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ISOFORM DISTRIBUTION OF PARVALBUMINS AND OF SOME MYOFIBRILLAR PROTEINS IN ADULT AND DEVELOPING *CHRYSICHTHYS AURATUS* (GEOFFROY ST. HILAIRE, 1808) (PISCES, CLAROTEIDAE)

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KEYWORDS*: Catfish,* Chrysichthys auratus*, development, muscle, myosin light chains, parvalbumin isoforms, polyacrylamide gel electrophoresis, troponin I.*

ABSTRACT

Polyacrylamide gel electrophoresis was used to analyse the distribution of parvalbumin, myosin light chain, and troponin I isoforms in white muscles of larval, juvenile, and adult Chrysichthys auratus *(catfish, siluriforms) and to study the kinetics of their synthesis. Parvalbumin isoform PA II was first detected from day 5 post-hatching and was the main "larval" isoform in this species. PA III appeared at the beginning of the juvenile stage but always remained the minor isoform, even in adult fish. Young mature specimens (approximately 12 cm long) displayed the highest total parvalbumin content. Adult-type myosin light chains were detected from day 8. Densitometric analysis confirmed the light-chain distribution typical of fish muscles, with a relatively high amount of LC_3 and a low amount of LC_1. We evidenced a "larval" form of troponin-I and its progressive replacement by an "adult" form.*

INTRODUCTION

Fish fast white muscles are characterized by an abundance of sarcoplasmic proteins called parvalbumins (PA). Parvalbumins speed up relaxation in coldblooded vertebrates (Rall, 1996). These proteins are polymorphic and bind calcium with a high affinity. The various isoforms (up to five in fish) possess a low molecular mass (12 kD) and a high negative charge (reviewed in Gerday, 1982). Which isoforms are present depends on the species (Focant *et al.*, 1988), and different isoforms have recently been shown to appear sequentially during the development of several fish species, suggesting a special physiological role suited to the developmental stage (Focant *et al.*, 1992, 1996; Huriaux *et al.*, 1996, 1997).

Myofibrils contain structural and regulatory proteins such as myosin and troponin. A distinctive feature of fish myosin is the higher amount of LC_3 than LC_1 light chain (Huriaux & Focant, 1985). Sequential appearance of different isoforms of heavy and light chains has been demonstrated in some species (Scapolo *et al.*, 1988; Martinez *et al.*, 1991; Focant *et*

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al., 1992, 1994b, 1995, 1996). The regulatory protein troponin is a complex of three subunits (TN-T, TN-I, TN-C). Embryonic forms of TN-T and TN-I have been observed in Atlantic herring larval muscles (1- and 7-day-old larvae) (Crockford & Johnston, 1993).

We have here conducted screening for parvalbumins, myosin light chains, and troponin I during the development of a Claroteidae species, *Chrysichthys auratus* (Geoffroy St. Hilaire, 1808), in the framework of a multidisciplinary survey of African catfish.

Chrysichthys auratus represents a source of protein for a great number of African countries and has been the subject of several ecological and/or morphological investigations (Laleye, 1995; Vandewalle *et al.*, 1995). Research on *Chrysichthys auratus* demography and population dynamics in relation to environmental factors has revealed a *type r* demographic strategy characterized by a maximal breeding potential (early maturity, high fertility) (Laleye, 1995). This effort to reproduce independently of growth has favoured the preservation of populations in their environment as conditions have increasingly worsened. We have aimed here to contribute basic knowledge towards understanding the biology of *Chrysichthys auratus*. This is the first biochemical approach to this species. Muscle proteins were analysed by several electrophoretic techniques.

MATERIALS AND METHODS

With a view to breeding, mature couples of *Chrysichthys auratus* were caught in the lower valley of Ouémé river (Bénin) using the bamboo and PVC tube technique (Laleye, 1995). They were bred in aquaria (non-circulating aerated water, mean temperature: 27°C) until egg fertilisation. The larvae were reared on a diet of artemia nauplii until day 22 posthatching. Sets of 50 specimens were taken on days 5, 8, 10, 15, 18, 20, and 22 post-hatching (Fig. 1). The average total length in these sets was respectively 0.75, 0.9, 1.1, 1.2, 1.25, 1.3, and 1.35 cm. Juvenile and adult fish (3 to 25 cm) of the same species were caught in fishponds in Godomey (Cotonou-Bénin). All samples were frozen until analysed in Liège (Belgium).

