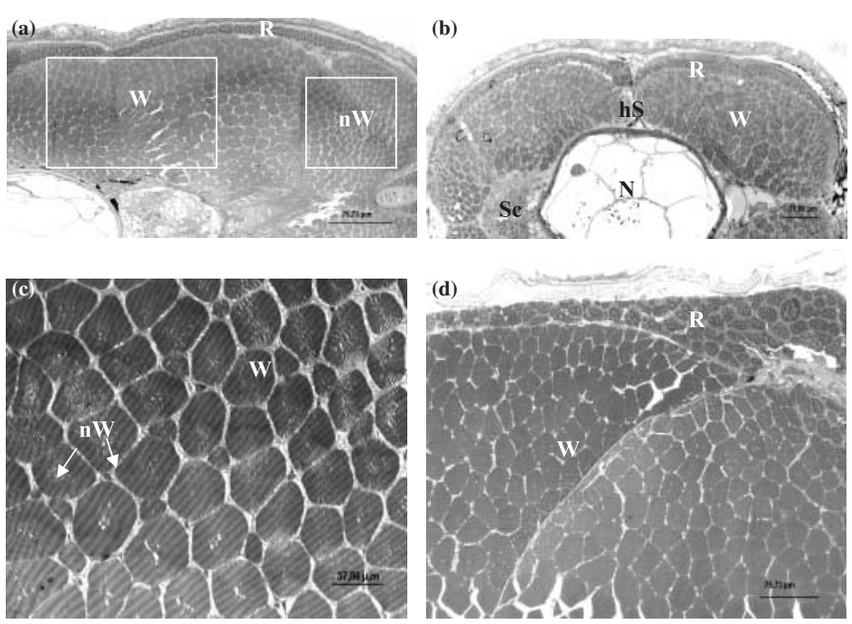

Fig. 6. Cross sections of the lateral musculature of sea bass larvae stained with Ontell's technique (1974). (a) and (b) correspond to 25-day larvae reared at 19°C and ambient temperature, respectively. (c) and (d) correspond to the end of larval metamorphosis at 19°C and ambient temperature, respectively. Squared area was used to estimate the average white muscle fibre diameter in specimens of 25 days and by the end of metamorphosis. W, white muscle; R, red muscle; N, notochord; Sc, spinal chord; hS, horizontal septum; nW, new white muscle fibres.

genetic and environmental factors as well as to the competition between growth and metabolism for yolk energy. The number of white and red muscle fibres almost remained stable during the pre-larval phase, being similar in both experimental groups, which coincides with previous results in *Dicentrarchus labrax* (Ayala et al., 2000, 2001) and *C. harengus* (Johnston et al., 1998). Considering results in this and previous works on sea bass larvae muscle hypertrophy and hyperplasia varied their sensitivities to environmental temperature depending on developmental stage. Similarly, in larvae of Atlantic salmon, Stickland et al. (1988) and Usher et al. (1994) observed that higher temperatures had a greater effect on muscle hypertrophy than on hyperplasia, and so, it would seem that temperatures had a differential effect on cell division and protein synthesis (Stickland et al., 1988).

After pre-larval phase, larval growth was rapid in both groups. High cultivation temperature positively influenced muscle growth and was associated with a significant increase in muscle fibre hyperplasia, that coincides with results found in turbot, *Scophthalmus maximus* (Gibson and Johnston, 1995), and salmon (Johnston and Mclay, 1997).

Temperature had a profound effect on the rate of development of sea bass. Thus, metamorphosis finished earlier at 19°C than at real ambient temperature (at 52 and 82 days, respectively). The different rate of development was accompanied by differences in the relative contribution of muscle hypertrophy and hyperplasia to the total muscle growth between both groups. However, as a consequence of the rapid development of larvae reared at 19°C, the total transverse area and the body length reached by this experimental group at the end of metamorphosis was similar to that reached by larvae reared at ambient temperature. These results show that development and growth are related, such that when development is accelerated more than growth it may result in a reduced size at a given stage (Atkinson, 1996). The shorter time period between developmental stages would reduce the total amount of growth.

The results of this study show that during larval development of sea bass muscle cellularity (size and number of fibres) is particularly sensitive to environmental temperature, whereas the final growth of larvae is not significantly influenced, confirming our previous results (Ayala et al., 2001).

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