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Myofibrillar proteins in white muscle of the developing African catfish *Heterobranchus longifilis* (Siluriforms, Clariidae)

F. Huriaux[1], P. Vandewalle[2], E. Baras[3], M. Legendre[4] and B. Focant[1]
[1]*Université de Liège, Laboratoire de Biologie Cellulaire et Tissulaire, rue de Pitteurs 20, B-4020 Liège, Belgique, (Phone: 32-4-366 51 65; Fax: 32-4-366 51 73; E-mail: B.Focant@ulg.ac.be);* [2]*Université de Liège, Laboratoire de Morphologie Fonctionnelle, B-4020 Liège, Belgique;* [3] *Université de Liège, Laboratoire de Démographie des Poissons et d'Aquaculture, B-4500 Tihange, Belgique;* [4]*IRD (ORSTOM), Groupe aquaculture continentale méditerranéenne et tropicale, F-34033 Montpellier, France*

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Abstract

Developmental changes in myofibrillar protein composition were investigated in the myotomal muscle of the African catfish, *Heterobranchus longifilis* (Clariidae), by several electrophoretic techniques. The main muscle fibres of larvae and the fast-white muscle fibres of juvenile and adult fish were found to express distinct myosin heavy chain and myosin light chain 2 (LC2) isoforms. Three myosin LC2 chains were successively detected, differing by their isoelectric points. In contrast, the alkali light chains remained qualitatively and quantitatively unchanged during fish growth. Actin, α-tropomyosin, and troponin-C (TN-C) were also similar in larval, juvenile, and adult white muscle, but an additional larval tropomyosin isoform was found in the first developmental stages. Two isoforms of troponin-T (TN-T) and troponin-I (TN-I) were synthesised in the course of fish growth. Transition from the larval to the adult isoform was much faster for TN-T than for TN-I. Slow-red muscle myofibrils from adult *H. longifilis* showed no common component (except actin) with larval, juvenile, or adult fast-white muscle myofibrils. Red myofibrils displayed a single TN-T and a single TN-I isoform, but two isoforms of TN-C. The myofibrillar protein isoforms synthesised at any given developmental stage almost certainly reflect changes in the functional requirements of swimming muscles in the course of fish development.

Introduction

Vertebrate muscle fibres are dynamic structures adapting to altered functional demands, hormonal signals, and changes in neural input. In higher vertebrates, multiple isoforms of the myofibrillar proteins are expressed in a tissue- and developmental-stage-specific manner (see reviews: Pette and Staron 1990; Schiaffino and Reggiani 1996). Muscle development in fish differs from that of mammals and birds. In most fish, the different fibre types of axial muscle develop in physically distinct areas and the production of new fibres continues throughout much of adult life in proportion to the continuous increase in body length (Weatherley and Gill 1985; Koumans and Akster 1995). Myotomes of adult fish contain a lateral zone with slow-red aerobic fibres. A zone of pink fibres separates the red fibres from the bulk of the musculature that consists of fast-white anaerobic fibres. Only red muscle is active at slow swimming speeds, whereas white muscle is used for bursts of rapid vigorous activity (Johnston et al. 1977; Bone 1978). As in terrestrial vertebrates, distinct isoforms of the myofibrillar proteins, chiefly myosin, have been described in fast-white and slow-red fish fibres. White-muscle myosin possesses three types of light chains while red-muscle myosin has only two, differing from the white-muscle types (Focant et al. 1976; Huriaux and Focant 1985; Rowlerson et al. 1985; Karasinski and Kilarski 1989; Huriaux et al. 1990; Martinez et al.

1990; Bassani and Dalla Libera 1991). White- and red-muscle myosins also differ in their heavy-chain subunits (Karasinski and Kilarski 1989; Chanoine et al. 1990; Martinez et al. 1990; Bassani and Dalla Libera 1991; Huriaux et al. 1991; Karasinski 1993). All these subunits show great species-related variability.

Fish muscle development is associated with sequential expression of a range of myofibrillar protein isoforms. Histochemical and immunohistochemical investigations have revealed developmental transitions in myosin composition in the sea bass *Dicentrarchus labrax* (L. 1758) (Scapolo et al. 1988): distinct myosin isoforms appear in early and late larvae, and these are different from those found in adult white or red muscle fibres. Myofibrillar isoforms have been studied more extensively in several other teleost species by a range of electrophoretic techniques. Martinez and collaborators (1991, 1993, 1994) analysed the development of two Salmonidae species, the Atlantic salmon, *Salmo salar* L. 1758 and the Arctic charr, *Salvelinus alpinus* (L. 1758). They found up to six different myosin heavy chains sequentially expressed in white skeletal muscle from the embryo to the adult stage. They observed qualitative and/or quantitative differences in expression of the alkali light chains LC1 and LC3 and in the relative proportions of the two tropomyosin subunits. Polymorphism of myosin isoforms has also been demonstrated during growth of the eel, *Anguilla anguilla* (L. 1758) (Chanoine et al. 1992). Changes in the composition of all myofibrillar proteins have been investigated in the myotomal muscle of the Atlantic herring, *Clupea harengus* L. 1758 reared at temperatures ranging from 5 °C to 15 °C. It was concluded that although the main muscle-fibre type in larvae shares some myofibrillar proteins with adult white muscle, it also contains characteristic isoforms of myosin heavy chains, myosin light chain LC2, troponin-I, and troponin-T and thus represents a distinct fibre type (Crockford and Johnston 1993). Embryonic isoforms of the aforementioned proteins were identified in presumptive white muscle, these being gradually replaced by other isoforms during the larval stages. The size range over which embryonic isoforms were present was inversely related to rearing temperature (Johnston et al. 1997). In a flatfish such as the plaice, *Pleuronectes platessa* L. 1758, metamorphosis does not correlate with changes in fibre types or myosin heavy chain composition (Brooks and Johnston 1993). In contrast, following metamorphosis and during the first year, inner-muscle fibres co-express

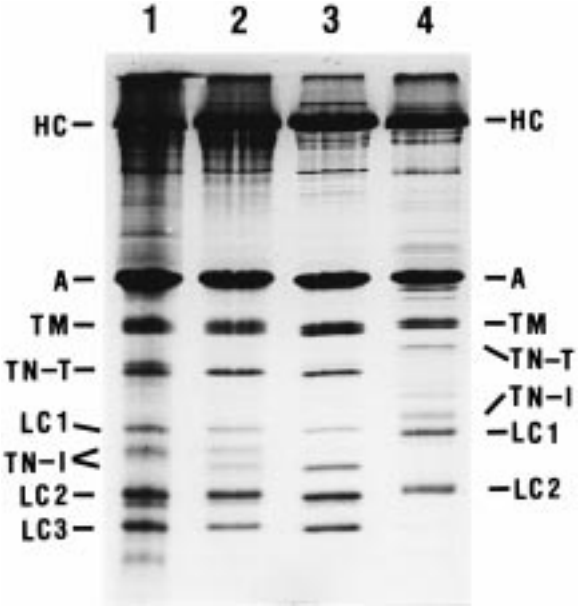

Figure 1. SDS-PAGE of myofibrillar proteins from (1) larval muscle (fish length: 1.5 cm), (2) juvenile muscle (11 cm), (3) adult white muscle (38 cm), and (4) adult red muscle (38 cm). HC: myosin heavy chain; A: actin; TM: tropomyosin; TN-T: troponin-T; LC1: myosin light chain 1; TN-I: troponin-I; LC2: myosin light chain 2; LC3: myosin light chain 3.

two LC2 isoforms characteristic of adult superficial fast muscle, in addition to the two larval isoforms.

Several freshwater fish studied by us seem to display a similar myofibrillar development pattern. In the barbel, *Barbus barbus* (L. 1758) and the rainbow trout, *Oncorhynchus mykiss* (Walbaum 1792), different myosin isoforms were identified in embryonic, larval, and adult white muscle, and the ratios of the alkali light chains were also found to change during development (Focant et al. 1992, 1994). In a recent work concerning an African catfish of the Claroteidae family, *Chrysichthys auratus* (Geoffroy Saint-Hilaire 1808), we described successive expression of two troponin-I isoforms (23.5 and 21.5 kDa). Both forms were present in equal amount in juvenile fish 5 cm long (Chikou et al. 1997).

The aim of the present study was to investigate the biochemical composition of the swimming muscles of the catfish, *Heterobranchus longifilis* Valenciennes 1840 (Clariidae), which is a key species in Western African fisheries and a candidate for tropical aquaculture because of its fast growth (Legendre et al. 1992; Hecht et al. 1996; Otémé et al. 1996). This biochemical approach should contribute further to knowledge on this species, chiefly to a better understanding of

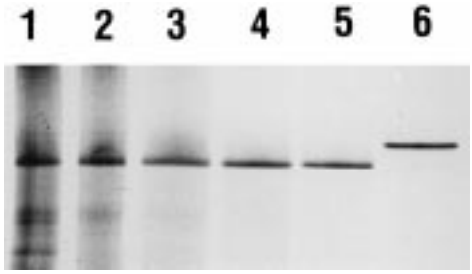

Figure 2. SDS-PAGE of myosin heavy chains. (1) larval muscle (fish length: 0.6 cm); (2) larval muscle (0.8 cm); (3) larval muscle (1.5 cm); (4) juvenile muscle (7.5 cm); (5) adult white muscle (38 cm); (6) adult red muscle (38 cm).