We used the dissection method of Focant *et al.* (1992). Total parvalbumins were isolated from white muscle sarcoplasmic extract by heating at 100°C for 5 min and centrifuging. Good separation of the different PA isoforms according to their electric charge was obtained by polyacrylamide gel electrophoresis (PAGE) at pH 8.6 in the presence of 10% glycerol (Focant *et al.*, 1992).

Myofibrillar components were obtained from the myofibril pellet resulting from centrifugation of muscles conserved in preservative solution. They were incubated at pH 6.8 with sodium dodecylsulfate (SDS) and separated according to molecular mass by electrophoresis on discontinuous SDS-polyacrylamide gels (Laemmli, 1970). Only myosin light chains and troponin I were examined.

Densitometer traces of the electrophoretograms (obtained with a *Biorad* Model GS-670 Imaging Densitometer) were computed with the Molecular Analyst/PC software. This yielded two types of quantitative data: the "relative concentration" of each isoform studied (i.e., the amount of isoform corresponding to a same total sarcoplasmic protein content; this quantity is expressed here in arbitrary units and is also called the "relative content" of the extract in the isoform studied) and the "stoichiometry" of the various isoforms (i.e., percentage of the total parvalbumin, myosin light chain or TN-I concentration represented by each isoform).

***Figure 1**. CHRYSICHTHYS AURATUS MORPHOLOGY AT DIFFERENT STAGES OF LARVAL*

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DEVELOPMENT (SCALE DRAWING: BAR 5 2 MM) AND AT THE ADULT STAGE. D: day, bp: pectoral fin bud, aan: beginning of the anal fin, bv: ventral fin bud, aad: beginning of the adipose fin, cc: beginning of the caudal fin hollow. There is no sample before 5-day because techniques used for other fish species showed no constituted muscular structures before this stage. 20- and 22-day larvae are not represented because they show no new morphological structure with regard to day 18.