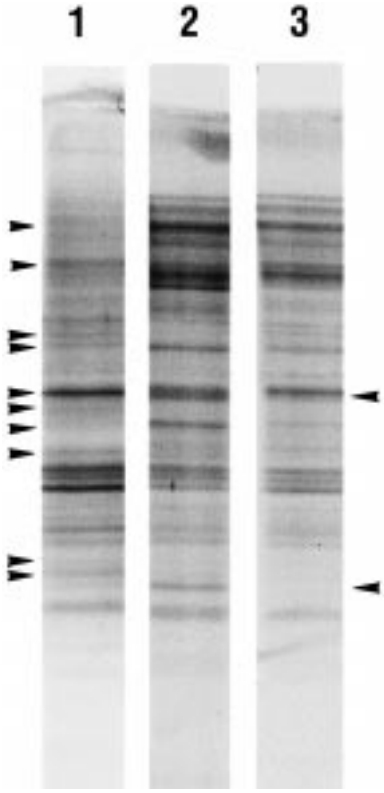

Figure 3. Peptide maps (SDS-PAGE) of myosin heavy chains from (1) larval muscle (fish length: 0.8 cm), (2) juvenile muscle (13 cm), and (3) adult white muscle (28 cm). Heavy chains were digested with *Staphylococcus aureus* V 8 protease for 1 min. Arrowheads to the left of the lanes indicate differences between larval and juvenile muscles, those to the right indicate differences between juvenile and adult muscles.

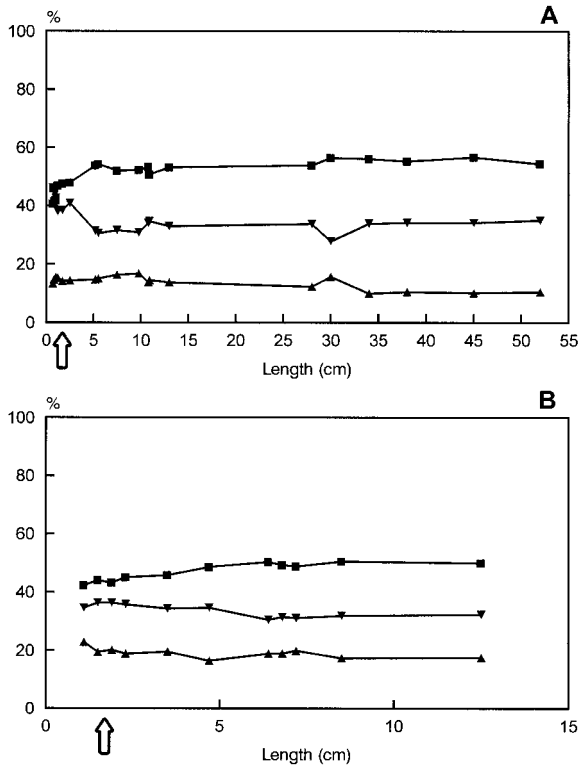

Figure 4. Evolution of the relative proportions of the three myosin light chains as a function of fish body length. (A) LDPA batch; (B) GAMET batch. Data were obtained from one-dimensional SDS-PAGE. ▲, LC1; ■, LC2; ▼, LC3. Arrow indicates the larval-to-juvenile stage transition (1.7 cm).

the relationships between biochemical and morphological (Legendre and Teugels 1991; Vandewalle et al. 1997) or behavioural (Baras 1999) changes during its ontogeny. In the present study, the expression of all myotomal myofibrillar proteins was investigated by high-resolution electrophoresis in the course of development. Red-muscle myofibrils from adult fish were also examined in order to detect the possible presence of red-fibre isoforms during white muscle ontogeny.

Materials and methods

Fish samples

H. longifilis specimens were obtained from two experimental hatcheries: LDPA (University of Liège, Tihange, Belgium) and IRD-GAMET (Montpellier, France). To make it easier to compare fish of different breeds (± 27 °C), growth stages were expressed in relation to standard length rather than age. The mor-

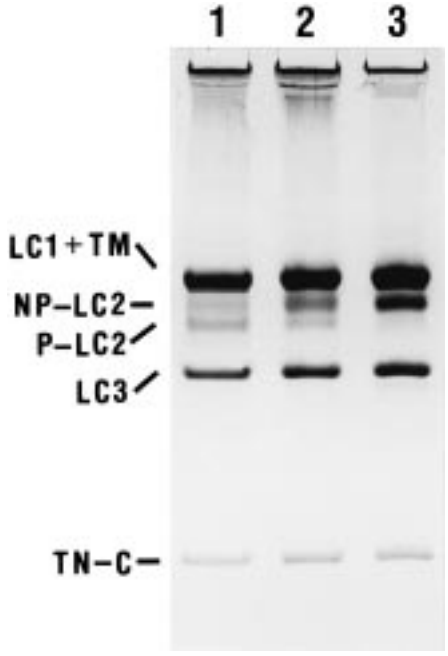

Figure 5. Alkali-PAGE of myofibrillar proteins incubated in the presence of 0.005 M EGTA, from (1) juvenile muscle (fish length: 5.2 cm), (2) juvenile muscle (10.9 cm), and (3) adult white muscle (45 cm). TM: tropomyosin; LC1: myosin light chain 1; NP-LC2: myosin non-phosphorylated light chain 2; P-LC2: myosin phosphorylated light chain 2; LC3: myosin light chain 3; TN-C: troponin-C.

phological stages of post-hatching development were established according to Legendre and Teugels (1991).

The LDPA batch of developing fish was sampled from 0.3 to 13 cm corresponding to day 1 post-hatching to day 100 (in graphs: 0.6 cm, day 4; 0.7 cm, day 5; 0.8 cm, day 8; 0.95 cm, day 10; 1.2 cm, day 15; 1.7 cm, day 20; 2.5 cm, day 30; 5.2 cm, day 40; 5.5 cm, day 50; 7.5 cm, day 60; 9.8 cm, day 70; 10.8 cm, day 80; 10.9 cm, day 90; 13 cm, day 100). Adult specimens (length 28 to 52 cm) were also obtained from this hatchery. The IRD-GAMET batch was sampled from 0.3 to 12.5 cm corresponding to day 1 post-hatching to day 70 (in graphs: 1.1 cm, day 10; 1.5 cm, day 13; 1.9 cm, day 18; 2.3 cm, day 21; 3.5 cm, day 25; 4.8 cm, day 29; 6.4 cm, day 33; 6.8 cm, day 37; 7.2 cm, day 41; 8.5 cm, day 47; 12.5 cm, day 70). The larvae became juvenile between 1.6 and 1.8 cm. All specimens were stored frozen.

Dissection of muscle samples was adapted to specimen size. For fish up to 1 cm, 30 specimens were pooled and the head, yolk-sac, and tail were removed. For fish up to 5 cm, 20 to 4 specimens were pooled and their trunk white muscle was collected after removal of the skin along with the superficial muscle fibres. For each length exceeding 5 cm, white muscle was dissected from a single specimen. For adult fish, only a piece of white muscle located in front of the dorsal fin was cut out. Red muscle was dissected at the level of the lateral line of adult fish (35–38 cm long); special care was taken to remove any pink or white fibres. Tibialis anterior rabbit muscle was used as a reference for fast-muscle tropomyosin isoforms. Samples were handled on ice for minimum proteolytic breakdown; they were minced, kept in glycerol preservative solution according to Focant et al. (1992), and conserved at −18 °C until required.

Preparation of myofibrils

Muscle preserved in glycerol was isolated by centrifugation and homogenised with Ultra-Turrax T25 (IKA) (3 × 25 s at 20,500 rpm) in 10 vol ice-cold buffer (pH 7.4) containing 0.05 M KCl, 0.01 M Tris-HCl, 0.005 M EDTA and the following proteolytic enzyme inhibitors (Sigma): 300 μM phenylmethylsulphonyl fluoride, 150 μM n-tosyl-L-phenylalanine chloromethyl ketone, 1 μM leupeptin, 1.5 μM pepstatin A, 0.1 μM aprotinin. The suspension was centrifuged for 30 min at 17,000 × g and the pellet was again treated under the same conditions. It was then suspended in 10 vol of 40% (w/v) sucrose, 0.1 M KCl, 0.005 M Tris-HCl, pH 7.4, homogenised for 25 s at 20,500 rpm, and centrifuged for 10 min at 17,000 × g. The final pellet containing washed myofibrils was incubated for gel electrophoresis or suspended in 50% (v/v) glycerol, 0.1 M KCl, 0.005 M Tris-HCl, pH 7.4 for preservation at −18 °C.

Incubation of proteins

Myofibril components were dissociated by mixing the myofibril pellets in at least 2 vol of one of the following three solutions: for alkali-PAGE, 0.02 M Tris, 0.12 M glycine, 8 M urea, 5% (v/v) 2-mercaptoethanol, pH 8.6 (with or without 0.005 M EGTA); - for SDS-PAGE, 0.0625 M Tris-HCl, 2% SDS, 10% (v/v) glycerol, 5% (v/v) 2-mercaptoethanol, pH 6.8, with heating for 3 min at 100 °C; - for isoelectric focusing (IEF) and non-equilibrium isoelectric focusing (NEIEF)-PAGE, 2% Servalyt ampholytes 3–10, 9 M urea, 2% (v/v) Nonidet P–40, 5% (v/v) 2-mercaptoethanol.