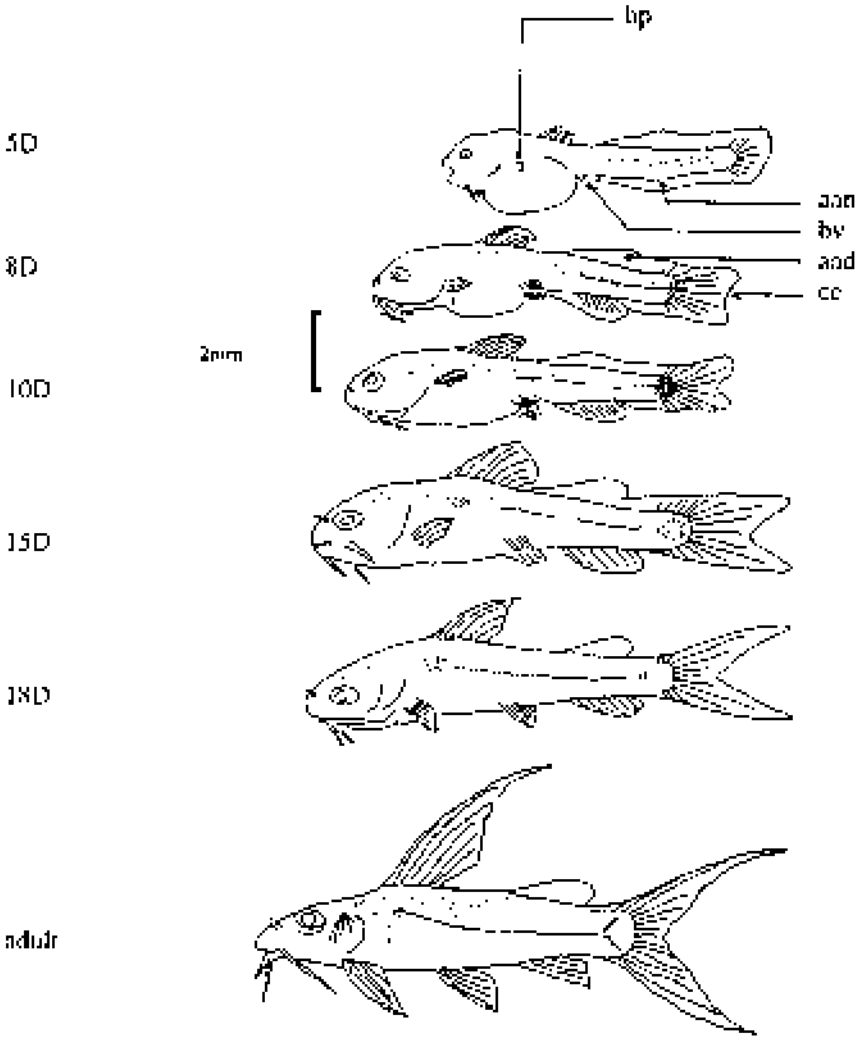

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***Figure 2**. 10% GLYCEROL PAGE (pH 8.6) OF SARCOPLASMIC EXTRACT HEATED TO 100°C (A, ADULT FISH) AND 20% ACRYLAMIDE SDS-PAGE (pH 8.4) OF MYOFIBRILLAR PROTEINS (B, 5 CM FISH). HC: myosin heavy chain, LC: myosin light chain, A: actin, TM: tropomyosin, TN: troponin.*

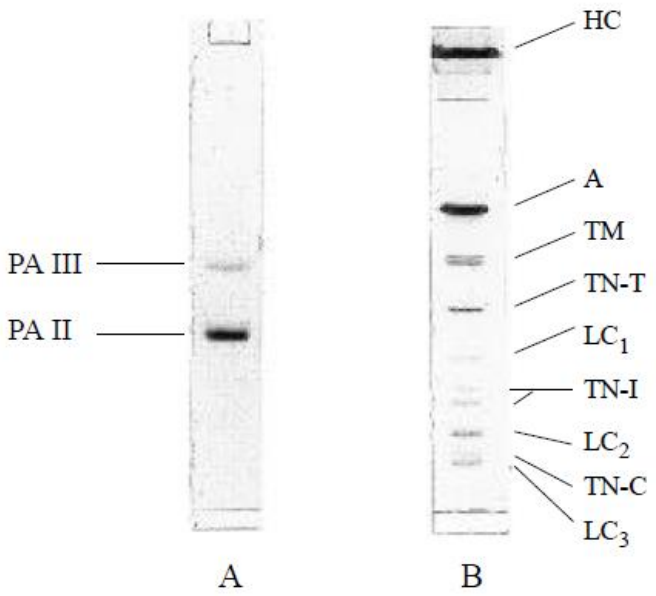
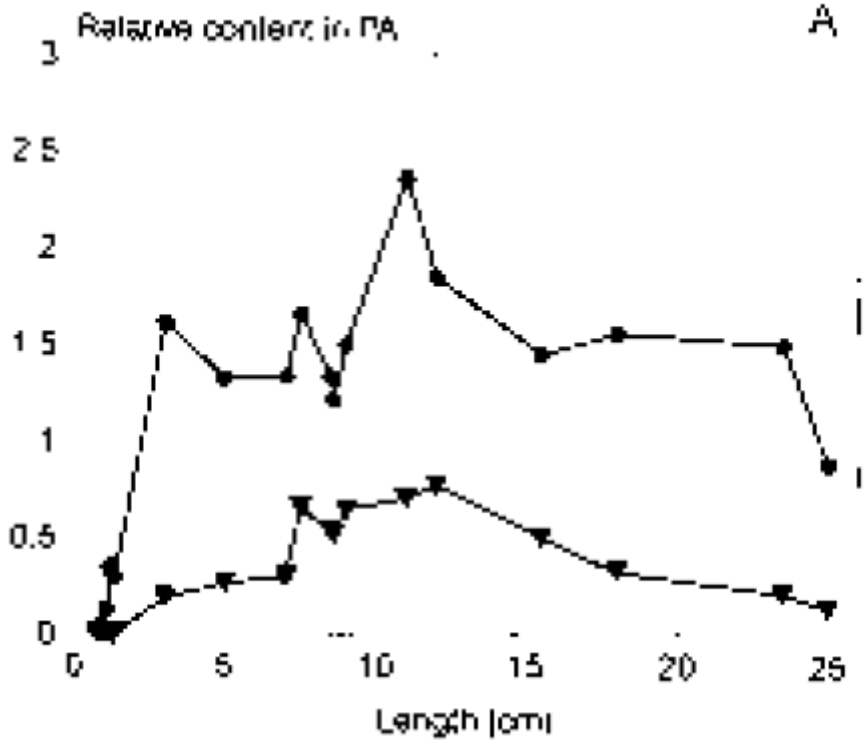


***Figure 3**. EVOLUTION OF THE CONCENTRATION OF THE TWO PARVALBUMIN ISOFORMS IN THE COURSE OF DEVELOPMENT FROM HATCHING TO A LENGTH OF 25 CM. A: relative content in arbitrary units; B: stoichiometry. ● PA II, ▼PA III.*



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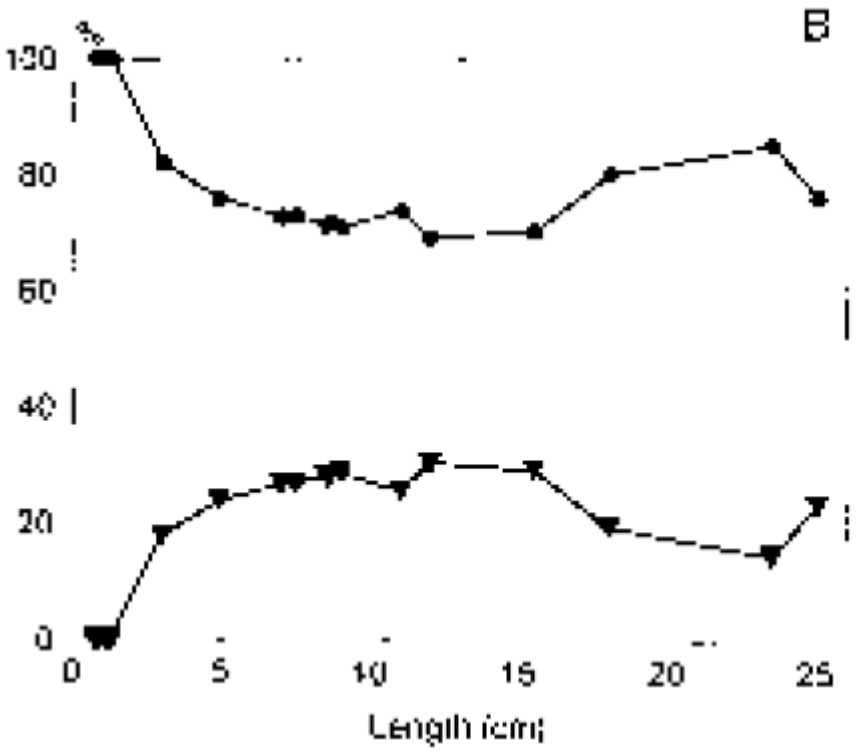

Figure 4. EVOLUTION OF MYOSIN LIGHT-CHAIN STOICHIOMETRY FROM HATCHING TO A LENGTH OF 25 CM. ▲: LC_1, ●: LC_2, ▼ : LC_3.