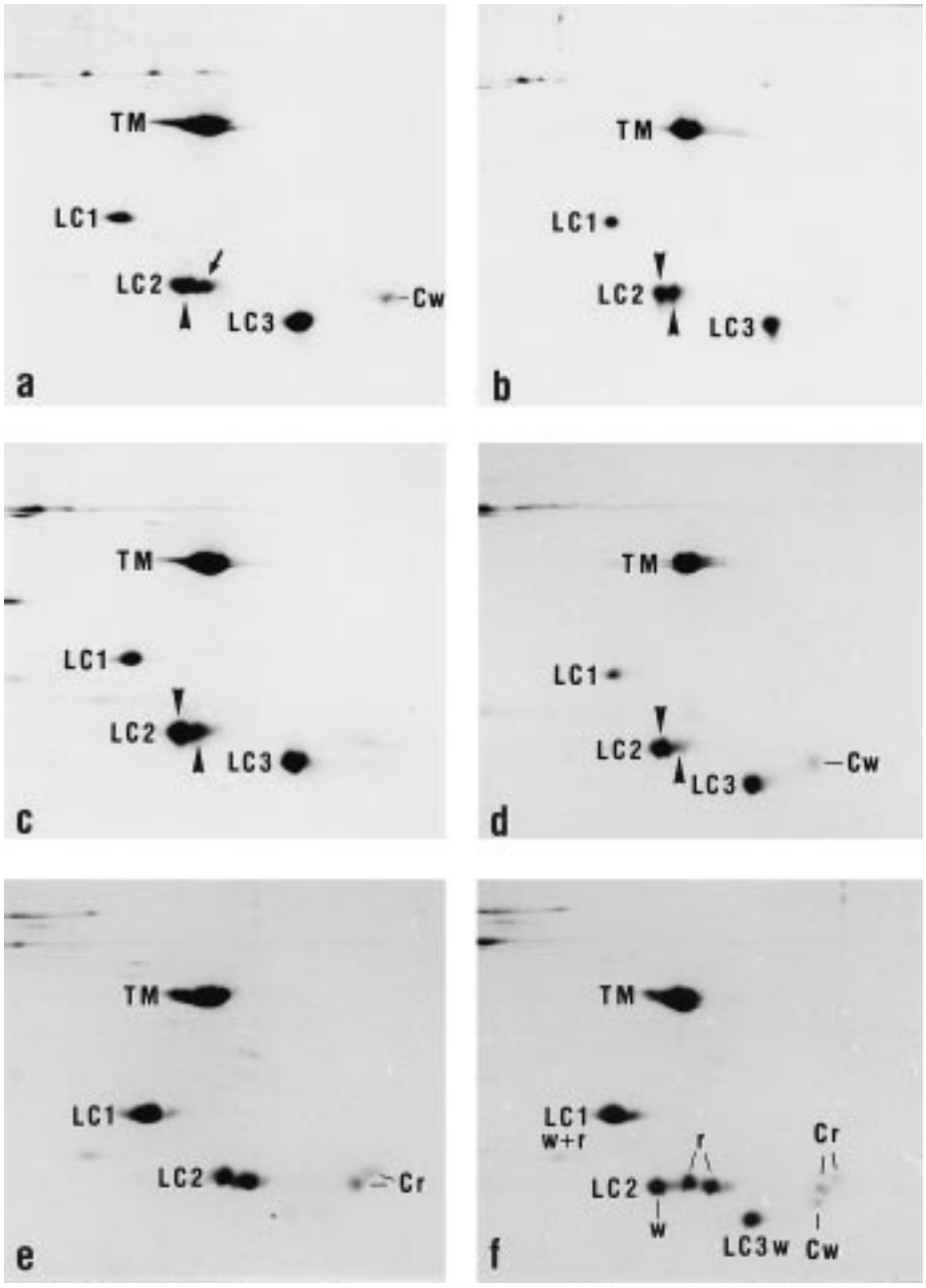

Figure 6. Two-dimensional PAGE of acidic myofibrillar proteins with an IEF gel as the first dimension (pH 5.4 on the left side and pH 4.3 on the right side of the window of the electrophoretogram). (a) larval muscle (fish length: 1.2 cm), (b) juvenile muscle (5.2 cm), (c) juvenile muscle (10.9 cm), (d) adult white muscle (35 cm), (e) adult red muscle (35 cm), (f) comigration of adult white (d) and red (e) muscle. TM: tropomyosin; LC1: myosin light chain 1; LC2: myosin light chain 2; LC3: myosin light chain 3; C: troponin-C; w: white muscle; r: red muscle. The arrow shows the position of larval LC2 and the arrowheads the positions of phosphorylated-LC2 (▲) and non-phosphorylated LC2 (▼).

Polyacrylamide gel electrophoresis (PAGE)

Analytical PAGE separations of myofibrillar proteins were performed in a Bio-Rad Mini-Protean II cell (vertical plate 8.3 × 6 × 0.075 cm) under various conditions. For whole myofibrils, discontinuous SDS-PAGE was done in gels containing 20% acrylamide and 0.1% bis-acrylamide according to Laemmli (1970), but at pH 8.4. For myosin heavy chains, discontinuous SDS-PAGE was done in gels containing 6% acrylamide, 0.1% bis-acrylamide, and 40% (v/v) glycerol according to Danieli Betto et al. (1986), but at pH 8.4 and for 5 h at 10 mA. IEF-PAGE was carried out on gel made with 7.1% acrylamide, 0.4% bis-acrylamide and usually containing 1.6% Servalyt 3–6, 0.4% Servalyt 3–10, 9.2 M urea, and 2% (v/v) Nonidet P–40 or 1.6% Servalyt 4–5, 0.4% Servalyt 3–10, 8 M urea, and 2% (v/v) Nonidet P–40 (for better discrimination of the three LC2 forms in two-dimensional PAGE), at 2,500 V h. NEIEF-PAGE was carried out on 7.1% acrylamide, 0.4% bis-acrylamide gels containing 1% Servalyt 7–9, 1% Servalyt 9–11, 9.2 M urea and 2% (v/v) Nonidet P–40 at 400 V h (O'Farrell et al. 1977). Alkali-PAGE was performed in gels containing 10% acrylamide, 0.26% bis-acrylamide, 8 M urea, at pH 8.6 (Focant et al. 1976). Two-dimensional electrophoresis was carried out using IEF-, NEIEF-, or alkali-PAGE as the first dimension (from left to right) and SDS-PAGE as the second dimension (from top to bottom). Vertical strips were cut from first-dimension gels and loaded horizontally on 1-mm-thick second-dimension gels as described by O'Farrell (1975).

SDS and alkali gels were routinely stained with Coomassie Brilliant Blue R-250 or, for detection of troponin-C in two-dimensional gels, with silver (Schleicher and Watterson 1983). IEF and NEIEF gels were stained with 0.04% Coomassie Brilliant Blue R-250, 0.05% Crocein Scarlet 7B, 0.5% $CuSO_4$, 27% isopropanol, 10% acetic acid. Apparent relative molecular masses (M_r) were estimated using protein standard kits covering the range 14.4 to 97.4 kDa (SDS-PAGE Standards, Low Range, Bio-Rad). Isoelectric points (pI) were determined using the *H. longifilis* parvalbumins PA II (pI 4.51), PA III (pI 4.76), and PA IV (pI 5.21) as standards (Focant et al. 1999). M_r and pI determinations were done from one-dimensional gels. Densitometry was performed with a Bio-Rad Model GS-670 densitometer combined with the Molecular Analyst Gelscan software. Measurements were done on at least three gels.

Identification of myofibrillar proteins

The different proteins were located by comparing their mobilities in the different gel systems: each band separated by IEF-PAGE or alkali-PAGE in the presence or absence of EGTA was cut out and subjected to SDS-PAGE and IEF-PAGE. Myosin heavy chains and actin were identified by their relative abundances and apparent M_r on SDS-polyacrylamide gels. Tropomyosin was identified on the basis of its anomalous migration on SDS-gels containing 8 M urea (Sender 1971). Myosin light chains were identified by their low apparent M_r and acidic pI (Huriaux and Focant 1977). The troponin complex was isolated by extracting the acetone powder in 0.6 M LiCl, 0.05 M Tris-HCl, 0.005 M 2-mercaptoethanol, pH 7.5, eliminating tropomyosin at pH 4.6, and fractionating the supernatant with $(NH_4)_2SO_4$ between 40 and 70% saturation (Mc Cubbin et al. 1982). This preparation was used to locate troponin-T, troponin-I, and troponin-C by one- and two-dimensional PAGE. Troponin-T and troponin-I have basic isoelectric points but different apparent M_r values (Crockford and Johnston 1993). Troponin-C is the most acidic component of myofibrils and was identified by its characteristic blue stain with 'Stains all' (Campbell et al. 1983).

Isolation and peptide mapping of myosin heavy chains

Heavy chains were isolated by discontinuous SDS-PAGE at pH 8.8 in gels 1.5 mm thick (Mini-Protean II) made with 6% acrylamide, 0.1% bis-acrylamide, 40% (v/v) glycerol. The heavy-chain bands were located by staining of external migration lanes. They were cut out and incubated in 1 ml buffer (pH 7.4) containing 0.05 M Tris-HCl, 0.001 M EDTA, 1% SDS. They were eluted with the Model 422 Electro-Eluter (Bio-Rad) in 0.05 M Tris-HCl, 0.001 M EDTA, 0.1% SDS, pH 7.4 (2 h at 10 mA/tube followed by 12 h at 5 mA/tube). The electrode buffer was changed once. Eluted heavy chains (0.4 ml) were collected in the membrane cap (membrane molecular weight cut-off 12–15 kDa). The pH of the protein-containing solution was adjusted to 7.5.

Six μg of *Staphylococcus aureus* V8 protease (Sigma) dissolved in water was added to 0.2 ml eluted heavy chains. Digestion was carried out at 20 °C for 1 and 10 min, then stopped by addition of 2 μl 2-mercaptoethanol, 20 μl 20% SDS and heating at 100 °C for 2 min (Crockford et al. 1991). The peptides produced were resolved on discontinuous SDS

gels (20% acrylamide) under the usual conditions. The gels were silver-stained.

Results

Identification of myofibrillar proteins

Myofibrillar protein bands were detected, after PAGE, in larvae 0.4 cm long (day 2), but densitometric evaluation was possible only from 0.6 cm (day 4 – LDPA batch) or from 1.1 cm (day 10 – GAMET batch) onward because of a protein background in first-stage samples. Specimens from both hatcheries displayed very similar electrophoretic patterns and developmental kinetics when results were related to the fish body length. One-dimensional SDS-PAGE of larval muscle, juvenile white muscle, adult white and adult red muscle revealed all myofibrillar components except troponin-C (TN-C), present in too-low amount (Figure 1). Only for myosin heavy chains and actin was the apparent relative molecular mass (M_r) the same in all fibre types (Figure 1 and Table 1). Myofibrils from larval, juvenile, and adult white muscle appeared similar except as regards troponin-T (TN-T) (presence of two close isoforms in larval samples) and especially troponin-I (TN-I). One TN-I isoform was found in larvae and another in adult fish; both forms were expressed in the juvenile muscle. The apparent M_r of TN-C was evaluated by cutting the EGTA-myofibril TN-C bands from alkali gels and subjecting their content to SDS-PAGE. Except myosin heavy chains and actin, adult red myofibrils shared no bands with white myofibrils, and two TN-C isoforms were detected in the red myofibrils.

Myosin heavy chains

These high-M_r ($\sim$ 200 kDa) myofibrillar components were analysed by SDS-PAGE at pH 8.8 or 8.4 and at several glycerol concentrations (Huriaux et al. 1991). The different myofibril samples showed a single band in all gel types. No mobility difference was observed when the glycerol concentration was 25% (v/v). Protein bands were best distinguished in 40% (v/v) glycerol, pH 8.4 (Figure 2). Myosin heavy chains from larval, juvenile, and adult white muscle migrated identically under these conditions, but the adult red-muscle isoform migrated more slowly, thus reflecting a different chemical composition. *Staphylococcus aureus* V8 protease peptide mapping revealed further differences among the myosin heavy-chain bands from larval, juvenile, and adult white myofibrils (Figure 3). It thus appears that these four muscle types express a different heavy chain.

Table 1. Apparent relative molecular masses (kDa) of the main myofibrillar proteins in *H. longifilis*

Protein	White muscle	Red muscle
Actin	44.0	44.0
Tropomyosin	36.0	36.2
Light chain 1	26.5	26.0
Light chain 2	19.5	20.0
Light chain 3	16.0	–
Troponin-T	31.5	33.0
	31.0	
Troponin-I	25.0	27.0
	23.0	
Troponin-C	18.0	20.0
		19.0

Myosin light chains

The relative proportions of the three myosin light chains were evaluated by SDS-PAGE in the course of development (Figure 4). No significant difference in light-chain 'stoichiometry' was found during fish growth. The LC2 content remained predictably near 50% in all but the first larval stages, where it appeared lower (Figure 4A), probably because of the greater instability of this regulatory light chain (larval LC2 – see below). The LC3 content varied inversely to the LC2 content, its titre always remaining higher than that of LC1 as in all fish myosins. When myofibril preparations are run in the presence of EGTA on alkali gels, only tropomyosin, myosin light chains, and TN-C enter the gel. Under these conditions, the phosphorylatable LC2 from specimens 5.2 cm long migrated as a double band (Figure 5, lane 1). According to its electrophoretic location, the slow band could be identified as the non-phosphorylated form (NP-LC2, pI 5.06) and the fast band as the phosphorylated form (P-LC2, pI 5.01) of this light chain (Pires et al. 1974; Focant and Huriaux 1976). Both bands appeared equally intense in this juvenile fish, the fast band diminishing during growth and becoming very minor in adult specimens (Figure 5, lanes 2 and 3). The two alkali light chains LC1 (pI 5.25) and LC3 (pI 4.68) displayed no change with age.

For better precision, myosin light chains were investigated in the course of fish development using the more discriminating two-dimensional PAGE with isoelectric focusing as the first dimension (Figure 6). There was no evidence of differences in myosin LC1 or LC3 from larvae to adults. Both NP-LC2 and P-LC2 were found in juvenile white muscle (Figure 6b, c) but the less acidic spot (NP-LC2) was almost alone when the extract was from an adult specimen 35 cm long (Figure 6d). Two LC2 spots were also visible on two-dimensional electrophoretograms obtained with myofibril preparations from larvae 1.2 cm long. In this case, however, the less acidic spot corresponded with the more acidic one observed in juvenile fish (P-LC2); the other spot was identified as larval LC2 (Figure 6a). Figure 7 shows the variation of the proportions of the three LC2 forms with respect to the body length. In larvae 0.8 cm long, larval LC2 and P-LC2 were in nearly equal amount. Larval LC2 decreased regularly and no longer appeared once the body length reached 3.5–5.0 cm. P-LC2 diminished from 2.5–3.5 cm onward but remained the major isoform until 5 cm. NP-LC2 appeared at 2.5 cm and increased until 13 cm, at which time it represented 85% of total LC2 light chain. The relative proportions of NP-LC2 and P-LC2 remained relatively constant during adult life.

On two-dimensional polyacrylamide gels, no difference was detected between adult white-muscle and adult red-muscle LC1 myosin light chains (pI of red muscle LC1: 5.23) (Figure 6e, f). Myosin light chain LC2 from red myofibrils displayed two spots (pI values 4.93 and 4.83), both distinct from white-muscle LC2.

Tropomyosin

In two-dimensional PAGE, two spots were obtained for tropomyosin from adult white and adult red myofibrils. In both cases, the two spots displayed the same pI (4.97) and very similar apparent M_r values (Figure 8a, b). The lighter component of red-muscle tropomyosin migrated at the level of the heavier component of white-muscle tropomyosin (Figure 1). All these subunits migrated close to rabbit α-tropomyosin (Figure 8c–e). The two white-muscle components displayed a constant 1:1 ratio during fish development. An additional spot with a more acidic pI (4.87) was detected in young larvae; its amount diminished with increasing age and disappeared at the end of the larval stage (Figure 8,f–h). It was thus identified as a larval tropomyosin isoform.

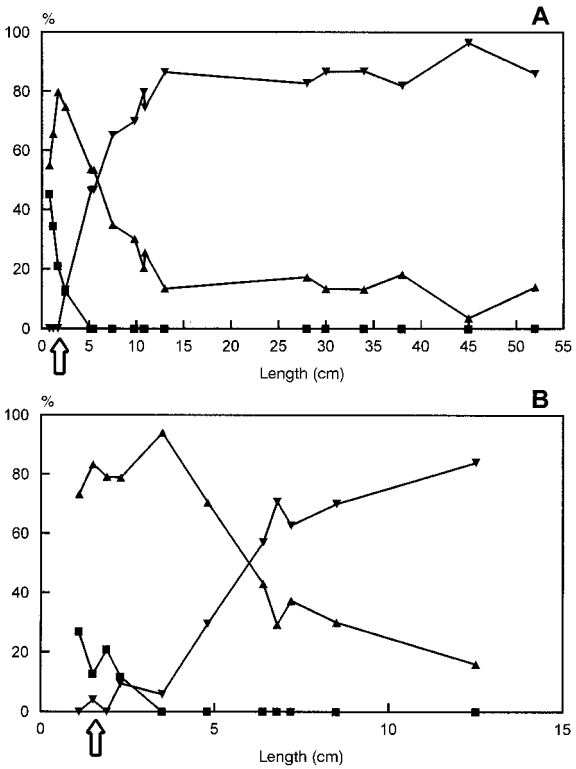

Figure 7. Evolution of the relative proportions of the three myosin LC2 chains as a function of fish body length. (A) LDPA batch; (B) GAMET batch. Data were obtained from two-dimensional PAGE. ■, larval LC2; ▲, phosphorylated LC2; ▼, non-phosphorylated LC2. Arrow indicates the larval-to-juvenile stage transition (1.7 cm).

Troponin-T and troponin-I

These basic proteins presented a problem when subjected to conventional two-dimensional PAGE. Even with ampholytes in the pH range of 9–11, TN-T and TN-I from *H. longifilis* appeared as streaks on the focusing gel. This problem was solved by using the method of O'Farrell et al. (1977), using a non-equilibrium pH gradient in the first dimension. The samples were applied on a gel containing ampholytes in the pH range of 7–11 and were electrophoresed for a short time so that the proteins did not reach their equilibrium positions but were separated by charge.