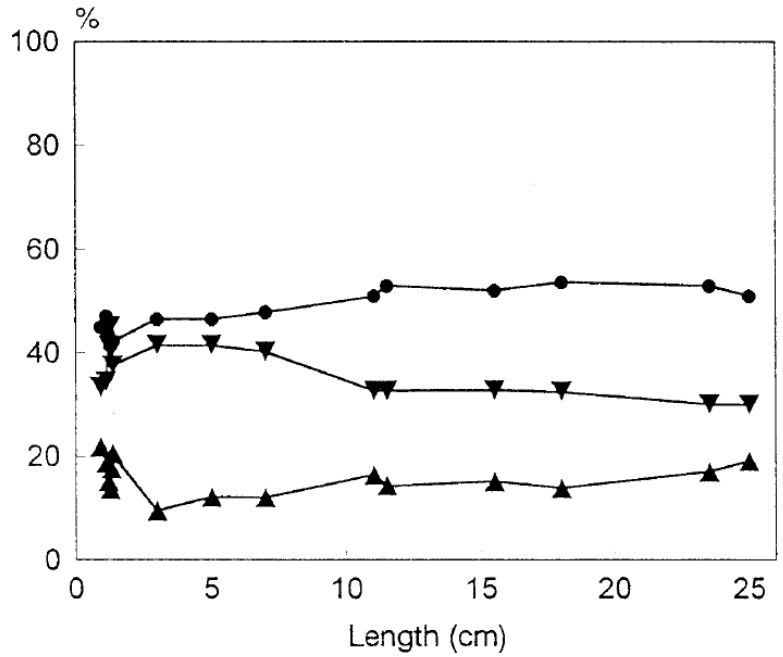

***Figure 5**. EVOLUTION OF THE STOICHIOMETRY OF THE TWO FORMS OF TN-I FROM*

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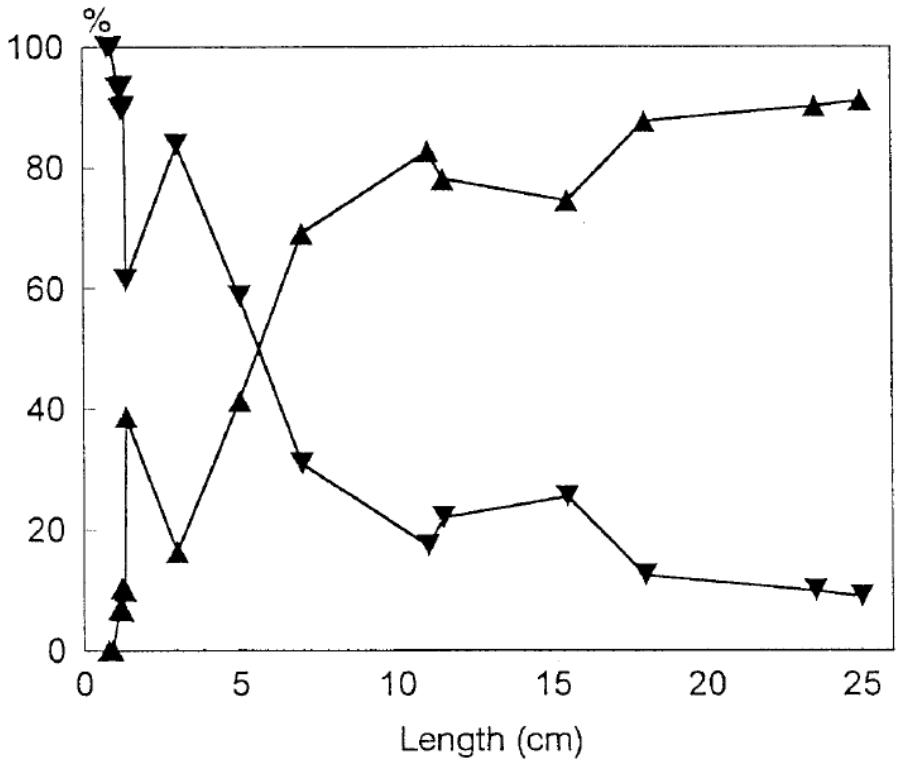
HATCHING TO A LENGTH OF 25 CM. ▼ "larval" TN-I, ▲ "adult" TN-I.



RESULTS

PARVALBUMINS

On the electrophoretograms of sarcoplasmic extracts from adult *Chrysichthys auratus* (25 cm), two isoforms were distinguishable: by order of decreasing mobility, PA II and PA III. These isoforms represented respectively 80 and 20% of the total PA content (Fig. 2A).

Sarcoplasmic extracts from specimens chosen at successive developmental stages were analysed under the same conditions. Figure 3 shows the evolution, during growth, of isoform relative concentrations (A) and stoichiometry (B). Isoform PA II appeared first and was detected as early as day 5 (0.75 cm); it accounted for 100% of the total PA content up to day 22 (1.35 cm), then decreased to 70% at 12 cm when its relative concentration was highest. This isoform then increased slowly in proportion, reaching 85% at 23.5 cm, but its relative concentration decreased by half. Isoform PA III was not detected until after day 22 (at 3 cm), reaching a maximum of 30% of the total parvalbumin content at 12 cm and stabilising in old fish at about 20%.

MYOFIBRILLAR PROTEINS

After SDS-PAGE of myofibril extracts from a juvenile specimen (5 cm), the gels displayed, from the cathode to the anode and by order of decreasing molecular mass (Fig. 2B): myosin heavy chains (HC), actin (A), the doublet of tropomyosin (TM), troponin T (TN-T), and six faster bands corresponding to myosin light chain LC_1, two bands of troponin I (TN-I), myosin light chain LC_2, troponin C (TN-C), and myosin light chain LC_3.