Two TN-T isoforms with close apparent M_r values (31.5 and 31.0 kDa) were co-expressed during fish development, as observed after SDS-PAGE (Figure 1 and Table 1). The 31.5 kDa isoform predominated in the early larval stages but it diminished rapidly in proportion during fish growth (Figure 9). The two isoforms appeared in equal amount at the end of the

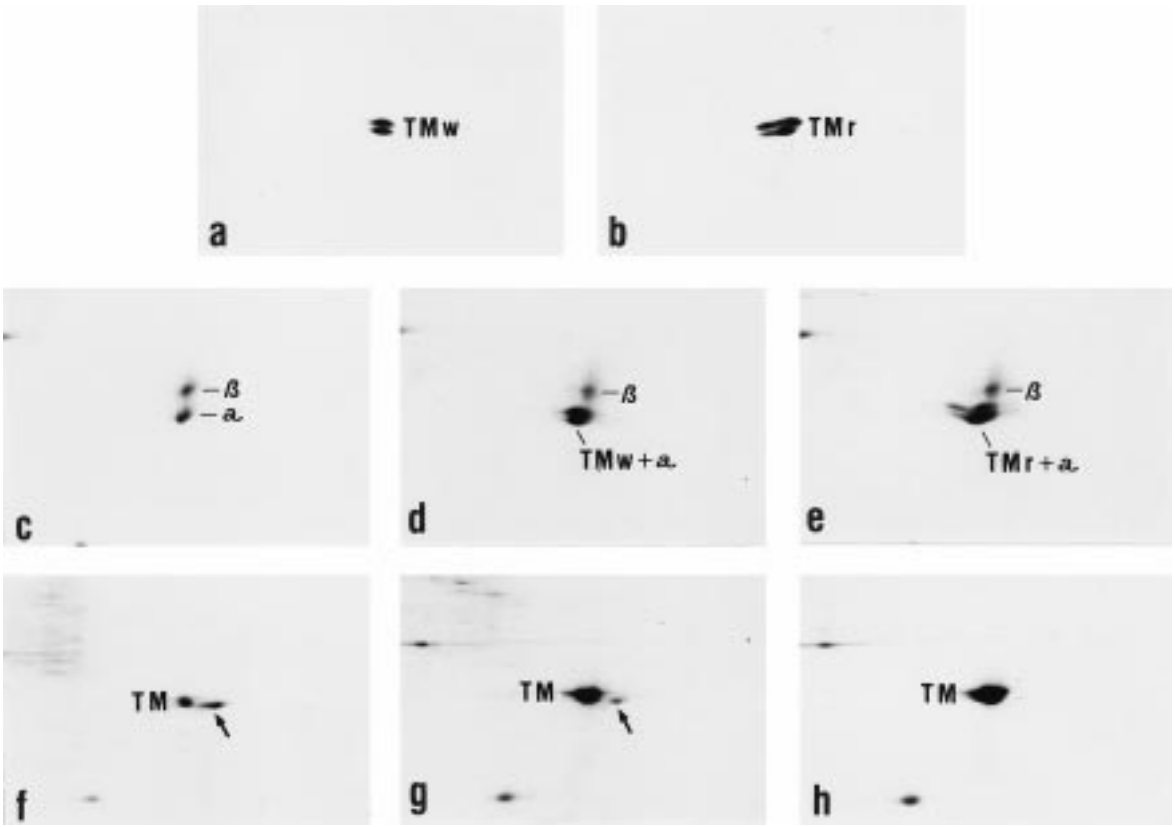

Figure 8. Two-dimensional PAGE of tropomyosin with an IEF gel as the first dimension (pH 5.4 on the left side and pH 4.5 on the right side of the window of the electrophoretogram). (a) *H. longifilis* adult white muscle (fish length: 35 cm); (b) *H. longifilis* adult red muscle (35 cm); (c) rabbit fast muscle; (d) comigration of proteins from *H. longifilis* adult white muscle (a) and rabbit fast muscle (c); (e) comigration of proteins from *H. longifilis* adult red muscle (b) and rabbit fast muscle (c); (f) *H. longifilis* larval muscle (fish length: 0.7 cm); (g) *H. longifilis* larval muscle (1.1 cm); (h) *H. longifilis* larval muscle (1.5 cm). TMw: *H. longifilis* white-muscle tropomyosin; TMr: *H. longifilis* red-muscle tropomyosin; α: α-chain of rabbit fast muscle tropomyosin; β: β-chain of rabbit fast muscle tropomyosin. Arrow in gels (f) and (g) indicates the presence of the larval isoform of *H. longifilis* tropomyosin.

larval stage. The 31.0 kDa isoform increased in proportion to reach 90% of the total TN-T in specimens 11–13 cm long; it stabilised at about 70-80% in adult fish. On two-dimensional gels, the major and minor TN-T spots were detected only from a length of 5.5–6.5 cm onward; they exhibited a slightly different electric charge at alkaline pH (Figure 10b, c). Adult red-muscle myofibrils had a single TN-T isoform with an apparent M_r (33 kDa) higher than those of both white-muscle isoforms (Figure 1, Table 1). This red-muscle isoform was not detected by two-dimensional PAGE (Figure 10 d).

SDS-PAGE revealed two successively appearing TN-I isoforms, varying in relative proportion as a function of fish development (Figure 1, Table 1). A larval TN-I isoform (25 kDa) was gradually replaced by an adult TN-I isoform (23 kDa) from 2.0–2.5 cm onward (Figure 11). The latter isoform represented 80% of the total TN-I in biggest fish. The two TN-I spots displayed distinct electrophoretic mobilities in NEIEF-PAGE experiments, corresponding very likely to different isoelectric points (Figure 10a–c). In these experiments the larval isoform was always accompanied by a minor spot migrating to a lower position, clearly visible in Figure 10a. A single TN-I spot was observed in red-muscle myofibrils. This spot displayed a higher electrophoretic mobility and a higher apparent M_r than its white-muscle counterparts (Figure 10d, e).

Troponin-C

This calcium-binding protein was examined by two-dimensional PAGE using IEF-PAGE (Figure 6) or alkali-PAGE in the presence of 0.005 M EGTA (Fig-

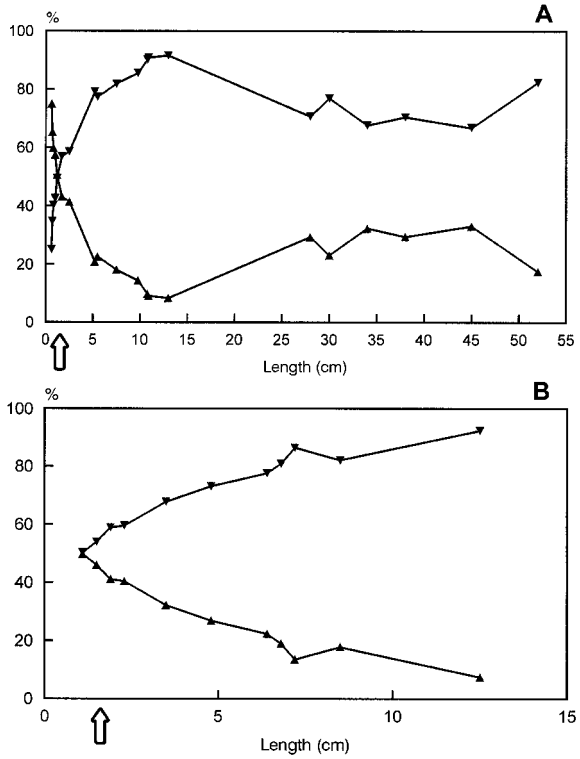

Figure 9. Evolution of the relative proportions of the two troponin-T isoforms as a function of fish body length. (A) LDPA batch; (B) GAMET batch. Data were obtained from one-dimensional SDS-PAGE. ▲, larval isoform; ▼, adult isoform. Arrow indicates the larval-to-juvenile stage transition (1.7 cm).

ure 12) as the first dimension. Both methods gave similar results but the alkali-PAGE yielded more readily detectable TN-C spots. Under these conditions, the specific complex formed by TN-I and TN-C was dissociated by EGTA and TN-C migrated as a very fast band (Syska et al. 1974). White-muscle preparations from fish at the various developmental stages consistently yielded a single spot, always characterised by the same isoelectric point, relative mobility on alkali gels, and apparent M_r. TN-C from adult red muscle, however, separated into two isoforms with slightly different isoelectric points, relative mobilities at alkaline pH, and apparent M_r values, both distinct from white-muscle TN-C. White-muscle TN-C had a pI of 4.41 and the two red-muscle TN-C isoforms averaged a pI of 4.37.

Discussion

Up to now, little is known about how the biochemical composition of myofibrils from catfish muscles evolves in the course of development. In a preliminary note concerning *Clarias gariepinus* (Burchell 1822), we found no structural difference between myosin isolated from larval and adult white muscle (Focant et al. 1996). In a multidisciplinary survey of *C. auratus*, we monitored expression of the three myosin light chains and two troponin-I isoforms during growth (Chikou et al. 1997). Here we have investigated developmental changes in the composition of all myofibrillar proteins in the swimming muscles of the Clariidae species *H. longifilis*. We have monitored these changes from hatching to the adult stage, using several different high-resolution electrophoretic techniques. The myofibrillar extracts were compared with extracts of adult red-muscle fibres. Specimens from two different breeds were examined. The GAMET batch only composed of larval and juvenile stages confirmed results obtained with the LDPA batch.