Myosin light chains were visible only from day 8 (0.9 cm). Densitometry indicated that the

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relative proportions of these light chains varied little during development (Fig. 4). The LC_2 content remained predictably at 50% while the amount of LC_1 started at 20%, dropped to 10% in fish 3 cm long, then again slowly increased to reach 20% in adult fish. The proportion of LC_3 varied inversely with respect to LC_1, its titre always remaining higher than that of LC_1, as in all fish myosins.

Two forms of TN-I appeared successively, varying in relative proportion according to fish development. A first form with a molecular mass of 23.5 kD was detected on day 5 (0.75 cm), rapidly decreasing in proportion as the fish progressed through the larval stages (Fig. 5). This "larval" form of TN-I was gradually replaced by another form with a slightly lower molecular mass (21.5 kD), called the "adult" form. Fish 5 cm long contained both forms in equal amount (cf. Fig. 2B). The adult form represented 90% of total TN-I in old fish.

DISCUSSION

Our data on parvalbumin isoforms in *Chrysichthys auratus* muscles show species-specific distribution and stoichiometry, characterized by the presence of a major isoform, PA II, and a minor one, PA III. Barbel (Focant *et al.*, 1992; Huriaux *et al.*, 1997), trout (Huriaux *et al.*, 1996), sea-bass (Huriaux *et al.*, 1996), several African cichlids (Focant *et al.*, 1994a), and the catfish *Clarias gariepinus* (Focant *et al.*, 1996) each show a distinctive qualitative and quantitative distribution of PA isoforms. Our observations confirm the species specificity of these proteins and their utility as biochemical indicators in systematic investigations.

As in barbel, trout, sea-bass, and *Clarias gariepinus*, the PA isoforms did not appear simultaneously but rather sequentially in the course of growth. In the fish just mentioned, isoform PA II always appeared in significant amount early in development, at stages corresponding to histologically well-structured myofibrils. PA II was called the "larval" isoform because its appearance is followed by that of other isoforms (PA III, PA IV, PA V) which become predominant in adult fish, where the proportion of "larval" form is much reduced. In *Chrysichthys auratus*, the trend is slightly different: the "larval" PA II persists during growth, still representing around 80% of the total PA pool in adults. The "adult" isoform PA III, synthesized from the juvenile stage onward, reaches a maximum of 30% at 12 cm, then decreases to remain at 20% in mature fish. In relation to total sarcoplasmic protein, synthesis of both forms appears to slow down steadily once the fish reach a length of about 12 cm. This slowing down is proportional to fish age.

In some previously studied fish, myosin light chains also appear to vary in molecular mass and proportion during development, studies having indicated the existence of "larval" forms (Focant *et al.*, 1992, 1994b). In *Chrysichthys auratus* muscles, myosin light chain distribution matches early in development the pattern usually encountered in adult fish (LC_3 in high amount). This does not necessarily mean a lack of transient myosin isoforms. Such forms might be revealed by analysis of myosin heavy chains (molecular mass and peptide mapping), not done here. We thus cannot role out the existence of "neonatal" myosins. The brief fluctuations in proportions of LC_1 and LC_3 between days 8 (0.9 cm) and 22 (1.35 cm) (Fig. 4) might be interpreted in this way.

Our results confirm the existence of two forms of TN-I (Crockford & Johnston, 1993) differing in molecular mass, one appearing at an early larval stage and one characteristic of maturity. At 6 cm both forms are present in equal amount. This is the first time that the polymorphism of the TN-I has been monitored in fish muscles in the course of development. Two isoforms of TN-T also occur from hatching to the adult stage, but their poor separation prevented their

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measurement.

In *Chrysichthys auratus*, the syntheses of parvalbumin, myosin light chain, and troponin-I isoforms clearly undergo stabilization when the fish reach a length of about 12 cm. At this time the parvalbumin titre peaks, light-chain stoichiometry becomes constant, and proportions of the two TN-I forms tend to stabilize, the "adult" form prevailing. These observations may correspond with maturity of the fish, in keeping with the observations of Laleye (1995) who established that *Chrysichthys auratus* is sexually mature at a length varying between 8 and 19 cm. The largest specimen examined (25 cm) can thus be considered old.

The sequential appearance of parvalbumin, myosin light chain, and troponin-I isoforms in the course of fish development reinforces the hypothesis that each particular isoform might play a role suited to a given developmental stage or stage-related physiological need (mobility, feeding, defence).

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