Myosin

Myosin heavy- and light-chain composition is a major determinant of muscle shortening velocity (Schiaffino and Reggiani 1996). The peptide maps obtained here with *H. longifilis* muscle preparations show that distinct myosin heavy-chain isoforms are synthesised in the white muscle of larvae, juveniles fishes and adults. Similar observations have been made on other teleost (Martinez et al. 1991, 1993; Chanoine et al. 1992; Focant et al. 1992; Brooks and Johnston 1993; Crockford and Johnston 1993; Johnston et al. 1997; James et al. 1998). One- and two-dimensional PAGE did not reveal any qualitative or quantitative difference in the alkali light chains LC1 and LC3 during development. At any stage, the LC3 titre is higher than the LC1 titre, as in all adult fish myosins. Both catfish previously examined showed similar characteristics (Focant et al. 1996; Chikou et al. 1997). In other teleosts such as the barbel (Focant et al. 1992), trout (Focant et al. 1994), or Arctic charr (Martinez et al. 1991), the proportion of LC3 is very low at hatching and increases regularly in the early larval stages.

On the other hand, there appear three forms of the phosphorylatable myosin light chain LC2 during *H. longifilis* development. These display the same apparent relative molecular mass but distinct isoelectric points. The first, expressed in the larval stage, disappears during the juvenile stage and was thus identified as ‘larval LC2’. The other two forms are clearly visible on alkali gels from the time the fish reach 5 cm. Their electrophoretic mobilities suggest that

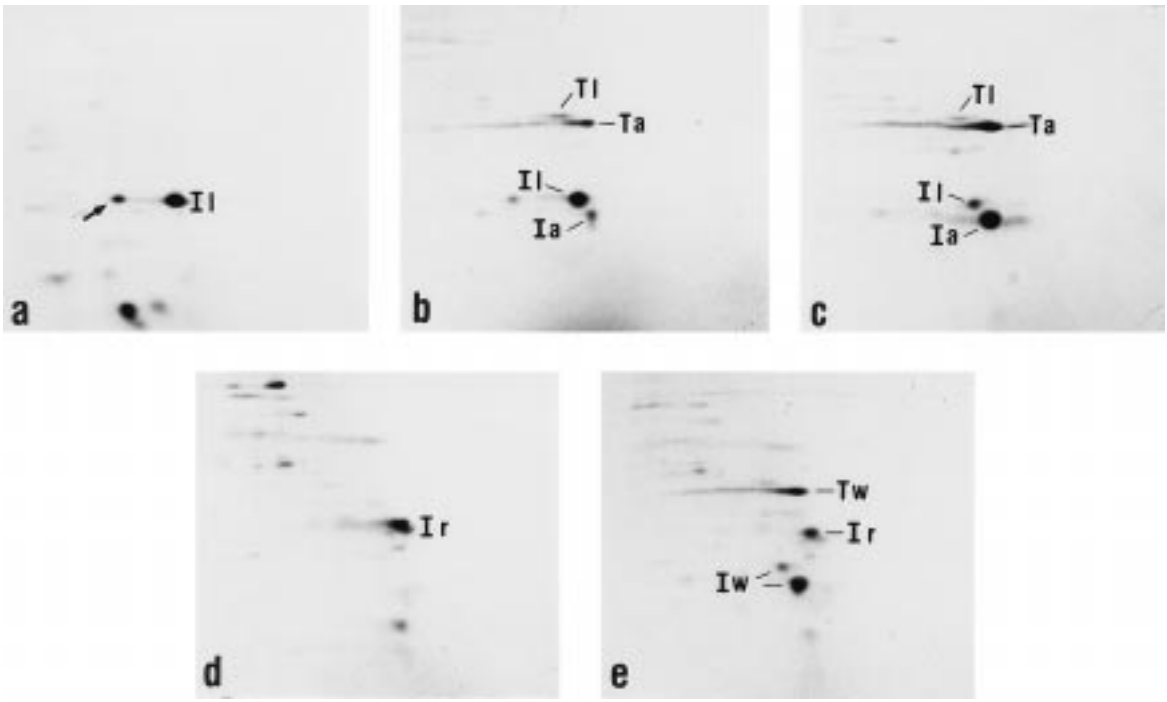

Figure 10. Two-dimensional PAGE of basic myofibrillar proteins with an NEIEF gel as the first dimension. In NEIEF-PAGE, proteins migrated to the cathode (right). (a) larval muscle (fish length 1.2 cm); (b) juvenile muscle (5.5 cm); (c) adult white muscle (35 cm); (d) adult red muscle (35 cm); (e) comigration of proteins from adult white (c) and red (d) muscle. T1: larval troponin-T; Ta: adult troponin-T; I1: larval troponin-I; Ia: adult troponin-I; Tw: white muscle troponin-T; Iw: white muscle troponin-I; Ir: red muscle troponin-I. Arrow in gel (a) indicates the satellite spot of the larval troponin-I.

they are the non-phosphorylated and phosphorylated forms of the same adult LC2. Phosphorylated LC2 diminishes during the juvenile stage and is very minor in the adult specimens. Distinct larval and adult LC2 forms have also been described in the Atlantic herring (Crockford and Johnston 1993) and plaice (Brooks and Johnston 1993), but in these fish the two isoforms display not only different isoelectric points as in *H. longifilis*, but also different apparent relative molecular masses, suggesting different chemical compositions. As the myosin light chain LC2 is located at the head-rod junction of the myosin molecule, it would appear to influence the mechanical properties of the muscle fibre (Moss et al. 1982). Successive synthesis of different myosin LC2 forms must presumably enable modulation of the contractile response of the myotomal musculature according to the developmental stage although the functional significance of LC2 phosphorylation has not been established in fish muscle (Yancey and Johnston 1982).

I filament proteins

Actin is the main protein of the I filament and appears unchanged during growth of *H. longifilis*.

Tropomyosin is involved, with the troponin complex, in calcium regulation of muscular contraction. In mammals, this protein consists of two subunits, α and β, whose ratio appears to be species- and muscle-specific (Pette and Staron 1990). By electrophoresis, white muscle from various fish species has been found to contain either α-type tropomyosin or β-type tropomyosin (Heeley and Hong 1994). *H. longifilis* tropomyosin splits into two spots (with very similar molecular masses) when subjected to two-dimensional PAGE. The complex appears identical throughout growth and corresponds with the α-tropomyosin subunit of mammals. An additional, acidic spot is present early in development, as in developing salmon fry (Heeley et al. 1995); it was identified as a larval α-tropomyosin isoform.

The troponin complex consists of three different subunits in equimolar proportions, two basic proteins, TN-T and TN-I, and an acidic calcium-binding pro-

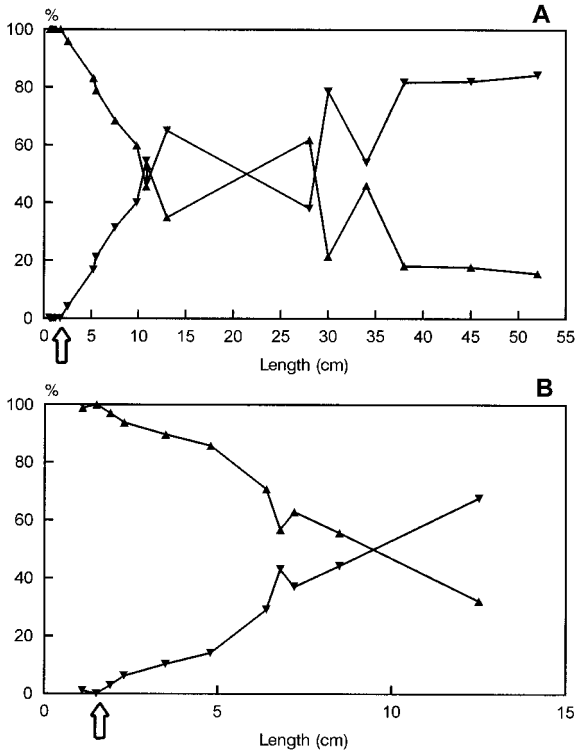

Figure 11. Evolution of the relative proportions of the two troponin-I isoforms as a function of fish body length. (A) LDPA batch; (B) GAMET batch. Data were obtained from one-dimensional SDS-PAGE. ▲, larval isoform; ▼, adult isoform. Arrow indicates the larval-to–juvenile stage transition (1.7 cm).

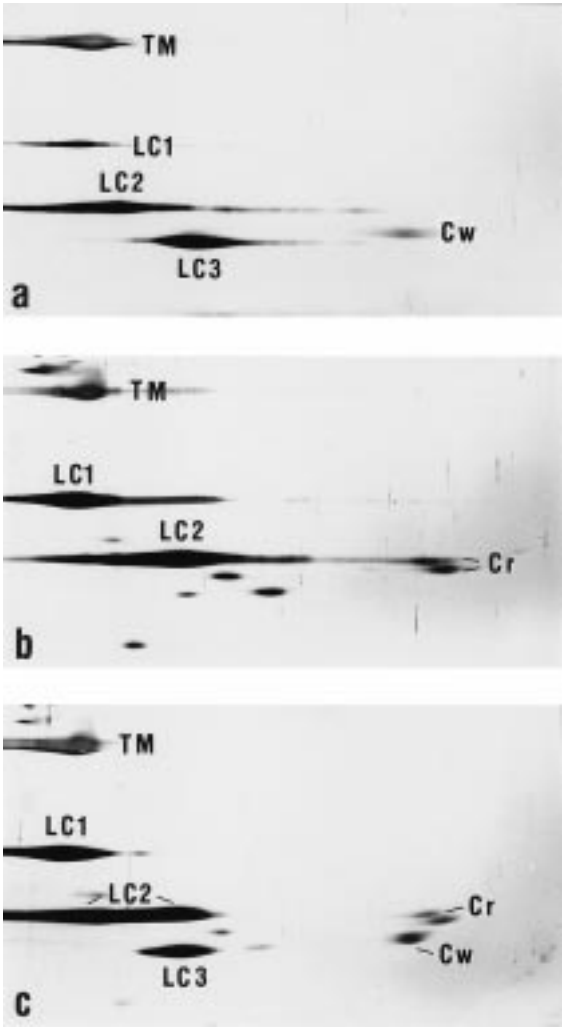

Figure 12. Two-dimensional PAGE of acidic myofibrillar proteins with alkali gel as the first dimension. In alkali-PAGE, proteins were incubated in the presence of 0.005 M EGTA and migrated to the anode (right). The gels were silver-stained. (a) adult white muscle (fish length: 35 cm); (b) adult red muscle (35 cm); (c) comigration of proteins from adult white (a) and red (b) muscle. Cw: white muscle troponin-C; Cr: red muscle troponin-C; TM: tropomyosin; LC1: myosin light chain 1; LC2: myosin light chain 2; LC3: myosin light chain 3.

tein, TN-C. Two isoforms of TN-T and two of TN-I appear during the development of *H. longifilis*, but transition from one form to the other occurs at different speeds for the two troponins. The larval form of TN-T is present already in the juvenile stage as the minor component. For TN-I, transition from the larval to the adult isoform is slower, some adult specimens still exhibiting equal amounts of the two forms. The different sequential synthesis of TN-T and TN-I isoforms suggests that the expression of the genes for the two subunits is under different control. Distinct embryonic, larval, and adult isoforms of TN-T and TN-I have been described in the development of the Atlantic herring (Crockford and Johnston 1993; Johnston et al. 1997). These authors find the existence of a larval TN-I isoform particularly interesting, since no similar isoform has ever been identified in higher vertebrates. In *H. longifilis* contrary to the herring, however, the larval TN-T and especially TN-I isoforms remain quite abundant in adult specimens. Changes in TN-T and TN-I isoforms might affect the regulation of the muscle contraction.

In our samples, *H. longifilis* displayed only one form of TN-C throughout growth. This calcium-binding protein was detected with difficulty by PAGE, being partly removed during myofibril sample preparation probably because of its higher solubility. For a few years we have been studying the synthesis of other calcium-binding proteins, the parvalbumins, in the course of development in several fish (Focant et al.

1992; Huriaux et al. 1996, 1997; Chikou et al. 1997). In *H. longifilis*, five isoforms are sequentially synthesised during growth (Focant et al. 1999). In cold-blooded vertebrates, parvalbumins are involved in Ca^{++} transfer from the myofibrils to the sarcoplasmic reticulum during relaxation of white muscles (Gillis and Gerday 1977). In addition to TN-C, the various parvalbumin isoforms could modulate the calcium flux during the contraction-relaxation cycle, according to the physiological needs of the fish.

Adult red muscle

Myofibrils from adult *H. longifilis* red muscle share no common component with larval, juvenile, or adult white muscle, except actin which is known to be highly conserved in vertebrate skeletal muscle (Vandekerckhove and Weber 1984). Myosin heavy and light chains appeared distinct from their white-muscle counterparts. As previously described (Huriaux et al. 1991), the relative electrophoretic mobility of red-muscle and white-muscle myosin heavy chains depends on the glycerol content of the gels and on the fish species studied. Under the experimental conditions giving the best separation of barbel red- and white-muscle heavy chains, the corresponding *H. longifilis* isoforms migrate together. Heavy-chain molecular mass estimates based on this electrophoretic method must thus be taken with caution. Although both red-muscle and white-muscle tropomyosins were classified as α-type tropomyosins, the former has a slightly higher molecular mass. TN-T and TN-I each exhibited only one isoform; the molecular masses determined for these isoforms were again somewhat higher than those determined for their white-muscle counterparts. Even TN-C, which appeared very constant throughout fish development, displayed a different isoform pattern in red and white muscle: two components were discerned in red myofibrils, differing in both molecular mass and electric charge from white-myofibril TN-C. These results tally with the division of labour between white and red fibres in fish.

In summary, the myofibrillar isoform pattern of *H. longifilis* varies substantially during its ontogeny and the transition between isoforms does not systematically follow ontogeny steps. No isoform characteristic of adult slow-red muscle appears in fast-white muscle, whether larval, juvenile, or adult stage. Sequential expression of specific isoforms at different life stages presumably reflects variations in the contractile properties of the muscle fibres, required by changes in functional demands. To this respect, it is worth noticing that a significant biochemical change in myofibrillar pattern takes place at the larva-juvenile transition characterized by a major expression of phosphorylated LC2, the disappearance of larval tropomyosin, an equal expression of larval and adult TN-T, and the emergence of adult TN-I. This biochemical transition coincides with changes in morphology, swimming and feeding behaviour of this catfish (Legendre and Teugels 1991; Vandewalle et al. 1997; Baras 1999). The present results correlate with the recent description of the successive appearance of three larval and two adult parvalbumin isoforms in the developing muscle of *H. longifilis* (Focant et al. 1999). These muscle proteins can constitute valuable tools in monitoring fish development.

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References

Baras, E. 1999. Sibling cannibalism among juvenile vundu under controlled conditions. I. Cannibalistic behaviour, prey selection and prey size selectivity. J. Fish Biol. 54: 82–105.

Bassani, V. and Dalla Libera, L. 1991. Myosin isoforms in white, red and pink muscles of the teleost sea bass (*Dicentrarchus labrax* L.). BAM 1: 153–156.

Bone, Q. 1978. Locomotor muscle. *In*: Fish Physiology. Vol. 7, pp. 361–424. Edited by W.S. Hoar and D.J. Randall. Academic Press, New York.

Brooks, S. and 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*. Mar. Biol. 117: 501–513.

Campbell, K.P., MacLennan, D.H. and Jorgensen, A.O. 1983. Staining of the Ca^{2+}-binding proteins, calsequestrin, calmodulin, troponin C, and S-100, with the cationic carbocyanine dye 'Stains-all'. J. Biol. Chem. 258: 11267–11273.

Chanoine, C., Saadi, A., Guyot-Lenfant, M., Hebbrecht, C. and Gallien, C.L. 1990. Myosin structure in the eel (*Anguilla anguilla* L.). Demonstration of three heavy chains in adult lateral muscle. FEBS Lett. 277: 200–204.

Chanoine, C., Guyot-Lenfant, M., El Attari, A., Saadi, A. and Gallien, C.L. 1992. White muscle differentiation in the eel (*Anguilla anguilla* L.): changes in the myosin isoforms pattern and ATPase profile during post-metamorphic development. Differentiation 49: 69–75.

Chikou, A., Huriaux, F., Laleye, P., Vandewalle, P. and Focant, B. 1997. Isoform distribution of parvalbumins and of some myofibrillar proteins in adult and developing *Chrysichthys auratus* (Geoffroy St Hilaire, 1808) (Pisces, Claroteidae). Arch. Physiol. Biochem. 105: 611–617.

Crockford, T. and Johnston, I.A. 1993. Developmental changes in the composition of myofibrillar proteins in the swimming muscles of Atlantic herring, *Clupea harengus*. Mar. Biol. 115: 15–22.

Crockford, T., Wommack, K.E., Johnston, I.A., McAndrew, B.J., Mutungi, G. and Johnson, T.P. 1991. Inter- and intra-specific variation in myosin light chain and troponin I composition in fast muscle fibres from two species of fish (Genus *Oreochromis*) which have different temperature-dependent contractile properties. J. Muscle Res. Cell Motil. 12: 439–446.

Danieli Betto, D., Zerbato, E. and Betto, R. 1986. Type I, 2A and 2B myosin heavy chain electrophoretic analysis of rat muscle fibers. Biochem. Biophys. Res. Commun. 138: 981–987.

Focant, B. and Huriaux, F. 1976. Light chains of carp and pike skeletal muscle myosins. Isolation and characterization of the most anodic light chain on alkaline pH electrophoresis. FEBS Lett. 65: 16–19.

Focant, B. Huriaux, F. and Johnston, I.A. 1976. Subunit composition of fish myofibrils: the light chains of myosin. Int. J. Biochem. 7: 129–133.

Focant, B., Huriaux, F., Vandewalle, P., Castelli, M. and Goessens, G. 1992. Myosin, parvalbumin and myofibril expression in barbel (*Barbus barbus* L.) lateral white muscle during development. Fish Physiol. Biochem. 10: 133–143.

Focant, B., Vandewalle, P. and Huriaux, F. 1994. Myosin polymorphism during the development of the trout, *Oncorhynchus mykiss*. Arch. Int. Physiol. Biochim. Biophys. 102: B54.

Focant, B., Mélot, F., Collin, S., Vandewalle, P. and Huriaux, F. 1996. Distribution of myosin and parvalbumin isoforms during the development of the catfish. Arch. Physiol. Biochem. 104: B18.

Focant, B., Mélot, F., Collin, S., Chikou, A., Vandewalle, P. and Huriaux, F. 1999. Muscle parvalbumin isoforms of *Clarias gariepinus*, *Heterobranchus longifilis* and *Chrysichthys auratus*: isolation, characterization, and expression during development. J. Fish Biol. 54: 832-851.

Gillis, J.M. and Gerday, Ch. 1977. Calcium movements between myofibrils, parvalbumins and sarcoplasmic reticulum in muscle. *In*: Calcium-binding Proteins and Calcium Function. pp. 193–196. Edited by R.H. Wasserman, R.A. Corradino, E. Carafoli, R.H. Kretsinger, D.H. MacLennan and F.L. Siegel. Elsevier, Amsterdam, North-Holland.

Hecht, T., Oellermann, L. and Verheust, L. 1996. Perspectives on clariid culture in Africa. *In*: The Biology and Culture of Catfishes. Edited by M. Legendre and J.-P. Proteau. Aquat. Living Resour. 9 Special Issue: 197–206.

Heeley, D.H. and Hong, C. 1994. Isolation and characterization of tropomyosin from fish muscle. Comp. Biochem. Physiol. 108B: 95–106.

Heeley, D.H., Bieger, T., Waddleton, D.M., Hong, C., Jackman, D.M., McGowan, C., Davidson, W.S. and Beavis, R.C. 1995. Characterization of fast, slow and cardiac muscle tropomyosins from salmonid fish. Eur. J. Biochem. 232: 226–234.

Huriaux, F. and Focant, B. 1977. Isolation and characterization of the three light chains from carp white muscle myosin. Arch. Int. Physiol. Biochim. 85: 917–929.

Huriaux, F. and Focant, B. 1985. Electrophoretic and immunological study of myosin light chains from freshwater teleost fishes. Comp. Biochem. Physiol. 82B: 737–743.

Huriaux, F. Vandewalle, P., Philippart, J.C. and Focant, B. 1990. Electrophoretic comparison of myosin light chains and parvalbumins of trunk and head muscles from two barbel (*Barbus barbus*) populations. Comp. Biochem. Physiol. 97B: 547–553.

Huriaux, F., Vandewalle, P. and Focant, B. 1991. Myosin heavy chain isoforms in white, red and ventricle muscles of barbel (*Barbus barbus* L.). Comp. Biochem. Physiol. 100B: 309–312.

Huriaux, F., Mélot, F., Vandewalle, P., Collin, S. and Focant, B. 1996. Parvalbumin isotypes in white muscle from three teleost fish: characterization and their expression during development. Comp. Biochem. Physiol. 113B: 475–484.

Huriaux, F., Collin, S., Vandewalle, P., Philippart, J.C. and Focant, B. 1997. Characterization of parvalbumin isotypes in white muscle from the barbel and expression during development. J. Fish Biol. 50: 821–836.

James, R.S., Cole, N.J., Davies, M.L.F. and Johnston, I.A. 1998. Scaling of intrinsic contractile properties and myofibrillar protein composition of fast muscle in the fish *Myoxocephalus scorpius* L. J. Exp. Biol. 201: 901–912.

Johnston, I.A., Davison, W. and Goldspink, G. 1977. Energy metabolism of carp swimming muscles. J. Comp. Physiol. 114: 203–216.

Johnston, I.A., Cole, N.J., Viera, V.L.A. and Davidson, I. 1997. Temperature and developmental plasticity of muscle phenotype in herring larvae. J. Exp. Biol. 200: 849–868.

Karasinski, J. 1993. Diversity of native myosin and myosin heavy chain in fish skeletal muscles. Comp. Biochem. Physiol. 106B: 1041–1047.

Karasinski, J. and Kilarski, W. 1989. Polymorphism of myosin isoenzymes and myosin heavy chains in histochemically typed skeletal muscles of the roach (*Rutilus rutilus* L., Cyprinidae, Fish). Comp. Biochem. Physiol. 92B: 727–731.

Koumans, J.T.M. and Akster, H.A. 1995. Myogenic cells in development and growth of fish. Comp. Biochem. Physiol. 110A: 3–20.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, Lond. 227: 680–685.

Legendre, M. and Teugels, G.G. 1991. Développement et tolérance à la température des œufs de *Heterobranchus longifilis*, et comparaison des développements larvaires de *H.longifilis* et de *Clarias gariepinus* (Teleostei, Clariidae). Aquat. Living Resour. 4: 227–240.

Legendre, M., Teugels, G.G., Cauty, C. and Jalabert, B. 1992. A comparative study on morphology, growth rate and reproduction of *Clarias gariepinus* (Burchell, 1822), *Heterobranchus longifilis* Valenciennes, 1840, and their reciprocal hybrids (Pisces, Clariidae). J. Fish Biol. 40: 59–79.

Martinez, I. Ofstad, R. and Olsen, R.L. 1990. Myosin isoforms in red and white muscles of some marine teleost fishes. J. Muscle Res. Cell Motil. 11: 489–495.

Martinez, I. Christiansen, J.S., Ofstad, R. and Olsen, R.L. 1991. Comparison of myosin isoenzymes present in skeletal and cardiac muscles of the Arctic charr *Salvelinus alpinus* (L.). Sequential expression of different myosin heavy chains during development of the fast white skeletal muscle. Eur. J. Biochem. 195: 743–753.

Martinez, I., Bang, B., Hatlen, B. and Blix, P. 1993. Myofibrillar proteins in skeletal muscles of parr, smolt and adult Atlantic salmon (*Salmo salar* L.). Comparison with another salmonid, the Arctic charr *Salvelinus alpinus* (L.). Comp. Biochem. Physiol. 106B: 1021–1028.

Martinez, I. and Christiansen, J.S. 1994. Myofibrillar proteins in developing white muscle of the Arctic charr, *Salvelinus alpinus* (L.) Comp. Biochem. Physiol. 107B: 11–20.

McCubbin, W.D., Oikawa, K., Sykes, B.D. and Kay, C.M. 1982. Purification and characterization of troponin C from pike muscle: a comparative spectroscopic study with rabbit skeletal muscle troponin C. Biochemistry 21: 5948–5956.

Moss, R.L., Giulian, G.G. and Greaser, M.L. 1982. Physiological effects accompanying the removal of myosin LC2 from skinned skeletal muscle fibers. J. Biol. Chem. 257: 8588–8591.

O'Farrell, P.H. 1975. High resolution two-dimensional electrophoresis of proteins. J. Biol. Chem. 250: 4007–4021.

O'Farrell, P.Z., Goodman, H.M. and O'Farrell, P.H. 1977. High resolution two-dimensional electrophoresis of basic as well as acidic proteins. Cell 12: 1133–1141.

Otémé, Z., Hem, J.S. and Legendre, M. 1996. Nouvelles espèces de poissons-chats pour le développement de la pisciculture africaine. In: The Biology and Culture of Catfishes. Edited by M. Legendre and J.-P. Proteau. Aquat. Living Resour. 9 Special Issue: 207–217.

Pette, D. and Staron, R.S. 1990. Cellular and molecular diversities of mammalian skeletal muscle fibers. Rev. Physiol. Biochem. Pharmacol. 116: 1–76.

Pires, E., Perry, S.V. and Thomas, M.A.W. 1974. Myosin light-chain kinase, a new enzyme from striated muscle. FEBS Lett. 41: 292–296.

Rowlerson, A., Scapolo, P.A., Mascarello, F., Carpenè, E. and Veggetti, A. 1985. Comparative study of myosins present in the lateral muscle of some fish: species variations in myosin isoforms and their distribution in red, pink and white muscle. J. Muscle Res. Cell Motil. 6: 601–640.

Scapolo, P.A., Veggetti, A., Mascarello, F. and Romanello, M.G. 1988. Developmental transitions of myosin isoforms and organisation of the lateral muscle in the teleost *Dicentrarchus labrax* (L.). Anat. Embryol. 178: 287–295.

Schiaffino, S. and Reggiani, C. 1996. Molecular diversity of myofibrillar proteins: gene regulation and functional significance. Physiol. Rev. 76: 371–423.

Schleicher, M. and Watterson, D.M. 1983. Analysis of differences between Coomassie blue stain and silver stain procedures in polyacrylamide gels: conditions for the detection of calmodulin and troponin C. Anal. Biochem. 131: 312–317.

Sender, P.M. 1971. Muscle fibrils: solubilization and gel electrophoresis. FEBS Lett. 17: 106–110.

Syska, H., Perry, S.V. and Trayer, I.P. 1974. A new method of preparation of troponin I (inhibitory protein) using affinity chromatography. Evidence for three different forms of troponin I in striated muscle. FEBS Lett. 40: 253–257.

Vandekerckhove, J. and Weber, K. 1984. Chordate muscle actins differ distinctly from invertebrate muscle actins. The evolution of the different vertebrate muscle actins. J. Mol. Biol. 179: 391–413.

Vandewalle, P., Gluckmann, I., Baras, E., Huriaux, F. and Focant, B. 1997. Postembryonic development of the cephalic region in *Heterobranchus longifilis*. J. Fish Biol. 50: 227–253.

Weatherley, A.H. and Gill, H.S. 1985. Dynamics of increase in muscle fibers in fishes in relation to size and growth. Experientia 41: 353–354.

Yancey, P.H. and Johnston, I.A. 1982. Effect of electrical stimulation and exercise on the phosphorylation state of myosin light chains from fish skeletal muscle. Pflügers Arch. 393: 334–339.